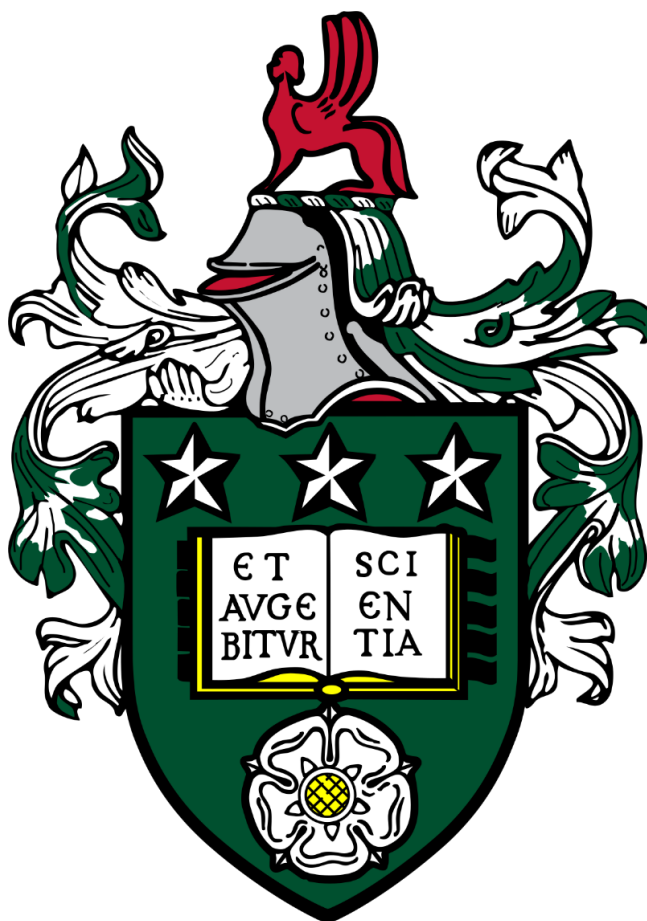


Pathway-dependent block copolymer self-assembly



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Declaration

The work described in this thesis was carried out at the University of Leeds between July 2018 and June 2023 and has not been submitted, either wholly or in part, for this or any other degree. All the work is the original work of the author, except where acknowledged by references.

Signature: _____

Adan Abdilahi Yusuf

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It's not about the journey or the destination; it's about the company along the way.

Abstract

Polymer nanoparticles are key for a wide range of applications, such as nano-reactors, encapsulating agents, and biological drug delivery. The synthesis and self-assembly of block copolymers have been studied previously, but a holistic understanding of how the self-assembly pathway affects the size, shape, and morphology of the polymer nanoparticles has yet to be reported. This work takes a functionalisable block copolymer (polydimethyl acrylamide-b-polydiacetone acrylamide) and synthesises it via Polymerisation Induced Self Assembly (PISA) for a range of block copolymer molecular weights. This results in well-defined block copolymers characterised by gel permeation chromatography (GPC), nuclear magnetic resonance spectroscopy (NMR), dynamic light scattering (DLS), and transmission electron microscopy (TEM). The PISA syntheses suggest a hydrophobic block length of 100 self-assembles into a mixed morphology of nano-objects, but increasing this to 200 results in pure vesicular phases. The pH and salt concentration were varied to physiological conditions (pH 7.5, 140 mM NaCl), and polymer vesicles persisted at neutral pHs and with added electrolyte concentration. The next step was to investigate alternative self-assembly pathways, such as Thin Film Rehydration (TFR) and Solvent Exchange (SE), with the same block copolymer used for the PISA syntheses. TFR proved to be unsuccessful with the targeted block copolymers at pH 2.5. Differential Scanning Calorimetry (DSC) was employed to identify any phase changes of the block copolymer in the solid state. SE was studied as a potential self-assembly pathway for the block copolymer. Methanol was chosen as a co-solvent, and low methanol (5-15%) SE experiments yielded spherical micelles. Upon increasing the methanol concentration to 50%, cylindrical micelles were observed. Upon methanol evaporation, the PDAM₅₀-b-PDAAM₃₀₀ sample produced a mixed phase of vesicles and kinetically trapped spheres. Increasing the pH led to the production of cylindrical micelles and spherical micelles; however, when salt was added, the charge was screened, leading to distorted vesicles. A key finding was that the volume of the hydrophobic block is affected when changing self-assembly conditions, which in turn changes the interfacial curvature of the block copolymer, leading to different morphologies being formed during SE. When methanol is increased to 50%, the added solvent plasticises the hydrophobic block, increasing its volume and decreasing the block-copolymer curvature, leading to worms. When 140

mM of NaCl was added to the SE mixture, this result suggests that the NaCl screens the charge on the polymer enough for the polymer to decrease the hydrophilic volume and reduce the interfacial curvature to form larger order nano-objects such as membrane-bound vesicles.

To better understand the morphological changes occurring during methanol evaporation, a three-hour SE evaporation was undertaken at various pHs and ionic strengths, monitoring the methanol content via benchtop NMR. The main morphological changes were from worms to spherical micelles before aggregation occurred. A membrane directing lipid (POPC) was used with the block copolymer during SE experiments to see if this could alter the morphology towards vesicles. This led to a range of spherical micelles being produced at various POPC block copolymer ratios. The self-assembly pathways studied prove that you can get a variety of morphologies from one starting material.

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Figure 6.1. Overview and the pitfalls of the inverted emulsion method. a) Schematic representation of the five steps of the inverted emulsion method with the ideal GUV production. Parameters, such as b) low lipid concentrations and insufficient incubation times, may result in an incomplete interfacial lipid monolayer that can compromise overall GUV yield and size. c) Centrifugation speed and duration also have an impact on the morphology and yield of the GUVs, as the droplets may remain in the oil phase or at the interface. ¹

Figure 6.2. Chemical structure of 6-carboxyfluorescein.

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Chapter 1

1.1. Introduction

1.1.1 Polymeric Nanoparticles

Polymeric nanoparticles (NPs) are colloidal particles within the size range from 1 to 1000 nm. These particles are made up of polymers, which can differ widely in terms of chemical and physical properties. This thesis explores the fabrication and self-assembly of polymer nanoparticles in aqueous conditions.

The term polymer has Greek origins, with the word meaning “many parts”. IUPAC defines a polymer as “A molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass.” The elongated structure of polymers was first discovered in 1920 by Herman Staudinger, who subsequently won the Nobel Prize in 1953 for his work. The continuously linked backbone of a polymer consists mainly of carbon atoms. Oxygen is also commonly present in polymer backbones, such as those of polyethylene glycol, polysaccharides (in glycosidic bonds), and DNA (in phosphodiester bonds). Polymer backbones can contain a variety of atoms, including Oxygen, Nitrogen, and Silicon, but the majority of synthetic polymers comprise a carbon backbone.

Polymers can be categorised into naturally occurring and synthetic polymers. Many materials that exist in nature are made from polymers, for example, materials such as hemp, shellac, amber, wool, silk and some natural rubbers. Natural polymers also play a part in life, as many biological macromolecules, such as proteins, starch, cellulose, and even DNA, are made up of smaller repeating units. It is no surprise, then, that the fundamental science behind natural polymers is being utilised to inspire new polymeric materials on both the macro and microscale. Synthetic polymers now constitute one of the most successful and useful classes of materials and possess a broad range of advantageous physical properties over natural polymers and lipids, such as good strength, flexibility, resistivity, and chemical inertness.¹

Synthetic polymers can have many different architectures, depending on the monomers utilised; some are presented below, Figure 1.1. These polymers are schematic representations of sequence-controlled copolymers, where copolymers are made up of two (diblock) and sometimes three (triblock) different monomers polymerised together.

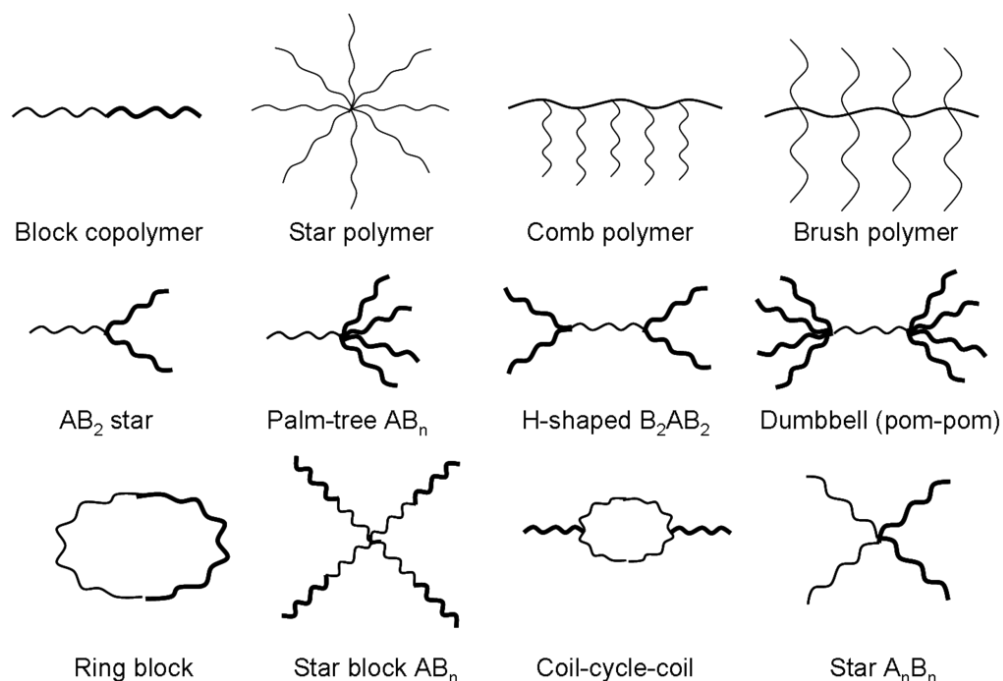


Figure 1.1: Examples of common architectures of sequence-controlled block copolymers.

Polymer Synthesis

Polymerisation can be defined as the process of combining many small molecules known as monomers into a covalently bonded chain or network. In nature, enzymes mediate many polymerisations, an example being the polymerisation of DNA by DNA polymerase.² Macromolecules with high molecular weight have numerous advantages in chemical and physical properties over traditional small molecular materials. A general method for the preparation of macromolecules is to convert small molecules into high molecular weight polymers by simple organic reactions, such as coupling reactions and polymerisation.^{3,4} condensation polymerisation^{5,6} and radical polymerisation⁷.

There are many different protocols to make polymers synthetically; free radical polymerisation has established itself as a relatively simple type of chain polymerisation, which can be applied to a wide range of monomers with good tolerance. In addition to this, free radical polymerisation can be effective in the presence of water and protic impurities. Also, free radical polymerisation can be carried out under relatively undemanding reaction conditions, provided that oxygen is eliminated. Oxygen is paramagnetic with two unpaired electrons, which can deactivate radical initiators. Oxygen is typically removed from the

solution prior to radical polymerisation by purging with an inert gas, such as high-purity nitrogen (N₂), to prevent inhibition of the polymerisation reaction. Most of the world's synthetic polymers are manufactured using this technique or variations of it, where polymerisation can be achieved in bulk, solution, dispersion, and emulsion. Free radical chain polymerisation consists of three fundamental steps, initiation, propagation, and termination, which are illustrated below, Figure 1.2.

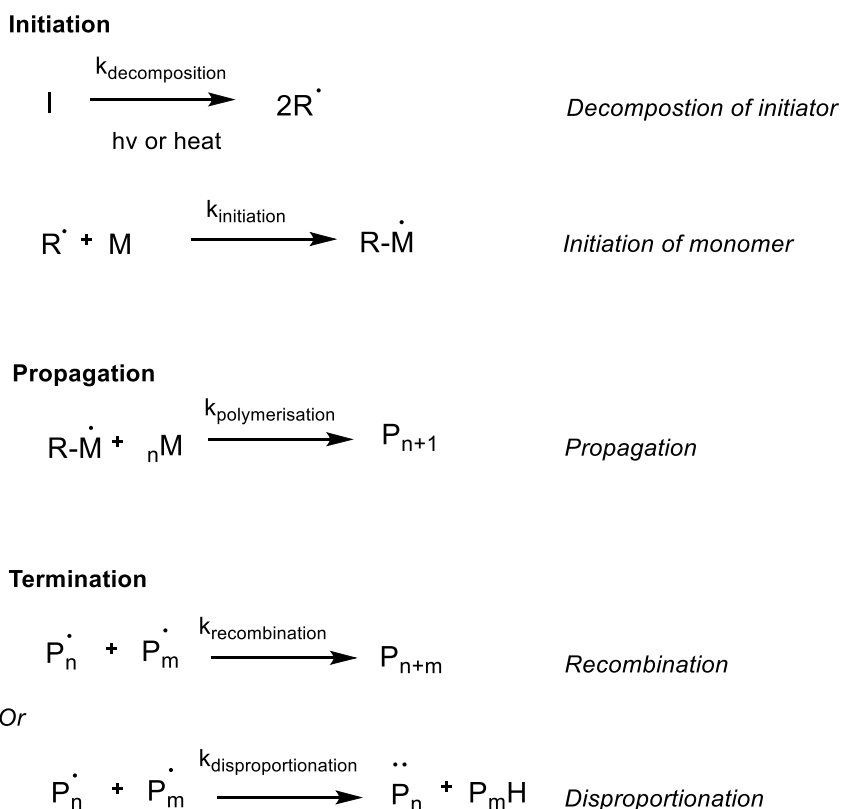


Figure 1.2: Elementary steps in traditional free radical polymerisation.

The first step of polymerisation reactions is initiation, which encompasses two separate processes. Initially, the radical initiator produces the original source of radicals when exposed to thermal or UV radiation. Common initiators often have a relatively weak bond that is susceptible to homolytic cleavage, such as the weak oxygen-oxygen single bond in peroxides.⁸ The radicals produced react to activate the monomer and form the R-M[·] species. This radical is now free to take part in propagation reactions. The ability of the primary radical to react with the monomer to initiate a polymer chain is defined as the initiator efficiency, *f*.⁹

During propagation, the R-M[•] Species react with additional monomer species repetitively to form a polymer radical chain P_n. Each addition creates a new polymer chain radical that has the same identity as the one previously, except that it is larger by one monomer unit until it undergoes termination. Termination typically occurs by a bimolecular reaction between radicals. This occurs via either a combination of two polymer chains or disproportionation, in which a hydrogen radical that is beta to one radical centre is transferred to another radical centre. This results in the formation of two polymer molecules, one saturated and one unsaturated.¹⁰

Kinetics of free radical chain polymerisation

Monomer consumption occurs during the initiation and propagation reactions. The rate of monomer consumption, which is synonymous with the rate of polymerisation, is given by equation 1.

$$\frac{-d[M]}{dt} = R_i + R_p \quad \text{equation 1.1}$$

Where R_i and R_p are the rates of initiation and propagation, respectively, however, the number of monomer molecules reacting in the initiation step is far less than the number in the propagation step, and is neglected. Hence, the polymerisation rate can be simply represented by the rate of propagation.¹¹

$$\frac{-d[M]}{dt} = R_p \quad \text{equation 1.2}$$

The rate of propagation, and therefore the rate of polymerisation, is the sum of many individual propagation steps. Since the rate constants for all the propagation steps are the same, one can express the polymerisation rate by

$$R_p = k_p [RM\cdot][M] \quad \text{equation 1.3}$$

Where [M] is the monomer concentration and [RM[•]] is the total concentration of all chain radicals.

Equation 3 for the polymerisation rate is not directly usable because it contains a term for the concentration of radicals. Radical concentrations are difficult to measure quantitatively, since they are often very low (and also present for short periods of time) (~10⁻⁸ M), and it is therefore desirable to eliminate [RM[•]] from equation 3. In order to do this, the steady-state assumption is made, where the

concentration of radicals increases initially, but almost instantaneously reaches a constant steady-state value.¹¹ The rate of change of the concentration of radicals quickly becomes and remains zero during the course of the polymerisation. This is equivalent to stating that the rates of initiation, R_i , and termination, R_t , of radicals are equal or

$$R_i = R_t = 2K_t[RM\cdot]^2 \quad \text{equation 1.4}$$

Rearrangement of Equation 4 and substitution into Equation 3 yields the rate of polymerisation as

$$R_p = k_p[M] \left(\frac{R_i}{2k_t} \right)^{\frac{1}{2}} \quad \text{equation 1.5}$$

Here, it is shown that the rate of polymerisation increases linearly with monomer concentration.¹¹

Living polymerisation

An important example of living polymerisation is the anionic polymerisation of styrene. Anionic polymerisation takes place with monomers possessing electron-withdrawing groups such as nitrile, carboxyl, phenyl, and vinyl. These polymerisations are initiated by nucleophilic addition to the double bond of the monomer. This nucleophile can be an ion such as hydroxide, alkoxide, cyanide, or a carbanion. The mechanism of the living anionic polymerisation of styrene is given in Figure 1.3. The carbanion can originate from an organo-metallic species such as an alkyl lithium or Grignard reagent. The coupling and disproportionation terminations for free radical polymerisations are not possible with anionic polymerisations due to coulombic repulsion of two negatively charged species. Termination has to involve a proton transfer from another species, such as from solvent, monomer, polymer, water, etc. Non-terminated polymeric species are referred to as “living” polymers. A “living” polymer does not grow indefinitely, nor does its molecular weight increase beyond certain limits. If the supply of monomer is depleted, then the growth is stopped. The chain end is still active, so if more monomer is added to the system, then the polymer will continue to propagate.

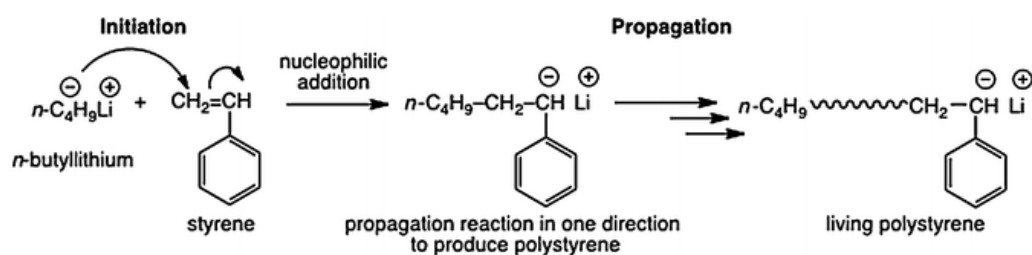


Figure 1.3. Mechanism for the anionic polymerisation of styrene. Figure adapted from Ishizone, T., Goseki, R. (2015)¹²

In addition to the aforementioned elementary reactions involved in traditional free radical polymerisations, there is also an inclination for polymer radicals to undergo chain transfer. Chain transfer is a polymerisation reaction by which the activity of a growing polymer chain is transferred to another molecule, as below.



In reality, this transfer can be to either a monomer, a polymer, a solvent, or a transfer agent. In an ideal living polymerisation, all chains are initiated at the beginning of the reaction, grow at a similar rate, and survive the polymerisation.¹¹ Some free radical polymerisation techniques can be controlled to the same extent as living polymerisation techniques. In these polymerisation techniques, chain transfer and chain termination elementary steps are minimised, which technically means they are pseudo-living. In a living polymerisation, the growing chain can only react with monomer, and no new chains are formed via chain transfer. One polymerisation technique that utilises this phenomenon is RAFT (Reversible Addition Fragmentation chain Transfer) polymerisation. Using this technique, complex polymeric architectures with controlled molecular weight, low dispersity, and varying functionality have been constructed. In such polymerisation reactions, not all chains can be simultaneously active. In RAFT polymerisation, there are chain transfer agents that are capable of reversibly deactivating propagating radicals. Because of this, the majority of living chains are maintained in a dormant form, with reaction conditions supporting a rapid equilibrium between the active and dormant chains (Figure 1.4)

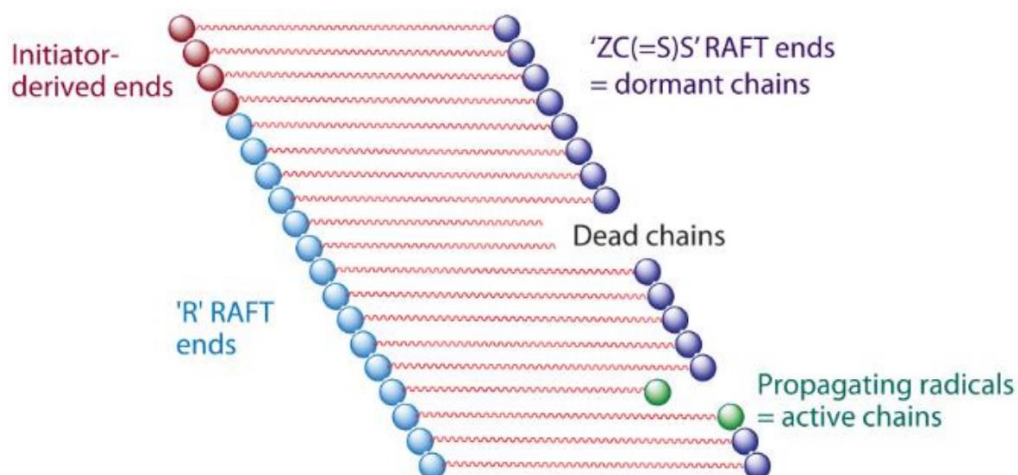


Figure 1.4: Schematic representation of RAFT Polymerisation.⁴ The number of chains of each type shown here is not in proportion to that expected for a well-designed experiment. On average, all living chains grow simultaneously and have equal chain length because equilibration of the dormant and active chain ends is rapid with respect to propagation. A RAFT agent is represented as 'ZC(=S)S'.¹³

A RAFT chain transfer agent typically has a dithioester group, comprising a sulphur-carbon double bond adjacent to a second sulphur atom. The carbon substituents R and Z affect the polymerisation reaction kinetics and, importantly, the degree of structural control. Initiation of the polymerisation reaction can be accomplished using thermal, photochemical, or redox methods. It is the proper choice of the RAFT reagents appropriate for a particular monomer and reaction medium that will largely determine the success of a RAFT polymerisation experiment.

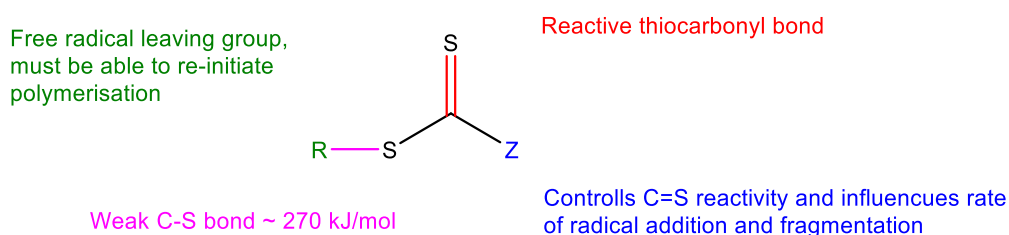
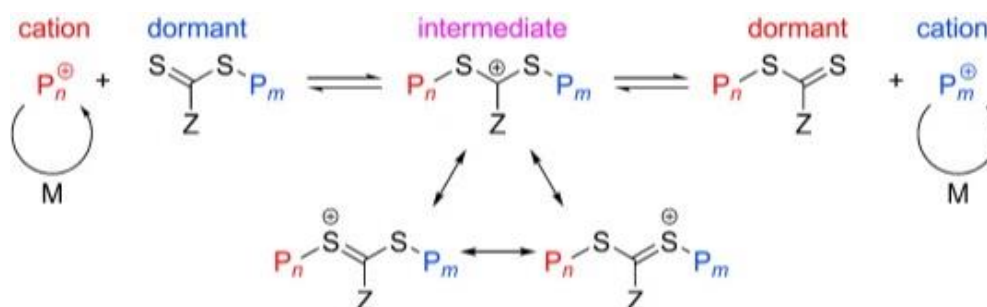


Figure 1.5: Typical chemical structure of RAFT chain transfer agents.

The reactions associated with RAFT equilibria are in addition to those (i.e., initiation, propagation, and termination) that occur during conventional radical polymerisation. In an ideal RAFT process, the chain transfer agent should

undergo rapid, reversible transfer between propagating polymer chains (Figure 6).



M= monomer , **P_n** = Polymer chain n, **P_m** = Polymer chain m

Figure 1.6. Mechanism of reversible chain transfer during RAFT polymerisation. Figure adapted with permission from Uchiyama, M.; Satoh, K.; Kamigaito, M. Cationic RAFT Polymerisation Using ppm Concentrations of Organic Acid. *Angew. Chem. Int. Ed.* 2015, 54, 1924–1928

Remarkably, the RAFT process does not suppress termination. Retention of the thiocarbonylthio groups in the polymeric product is responsible for the living character of RAFT polymerisation and renders the process suitable for synthesising block copolymers and end-functional polymers. Removal or transformation of the thiocarbonylthio group may be required for some applications. For example, end group functionalisation has been reported by Moad and co-workers in a review, which includes reactions with nucleophiles, radical-induced reactions, thermolysis, electrocyclic reactions and 'click' processes.¹³

Moad and Rizzardo first reported RAFT polymerisation in 1998.¹² RAFT polymerisation has many advantages over traditional polymerisation, which include the ability to control polymerisation of most monomers polymerisable by radical polymerisation.¹⁴ These include methacrylates¹⁵, methacrylamides,¹⁶ acrylonitrile,¹⁷ styrenes,¹⁸ dienes¹⁹ and vinyl monomers. RAFT polymerisation has been compared to anionic polymerisation and has some advantages. For example, anionic polymerisation suffers from the reactivity of the carbanionic group, which reacts readily with electrophiles in competition with monomer propagation, a caveat not experienced by RAFT polymerisation.²⁰

RAFT polymerisation is tolerant of unprotected functionality in the monomer or solvent (e.g., OH, NR₂, COOH, CONR₂, SO₃H). Additionally, RAFT

polymerisation is compatible with a wide range of reaction conditions, for example, bulk, organic or aqueous solution, emulsion and dispersion. RAFT polymerisation is also easy to implement and a relatively cheap methodology to produce well-controlled polymers. This highly controlled polymerisation technique allows for synthetic tailoring of copolymers, which can assemble into a multitude of complex architectures.

RAFT agents are classified into four major categories: aromatic dithioesters, trithiocarbonates, dithiocarbamates, and xanthates.²¹ The selection of an appropriate RAFT agent depends significantly on the monomer being polymerised. Aromatic dithioesters and trithiocarbonates are typically employed for "More Activated Monomers" (MAMs), including styrene, vinylpyridine, (meth)acrylates, and (meth)acrylamides.²¹ This compatibility arises from their ability to stabilise the intermediate radical formed when MAMs radicals add to the C=S bond.

3-((((1-carboxyethyl)thio)carbonothioyl)thio)propanoic acid (BM1429), which will be utilised for the RAFT polymerisations in this work, has the chemical formula $C_7H_{10}O_4S_3$ and molecular weight of 254.35 g/mol. BM1429 represents a specialised RAFT agent belonging to the trithiocarbonate class.²²

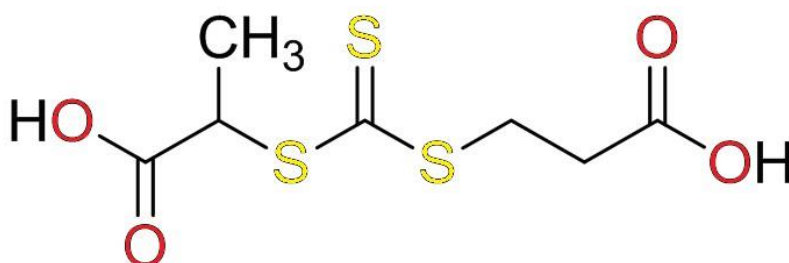


Figure 1.7: Chemical structure of BM1429 RAFT agent used in this work

Its structural composition features a trithiocarbonate core, linked to a 1-carboxyethyl group and a terminal propanoic acid, a unique chemical arrangement which offers properties that make BM1429 especially valuable in controlled radical polymerisation, particularly PISA. A distinctive characteristic of BM1429 lies in its ability to enable controlled radical polymerisation under acidic conditions, producing polymers with remarkably narrow polydispersities ($\bar{D} < 1.2$) in a range of polar solvents such as water, dioxane, and dimethyl sulfoxide (DMSO).

The effectiveness of BM1429 in controlling polymerisation parameters has been corroborated by experimental findings. Mathematical modelling of RAFT polymerisation kinetics has shown agreement with literature examples of acrylamide (used in this work) and acrylate monomers polymerisation in batch reactors.²³

Here, Wilding and coworkers found informative kinetic profiles for the RAFT polymerisation modelled using BM1429, including a delayed onset of polymerisation followed by rapid conversion, which can be attributed to heat transfer dynamics and exothermic reactions during the polymerisation process. When compared to other RAFT agents, BM1429 offers distinct advantages. Its carboxylic acid functionalities enhance its solubility in aqueous and polar media, making it particularly suitable for environmentally friendly polymerisation processes. Additionally, the commercial availability of BM1429 at 95% purity facilitates its adoption in both research and industrial contexts, potentially logistically allowing for future industrial scale-up of the work completed in this thesis.

BM1429 demonstrates excellent compatibility with various monomers, particularly those belonging to the MAMs category. The RAFT agent selection guide indicates that trithiocarbonates like BM1429 are particularly effective for controlling the polymerisation of acrylates, acrylamides, and related compounds.²⁴ This compatibility stems from the ability of the trithiocarbonate moiety to stabilise the radical intermediates formed during polymerisation of these monomers.

In practical applications, BM1429 has been successfully employed in polymerising monomers such as dimethylacrylamide (DMAm), diethylacrylamide (DEAm), and 4-acryloylmorpholine (NAM). These polymerisations yield well-defined polymers with controlled molecular weights and narrow dispersities, confirming the efficacy of BM1429 in mediating controlled radical polymerisation of acrylamide-based monomers.

1.1.2. Polymer self-assembly

Self-assembly is a process where a disordered system becomes an organised structure or pattern due to the local interaction within the system without external support.²⁵ Polymers are a great example of where this type of process might be found. Self-assembly processes involving amphiphilic polymers provide unique and new opportunities for designing novel materials for advanced applications in nanotechnology.²⁶

Amphiphilic molecules

Amphiphilic molecules are a general term that describes any compound that contains two distinct covalently bonded components with different affinity for the solvent in the same molecule, in which one part possesses a high affinity for polar solvents (such as water), and another part has a strong affinity for nonpolar solvents, such as hydrocarbons, ethers, and esters. Surfactants, polymer amphiphiles, and some lipid molecules, containing both hydrophilic and hydrophobic components, are typical examples of amphiphilic molecules.²⁷

Block copolymer self-assembly in bulk.

On a basic level, different polymers do not like to mix.²⁸ The thermodynamic incompatibility between the different blocks causes a spatial organisation into ordered morphologies on the nanoscale, with the production of novel structural features. Despite this diversity of macromolecular architectures, all block copolymers share a common physics: translational entropy drives the blocks to mix, while the enthalpic repulsion between the chemically different A and B units drives the blocks to segregate. Bulk self-assembly is due to the incompatibility between two differing blocks. As per the Second Law of Thermodynamics, the entropic contribution is weighted by temperature, so for a suitably designed polymer, it is possible for the blocks to intermix at sufficiently high temperature, generating the “disordered” structure shown in figure 1.7, while at lower temperature, the blocks spontaneously self-assemble into one of a diversity of mesophases, with the size scale governed by the polymer’s radius of gyration (order tens of nanometres).

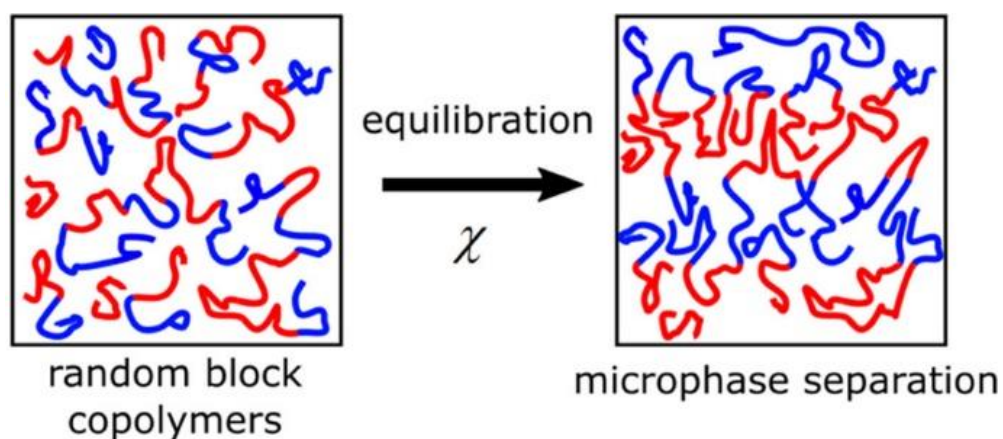


Figure 1.8. Depiction of disordered and ordered states of block copolymers in bulk, due to microphase separation. Figure adapted from V. Yu. Rudyak, D. E. Larin and E. N. Govorun, *Macromolecules*, 2022, **55**, 9345–9357.

Below the order-disorder transition (ODT), the mesophases exist as distinct “microdomains” made up purely of one of the copolymer ‘blocks’. The covalent bonds linking the dissimilar blocks are thus localised at the microdomain interfaces. While the schematic example in Figure 1.7 illustrates the case where the A and B blocks are of comparable lengths, the block ratio is easily varied during polymer synthesis to alter the mesophase structure. This results in the potential to form a multitude of ordered morphologies, including lamellae, hexagonally-packed cylinders, gyroid phases, and body-centred arrays of spheres (Figure 1.8). Furthermore, adding additional blocks to the copolymer can yield some remarkable morphologies due to the requirement for multiple blocks to phase separate.^{29,30}

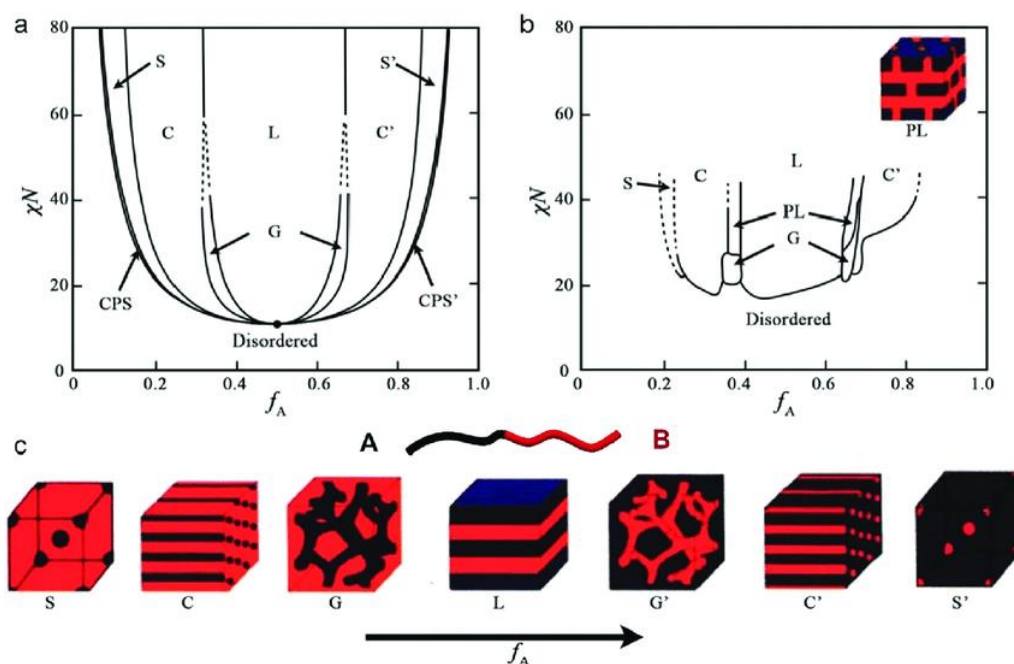


Figure 1.9. Self-assembly of BCPs in the bulk. (a) Theoretical phase diagram of AB diblock copolymers predicted by the self-consistent mean-field theory, depending on volume fraction (f) of the blocks and the segregation parameter, wN , where w is the Flory-Huggins parameter and N is the degree of polymerisation. (b) Experimental phase portrait of PI-*b*-PS copolymers, in which f_A represents the volume fraction of polyisoprene, PL = perforated lamellae. (c) Equilibrium morphologies of AB diblock copolymers in the bulk. In these figures, CPS and CPS₀ = closely packed spheres, S and S₀ = body-centred cubic spheres, C and C₀ = hexagonally packed cylinders, G and G₀ = bi-continuous gyroids, and L = lamellae. Reproduced with permission from Y. Mai and A. Eisenberg, *Chem. Soc. Rev.*, 2012, **41**, 5969–5985.²⁷

1.1.3. Self-assembly of amphiphilic block copolymers in water

Water is an inorganic, transparent, tasteless, odourless, and nearly colourless chemical substance. Its chemical formula, H₂O, indicates that each of its molecules contains one oxygen and two hydrogen atoms, connected by covalent bonds. The hydrogen atoms are attached to the oxygen atom at an angle of 104.45 °. Due to its polarity, a molecule of water in the liquid or solid state can form up to four hydrogen bonds with neighbouring molecules, resulting in a tetrahedral arrangement (Figure 1.9). Each of these intermolecular

interactions, which are present in most other liquids, result in elevated melting and boiling points compared to analogous compounds such as hydrogen sulphide.

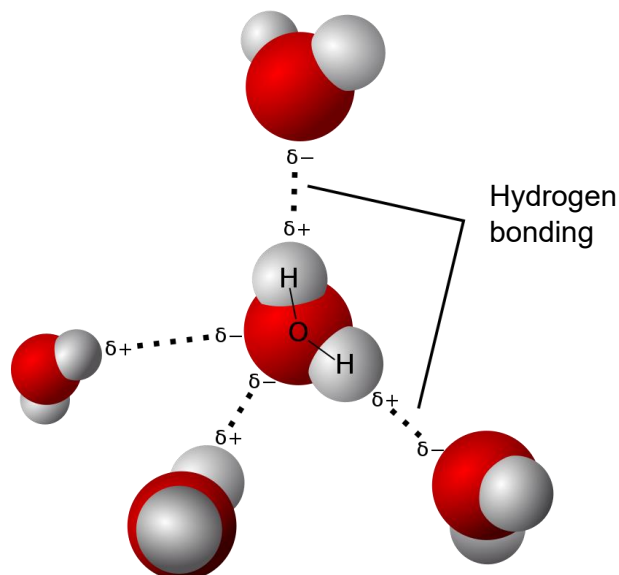


Figure 1.10. Tetrahedral arrangement of water molecules as a result of hydrogen bonding due to dipole moments in water molecules. Reproduced from https://en.wikipedia.org/wiki/Hydrogen_bond (Date accessed 01/01/23)

The hydrogen bonds in water also significantly influence it as a solvent. Hydrophilic or “water-loving” groups prefer to be in contact with water rather than with each other and, as a result, dissolve as a solute. These molecules have polar properties, such as the presence of electronegative atoms or groups in the structure. This can include simple dipole moments or the presence of charge. This enables the formation of dipoles, which can integrate into the hydrogen bonding network in water. Conversely, hydrophobic molecules tend to be non-polar molecules that are incapable of interacting with water molecules via hydrogen bonding. If a single non-polar molecule is placed into water, the hydrogen bonding within the water is disrupted. At least one of the dipoles on the water molecules in direct proximity must face the non-polar molecule, resulting in the loss of a hydrogen bond. However, in principle, the water molecules can rearrange themselves into a cage-like structure around the non-polar molecule in order to maximise the number of hydrogen bonds per water molecule. This reorganisation is highly unfavourable due to the reduction in

entropy. In real systems, this would result in phase separation (e.g., water and oil). This phenomenon is known as the hydrophobic effect.²⁹

$$\text{Equation 1.7} \quad \Delta G = \Delta H - T\Delta S$$

Where ΔG is Gibbs free energy, ΔH is enthalpy, and ΔS is the entropy of the system.

Amphiphilic block copolymers consist of both soluble and insoluble components connected via a covalent bond, so rather than phase separation, these undergo self-assembly, which accommodates the requirement for the hydrophilic block to dissolve and the hydrophobic block to precipitate. It is one of the most widely used template-based strategies for preparing and dispersing polymer nanomaterials. The formed aggregates are protected by hydrophilic coronae in aqueous solutions, which provides steric stabilisation as well as minimising the contact of the hydrophobic block with water. The most commonly reported nano-objects are spherical micelles, cylindrical micelles, and polymersomes, but more exotic phases such as bicontinuous structures, lamellae, and inverse structures are also possible. Figure 8. A crude prediction of the final morphologies can be made using the packing parameter principle (which is crudely related as p was originally designed for small molecule surfactants by Israelachvili), p , which relies on the relative composition of each block.²⁷

$$\text{equation 3.8} \quad P = \frac{v}{a_0 l_c}$$

Where v and l_c represent the volume and length of the hydrophobic segment, respectively, while a_0 denotes the contact area of the hydrophilic block, in dilute solution, PP indicates isotropic phases of spherical nano-objects (a.k.a spheres) are produced when $p = 1/3$, cylindrical micelles (a.k.a worms or rods) when $1/3 < p < 1/2$ and bilayer structures such as vesicles or polymersomes when $1/2 < p < 1$. The more complex and inverse morphologies tend to be formed when $p > 1$. The general principle can also be represented using the cone-column geometric change mechanism (Figure 1.10), which is also applicable to microphase separation of BCPs in the bulk. In reality, the packing parameter was designed for small-molecule amphiphiles, and in polymers, there is a significant contribution from chemical composition, overall molecular weight, and presence of charge. The adjustments of these influencing factors

afford flexible control over the morphology and dimensions of block copolymer aggregates. During self-assembly, the effects of the added precursors on the morphology of the resulting aggregates and the resulting pore architectures primarily originate from the variations in the interactions among corona chains in the presence of the precursors.

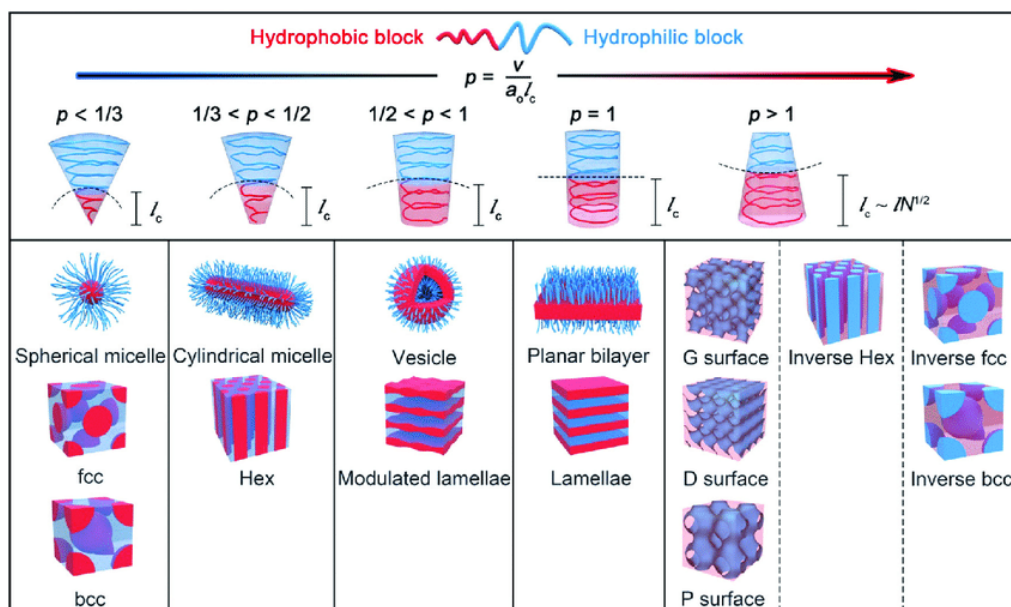


Figure 1.11. Schematic phase diagram of block copolymer self-assembly in solution, which presents the major morphologies. The self-assembled structures are determined by the packing parameter (p) of the copolymer chains, which correlates with their conical or columnar arrangements in the assemblies. Reproduced with permission from C. Li, Q. Li, Y. V. Kaneti, D. Hou, Y. Yamauchi, and Y. Mai, *Chem. Soc. Rev.*, 2020, **49**, 4681–4736.³²

1.1.4. Solvent Exchange

Solvent Exchange (SE) is a well-established post-polymerisation procedure that is based on the addition of a selective solvent to a dilute block copolymer solution.³³ The addition of this selective solvent leads to the insolubility of one of the polymer blocks. This process triggers block copolymer self-assembly into various nanoparticle morphologies once a certain solvent ratio is attained. Consequently, extensive dialysis is required to remove the suitable initial solvent. This approach is usually performed at a polymer concentration of ~ 1 –

3% w/w and might, hence, seem inefficient compared with other self-assembly techniques to be discussed later in this chapter. However, the solvent exchange method allows a wider variety of polymers than PISA since this method is independent of the polymerisation technique. In other words, the preferred polymer is not always easily accessible with a polymerisation technique that is well established for PISA. Therefore, the solvent exchange method is still widely used for the preparation of various application-oriented functional nanoparticles.^{34,35}

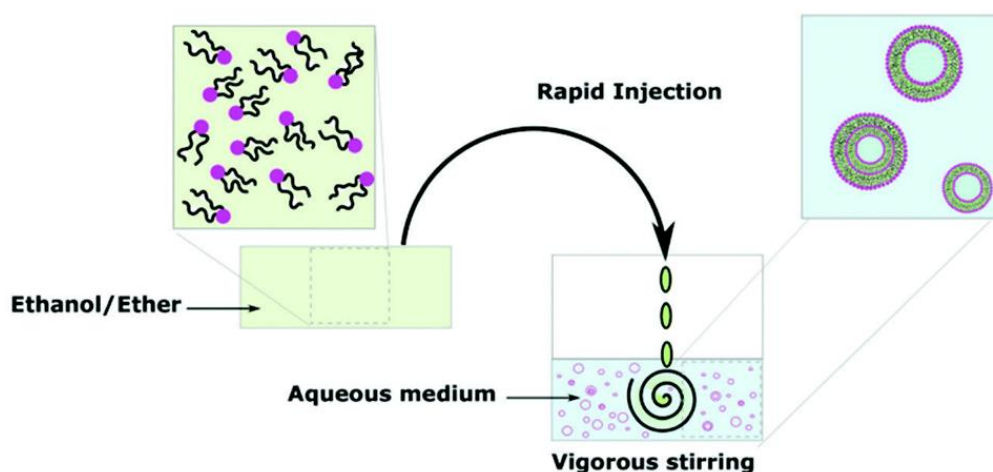


Figure 1.12. Illustration depicting the process of Solvent Exchange (SE) for an amphiphilic molecule. Reprinted with permission from E. Rideau et al., *Chem. Soc. Rev.*, 2018, **47**, 40.

The most common of the solvent displacement methods, Solvent Exchange (SE), exploits the differences in solubility of the polymer in organic solvent and in aqueous solution. Similar to thin film rehydration, SE is an easy and inexpensive process to perform, hence its popularity in the literature.³⁶⁻³⁸

An organic solvent that solvates all blocks of the amphiphile is selected to dissolve the block copolymer. Next, the polymer-solvent solution is injected into an aqueous solution where the hydrophobic block becomes insoluble due to an increase in interfacial tension between the block and surrounding water molecules. This interaction triggers the self-assembly of polymer nano-objects. The reverse process can also produce nanoparticles where water is injected into the polymer-solvent solution to trigger self-assembly. SE can produce a broad distribution of particle sizes and morphologies. However, the size

distribution can be homogenised by extrusion as well as changing the solvent and concentration of the solvent.

Variables in Solvent Exchange

One variable that is experimented with during SE is the addition method. The traditional approach of adding the amphiphile-solvent mixture directly into the aqueous environment is different from the other alternative, i.e., the reverse procedure of adding water into the amphiphile-solvent mixture.^{39,40}

The choice of co-solvent is also an important variable in the SE method. The selected solvent must fully dissolve the whole amphiphile, i.e., for a block copolymer, both the hydrophobic and hydrophilic blocks must be solvated by the organic solvent. The solvent must also be miscible with water and easily removed from the dispersion by evaporation or dialysis. Typically, in the literature, a wide range of solvents have been previously employed for SE, including methanol,⁴¹ tetrahydrofuran⁴²⁻⁴⁴ and acetonitrile⁴⁵.

Finally, the choice of solvent removal method is a key factor that should be discussed. For SE to work, the cosolvent must be volatile and be easily separated from the water-polymer mixture. There are two main methods to achieve this in the literature: dialysis,^{46,47} and evaporation.⁴¹ However, there seems to be a gap in the literature for the effective monitoring of cosolvent levels during evaporation or dialysis. This is needed to ensure that all the cosolvent has been removed from the system and to monitor any residual cosolvent still left in the system.

Throughout the solvent exchange methodology, there have been a few instances of phase diagrams being made for some systems. Battaglia and co-workers have understood the solvent exchange mechanisms and in 2005 published a phase diagram for Poly(ethylene oxide)-b-poly(butylene oxide) (PEO-PBO) block copolymers upon aqueous dilution, Figure 1.12.⁴² Battaglia and co-workers managed to isolate a range of morphologies, which include lamellae and vesicles, and imaged these phases via TEM.

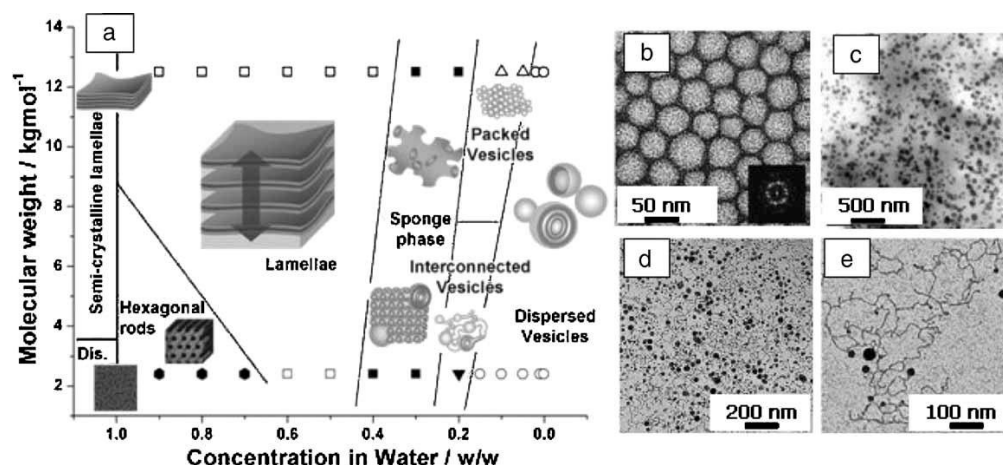


Figure 1.13. a) Isothermal phase diagram for bulk vesicle-forming PEO-PBO copolymers upon aqueous dilution. TEM images of b) hexagonally packed and c) dispersed vesicles, and d,e) interconnected vesicles. Reproduced with permission from G. Battaglia, A. J. Ryan, *Nat. Mater.* 2005, **4**, 869.

There has been a theme of kinetic control in these solvent exchange methodologies. Kinetic control is a term used to refer to chemical processes that are not governed by thermodynamic equilibria. This was showcased by Cui and co-workers, who investigated Block Copolymer Assembly via Kinetic Control.⁴⁷ In this work, through the kinetic manipulation of charged, amphiphilic PAA94-b-PMA103-b-PS44 block copolymers in solution, this group was able to generate different nanoscale structures with simple block copolymer chemistry (Figure 1.13). The technique relies on divalent organic counter ions and solvent mixtures to drive the organisation of the block copolymers down specific pathways into complex one-dimensional structures. This group illustrated how the effect of THF introduction causes a morphological transition from spherical micelles to disk-like micelles.

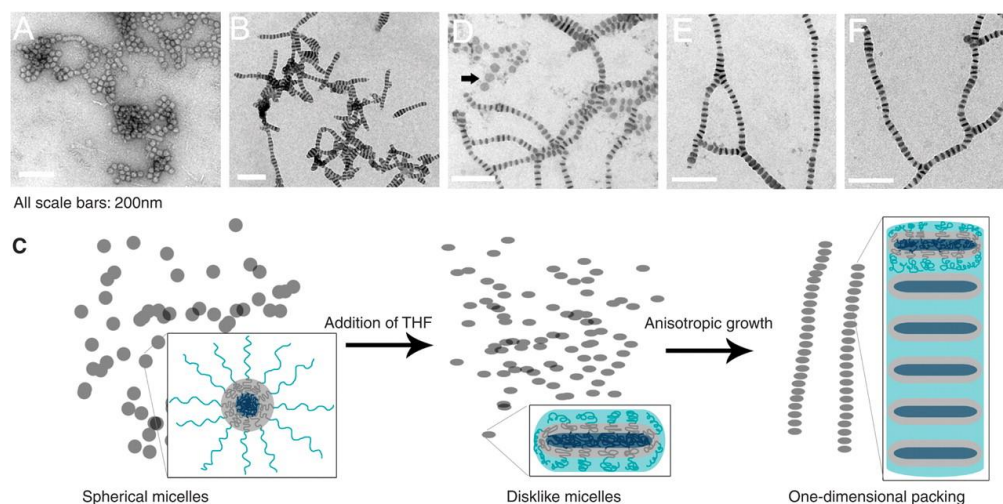


Figure 1.14. (A) Spherical micelles of PAA₉₄-*b*-PMA₁₀₃-*b*-PS₄₄ formed at the 1:4 ratio of THF to water in the presence of EDDA (molar ratio of amine groups: acid groups = 1:1). (B) TEM image of one-dimensional aggregation of spherical micelles immediately after introducing THF into the original solution to reach a 2:1 final ratio of THF to water. Further growth of these short structures led to a giant one-dimensional supra-assembly. (C) Growth mechanism of spherical micelles. The sphere-disk transition occurred first as THF was introduced. Anisotropic shape of disk-like micelles allows for one-dimensional preferred growth. The inserted schematic illustrates the proposed chain packing of spherical micelles, disklike micelles, and one-dimensional packing structures. (D) Separate disk-like micelles are marked with a black arrow. (E and F) Branches appear as a growth defect. The samples were stained with an aqueous solution of uranyl acetate. Reproduced with permission from H. Cui, Z. Chen, S. Zhong, K. L. Wooley, and D. J. Pochan, *Science*, 2007, **317**, 647-65.

In SE, mixed miscible solvents and their removal are a key stage of the polymer nanoparticle formation. The self-assembly is driven by solvent mixing. There are many reports of evaporation-induced self-assembly and self-assembly of BCPs from thin films; however, the reported use of solvent evaporation, specifically rotary evaporation, to form BCP nanoparticles is scarce.

Based on the available literature in the public domain on polymer nanoparticle formation, co-solvent evaporation can significantly impact nanoparticle morphology during solvent exchange processes like nanoprecipitation. One of the key effects includes particle size control dependent on the nature of the co-solvent and the rate of its evaporation during SE. The choice of solvents and

their evaporation rates can influence the final nanoparticle size. Faster evaporation of the organic solvent generally leads to smaller particle sizes, as it limits the time for polymer aggregation and growth. A lower polymer/solvent interaction parameter, indicating more unfolded polymer chains in the organic solvent, tends to lead to larger colloidal particles.³⁶

Shape anisotropy is also a key factor affected by co-solvent evaporation. Differential evaporation rates between co-solvents can induce anisotropic shapes. For example, elongated or rod-like particles may form if one solvent evaporates more rapidly, creating directional polymer nanoparticle self-assembly.⁴⁸

The specific effects of co-solvent evaporation depend on factors such as the polymer type, solvent choices, concentrations, and process conditions. Careful selection and control of co-solvents and their evaporation kinetics can be used to tune nanoparticle morphology for desired applications.⁴⁹

1.1.5. Thin-film Rehydration

First proposed in 1969, Reeves and colleagues used film rehydration with brain phospholipids, which consisted of simply hydrating a dry thin film of amphiphiles on glass or Teflon.⁵⁰ Thin-Film Rehydration (TFR), like solvent exchange, is a post-polymerisation self-assembly technique used to self-assemble a range of polymeric nano-objects. The first step in TFR is to dissolve the block copolymer into a good solvent for both the hydrophobic and hydrophilic blocks. This solvent must be volatile such that it can be fully removed from the mixture to leave a thin layer of film on the surface of the vessel. This film is rehydrated by adding water and leaving to equilibrate, often with gentle agitation/stirring. It is worth noting that encapsulants are added at this stage within the aqueous mixture used to rehydrate the thin film. Encapsulants can also be added to the solvent and then form part of the film. This would be the case for hydrophobic payloads.⁴⁰ This rehydration in aqueous environments causes swelling of the layers in the film, followed by budding to leave polymer vesicles detached from the surface of the film. This phenomenon is depicted in Figure 1.14.

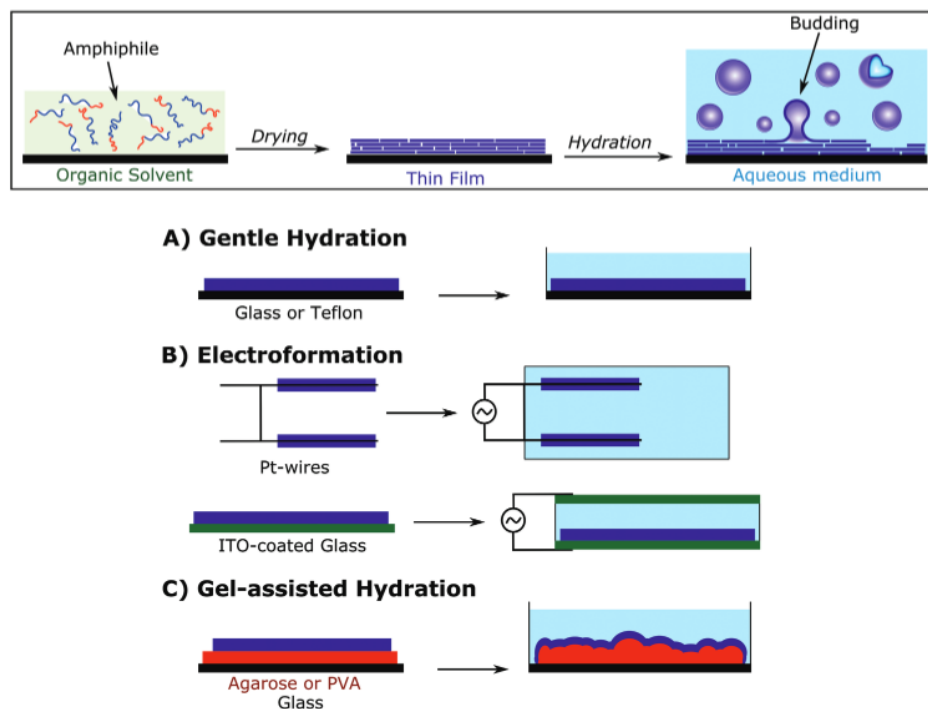


Figure 1.15. Depiction of the thin film rehydration method and its spin-off methodologies, gentle hydration, electroformation, and gel-assisted rehydration. Reproduced with permission from Rideau, E., Worm, F. R. & Landfester, K. Self-Assembly of Giant Unilamellar Vesicles by Film Hydration Methodologies. *Adv. Biosyst.*, 2019, **3**, 1–13 ⁴⁸

There are several variations in this method, including gentle rehydration, electroformation, and gel-assisted rehydration (Figure 1.14). The first solvent-free method and the simplest method to obtain solvent-free vesicles is called gentle hydration.

The mechanism behind gentle hydration is the entropically favoured self-assembly of amphiphiles into vesicles in an aqueous environment (Figure 1.15). This technique has been developed for phospholipids, generally with a lot of literature based on using gentle rehydration for the formation of liposomes. A typical alteration of this method is to use stirring or sonication to assist the rehydration process.⁴⁹

Electroformation is essentially a THR method aided by an AC current. Thin films are deposited on Pt electrodes, and an alternating current is applied, which causes repulsion in the thin films, which aids rehydration. By applying an alternating current to the electrodes, the rate of water diffusion across the

polymer film can be controlled. As a result, the degree of bulging and separation of the vesicles can be controlled to give a narrow distribution of polymersome sizes.⁴⁸ Despite electroformation being first reported 30 years ago and extensively reused since then, the exact underlying principles and mechanisms that induce the self-assembly of amphiphiles are not well understood. The assumption is that the electrical field, particularly the alternating current, facilitates bilayer separation and bending of the film by electro-osmotic effects.⁴⁹⁻⁵¹ Nonetheless, there has been a wide array of research into the use of electroformation to fabricate a range of polymeric nanoparticles, mostly polymer vesicles. Kunzler and coworkers managed to self-assemble giant biodegradable poly(ethylene glycol)-b-poly(ϵ -caprolactone) polymersomes via electroformation.⁵⁰

The final variation in the TFR protocol is called Gel-assisted rehydration. Gel-assisted rehydration was established quite recently in 2009, naturally initially for liposomes.⁵² The technique was developed to account for the then-poor performance of electroformation in physiological ionic strength buffers.⁶⁰ This method has shown great potential for forming solvent-free Giant Unilamellar Vesicles (GUVs), as it does not require any elaborate equipment; it is an economic, simple, fast, and efficient method.

Now, like the other two variations, the first stage in Gel-assisted rehydration is to dissolve the block copolymer in a good solvent for both the hydrophobic and hydrophilic blocks. Gel-assisted hydration consists of hydrating a dry amphiphile film above a dehydrated hydrophilic polymeric gel. Contrary to electroformation, the gel-assisted hydration's principles are better understood. Upon hydration of the polymeric support on the glass surface, the amphiphile film mechanically swells and is subjected to a higher surface exposure to hydration, facilitating self-assembly into GUVs. The amphiphile solution (in chloroform) is manually spread above the dehydrated hydrogel layer and is dried. The hydrating chamber, created with vacuum grease, O-rings, or commercially available cover wells, is filled with the desired aqueous solution (deionized water, buffer, etc.) and is hydrated over not more than 1 hour. For this technique, no special equipment is necessary (two glass slides and a chamber for hydration), contrary to microfluidics and electroformation. Thus, gel-assisted hydration requires only a few parameters, such as the time of hydration, concentration of amphiphile, spreading technique, and the gel used. In 2016, Greene and co-workers understood how the size distribution of giant

poly(ethylene glycol)-poly(butadiene) (PEO-PBD) polymersomes formed by gel-assisted rehydration may be controlled based on membrane fluidisation.⁵⁶

1.1.6. Polymerisation Induced Self-Assembly (PISA).

As polymer nanoparticles become more pertinent, the demand for their efficient synthesis has expanded. Previous methods, such as SE and THR, are limited because they operate on smaller weight percentages of polymer (1-3%).²⁹ Also, using these methods, it is not possible to synthesise and self-assemble the polymer in one pot without the use of solvents, unlike for PISA. Polymer synthesis methods that induce nanoparticle formation are highly sought after. Numerous polymerisation techniques that enable in situ nanoparticle preparation are precipitation polymerisation, dispersion polymerisation, and emulsion polymerisation.^{62,63} The previous two employ miscible monomers; the final two employ a polymer stabiliser or surfactant. These three techniques are based on a growing insoluble polymer chain or network that is stabilised by various means, and therefore, remains dispersed in the preparation media. An inherent restriction of these methods is that the resulting nanoparticle morphology is mainly spherical, lacking diversity in nanoparticle morphology. Furthermore, in the last two cases, stabiliser polymers or surfactants are not always desired in the final polymer product, and removal is regularly problematic.

Other commonly used nanoparticle preparation methods include polymerisation-induced self-assembly (PISA).^{64,65} This method employs block copolymers consisting of soluble blocks and insoluble blocks. The resulting spherical particles can, therefore, also be seen as polymer micelles; higher-order morphologies can also be achieved. To some extent, PISA is a type of dispersion (or emulsion) polymerisation—an insoluble polymer block formed from a soluble (or immiscible) monomer. Alternatively, conventional dispersion or emulsion polymerisation employs a separate polymer stabiliser or surfactant for the preparation of latex particles. These stabilisers are, therefore, not chemically attached to the “core-forming” polymer. PISA is based on the chain extension of an initial soluble precursor block, which acts as a steric stabiliser, with a second insoluble polymer block that forms the nanoparticle core in situ.

This polymerisation reaction triggers diblock copolymer self-assembly once a sufficiently high degree of polymerisation (DP) of the core-forming block is attained. PISA is efficient because polymer synthesis and assembly occur simultaneously. Furthermore, PISA can be performed at a range of final polymer concentrations (5–50% w/w).⁶³

The most observed nanoparticle morphologies obtained from PISA are spheres, worms, and vesicles. An example of PISA is shown below in Figure 1.15.

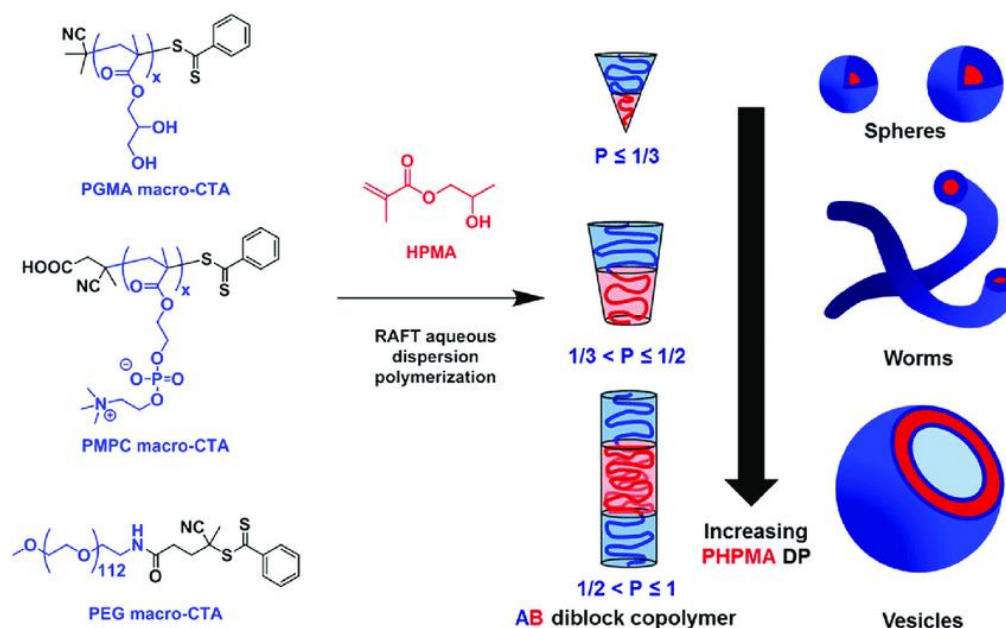


Figure 1.16. Polymerisation-induced self-assembly (PISA) of 2-hydroxypropyl methacrylate (HPMA). A variety of macromolecular chain transfer agents (macro-CTAs) were used to produce various assemblies depending on the degree of polymerisation of HPMA. Reproduced with permission from N. J. Warren and S. P. Armes, *J. Am. Chem. Soc.*, 2014, 136(29), 10174-10185.⁶²

1.1.7. Processing methods and self-assembly

The self-assembly of block copolymers into nano-objects can be of two types: equilibrium structures and non-equilibrium structures. A key feature of these materials is their tendency to become kinetically trapped in nonequilibrium states, meaning that the structures they adopt depend sensitively on the

processing route taken. While this makes it challenging to fully explore the landscape of possible morphologies, it also means that careful attention to the pathway of self-assembly can allow for remarkable control over the resulting nonequilibrium structures. Non-equilibrium structures depend greatly on the processing pathway used for their self-assembly. This phenomenon of pathway-dependent self-assembly was highlighted by Zhang and co-workers, who investigated two polymersome self-assembly pathways from one PISA formulation.⁵⁹ This group isolated a variety of exotic polymer nanoparticle morphologies from the same starting material (Figure 1.16)

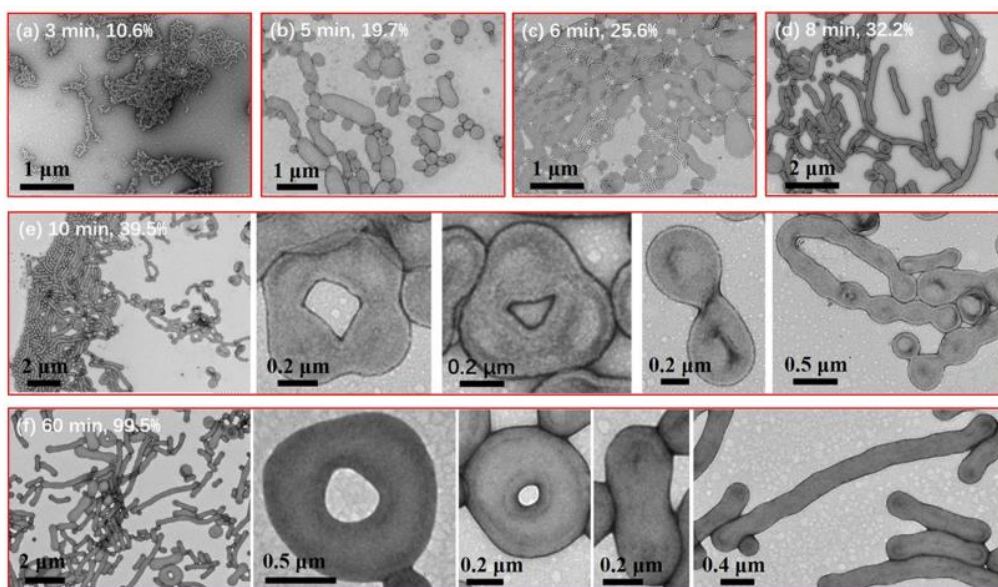


Figure 1.17. TEM images of Poly(Glycidyl Methacrylate)-b- N-(2-Hydroxypropyl) methacrylamide PGMA₆₂-PHPMA diblock copolymer nano-objects withdrawn during the kinetic study of aqueous photoinitiated RAFT-mediated dispersion polymerisation of HPMA (20% w/w, target DP of 1000) at 25 °C mediated by PGMA₆₂-CTA. Reproduced with permission from Q. Zhang, R. Zeng, Y. Zhang, Y. Chen, L. Zhang, and J. Tan, *Macromolecules*, 2020, **53**, 8982–8991.⁶³

This group found that the growth of polymersomes could be driven by temperature via two pathways: (i) at high temperatures from polymersomes with a thin membrane to polymersomes with a thick membrane, and (ii) at low temperatures from spherical polymersomes to tubular and donut-like polymersomes. Different from the typical polymersome growth mechanism, where polymersomes are prepared one by one in PISA at high temperatures, it was found that polymersomes grew both inward and outward during the kinetic

study. Furthermore, fusion between polymersomes in aqueous photo-PISA at room temperature encouraged the formation of tubular and donut-like polymersomes. Careful monitoring of the aqueous photo-PISA by TEM reveals a variety of novel intermediate morphologies, which provide important insights into the spherical polymersome-to-tubular and donut-like polymersome transition. Similar morphological evolutions were also observed when using a shorter macro-CTA, demonstrating that the polymersome growth mechanism reported in this study is general.²⁷ This is an example of how a processing variable, in this case temperature, can influence the size and morphology of polymeric nano-objects self-assembled in aqueous conditions.

Solvent Evaporation for Nanoparticle Formation

Solvent evaporation has emerged as a versatile and widely employed technique for the fabrication of polymer nanoparticles, particularly those derived from block copolymers. The process fundamentally relies on the dynamic non-equilibrium transition that occurs as solvent molecules depart from a polymer solution, inducing particle nucleation and subsequent growth.⁶⁹

This approach offers significant advantages over alternative methodologies, including relatively mild processing conditions, scalability, and precise control over particle characteristics. When combined with solvent evaporation techniques, BCPs can form nanoparticles with remarkably complex internal architectures and surface properties.

Fundamental Processes in Solvent Evaporation

The formation of polymer nanoparticles via solvent evaporation typically occurs through several distinct stages: initial dissolution of the polymer in an appropriate solvent, nucleation as the solvent begins to evaporate, growth of nascent particles, and eventual stabilisation of the final nanostructure.⁷⁰

For BCPs specifically, this process is further complicated by the microphase separation that occurs between distinct polymer blocks, leading to complex internal morphologies within the resulting nanoparticles.

Homogeneous nucleation represents a critical stage in particle formation, occurring when the polymer concentration exceeds its solubility limit in the evaporating solution. As described by Yabu and co-workers investigating spontaneous polymer nanoparticle formation, "Homogeneous nucleation and

successive growth of polymer particles takes place during the dynamic nonequilibrium process of solvent evaporation"⁶⁹. This nucleation phenomenon initiates the transformation from a homogeneous polymer solution to discrete nanoparticles with defined morphological characteristics.

Solvent Evaporation Techniques for Block Copolymer Nanoparticles

Emulsification-Solvent Evaporation

The emulsification-solvent evaporation technique represents one of the most established methods for preparing polymer nanoparticles. This approach follows a two-step process: first, emulsification of the polymer solution into an aqueous phase, and second, evaporation of the solvent to induce polymer precipitation and nanoparticle formation.⁷⁰ The technique offers versatility in terms of the types of polymers that can be processed and the morphologies that can be achieved.

For block copolymers specifically, this method enables the formation of structured nanoparticles with controlled internal architectures. The emulsification process creates droplets containing dissolved block copolymer chains, and as the solvent evaporates, these chains undergo self-assembly within the confined space of the droplet. Recent research by Shin and co-workers has demonstrated that the rate of solvent evaporation significantly influences the final morphology of the nanoparticles, with implications for their functional properties.⁷¹ This will be a factor studied within chapters 4 and 5 of this thesis.

Nanoprecipitation and Solvent Displacement

Nanoprecipitation has gained increasing attention as a method for producing block copolymer nanoparticles. In this approach, an organic phase containing the dissolved polymer is introduced to an aqueous phase, with the polymer being insoluble in the latter. The water-miscible organic solvent diffuses into the aqueous medium, leading to polymer precipitation and nanoparticle formation.⁷⁰

Recent investigations have revealed crucial insights into the role of solvent choice in determining nanoparticle size during nanoprecipitation. As elucidated in a comprehensive study by Bovone and colleagues in 2022. In the initial

stages of mixing, polymers assemble into dynamic aggregates that grow via polymer exchange. At later stages of mixing, further growth is prevented beyond a solvent-specific water fraction.⁷² This finding highlights the critical relationship between solvent properties and the kinetics of nanoparticle formation.

Evaporation-Induced Self-Assembly (EISA)

Evaporation-induced self-assembly (EISA) represents a specialised approach for fabricating nanostructured materials from block copolymers. This method takes advantage of the natural tendency of block copolymers to self-assemble into ordered structures as solvent evaporation increases the effective concentration of the polymer. According to recent simulation studies by Dreyer and co-workers, at a certain point, the repulsive interaction of the different blocks of the copolymer is not sufficiently diluted by solvent, and microphase separation commences at the critical density of the order–disorder transition.⁷³

In the context of membrane fabrication, EISA has been utilised to create isoporous block copolymer membranes via a two-stage process. First, the evaporation of a volatile solvent creates a polymer skin with self-assembled structures, followed by exchange between a remaining non-volatile solvent and a non-solvent to generate a macroporous support structure.⁷⁴

This approach demonstrates how solvent evaporation can be integrated into more complex fabrication protocols to yield functional nanostructured materials, in the case of this thesis, potential polymersomes.

Critical Factors Influencing Nanoparticle Formation

The rate at which solvent evaporates from a block copolymer solution has emerged as a critical parameter controlling nanoparticle morphology. As demonstrated in a seminal study, "The evaporation rate of solvent from emulsions encapsulating symmetric polystyrene-*b*-polybutadiene (PS-*b*-PB) determines the shape and internal nanostructure of micron-sized BCP particles."⁷¹ This research revealed a distinct morphological transition from ellipsoids with striped lamellae to onion-like spheres with decreasing evaporation rate.

The mechanistic explanation for this phenomenon lies in the differential diffusion rates of the solvent in distinct polymer blocks. At rapid evaporation rates, the alignment of domains occurs perpendicular to the particle surface, resulting in ellipsoidal particles with axial lamellar stripes. Conversely, slower evaporation rates provide sufficient time for the block copolymer to organise into onion-like structures, with the more solvent-compatible block forming the outermost layer.⁷¹

Recent advances in understanding nanoprecipitation have highlighted the concept of a "critical water fraction" at which nanoparticle growth becomes "arrested". Experimental investigations have demonstrated that "The nanoparticle size correlated to the experimental critical water fraction for all solvents, block copolymers, and mixing kinetics tested".⁷² This critical water fraction represents the point at which the polymer aggregates transition from a dynamic state to kinetically trapped nanoparticles.

The solvent-dependent nature of this critical water fraction explains why different solvents yield nanoparticles of varying sizes under otherwise identical conditions. This insight provides a physical basis for solvent selection in nanoprecipitation protocols and offers a predictive framework for controlling nanoparticle dimensions. The specific composition and architecture of block copolymers significantly influence their self-assembly behaviour during solvent evaporation. Factors such as the relative lengths of different blocks, their chemical compatibility with various solvents, and the presence of functional groups all contribute to the final nanoparticle morphology. For instance, molecular weight has been shown to affect the critical evaporation rate corresponding to morphological transitions in block copolymer particles, as well as the aspect ratio of ellipsoidal nanoparticles.⁷¹ This highlights the importance of polymer design in dictating nanoparticle characteristics and underscores the need for tailored synthesis approaches to achieve desired morphologies. For instance, one would expect a lipid, which is significantly smaller than the BCPs used in this thesis, to have different nanoparticle properties than those of pure BCP nanoparticle self-assembled by solvent evaporation.

Effect of ionic strength (salt) on Polymersomes morphology

The morphology of polymersomes can be sensitive to the ionic environment in which they are formed and maintained. Variations in ionic strength, particularly

through the addition of salts such as sodium chloride, can significantly influence the structural evolution of these vesicular assemblies. Understanding these effects is crucial, as they not only impact the physical characteristics of polymersomes but also their functional performance in applications such as drug delivery and nanoreactors.

Recent investigations have highlighted that increasing the ionic strength of the synthesis medium can induce pronounced morphological transitions in polymersomes. Such transformations are often attributed to osmotic imbalances arising from the interaction between the internal aqueous core of the polymersome and the surrounding salt-rich environment. As salt ions accumulate in the external solution, water molecules are drawn out from the polymersome core to hydrate these ions, leading to a reduction in internal volume and subsequent shape changes.

This was highlighted in recent literature from Abdelmohsen and co-workers based on biodegradable polymersomes comprising poly(ethylene glycol)-b-poly(D,L-lactide).⁷⁵ In this study, this group demonstrated the osmotically induced shape transformation of spherical polymersomes into high-aspect-ratio tubular rods upon increasing the ionic strength of the solution to 50 mM NaCl.

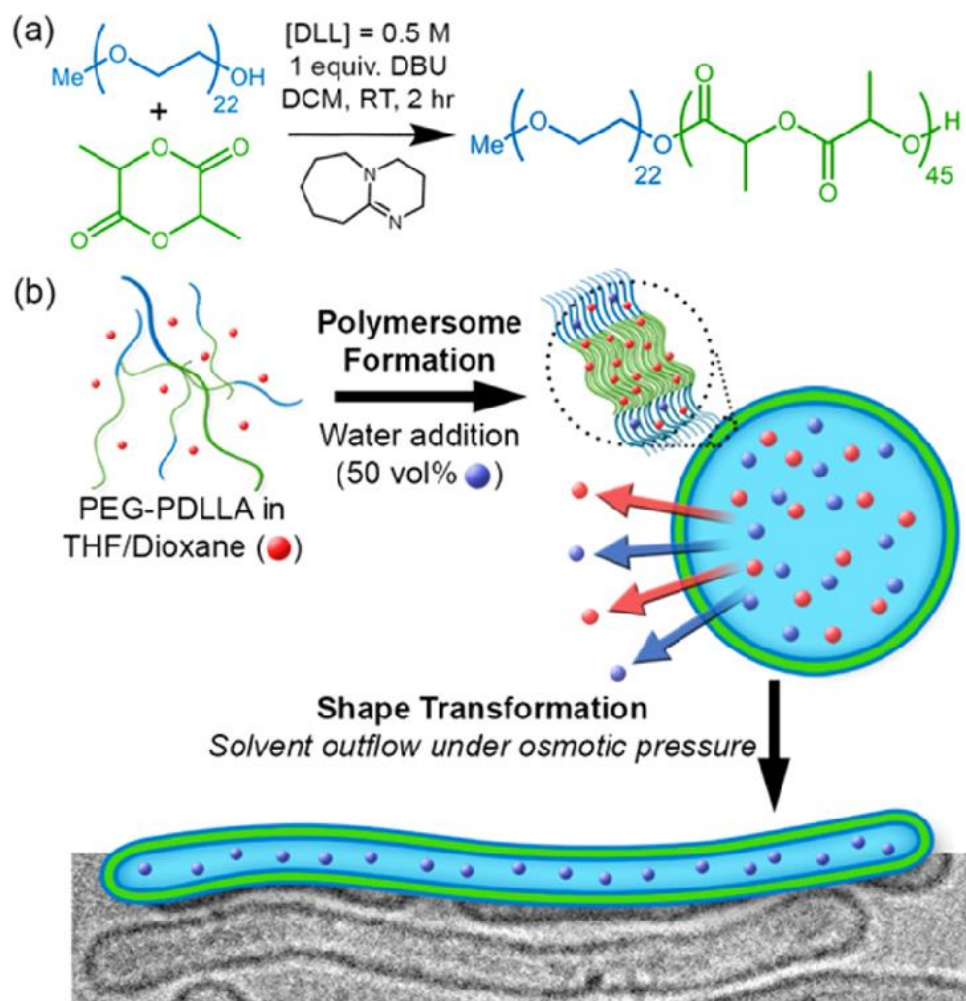


Figure 1.18: Schematic outlining (a) the organobase-catalysed synthesis of PEG-PDLLA and (b) the osmotically induced shape transformation of spherical polymersomes into nanotubes.⁷⁵

1.1.8. PISA of an Acrylamide-based block copolymer

Throughout this thesis, the main amphiphile of choice will be a copolymer of Polydimethylacrylamide and Polydiacetoneacrylamide (PDMAm-PDAAm). This is an amphiphilic block copolymer with PDMAm being the hydrophilic block and PDAAm being the hydrophobic block (Figure 1.18)

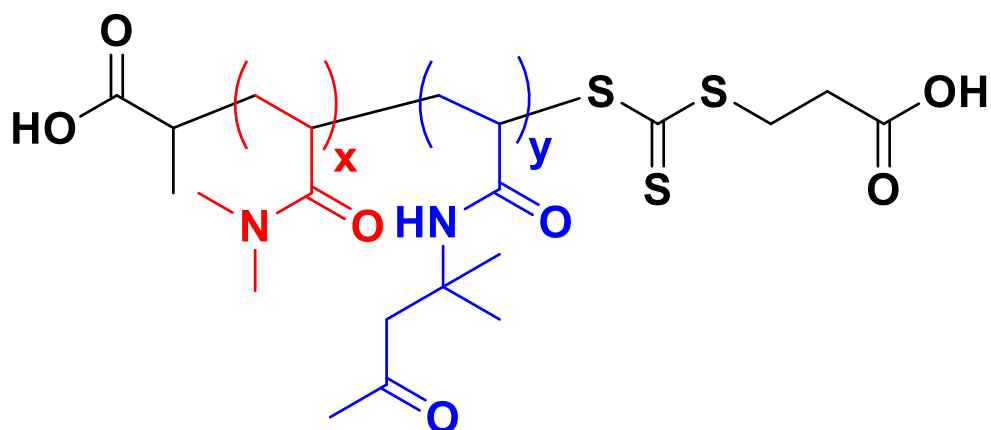


Figure 1.19 Chemical structure for PDMA_m-b-PDAA_m block copolymer used in this thesis.

The aqueous PISA of this block copolymer has been well studied in the recent literature. The earliest entry was from Byard and co-workers in 2017, who studied this polymer and its self-assembly behaviour via PISA in aqueous pH 2.5 conditions.⁷⁶ Byard and co-workers managed to produce a phase diagram for the PISA of this polymer by chain extending a range of different sized macro-CTAs with different lengths of DAAm, Figure 1.20. This included pure phases of polymer vesicles, worms and spheres. In addition to this, Byard and co-workers managed to cross-link the PDMA_m-b-PDAA_m polymer with a crosslinking agent, Adipic Acid Dihydrazide (ADH).

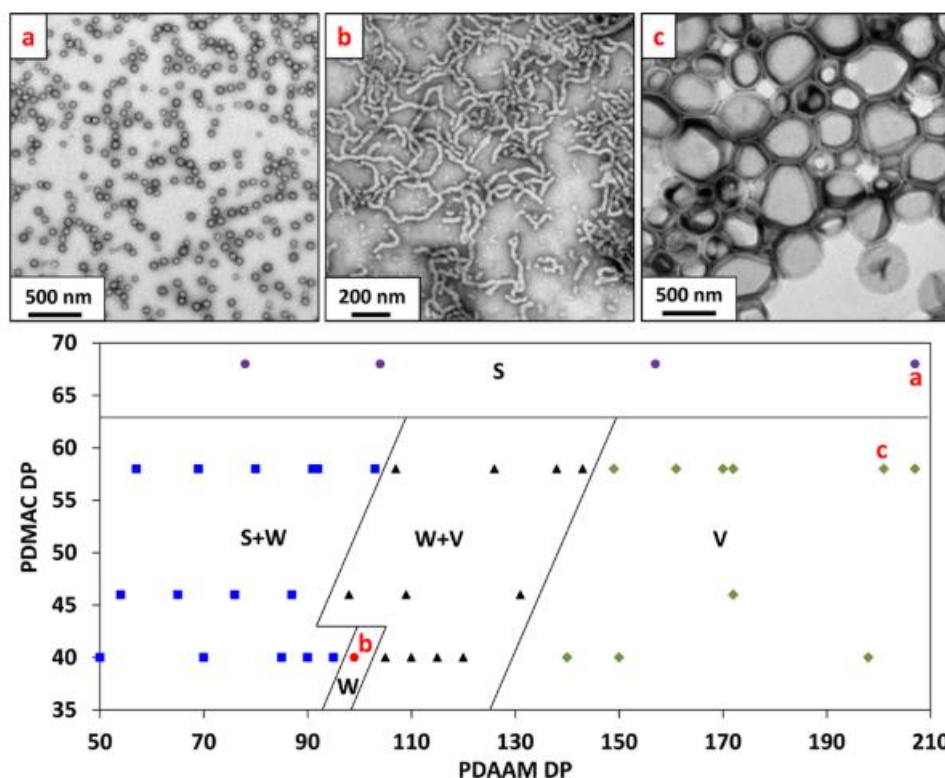


Figure 1.20. Representative transmission electron microscopy images showing pure sphere, worm, and vesicle morphologies obtained for 0.1% w/w aqueous dispersions of PDMAm_x-PDAAm_y diblock copolymer nano-objects at pH 2.5: (a) PDMAm₆₈-PDAAm₂₀₇; (b) PDMAm₄₀-PDAAm₉₉; (c) PDMAm₅₈-PDAAm₂₀₁. A phase diagram was constructed for a series of PDMAm_x-PDAAm_y diblock copolymer nano-objects. S = spheres, S + W = mixed spheres and worms, W = worms, W + V = mixed worms and vesicles and V = vesicles. Reproduced with permission from S. J. Byard, M. Williams, B. E. McKenzie, A. Blanz and S. P. Armes, *Macromolecules*, 2017, **50**, 1482–1493.

Following on from this early work into the PDMAm-PDAAm system, Wang and co-workers investigated temperature-induced morphological transitions of the PDMAm-PDAAm polymer made via aqueous PISA.⁷⁷ Wang and co-workers accomplished the polymerisation of the core-forming PDAAm, leading to the formation of pure lamellae, mixed lamellae/ vesicles, and pure vesicles as revealed by dynamic light scattering (DLS), transmission electron microscopy (TEM), atomic force microscopy (AFM), and scanning electron microscopy (SEM), Figure 1.20. They concluded that the PDMAm₃₀-PDAAm_x lamellae were found to span a wide space in the morphology phase diagram. Furthermore, in situ cross-linking of lamellae via statistical copolymerisation of DAAM with an asymmetric cross-linker allyl acrylamide and the effect of cross-linking density

on the colloidal and morphological stabilities were studied, representing the first report on in situ cross-linking of lamellae during PISA, Figure 1.21.

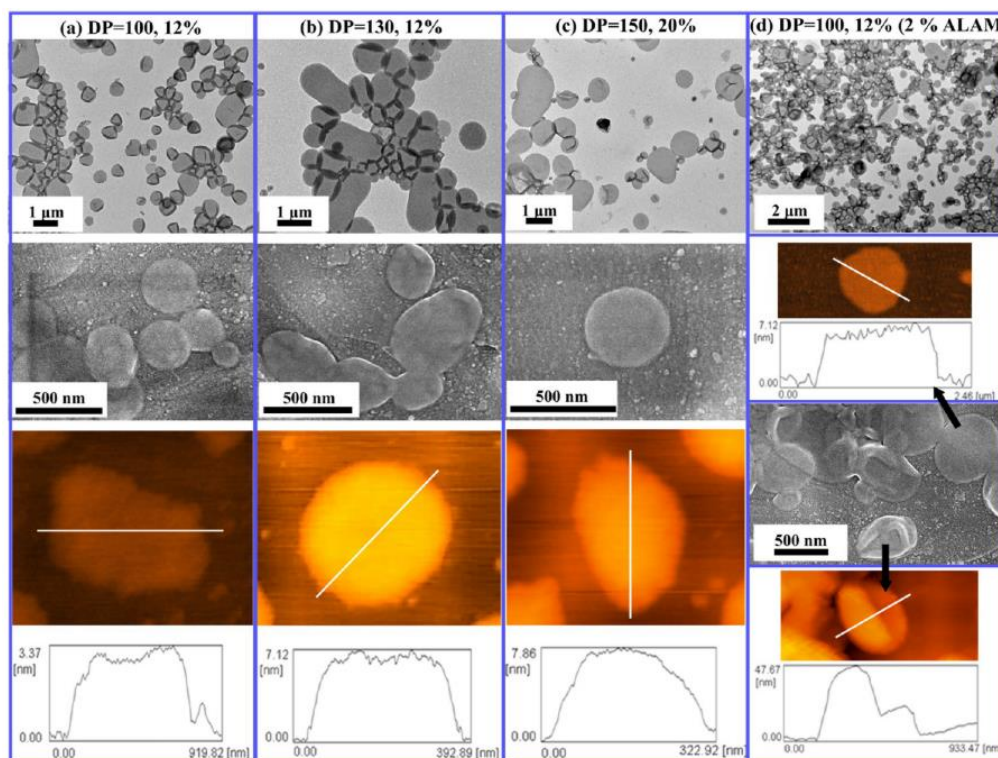


Figure 1.21. TEM, SEM, and AFM images for (a–c) linear PDMAm₃₀–b-PDAAm_x dispersions at various PDAAm DPs and solids (w/v), and (d) cross-linked PDMAm₃₀–P(DAAm₁₀₀–ALAM₂) dispersion. Reproduced with permission from X. Wang, J. Zhou, X. Lv, B. Zhang and Z. An, *Macromolecules*, 2017, **50**, 7222–7232.

Also, reversible, temperature-induced morphological transitions from lamellae to worms/spheres on cooling were investigated by DLS, TEM, ¹H NMR spectroscopy, and rheology. Finally, Wang and co-workers established that the kinetics of the temperature-dependent morphological transitions and the rheological properties could be tuned by the cross-linking density.

In 2018, Biais and co-workers experimented with this polymer further.⁷⁶ Biais and co-workers proposed a straightforward strategy to synthesise loop-stabilised particles in water via PISA. These structures can, in theory, be obtained through the synthesis of amphiphilic BAB triblock copolymers, starting from a hydrophilic middle block A, which is chain extended in an aqueous PISA process with two hydrophobic external blocks B. For this, symmetrical bifunctional poly(N, N-dimethylacrylamide) macro-RAFT agents with a central benzoic acid group and an alkyl chain as the Z group are used in the aqueous

dispersion polymerisation of diacetone acrylamide. For the first time, stable BAB flower-like particles were formed via PISA in pure water. Biais and co-workers highlighted that the colloidal stability and the resulting particle morphology (spheres, worms, vesicles) strongly depend on the degree of ionisation in the stabiliser loop, and thus on the pH at which PISA is performed. Furthermore, the influence of the length of the alkyl Z group (dodecyl and butyl alkyl chains) on the colloidal stability was studied; TEM images from this study are highlighted in Figure 1.22.

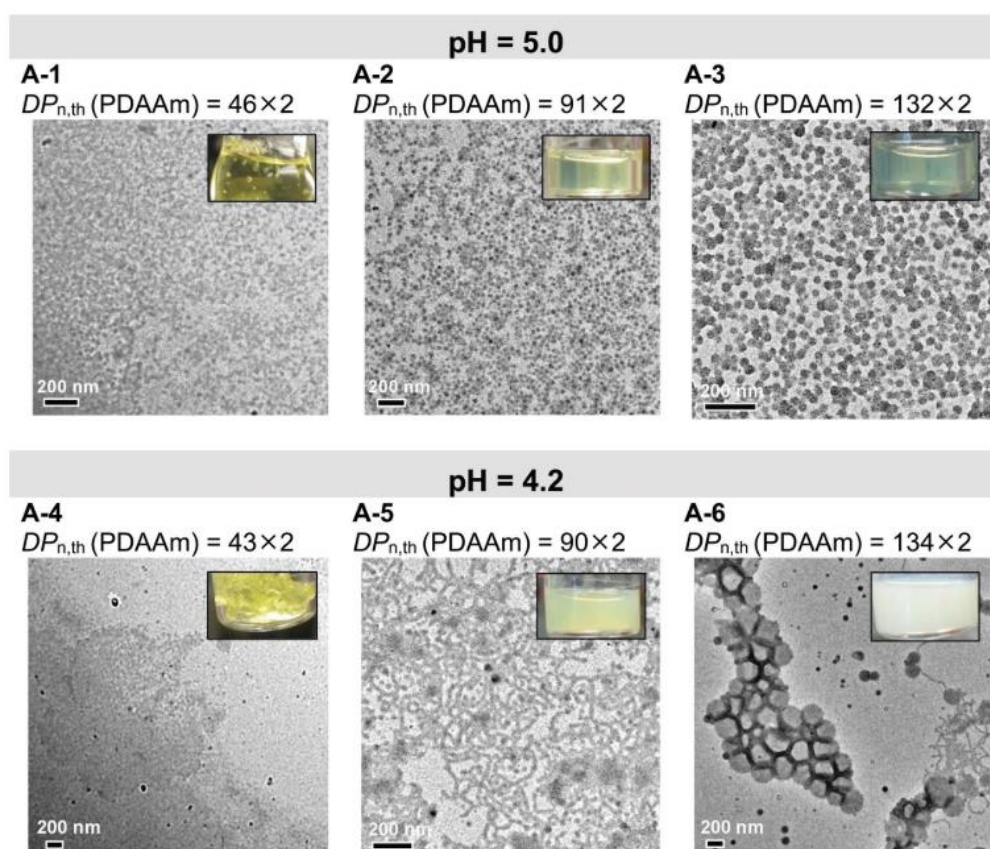


Figure 1.22. TEM images and photographs (inserts) for samples prepared by the dispersion polymerisation of DAAM with macroRAFT agent A (C12-TTC-PDMAc)2-BA at pH = 5.0 and pH = 4.2. Reproduced with permission from P. Biais, P. Beaunier, F. Stoffelbach and J. Rieger, *Polym. Chem.*, 2018, **9**, 4483–4491.

Further research into this polymer was accomplished in the following year by Parkinson and co-workers, who combined PISA and continuous flow to form a range of polymer nanoparticles.⁷⁹ Parkinson and co-workers reported the scalable, all-aqueous synthesis of polydimethyl acrylamide)–polydiacetone acrylamide) diblock copolymer spheres, worms and vesicles by RAFT aqueous

dispersion polymerisation in a low-cost continuous-flow reactor. During this polymerisation, the chains underwent PISA, producing block copolymer spheres. Parkinson and coworkers found that this reaction also proceeded faster than in batch, and the high-resolution kinetics enabled a clear observation of the rate enhancement, which is characteristic of PISA systems (Figure 1.23). Parkinson and co-workers also demonstrated that the PDMA_{m113} macro-CTA could be used to prepare PDMA_{m113}–PDAAm_x block copolymer spheres (where $x = 50, 100$ and 200) with systematically increasing particle diameters. Finally, by reducing the PDMA_m macro-CTA DP to 50 and increasing total solids to 20% w/w, Parkinson and co-workers found that it was possible to prepare worms and vesicles in the tubular reactor by tailoring the residence time to achieve specific degrees of polymerisation of the PDAAm block.

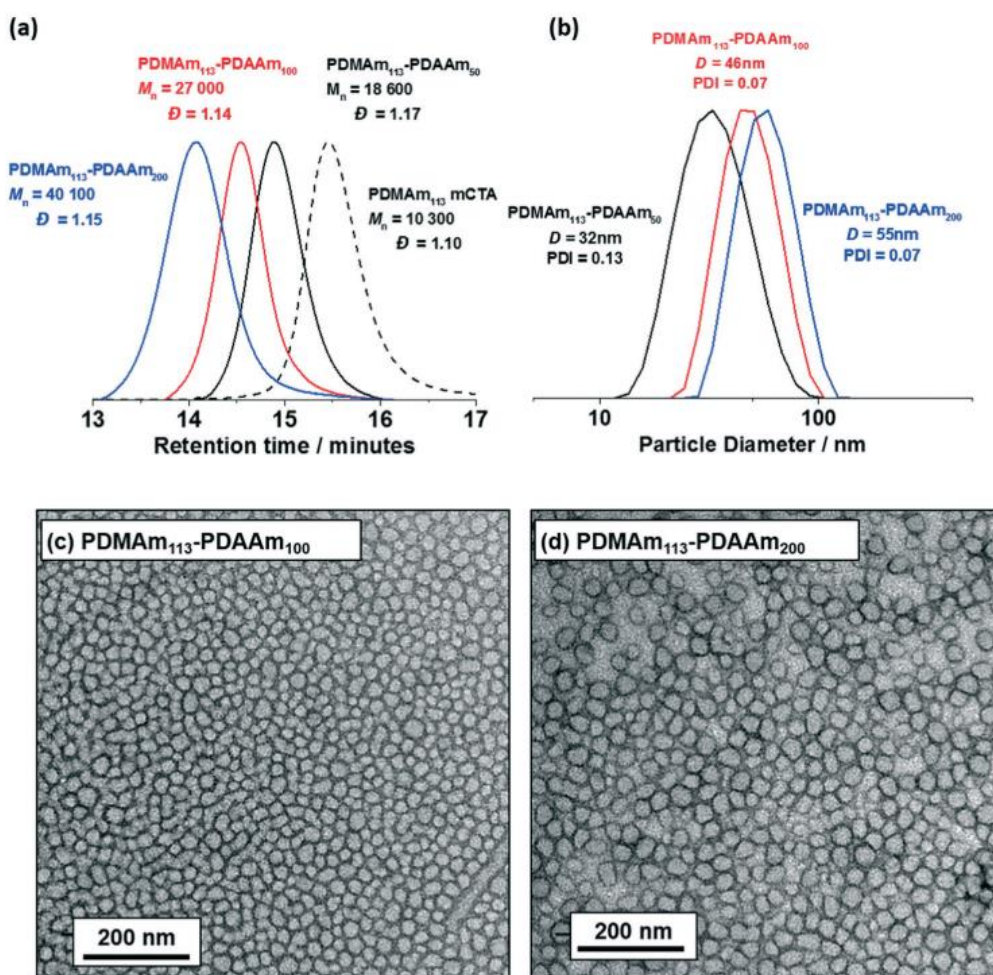


Figure 1.23. (a) GPC chromatograms and (b) DLS size distributions obtained for the chain extension of PDMA₁₁₃ with DAAM conducted using the continuous-flow reactor. (c) and (d) Show TEM images obtained for PDMA₁₁₃–PDAAm₁₀₀ and PDMA₁₁₃–PDAAm₂₀₀ diblock copolymer spheres. For all reactions, total

solids concentration of 10% w/w and [CTA] : [initiator] = 1: 0.1. Reproduced with permission from S. Parkinson, N. S. Hondow, J. S. Conteh, R. A. Bourne and N. J. Warren, *React. Chem. Eng.*, 2019, **4**, 852–861.

More research was conducted in 2019 into this polymer, with Shi and co-workers publishing a paper on “ABA-type triblock copolymer micellar system with lower critical solution temperature-type sol-gel transition”.⁷⁸ Shi and co-workers demonstrated a two-step, reversible addition-fragmentation chain transfer (RAFT) polymerisation of an ABA-type copolymer consisting of poly(N, N-dimethylacrylamide)-b-poly(diacetoneacrylamide)-b-poly(N, N-dimethylacrylamide) (PDMAm-b-PDAAm-b-PDMAm). Shi and co-workers found that this copolymer could be easily dispersed in water, and this dispersion is critical for its lower critical solution temperature (LCST)-type sol-gel transition, which was monitored using DLS, TEM, and rheology analysis, in addition to temperature-dependent ¹H NMR and FTIR. Finally, the loading and controllable release of the model drug paracetamol into and out of a copolymer gel was further determined by Shi and co-workers (Figure 1.24).

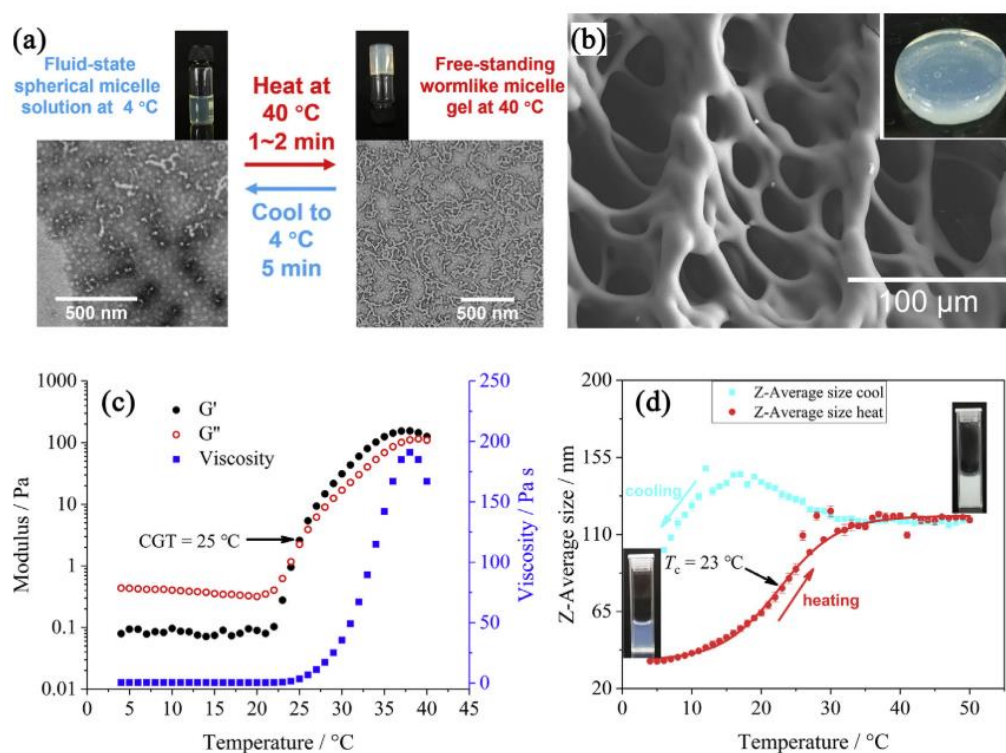


Figure 1.24. The thermal-triggered sol-gel transition of (PDMAm-b-PDAAm-b-PDMAm) (a) macroscopic sol-gel transition and corresponding TEM images at 4 and 40 °C, respectively; (b) SEM images of the freeze-dried hydrogel network; (c) rheological profiles of the heating process; (d) evolution of Z-average particle

sizes in a healing-cooling cycle at a rate of 1 °C/min. The data on particle sizes was averaged over three runs. Reproduced with permission from H. Shi, T. Qiu, H. D. Ou-Yang, H. Xu, Q. Lu, Y. Zheng, K. Liu, L. He, L. Guo and X. Li, *J. Colloid Interface Sci.*, 2019, **545**, 220–230.

In 2020, research into this polymer continued, with Byard and co-workers studying the unique self-assembly behaviour of this polymer in water.⁸¹ Byard and co-workers reported the one-pot PISA synthesis of a new amphiphilic PDMAm₅₆–P(Polyhydroxybutyrate-stat-DAAM)₂₆₄ diblock copolymer that can form spheres, worms or vesicles rapidly and reversibly in aqueous solution at copolymer concentrations as low as 0.10% w/w. In addition, Byard and co-workers found that a vesicle-to-lamellae transition also occurs on heating to around 70 °C, but the complementary lamellae-to-vesicle transition suffers from significant hysteresis on cooling. This new system proposed by Byard and co-workers was expected to provide a suitable model for studying the kinetics and mechanism of block copolymer self-assembly in solution, as well as offering a highly convenient model system for theoretical studies of thermoresponsive block copolymer nano-objects. Finally, Byard and co-workers theorised that the exceptional thermoreversibility exhibited by this new system is expected to enable the in situ loading of vesicles with either nanoparticles or globular proteins and enzymes, with subsequent release of such payloads being feasible simply by lowering the solution temperature to induce a vesicle-to-worm (or vesicle-to- sphere) morphological transition, Figure 1.25.

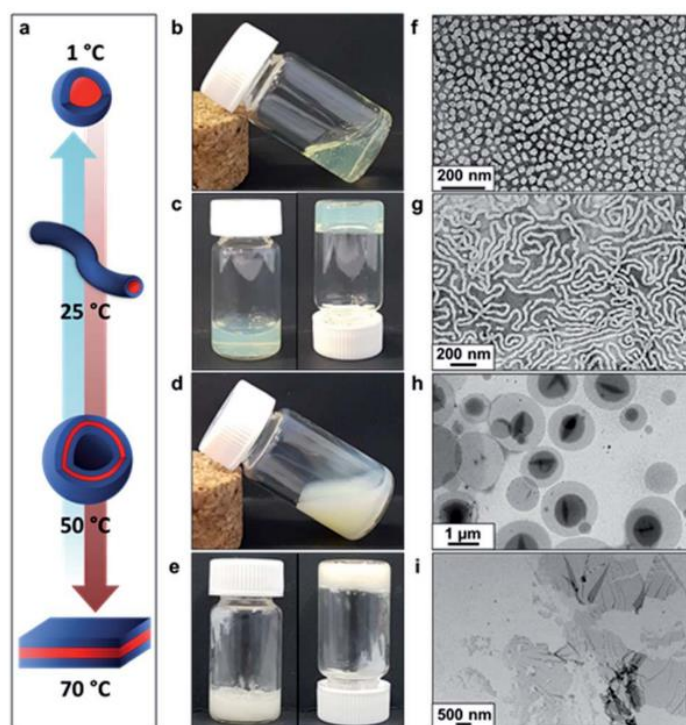


Figure 1.25. Thermally-induced diblock copolymer morphology transitions in aqueous solution. (a) Schematic representation of the reversible morphological transitions that occur for a 20% w/w aqueous dispersion of PDMAC₅₆–P(HBA-stat-DAAM)₂₆₄ diblock copolymer nano-objects on varying the temperature from 1° to 70 °C. Digital images show the physical appearance of this aqueous dispersion: (b) on cooling to 1 °C for 30 min, (c) at ambient temperature (25 °C), (d) on heating to 50 °C for 30 min and (e) on heating to 70 °C for 30 min. TEM images recorded for 0.10% w/w aqueous dispersions of PDMAC₅₆– P(HBA-stat-DAAM)₂₆₄ using uranyl formate as a negative stain after covalent stabilization at the desired temperature using adipic acid dihydrazide (ADH) at a DAAM/ADH molar ratio of 1.0: (f) spheres (crosslinked at 1 °C), (g) worms (crosslinked at 25 °C), (h) vesicles (crosslinked at 50 °C) and (i) lamellae (crosslinked at 70 °C). Reproduced with permission from S. J. Byard, C. T. O'Brien, M. J. Derry, M. Williams, O. O. Mykhaylyk, A. Blanazs and S. P. Armes, *Chem. Sci.*, 2020, **11**, 396–402.

Biais and co-workers investigated the thermoresponsive dynamic BAB block copolymer networks synthesised by aqueous PISA in one pot.⁸² In this work, Biais and co-workers reported a straightforward process to produce well-defined dynamic networks of associative BAB triblock copolymers through two successive polymerisation steps conducted in one-pot and exclusively in water. The copolymers are composed of a hydrophilic poly(N, N-dimethylacrylamide)

A block extended in a typical RAFT-PISA dispersion formulation with two lateral associative statistical poly(diacetone acrylamide-co-N, N-dimethylacrylamide) B blocks. Biais and co-workers found that the viscosity of the samples is strongly modified by the N, N-dimethylacrylamide (DMAm) content in the B block, Figure 1.26. This was attributed to the formation of a transient network for which the exchange dynamics of the B blocks between micelles and/or the number of bridges within the network depend on the DMAm content. Biais and co-workers used Rheology analyses, which showed that transient networks are formed that exhibit thermoresponsive behaviour with a maximum viscosity as a function of temperature, presumably caused by a maximum bond lifetime and/or the number of elastically active chains.

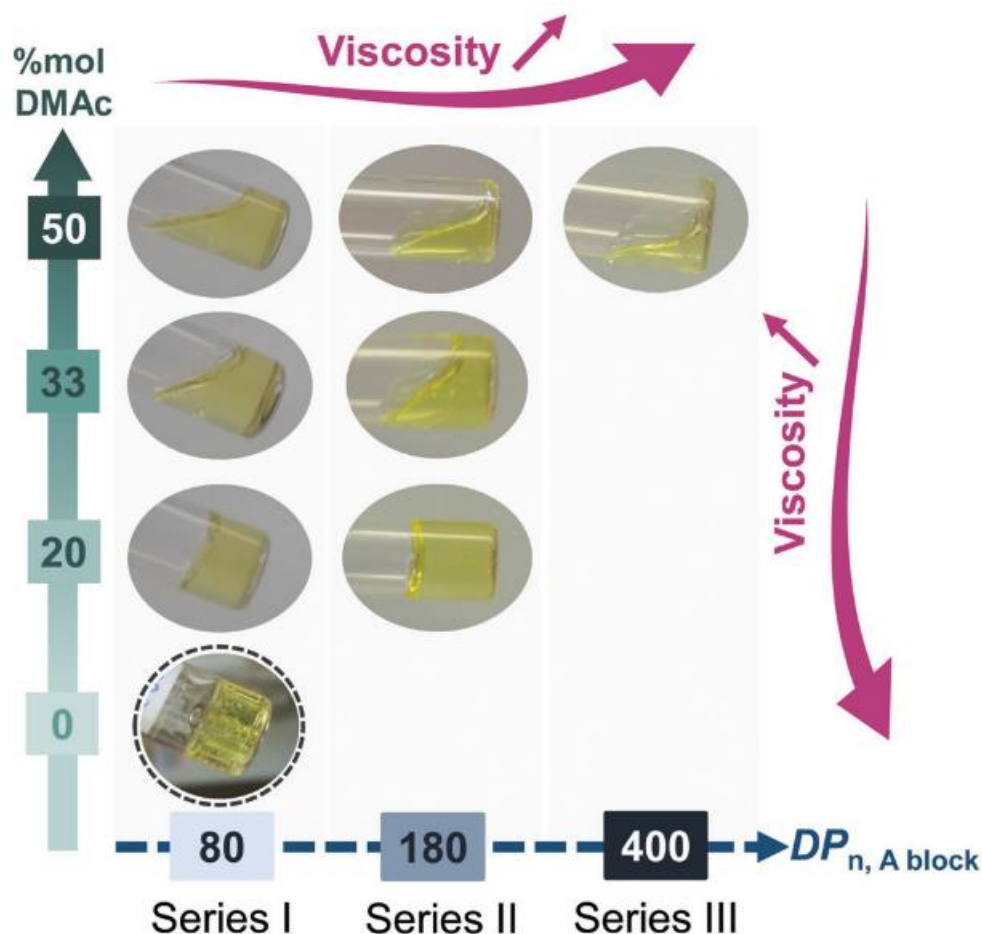


Figure 1.26. Macroscopic aspect of triblock copolymers with total DP_{n, B blocks} ~ 100 at 18 wt% in water, at 25 °C, observed 5 s after vial tilting. The dashed circle indicates a gel-like dispersion. Reproduced with permission from P. Biais, M. Engel, O. Colombani, T. Nicolai, F. Stoffelbach and J. Rieger, *Polym. Chem.*, 2021, **12**, 1040–1049.

In 2022, Fan and co-workers used PISA to self-assemble micelle-crosslinked tough and ultra-stretchable hydrogels.⁸³ They used polymerisation-induced self-assembly (PISA) and demonstrated it to be a general and powerful platform for micellar crosslinkers, affording micelle-crosslinked hydrogels with tailorable chemical structures, mechanics, and functionality. Poly(N, N-dimethylacrylamide)-b-poly(diacetone acrylamide) (PDMAm-b-PDAAm) micellar crosslinkers with a controlled nanostructure and uniform size distribution were prepared by this group via PISA and one-step post-polymerisation modification at high concentrations. Copolymerisation of these micellar crosslinkers with acrylamide generated tough and ultra-stretchable hydrogels, whose mechanical properties were found to be correlated with the concentration, nanostructure, and chemical composition of the micelles.

Also in 2022, Xiong and co-workers investigated the self-assembly evolution of ABA-type block copolymers in water during the gelation process using infrared spectroscopy and near-infrared spectroscopy.⁸⁴ During this research, a well-controlled triblock copolymer poly(N,N-dimethylacrylamide)-b-poly(diacetone acrylamide)-b-poly(N,N-dimethylacrylamide) (PDMAm-b-PDAAm-b-PDMAm) was synthesised. Its optical micro-rheology results suggest a gelation temperature range from 42 to 50 °C, showing a transition from viscosity to elasticity.

Here, this group observed a sol-gel transition from spheres to worms as the temperature increased. This suggested that initially this triblock copolymer tended to form spherical, kinetically trapped micelles. But as the temperature was increased to 50 °C, there was a morphological transition which could be thought of as a combination of spherical kinetically trapped micelles coming together end-on to form worms or cylindrical micelles.

Temperature-dependent IR spectra through two-dimensional correlation spectroscopy (2D-COS) and the Gaussian fitting technique were analysed to obtain the transition information of the molecular structure within the triblock copolymer, Figure 1.27. The N-way principal component analysis (NPCA) on the temperature-dependent NIR spectra was performed to understand the molecular interaction between water and the copolymer. The intramolecular hydrogen bonds within the hydrophobic PDAAm block tend to dissociate with temperature, resulting in improved hydration and a relative volume increase of

the PDAAm block. The dissociation of intermolecular hydrogen bonds within the PDAAm block was the driving force for the morphological transition. Moreover, the hydrophilic PDMAm block dehydrates with temperature, and three stages can be found. The dehydration rate of the second stage with temperature from 42 to 50 °C was obviously higher than those in the lower (first stage) and higher (third stage) temperature ranges.

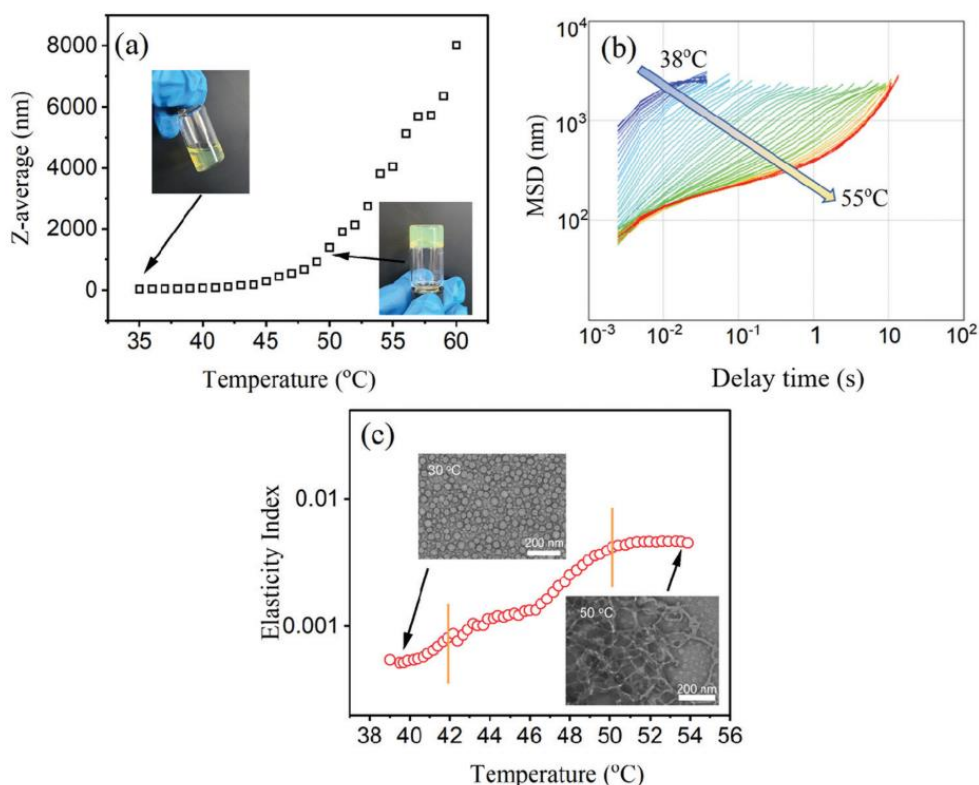


Figure 1.27. Characterisation results of PDMAm-b-PDAAm-b-PDMAm 20 wt% aqueous solution from 38 to 55 °C: (a) plots of Z-average size; (b) MSD curves measured by microrheology; (c) plots of EI calculated by MSD curves. Reproduced with permission from C. Xiong, B. Ma, T. Qiu, X. Li, X. Shao and L. Guo, *Phys. Chem. Chem. Phys.*, 2022, **24**, 17004–17013.

In 2023, Scheutz and co-workers investigated Gradient Copolymer Synthesis through Self-Assembly and demonstrated the use of PISA as a synthetic tool to direct gradient copolymer synthesis.⁸⁵ Specifically, this group leveraged hydrophobicity-induced reaction selectivity and the rate acceleration typically associated with polymer compartmentalisation upon assembly during PISA to bias reaction selectivity. In the chain extension of a poly(ethylene glycol) macrochain transfer agent, the selectivity of diacetone acrylamide (DAAm) and

N,N-dimethylacrylamide (DMA), two monomers with near-identical reactivity in water, can be modulated in situ such that DAAM is preferentially incorporated over DMA upon self-assembly. By increasing the feed ratio of DAAM, monomer differentiation can be further biased toward DAAM due to the locus of polymerisation becoming increasingly hydrophobic. This change in selectivity affords the autonomous generation of PDAAm–PDMAM gradient sequences, otherwise inaccessible without outside intervention. Finally, a mild hydrolysis protocol can then be employed to harvest PDAAm-b-PDMAM sequences, yielding compositionally unique gradient copolymers.

The unique aspect of this research was the focus on gradient copolymer synthesis through self-assembly. Gradient copolymers have a gradual change in composition along the polymer chain, which leads to interesting self-assembly behaviours, namely Composition-dependent assembly. The gradual change in composition along the polymer chain can result in unique self-assembly pathways, potentially leading to novel nanostructures. The gradient nature of the copolymers allows for fine-tuning of the resulting nanoparticle properties.

Overall, this PDMAM-b-PDAAm PISA system is well studied in aqueous environments, with a lot of literature showing the capabilities of this polymer to form a range of nanoparticles in water.

1.1.9. Encapsulation of foreign molecules inside polymersomes

One of the most obvious applications for polymer nanoparticles is the encapsulation of foreign entities inside the polymer nanoparticles. The encapsulation of foreign cargo is routinely done for polymer vesicles that have an internal space which is enclosed by the polymer membrane. Thus, encapsulation of foreign entities depends greatly on the permeability of the polymer membrane.⁸⁶

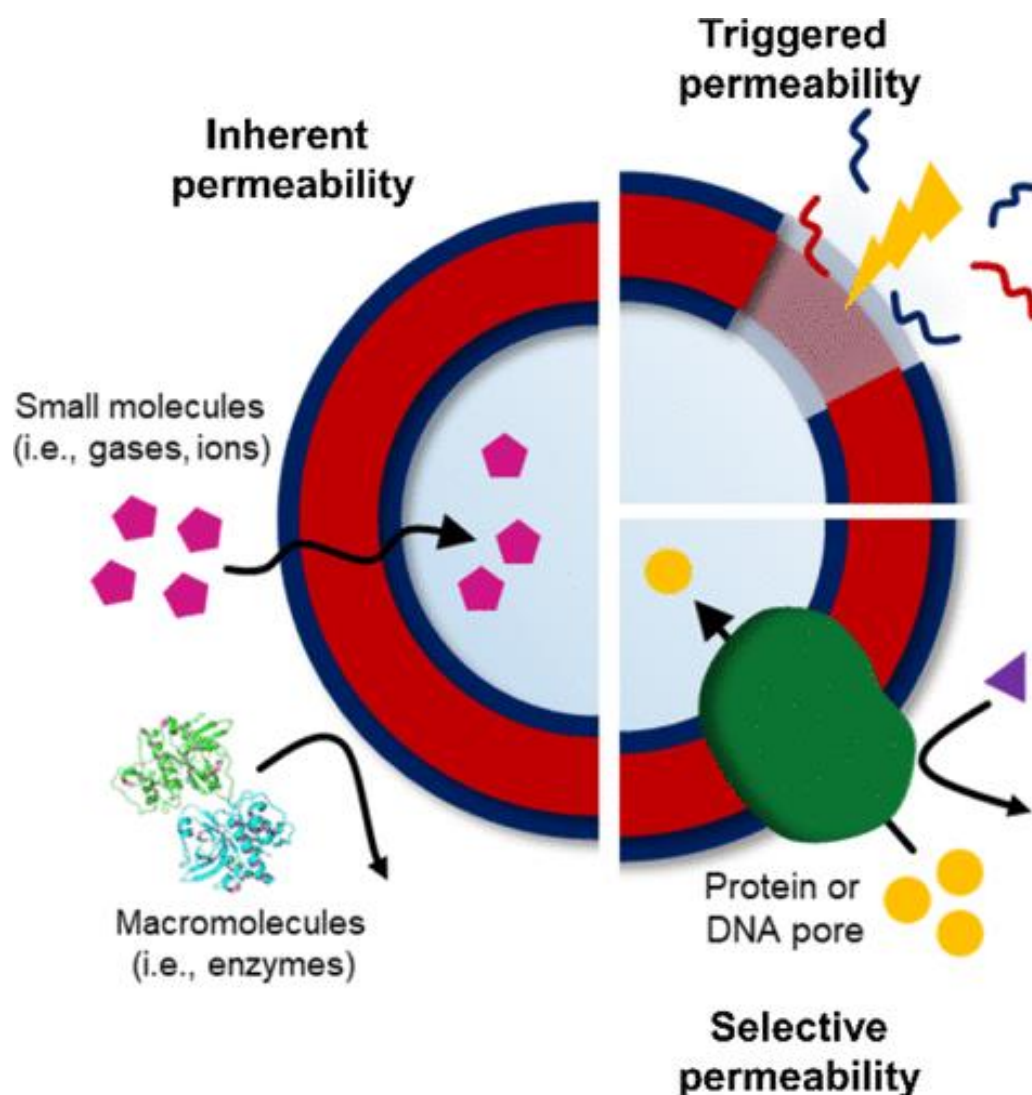


Figure 1.28. Manipulating polymersome permeability. Reproduced with permission from A. J. Miller, A. K. Pearce, J. C. Foster, and R. K. O'Reilly *ACS Central Science* 2021 **7** (1), 30-38

A simple approach is to theorise polymersomes with intrinsic permeability. This means that small molecules are free to diffuse across the polymeric membrane. This is because of membrane polymer chemistry, composition, or polymerisation technique. Membrane permeability can be controlled as a function of membrane thickness, dictated by polymer chain length or hydrophilic-to-hydrophobic balance, which in turn influences diffusion in agreement with Fick's first law.⁸⁷ Increasing polymer molecular weight also favours chain entanglements, thereby limiting the amount of diffusion.⁸⁸

Finally, the hydrophobicity and morphology of core-forming polymer blocks can influence diffusion, whereby glassy membranes slow rates of diffusion, and highly hydrophobic membranes are less hydrated and limit passage of polar species.⁸⁹

Hence, polymersomes are permeable to a wide range of small molecules depending on the polymer used in self-assembly. Encapsulation of larger macromolecules, such as enzymes and proteins, is more trivial, with encapsulation occurring in sync with self-assembly. Having enzymes present in the aqueous environment during self-assembly will lead to the enzymes being encapsulated by the polymersome. With little to no permeability of enzymes for the polymer membrane, the encapsulated enzyme remains present in the final polymersome suspension.

There have been many examples of successful enzyme encapsulation within polymersomes in recent and historic literature. An early example of this is highlighted in the work of Van Dongen and co-workers, who investigated a three-enzyme cascade reaction through positional assembly of enzymes in a polymersome nanoreactor.⁹⁰ In this work, Porous polymersomes based on block copolymers of isocyanopeptides and styrene have been used to anchor enzymes at three different locations, namely, in their lumen (glucose oxidase, GOx), in their bilayer membrane (Candida antarctica lipase B, CalB) and on their surface (horseradish peroxidase, HRP). The surface coupling of the polymersome to the HRP was achieved by click chemistry between acetylene-functionalised anchors on the surface of the polymersomes and azido functions of HRP. To determine the encapsulation and conjugation efficiency of the enzymes, they were decorated with metal-ion labels and analysed by mass spectrometry. This revealed an almost quantitative immobilisation efficiency of HRP on the surface of the polymersomes and a more than statistical incorporation efficiency for CalB in the membrane and for GOx in the aqueous compartment. The enzyme-decorated polymersomes were studied as nanoreactors in which glucose acetate was converted by CalB to glucose, which was oxidised by GOx to gluconolactone in a second step. The hydrogen peroxide produced was used by HRP to oxidise 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid). Kinetic analysis revealed that the reaction step catalysed by HRP is the fastest in the cascade reaction.

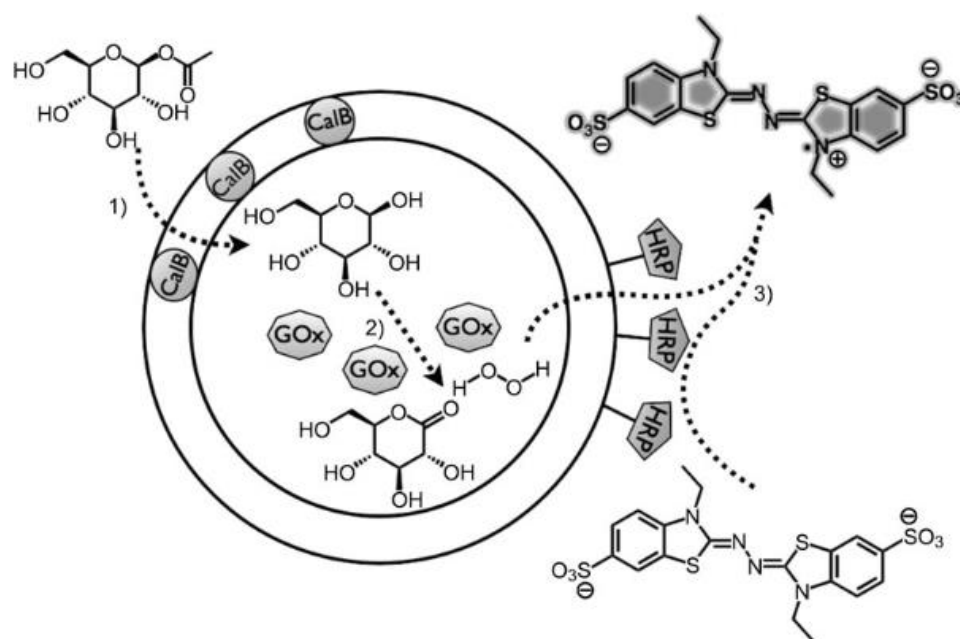


Figure 1.29 Schematic representation of the multistep reaction. 1) Monoacetylated Glucose (4) is deprotected by CalB, which is embedded in the polymersome membrane. 2) In the inner aqueous compartment, GOx oxidises glucose to gluconolactone, providing a molecule of hydrogen peroxide. 3) Hydrogen peroxide is used by HRP to convert ABTS to ABTSC⁺. HRP is tethered to the polymersome surface. Reproduced with permission from S. F. M. Van Dongen, M. Nallani, J. J. L. M. Cornelissen, R. J. M. Nolte and J. C. M. Van Hest, *Chem. - A Eur. J.*, 2009, **15**, 1107–1114.

This work highlights the ability to encapsulate enzymes within the lumen of the block copolymer polymersomes and also within the polymer membrane itself. The polymersome here shows permeability towards the small molecules, and a cascade reaction occurs. Also, this research highlights the ability to use encapsulation to make polymersome nano-reactors capable of multiple enzymatic reactions in one pot.

Hybrid lipid block-copolymer systems

Lipid-polymer hybrid nanoparticles (LPHNPs) have emerged as a promising next-generation nanoparticle system and drug delivery platform, combining the advantages of both polymeric nanoparticles and liposomes. These innovative

structures typically comprise a polymeric core, a lipid shell, and an outer lipid-PEG layer, though variations exist.⁹¹ The most common type is the polymer core-lipid shell nanoparticle, but other designs include polymer-caged nanobins, core-shell type hollow lipid-polymer-lipid nanoparticles, monolithic or mixed polymer-lipid hybrid nanoparticles, lipid bilayer-coated nanoparticles, and biomimetic LPHNPs.⁹²

The preparation methods for LPHNPs include single-step and two-step nanoprecipitation, emulsion-based methods, and microfluidic rapid mixing.^{93,94} The choice of method depends on the desired structure and properties of the nanoparticles. The composition of LPHNPs typically includes a hydrophobic polymeric core, a hydrophilic polymeric shell, and a lipid monolayer at the interface.⁷⁴ Both natural and synthetic lipids can be used, each offering unique advantages in terms of targeting ability, immune evasion, controlled drug release, and surface functionalisation.⁹⁵

LPHNPs have demonstrated superior in vivo cellular delivery efficacy compared to polymeric nanoparticles and liposomes alone. They exhibit high stability, biocompatibility, selective targeting, prolonged circulation time, greater drug encapsulation efficiency, and significant biological response. These properties make LPHNPs potentially applicable in various fields, including anticancer therapy, gene delivery, vaccine delivery, and bioimaging.⁹⁶

Recent research has focused on optimising LPHNP formulations and exploring new applications. For instance, studies have investigated the impact of lipid-to-polymer ratio on colloidal stability and the use of different lipid compositions to enhance drug delivery efficacy. Additionally, researchers have explored the use of natural, semi-synthetic, and synthetic polymers in LPHNP formulations, expanding the range of potential applications and tailoring the nanoparticles to specific therapeutic needs.⁹⁷

LPHNPs have emerged as a highly potent and promising nanocarrier for a variety of biomedical uses, including drug delivery and biomedical imaging, owing to recent developments in nanomedicine. In contrast with other forms of drug delivery systems, PLNs have been regarded as stable because they are simple to prepare and exhibit excellent stability. Natural, semi-synthetic, and synthetic polymers have been used to make these nanocarriers. Due to their small scale, LPHNPs can be used in several applications, including anticancer therapy, gene delivery, vaccine delivery, and bioimaging. These nanoparticles

are also self-assembled in a reproducible and predictable manner using a single or two-step nanoprecipitation process, making them significantly scalable.⁹⁹

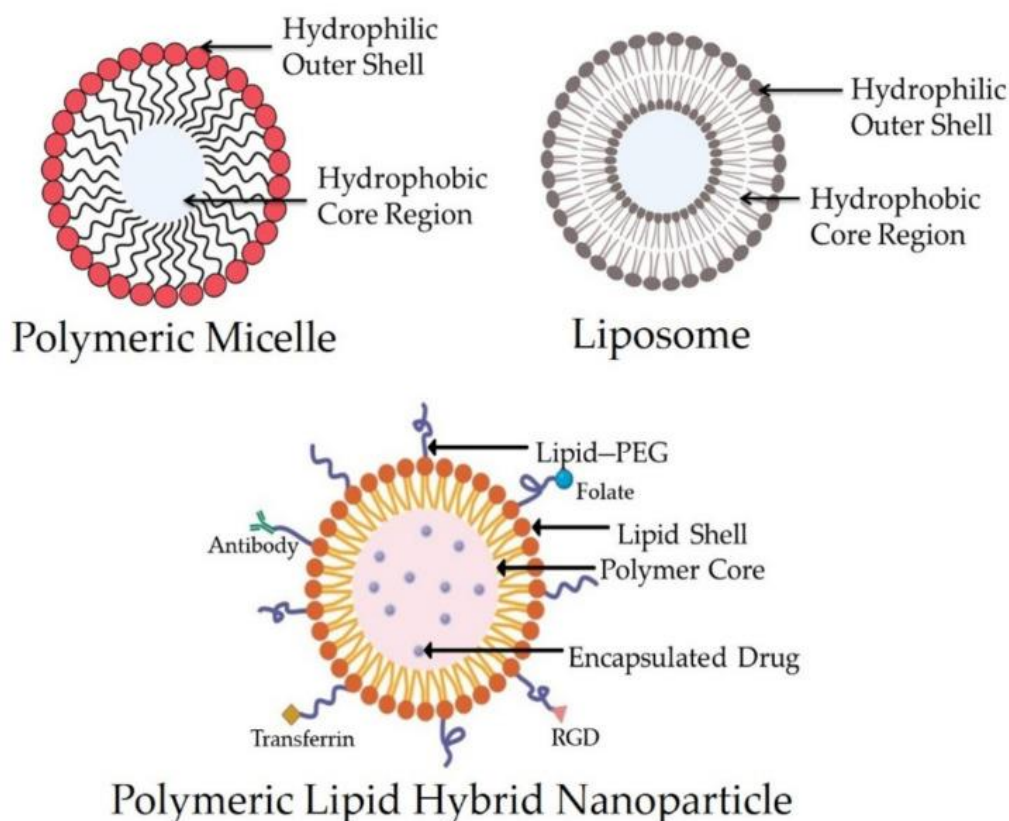


Figure 1.30. Structure of a polymeric micelle, liposome, and polymeric lipid hybrid nanoparticle (PLN) displaying the organisation of hydrophobic and hydrophilic cores. Reproduced with permission from Sivadasan, D., Sultan, M. H., Madkhali, O., Almoshari, Y., & Thangavel, *Pharmaceutics*, 2021, **13**(8), 1291.

Most notably, there have been a few reviews published that discuss the nuances of combining both lipids and polymers into hybrid nanoparticles. A review by Go and Leal explored polymer-lipid hybrid materials, highlighting their unique properties and potential applications.⁹⁸

Hierarchical Self-Assembly is discussed thoroughly in this work within the complex self-assembly behaviour of polymer-lipid hybrids. These materials can form a variety of structures, including vesicles, micelles, and more complex morphologies. The authors highlight how the interplay between polymer and lipid components leads to unique assembly pathways.

The emergence of synergistic properties in hybrid membranes is an important focus when discussing polymer-lipid hybrids. One example is the enhanced

permeability observed in phase-separated hybrid membranes. Rideau et al. showed that hybrid vesicles composed of DPPC lipids and PBd-PEO polymers exhibit increased permeability to small molecules at the phase boundaries between lipid-rich and polymer-rich domains.⁹⁹

Moreover, Go and Leal investigated how polymer incorporation could modulate membrane mechanics. For example, hybrid membranes often exhibit increased bending rigidity compared to pure lipid membranes, which can be advantageous for certain applications such as drug delivery, with a plethora of work being done to unlock the potential of these polymer-lipid hybrids to be used in biomedical applications. An example is Zhang et al., who reported that lipid-polymer hybrid nanoparticles offer high drug encapsulation yield, tunable and sustained drug release profiles, excellent serum stability, and potential for differential targeting of cells or tissues.¹⁰⁰

In the potential application towards targeted drug delivery, these hybrid materials offer several advantages compared to their counterparts.

- *Enhanced stability:* The polymer component can increase the structural integrity of the lipid vesicles, leading to improved circulation times for in-vivo applications.
- *Controlled release:* The phase behaviour of hybrid membranes can be exploited to achieve controlled drug release profiles.
- *Targeting capabilities:* The polymer component can be functionalised with targeting moieties for improved drug delivery to specific tissues.

Monolithic LPHNPs

Monolithic hybrid systems, also known as mixed lipid-polymer hybrid NPs, have a unique architectural dimension in which molecules of lipid are randomly distributed and form the core into which hydrophobic drugs can be loaded.

Such a combination strategy of nanoparticulate systems serve as a colloidal vehicle for encapsulation of hydrophobic drugs. Phospholipids are an important part of the hybrid structure that forms the carrier-like structure in this case. These LPHNPs can be used to entrap extremely lipophilic drug molecules that would otherwise not be entrapped inside the polymer. The mixing ratios of the lipid and polymer can be altered and optimised during manufacturing to prevent systemic toxicity throughout the body.⁹⁹

Interestingly, the structural dynamics of monolithic hybrid systems have been intensively studied, most recently by Seneviratne and co-workers who used small angle X-ray scattering (SAXS) and cryo-electron tomography (cryo-ET) to obtain detailed structural information about monolithic hybrid vesicles with different ratios of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and poly(1,2-butadiene-block-ethylene oxide) (PBd₂₂-PEO₁₄, Ms = 1800 g mol⁻¹).¹⁰² In this study, Seneviratne and colleagues found that increasing PBd₂₂-PEO₁₄ mole fraction increases the membrane thickness from 52 Å for a pure lipid system to 97 Å for pure PBd₂₂-PEO₁₄ vesicles. Two vesicle populations with different membrane thicknesses in hybrid vesicle samples are found, as these lipids and polymers are reported to homogeneously mix. The authors conclude that, by combining biophysical methods, accurate determination of the influence of composition on the structural properties of monolithic hybrid membranes is achieved, revealing that two distinct membrane structures can coexist in homogeneously mixed lipid-polymer hybrid vesicles. This was the first study of its kind to shed light on high-level non-invasive characterisation techniques and the structure of monolithic hybrid systems.

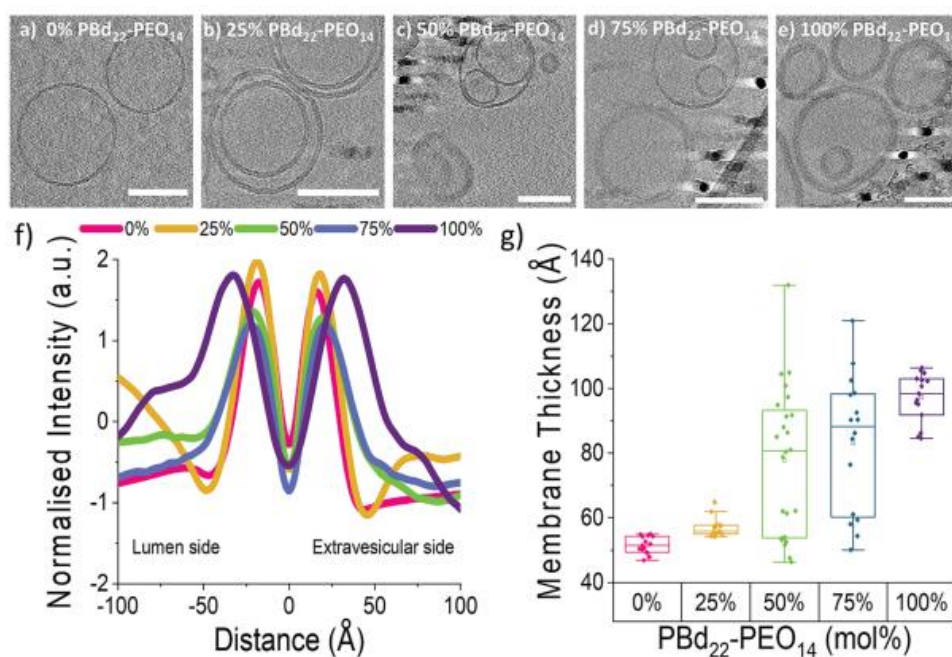


Figure 1.31. Electron intensity profiles (EIPs) of hybrid vesicle membranes obtained by cryo-ET. Cryo-ET images of a) POPC, b) 25%, c) 50%, d) 75%, and e) 100% PBd₂₂-PEO₁₄ vesicle compositions (scale bar is 750 Å). The graphs

show f) the average electron intensity profile across the vesicle membrane, and g) membrane thickness measurements (outer edges of contrast images in (a–e)) of individual membranes within 0–100% PBd₂₂-PEO₁₄ vesicle samples.¹⁰⁰

1.1.10. This Project

This project aims to explore the self-assembly behaviour of PDMAm-*b*-PDAAm block copolymers in aqueous environments. The aim is to use a fixed polymer length, such as PDMAm₅₀-*b*-PDAAm₃₀₀, and explore various self-assembly mechanisms. These include PISA, Thin Film Rehydration (TFR), and Solvent Exchange (SE). By investigating the self-assembly pathways for each method, a key understanding of what is happening during self-assembly will be gained. The self-assembly pathways will also be tailored towards biological conditions with the addition of electrolytes and a pH of around 7.4. This is done to allow for potential encapsulation of biologically relevant cargo such as enzymes and proteins into the core of some of the polymer nanoarchitectures.

1.1.11. Objectives

Chapter 3

The main objective in chapter 3 of this thesis was to use RAFT-PISA to form a range of polymeric nanoparticles from a PDMAm macro-CTA and chain extend with DAAM. These nanoparticles were characterised via DLS, NMR, GPC, and TEM to understand how varying the block length of the hydrophobic block of the polymer alters the morphology of the nano-objects self-assembled. The pH and salt concentrations were varied during PISA to see if there is an effect on the self-assembly pathway and to be able to fabricate these polymer nanoparticles under biological conditions.

Chapter 4

The aims of Chapter 4 were to explore alternative self-assembly particles. The aim of this was to answer the question: Can the processing techniques be used to take the *same starting material and make different structures*? This research question was answered by using TFR to self-assemble a range of polymeric nanoparticles with the same polymer used with the PISA formulations and

analyse the TFR process by looking at the solid polymer characteristics via DSC. As well as TFR, the aim is to use SE to make polymeric nanoparticles with the same polymer used with the PISA formulations. This was achieved by analysing a range of variables such as co-solvent% and addition rate to see if they have an effect on the size and morphology of polymer nano-particles made via SE.

Chapter 5

The aims of Chapter 5 were to expand upon the SE self-assembly pathway presented in Chapter 4. The main research question is to explore differences between equilibrium self-assembled structures and kinetic self-assembled structures made via PISA and SE, respectively. This was achieved by taking an in-depth view into the SE self-assembly pathway by taking aliquots during co-solvent evaporation and studying them via DLS and TEM. As well as this, the effect of pH and electrolyte concentration on SE was studied by trying to reproduce the results in chapter 4 under biological conditions. Also, the question of charge on the polymer and how it affects self-assembly pathways will be studied by varying the overall charge on the polymer and seeing if this influences the SE self-assembly pathway. Finally, this chapter aims to understand the incorporation of a membrane directing lipid, namely POPC, to try to alter the self-assembly pathway during SE towards vesicles and their aggregates.

1.1.12. References

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Chapter 2

2.1 Methodology

This chapter looks at common methodologies used throughout this thesis to understand and characterise the polymer nanoparticles made throughout this work. The first step of this work is to synthesise the block copolymer via RAFT-mediated PISA. To characterise if this reaction has been successfully undertaken, the polymer must be characterised based on its monomer conversion via NMR and its size via GPC.

2.1.2. ^1H Nuclear Magnetic Resonance (NMR) spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is a powerful analytical technique used to study the structure and dynamics of molecules. It works by exploiting the magnetic properties of atomic nuclei, which can be used to determine the chemical environment and interactions of molecules in a sample. NMR is widely used in chemistry, biochemistry, and materials science, and has contributed to numerous discoveries in these fields.

The basic principle behind NMR spectroscopy is that atomic nuclei with an odd number of protons or neutrons have a magnetic moment, which can be used to study their interactions with other atoms in a molecule. When a sample is placed in a strong magnetic field, the magnetic moments of the nuclei will align either with or against the field, depending on the spin state of the nucleus. A radio frequency pulse is then applied to the sample, causing some of the nuclei to flip their spin state. As the nuclei relax back to their original state, they emit a radio frequency signal that can be detected and analysed.

The resulting NMR spectrum provides information about the chemical structure and environment of the molecules in the sample. The position of the peaks in the spectrum corresponds to the resonance frequency of the nuclei, which is affected by the local magnetic field and the chemical environment of the nucleus. By analysing the shape and intensity of the peaks, it is possible to determine the number and type of nuclei present in the sample, as well as their interactions with other atoms in the molecule.

One of the key advantages of NMR spectroscopy is its ability to study the dynamics of molecules in solution. By analysing the relaxation times of the nuclei in the sample, it is possible to determine the rate at which molecules rotate or undergo conformational changes. This information can be used to

study protein folding, enzyme kinetics, and other dynamic processes in biological systems.

Another advantage of NMR spectroscopy is its ability to study the structure of large molecules and complexes. While other techniques, such as X-ray crystallography or electron microscopy, are limited in their ability to study large molecules, NMR spectroscopy can provide detailed information about the structure and dynamics of proteins, nucleic acids, and other macromolecules in solution.

In conclusion, NMR spectroscopy is a powerful analytical technique that has revolutionized the understanding of the structure and dynamics of molecules. With its ability to study molecules in solution, NMR spectroscopy has contributed to numerous discoveries in chemistry, biochemistry, and materials science.

During NMR spectroscopy, the sample is placed in a magnetic field, and the NMR signal is produced by excitation of the nuclei in the sample with radio waves into nuclear magnetic resonance, which is detected with sensitive radio receivers. The intramolecular magnetic field around an atom in a molecule changes the resonance frequency, thus giving access to details of the electronic structure of a molecule and its individual functional groups. As the fields are unique or highly characteristic of individual compounds, in modern organic chemistry practice, NMR spectroscopy is the definitive method to identify monomolecular organic compounds.

The principle of NMR spectroscopy usually involves three sequential steps:

1. The alignment (polarization) of the magnetic nuclear spins in an applied, constant magnetic field B_0 .
2. The perturbation of this alignment of the nuclear spins by a weak oscillating magnetic field, usually referred to as a radiofrequency (RF) pulse.

3. Detection and analysis of the electromagnetic waves emitted by the nuclei of the sample as a result of this perturbation.

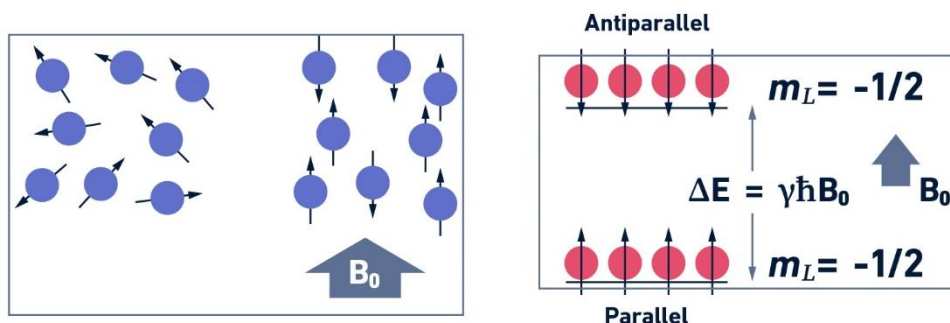


Figure 2.1. Nuclear spin orientations of a sample aligned (parallel and antiparallel) with the direction of an external magnetic field B_0 (left). Distribution of nuclear spin populations in the two possible energy levels in nuclei with $I = \frac{1}{2}$ (right). Image sourced from <https://www.technologynetworks.com/analysis/articles/nmr-spectroscopy-principles-interpreting-an-nmr-spectrum-and-common-problems-355891> (Date accessed 01/02/2023)

As NMR spectroscopy is used to identify compounds, in polymer chemistry, NMR can be used to determine monomer conversion during polymer synthesis. Monomer conversion (% conv.) can be calculated as follows,

$$\text{Equation 2.1} \quad \% \text{ conv.} = \frac{\text{integral of monomer peak}}{\text{integral of polymer peak} + \text{monomer peak}}$$

For the PDMAm polymerisation, conversion was calculated by integrating known monomer vinyl peaks at > 6 ppm, and integrating known polymer peaks at 3 ppm, as shown in Figure 1.

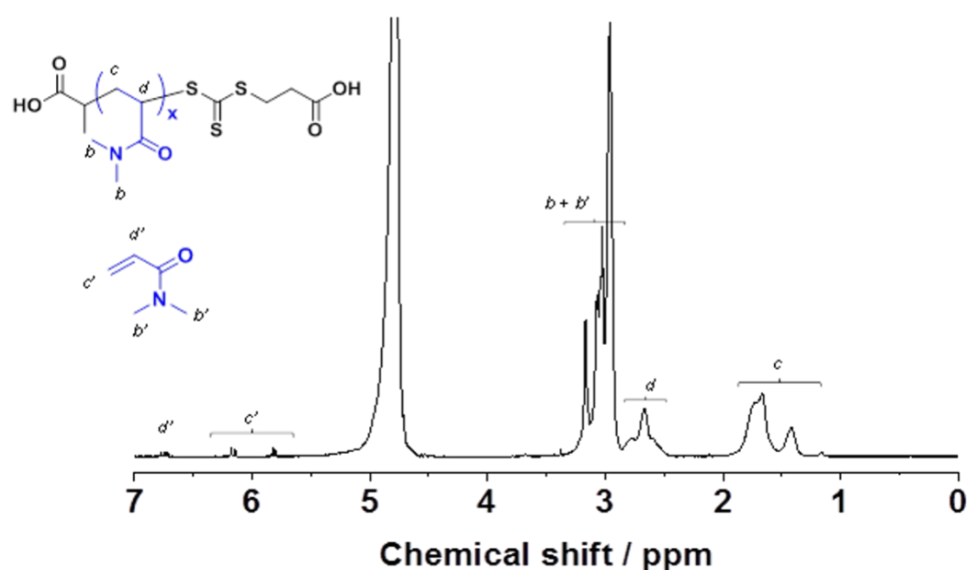


Figure 2.2: NMR spectrum and proton assignments for PDMAm_x macro-CTA synthesis. Reproduced with permission from S. J. Byard, M. Williams, B. E. McKenzie, A. Blanz and S. P. Armes, *Macromolecules*, 2017, **50**, 1482–1493.

For the conversion of the chain extension with PDAAm, conversion was calculated by integrating known monomer vinyl peaks at 6.18 ppm, and integrating known polymer and monomer ketone peaks at 1.99 ppm. The methodology for NMR analysis of block copolymers is presented below.

¹H Nuclear Magnetic Resonance (NMR) spectroscopy

All ¹H NMR spectra were recorded using a Bruker 400 MHz spectrometer. Samples were lyophilized and dissolved in deuterated methanol at 25°C. All chemical shifts were reported in ppm (δ). 32 scans were typically employed per spectrum. MestreNova software was used to analyse all NMR spectra.

Benchtop ¹H Nuclear Magnetic Resonance (NMR) spectroscopy

Initially, a calibration curve was obtained for a range of water and methanol mixtures ranging from 50% MeOH to 0.02% MeOH. Each sample was transferred into a standard NMR tube and characterised via low-field ¹H NMR Spectroscopy using a 60 MHz benchtop NMR (Magritek). The absolute integral of the peak due to the methanol protons at 3.18 ppm was determined for each sample and plotted as a function of the solution concentration.

A water-methanol mixture was prepared in a round-bottom flask. 10 mL of pH 7.5 water was added with 10 mL of methanol to give a 50% Methanol 50% water mixture by volume. The mixture was allowed to evaporate for 3 hours under reduced pressure at 150 RPM via rotary evaporation. 1 mL Aliquots were taken at regular intervals during the evaporation and replaced with 1mL of the aqueous buffer. Aliquots were characterised via benchtop ^1H NMR.

2.1.3 Gel-Permeation Chromatography (GPC)

Gel Permeation Chromatography (GPC) is a powerful analytical technique used to separate and characterise macromolecules based on their size and molecular weight. It is widely used in the fields of polymer chemistry, materials science, and biochemistry, and has contributed to numerous discoveries in these fields. Gel permeation chromatography (GPC) is a type of size-exclusion chromatography (SEC) that separates analytes on the basis of size, typically in organic solvents. The technique is often used for the analysis of polymers. When characterizing polymers, it is important to consider the dispersity ($\mathcal{D}$) as well as the molecular weight. Polymers can be characterised by a variety of definitions for molecular weight, including the number average molecular weight (M_n), the weight average molecular weight (M_w) (see molar mass distribution), the size average molecular weight (M_z), or the viscosity molecular weight (M_v). GPC allows for the determination of $\mathcal{D}$ as well as M_v , and, based on other data, the M_n , M_w , and M_z can be determined. The basic principle behind GPC is that macromolecules in a sample will be separated based on their size as they pass through a porous column filled with a gel matrix. The gel matrix consists of beads with a specific pore size distribution, which allows molecules of different sizes to enter the pores and be separated based on their size and molecular weight. GPC separates based on the hydrodynamic volume (radius of gyration) of the analytes. This differs from other separation techniques, which depend upon chemical or physical interactions to separate analytes. The smaller analytes can enter the pores more easily and therefore spend more time in these pores, increasing their retention time. These smaller molecules spend more time in the column and therefore will elute last. Conversely, larger analytes spend little if any time in the pores and are eluted quickly. All columns have a range of molecular weights that can be separated. The elution time of each molecule is recorded,

and a chromatogram is generated that shows the distribution of molecular weights in the sample. One of the key advantages of GPC is its ability to separate and characterise complex mixtures of macromolecules. Another advantage of GPC is its ability to be used in conjunction with other analytical techniques, such as mass spectrometry or light scattering, to provide additional information about the sample. By combining GPC with other techniques, it is possible to obtain a more complete picture of the sample's composition and properties.

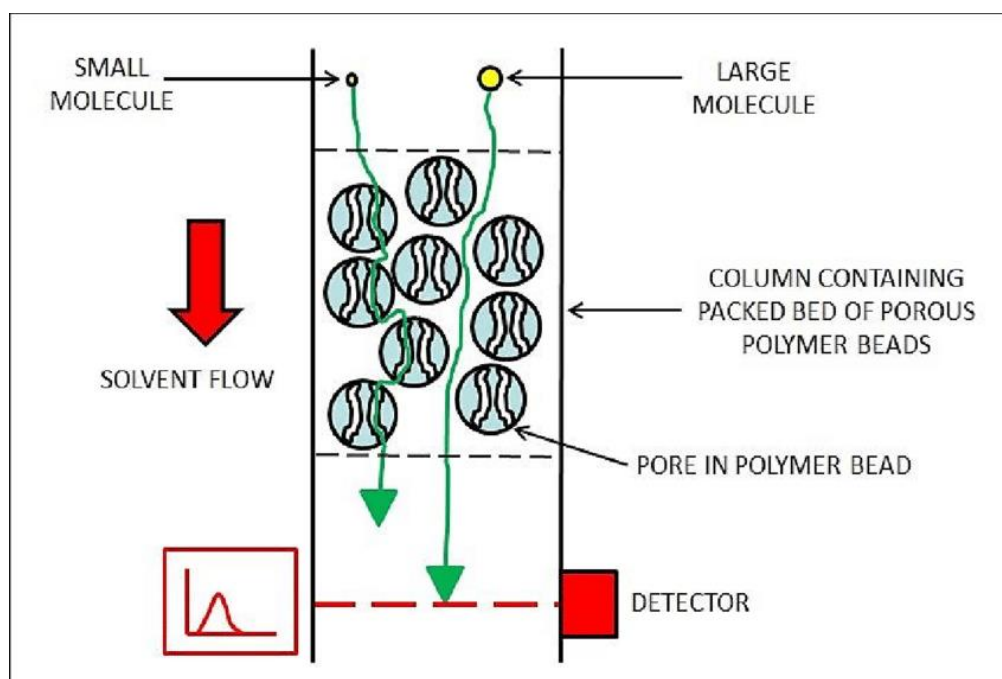


Figure 2.3. Schematic diagram of the process that occurs during a GPC measurement. Image reproduced from https://www.researchgate.net/figure/Schematic-diagram-of-the-process-which-occurs-during-a-GPC-measurement_fig4_305473975 (Date accessed 01/01/2023)

The general GPC methodology for polymers used in this thesis is presented below,

Experimental protocol for Gel-Permeation Chromatography (GPC)

Copolymer molecular weight distributions were assessed using DMF GPC. The set up was comprised of two Agilent PL gel 5 μm mixed-C column and a guard column connected in series to an Agilent 1260 Infinity GPC system fitted with both refractive index (RI) and a UV-vis detector operating at 309 nm. The GPC eluent was HPLC grade DMF containing 0.1 w/v % lithium bromide (LiBr) at a flow rate of 1.0 mL/min. The temperature of the column oven and RI detector was set to 60 °C. DMSO was used as a flow-rate marker. Calibration was achieved using a series of ten near-monodisperse poly (methyl methacrylate) standards (Mp ranging from 800 to 2,200,000 g mol⁻¹). Chromatograms were analysed using Agilent GPC/SEC software.

Once the polymer has been made successfully, the next step in the characterisation protocol is to measure and characterise the nanoparticle formed during the self-assembly. The main characteristics to characterise the nanoparticle are its size, via DLS, and its morphology via TEM.

2.1.4. Dynamic Light scattering (DLS).

Dynamic light scattering is a powerful technique used to measure the size distribution of particles in a sample. It works by measuring the fluctuations in the intensity of scattered light caused by the Brownian motion of particles in a solution. This technique is non-invasive, fast, and requires only small amounts of sample, making it ideal for a wide range of applications. The basic principle behind dynamic light scattering is that particles in a solution will move around randomly due to the thermal energy of the solution. As they move, they will collide with other particles, causing them to move as well. This movement is known as Brownian motion, and it results in fluctuations in the intensity of light scattered by the particles. These fluctuations can be measured using a photodetector, and the resulting data can be analysed to determine the size distribution of the particles in the sample.¹

To perform a dynamic light scattering experiment, a sample is first loaded into a cuvette and placed in a scattering instrument. A laser beam is then directed through the sample, and the scattered light is collected at a specific angle using a detector. The intensity of the scattered light is measured over time, and the

resulting data is analysed using a computer program to calculate the size distribution of the particles in the sample. One of the key advantages of dynamic light scattering is that it is a non-invasive technique.¹ Unlike other methods used to measure particle size, such as electron microscopy or scanning probe microscopy, dynamic light scattering does not require the sample to be fixed or stained in any way. This means that the sample can be analysed without altering its properties, making it ideal for studying biological samples or other delicate materials. Another advantage of dynamic light scattering is its speed. A typical experiment can be completed in a matter of minutes, making it ideal for high-throughput applications. Additionally, dynamic light scattering requires only small amounts of sample, typically on the order of microliters, which means that it can be used to analyse precious or limited samples. In conclusion, dynamic light scattering is a powerful technique for measuring the size distribution of particles in a sample.¹

In the time domain analysis, the autocorrelation function (ACF) usually decays starting from zero delay time, and faster dynamics due to smaller particles lead to faster decorrelation of the scattered intensity trace. DLS can also be used to probe the behaviour of complex fluids such as concentrated polymer solutions.

In DLS, the fluctuations in light intensity measured over time are quantified via a second-order correlation function $g^{(2)}(\tau)$. The function of intensity is shifted by a delay time (τ), and the autocorrelation function $g(\tau)$ is calculated. Qualitatively, the autocorrelation function is a measure of how similar the intensity function is to itself when shifted by time τ . As the value of τ increases, the function reaches a baseline of 1.

More specifically, and as described in various light scattering texts² the correlation function for a monodisperse sample can be analysed by the equation:

$$\text{Equation 2.2} \quad g^{(2)}(\tau) = 1 + \beta^{(-2\Gamma\tau)}$$

Where β is the correlation function amplitude at zero delay, Γ is the decay rate, and the baseline of the correlation function relaxes to a value of 1 at infinite delay.²

A nonlinear least squares fitting algorithm is used to fit the measured correlation function to equation 2 to retrieve the correlation function decay rate, Γ . From

this point, Γ can be converted to the translational diffusion coefficient D_t for the particle via the relation:²

$$\text{Equation 2.3} \quad D_t = \Gamma / q^2$$

Here, q is the magnitude of the scattering vector, and is given by:

$$\text{Equation 2.4} \quad q = \frac{4\pi n_0}{\lambda} \sin\left(\frac{\theta}{2}\right)$$

Where n_0 is the solvent index of refraction, λ_0 is the vacuum wavelength of the incident light, and θ is the scattering angle.

Finally, the diffusion coefficient can be interpreted as the hydrodynamic radius R_h of a diffusing sphere via the Stokes-Einstein equation:²

$$\text{Equation 2.5} \quad R_h = \frac{kT}{6\pi\eta D_t}$$

Where k is Boltzmann's constant, T is the temperature in K, and η is the solvent viscosity.

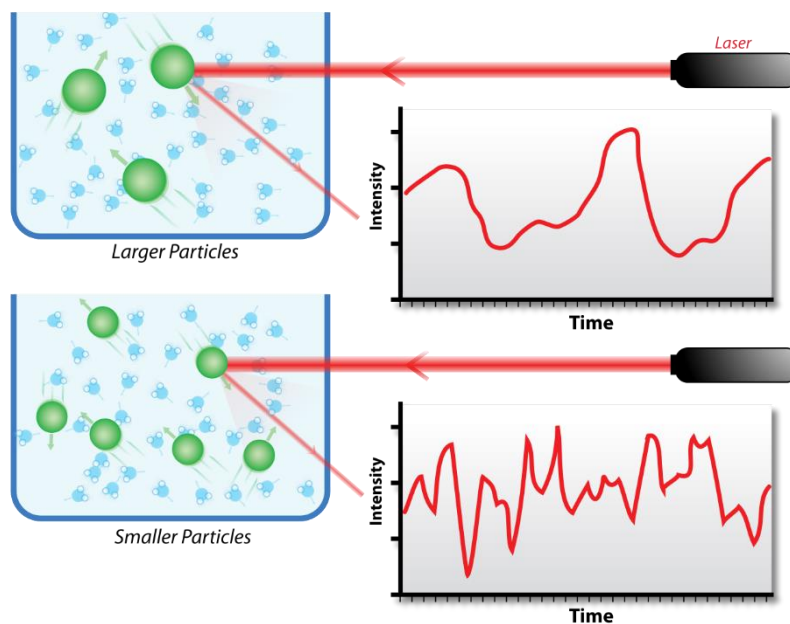


Figure 2.4: DLS operation deception highlighting the difference between larger and smaller particles. Image sourced from reference https://en.wikipedia.org/wiki/Dynamic_light_scattering (Date accessed 01/02/23).

Concerning a Polydispersity Index (PDI) for an individual peak, this is much easier and requires no re-analysis: when displaying the intensity particle size distribution, each peak comes with a mean and a standard deviation. The PDI for that peak is the square of the standard deviation divided by the square of the mean.

DLS is used throughout this thesis by looking at the size and polydispersity of polymeric nanoparticles made via PISA and SE. The DLS methodology is presented below.

Experimental protocol for Dynamic Light Scattering (DLS)

Dynamic light scattering measurements were obtained at 25 °C using a Malvern Zetasizer Nano series instrument with a standard operating procedure (SOP) of 15 runs, with a measurement taken after every 10 s. These runs were then averaged to produce a single distribution. Light scattering was detected at 173° and hydrodynamic diameters were determined using the Stokes–Einstein equation, which assumes spherical, non-interacting particles. The concentration of polymer was varied from 10 wt% to 0.078 wt% by serial

dilutions of the PISA formulation in pH 2.5 milliQ water. A dilution factor of 16 corresponding to 1.25 wt% was typically employed in DLS measurements.

2.1.5. Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) is a powerful imaging technique that allows us to see the structure and properties of materials (size, morphology, composition) and biological samples at the nanoscale. It has revolutionized the understanding of the world around us and has contributed to numerous discoveries in fields such as materials science, biology, and physics. The basic principle behind TEM is that a beam of electrons is transmitted through a thin sample, and the electrons that pass through the sample are focused onto a screen or detector to produce an image. The electrons are accelerated to high energies using an electron gun, and then focused onto the sample using a series of lenses and apertures.³ The sample must be very thin, typically less than 100 nm in thickness, in order to allow the electrons to pass through.

One of the key advantages of TEM is its ability to provide high-resolution images of materials and biological samples. With a resolution of up to 0.1 nm, TEM can reveal the atomic structure and arrangement of materials, as well as the structure of biological molecules and complexes. This has allowed us to gain a deeper understanding of the properties and behaviour of materials at the nanoscale. Another advantage of TEM is its ability to provide detailed information about the composition and properties of materials. By analysing the way that electrons scatter as they pass through the sample, it is possible to determine the crystal structure, chemical composition, and defects in the material. This has led to numerous discoveries in materials science, such as the development of new materials with unique properties for use in electronics, energy storage, and other applications. In conclusion, transmission electron microscopy is a powerful imaging technique that has revolutionized the understanding of the world at the nanoscale. With its ability to provide high-resolution images of materials and biological samples, as well as detailed information about their composition and properties, TEM has contributed to numerous discoveries in fields such as materials science, biology, and physics..

TEM is capable of unparalleled resolution due to the small wavelength of the transmitted electrons. Unlike scanning electron microscopy (SEM), which

gathers the net intensity of secondary electrons in each point of the scan, TEM resolution is only limited by the wavelength of the individual electron and the quality of the electron optics. This means that transmission electron microscopes can routinely collect sub-nanometre-scale (if not atomic-resolution) images. TEM is used on block copolymer samples to gain insights into the size, shape, and morphology of the polymer nanoparticles.

Organic samples have contrasting issues and require external staining by a staining agent; in this work, uranyl acetate was used as a staining agent. Uranyl acetate provides a negative stain, meaning that it is used to enhance the contrast between the background and the sample's image. TEM is performed under vacuum, which means another caveat is that samples must be dried before TEM, which can lead to certain drying artefacts being present on the grid whilst imaging. These drying artefacts can be visualised in Figure 4. Negative effects of drying during TEM include increasing particle concentration and attractive inter-particle forces, net transport of particles towards the drop perimeter, and dewetting resulting in shrinking contact area.⁴

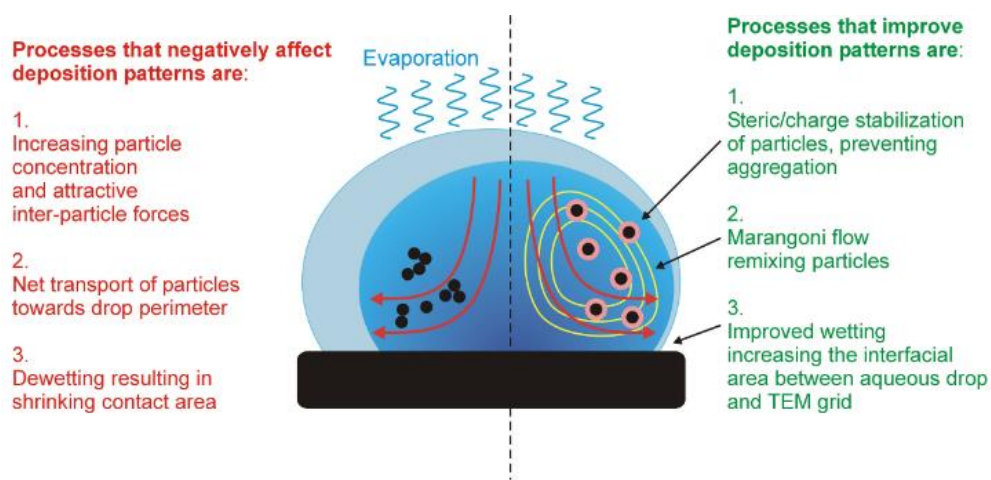


Figure 2.5. Illustration of an aqueous drop and the dominant processes influencing the deposition pattern of suspended particles onto a hydrophobic surface. Red arrows indicate effective particle flow due to the coffee ring effect. Yellow lines show counterflow introduced by the Marangoni flow. Reproduced with permission from B. Michen, C. Geers, D. Vanhecke, C. Endes, B. Rothen-Rutishauser, S. Balog, and A. Petri-Fink, *Sci. Rep.*, 2015, **5** DOI:10.1038/srep09793.

Experimental protocol for transmission electron microscopy (TEM)

Copper/palladium TEM grids were coated in-house to yield a thin film of amorphous carbon. The grids were then subjected to a glow discharge for 30 s. Individual 10.0 μ L droplets of 0.1% w/w aqueous copolymer dispersions were placed on freshly treated grids for 1 min and then carefully blotted with filter paper to remove excess solution. To ensure sufficient electron contrast, uranyl acetate (1% w/v) was absorbed onto the sample-loaded grid for 30 s and then carefully blotted to remove excess stain. Imaging was performed using a Tecnai F20 FEGTEM.

Size analysis of PDMAm₅₀-b-PDAAm_y block copolymer nano-objects was conducted using FIJI-Image J software. Individual nano-objects were selected from representative TEM images. The size was determined as the longest straight line through the centre of the nanoparticle for spherical nano objects (spherical micelles and polymersomes) and the contour and persistent length for worms and high aspect ratio tubules. The membrane characteristics of polymersomes were conducted on selected symmetrical polymer vesicles via two methods, the Line profile method and the Radial integration method. Details of both protocols are below.

Vesicle selection criteria

1. High spherical symmetry of the vesicle
2. Visible differences in intensity between the core and the membrane
3. Representative of the sample ethos

Line profile method:

Regions of interest were selected where membranes appeared in cross-sectional view, avoiding artefacts or overlapping structures. Using Fiji/ImageJ software, greyscale line profiles (width: 5 pixels) were drawn perpendicular to the membrane plane at a minimum of 10 randomly selected locations per polymersome. Intensity peaks corresponding to the polymer-rich membrane layers were identified, with the full width at half maximum (FWHM) of the combined peaks calculated to account for staining effects. Membrane thickness was derived by averaging the FWHM values across all measured profiles.

Radial integration method:

TEM Images were first calibrated to nm/pixel using Fiji ImageJ's Set Scale tool. Brightness and contrast were adjusted where necessary to enhance differentiation between the bilayer membrane and buffer. The Radial Profile Angle plugin (Plugins > Radial Profile Angle) was applied to individual vesicles. A 360° integration angle (default 180° setting) was used for intact vesicles, while partial angles were selected for vesicles with obscured membranes. The analysis circle was centred manually on each vesicle, and spatial calibration was enabled prior to profile calculation. Profile data (distance vs. intensity) were exported to OriginLab for further processing. To account for vesicle size variability, intensity profiles were horizontally translated (Analysis > Data Manipulation > Horizontal Translate) to align bilayer centre points (intensity minima) at the x-axis origin (0 nm). Columns were then normalised (Analysis > Mathematics > Normalise Columns) to standardise intensity ranges across datasets.

Membrane thickness was defined as the full width at half maximum (FWHM) of the normalised intensity dip corresponding to the bilayer. Measurements were repeated across at least five vesicles per sample to ensure reliability.

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2. B. Chu, *Laser Light Scattering: Basic Principles and Practice*, Academic Press, Boston, 1991.
3. A. M. Paredes, in *Encyclopedia of Food Microbiology*, ed. C. A. Batt and M. L. Tortorello, Academic Press, Oxford, 2nd edn., 2014, pp. 609–615.
4. B. Michen, C. Geers, D. Vanhecke, C. Endes, B. Rothen-Rutishauser, S. Balog and A. Petri-Fink, *Sci. Rep.*, **2015**, *5*, 14680.

Chapter 3

Synthesis and characterisation of PDMAm-PDAAm block-copolymer nano-objects via RAFT-mediated PISA aqueous dispersion polymerisation.

3.1.1. Introduction

The recent increase in the applicability of polymer nanoparticles has increased the demand for efficient and controlled synthetic pathways. Polymer nanoparticles have been utilised in various applications, which include drug delivery,¹ biosensors,² stimulus-responsive cargo delivery,³ nanocomposites,⁴ Agricultural and environmental applications. Recent advances have afforded Polymerisation-Induced Self-Assembly (PISA) as a highly promising self-assembly method to synthesise and self-assemble a wide range of polymer nanoparticles from amphiphilic block copolymers. Due to the method's ability to produce polymer nanoparticles at higher polymer concentrations, compared to traditional self-assembly pathways such as film rehydration techniques, this method has attracted interest as a method to synthesise and self-assemble amphiphilic block copolymers into nano-objects in an industrial setting, as highlighted in the recent review by György and colleagues.⁶

This, along with the attractiveness of in-situ polymer synthesis and self-assembly, has led PISA to be studied intensively, even recently, by the polymer self-assembly scientific community.^{7,8}

PISA has been applied to a range of monomer pairings and has produced vesicles routinely at high concentrations of up to 50% solids.⁹ This method is attractive synthetically because it also allows for a range of polymer nano-morphologies that can be tuned by alterations to the block length of the hydrophilic and hydrophobic segments of the block copolymer during PISA synthesis.⁹

This PDMAm-PDAAm polymer has been introduced by Byard and co-workers in 2017.¹⁰ This group synthesised and characterised a range of PDMAm/PDAAm-based block copolymers of varying lengths and hydrophobic/hydrophilic characteristics. In this work¹⁰, Byard and co-workers created a phase diagram which accurately predicted and characterised block-copolymer morphologies obtained by RAFT-mediated PISA, showing shifts in morphology from kinetically trapped spheres, worms, and vesicles, all depending on the relative lengths of both the hydrophilic and hydrophobic segments of the block-copolymers. Since 2017, this PDMAm/PDAAm block copolymer PISA system has been well-studied. Wang and co-workers added to the library of information available on this polymer. This group also highlighted

the ability of this polymer to form a wide range of nano-objects via PISA.¹¹ This chapter intends to expand on this work by focusing on obtaining vesicular morphologies with this PISA system, which can be used in a wide variety of applications, including Nano reactors, bio-nanotechnology, and drug delivery vehicles.^{12,13} In this chapter, it is intended to recreate vesicle self-assembly towards a range of biologically relevant conditions.

One of the main focuses of novelty in this chapter is tailoring syntheses towards biological conditions. Emphasis will also be on trying to alter the experimental conditions of the RAFT-mediated PISA synthesis towards milder conditions in temperature and pH, as well as mimicking natural biological conditions where there is the presence of background salt and increased ionic strength.

This polymer system was chosen because it has shown promising self-assembly behaviour during PISA and has yet to be explored via other traditional self-assembly routes. A systematic study of how different self-assembly routes and hence processing parameters affect the size, shape, and morphology of polymeric nano-objects made from this polymer has yet to be achieved. In doing so, this study could help inform future studies on designing polymeric nano-objects to achieve a specific task, such as encapsulation, under a wide range of biological or industrial conditions.

In this chapter the synthesis and characterisation of various PDMAm₅₀-b-PDAAm_y block copolymers will be discussed. A PDMAm₅₀ macro-chain transfer agent (m-CTA) was made and characterised. This m-CTA was chain extended with DAAM targeting a range of different block lengths. The aqueous phase of the PISA synthesis was altered to allow for polymer synthesis and self-assembly at biologically relevant conditions. The effect of both pH and ionic strength of the aqueous phase of the PISA synthesis was discussed and studied looking at both the size and morphology of nano-objects obtained as the synthesis conditions were altered. The effect of charge on the polymer and how these influences self-assembly was also studied.

3.1.2. Methodology

Synthesis of PDMAm₅₀ macro–Chain Transfer Agents by solution RAFT polymerisation.

The homo-polymerisation of dimethyl acrylamide to a targeted degree of polymerisation of 50 was achieved by dissolving dimethyl acrylamide (10.01 g, 0.1009 mol), 3-((((1-carboxyethyl)thio)carbonothioyl)thio)propanoic acid, (0.5125 g, 2.017x10⁻³ mol) and 2,2'-Azobis[2-(2-imidazolin-2-yl)propane] Dihydrochloride (0.0656 g, 2.017x10⁻⁴ mol) were weighed out and placed in a 40 mL vial. The solution was made up to 30% w/w by adding 24 mL of water at pH 2.5. The solution was degassed under nitrogen for 20 min before being heated for 12 minutes at 70°C in an oil bath. The polymerisation reaction was quenched by exposure to air. The macro-CTA polymer was used without further purification.

PISA synthesis of PDMAm₅₀-b-PDAAm_y diblock co-polymer nano-objects by RAFT Aqueous dispersion polymerisation at pH 2.5.

Sample Name	m-CTA (g)*	DAAm (g)	ACVA (g)	Total Solvent (g)
PDMAm ₅₀ -b-PDAAm ₁₀₀ A	2.05	2.00	0.003	10.5
PDMAm ₅₀ -b-PDAAm ₁₀₀ B	2.05	2.00	0.003	10.5
PDMAm ₅₀ -b-PDAAm ₁₀₀ C	2.05	2.00	0.003	10.5
PDMAm ₅₀ -b-PDAAm ₂₀₀ A	1.03	2.00	0.002	9.24
PDMAm ₅₀ -b-PDAAm ₂₀₀ B	1.03	2.00	0.002	9.24
PDMAm ₅₀ -b-PDAAm ₂₀₀ C	1.03	2.00	0.002	9.24
PDMAm ₅₀ -b-PDAAm ₃₀₀ A	0.684	2.00	0.001	8.83
PDMAm ₅₀ -b-PDAAm ₃₀₀ B	0.684	2.00	0.001	8.83
PDMAm ₅₀ -b-PDAAm ₃₀₀ C	0.684	2.00	0.001	8.83
PDMAm ₅₀ -b-PDAAm ₄₀₀ A	0.514	2.00	0.001	8.62
PDMAm ₅₀ -b-PDAAm ₄₀₀ B	0.513	2.00	0.001	8.62
PDMAm ₅₀ -b-PDAAm ₄₀₀ C	0.513	2.00	0.001	8.62

Table 3.1: Amounts of each reagent used for the RAFT synthesis of PDMAm₅₀-b-PDAAm_y nano-objects

The PDMAm₅₀ macro-CTA was chain extended with diacetone acrylamide to give targeted degrees of polymerisation of 100, 200, 300, 400, and 1200 at 20% w/w in pH ~2.5 water. The PDMAm₅₀ macro-CTA, Diacetone acrylamide, and 4,4'-Azobis(4-cyanovaleric acid) were added before dissolving in pH ~2.5 water, Table 3.1. The solution was degassed under nitrogen for 20 min before being heated for 4 hours at 70°C in an oil bath. The polymerisation reaction was quenched by exposure to air. The m-CTA was used for subsequent PISA reactions without further purification.

PISA synthesis of PDMAm₅₀-b-PDAAm_y diblock co-polymer nano-objects by RAFT Aqueous dispersion polymerisation at pH 2.5.

The PDMAm₅₀ macro-CTA was chain extended with diacetone acrylamide to a targeted degree of polymerisation of 300 at 20% w/w in pH 7.5 water with or without 140mM NaCl. The PDMAm₅₀ macro-CTA, Diacetone acrylamide, and 4,4'-Azobis(4-cyanovaleric acid) were added before dissolving in pH 7.5 water. The solution was degassed under nitrogen for 20 min before being heated for 4 hours at 70°C in an oil bath. The polymerisation reaction was quenched by exposure to air.

3.1.3. Synthesis and characterisation of PDMAm₅₀ macro-CTA via RAFT aqueous dispersion polymerisation

The homo-polymerisation of DMAm was conducted in batch, via RAFT polymerisation in pH 2.5 MilliQ water.

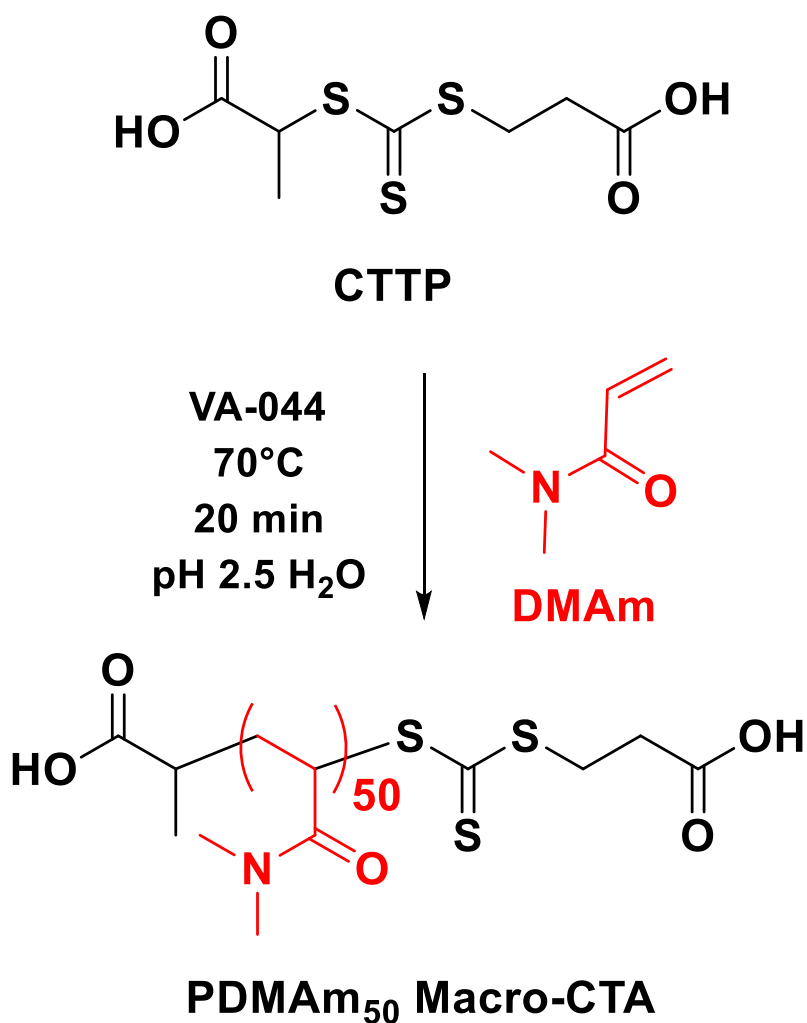


Figure 3.1. Chemical reaction scheme for the homopolymerisation of DMAm to form PDMAm₅₀ m-CTA

The RAFT agent utilised in the m-CTA synthesis is known as CTPP, which has bi-carboxyl functionality. In order to keep these end groups in their neutral,

protonated form, the synthesis of the PDMAm₅₀ m-CTA was performed in acidic MilliQ water. Va-044, or more precisely, 2,2'-Azobis[2-(2-imidazolin-2-yl)propane] Dihydrochloride was used as a source of radicals. This radical initiator is used widely due to its ability to produce radicals rapidly at 70°C.¹⁴

This is due to the instability of the N=N double bond in response to thermal energy, aided by the methyl groups α to the N=N double bond, which stabilise the resultant radicals via hyperconjugation, Figure 3.2.

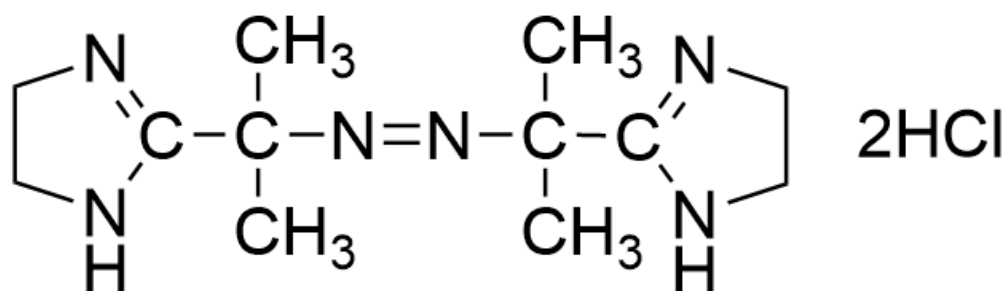


Figure 3.2. Chemical structure of VA-044 initiator used in the synthesis of PDMAm₅₀ m-CTA

Moreover, a different RAFT agent, compared to the one used by Byard and coworkers, CTPP (3-((((1-carboxyethyl)thio)carbonothioyl)thio)propanoic acid), was employed throughout syntheses in this chapter. This RAFT agent has two terminal carboxylic acid groups and a stable thioester group, typically used for RAFT polymerisations.

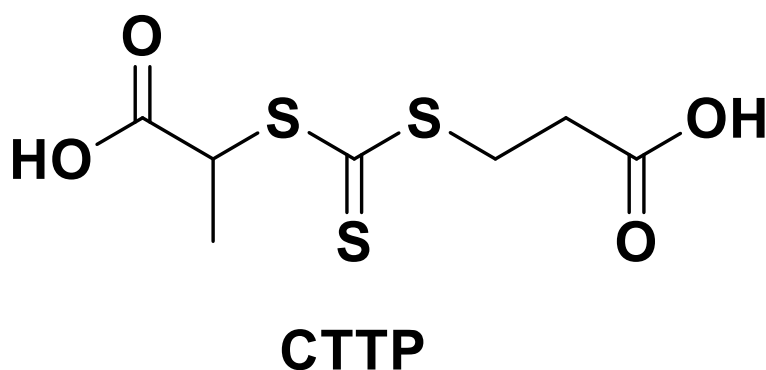
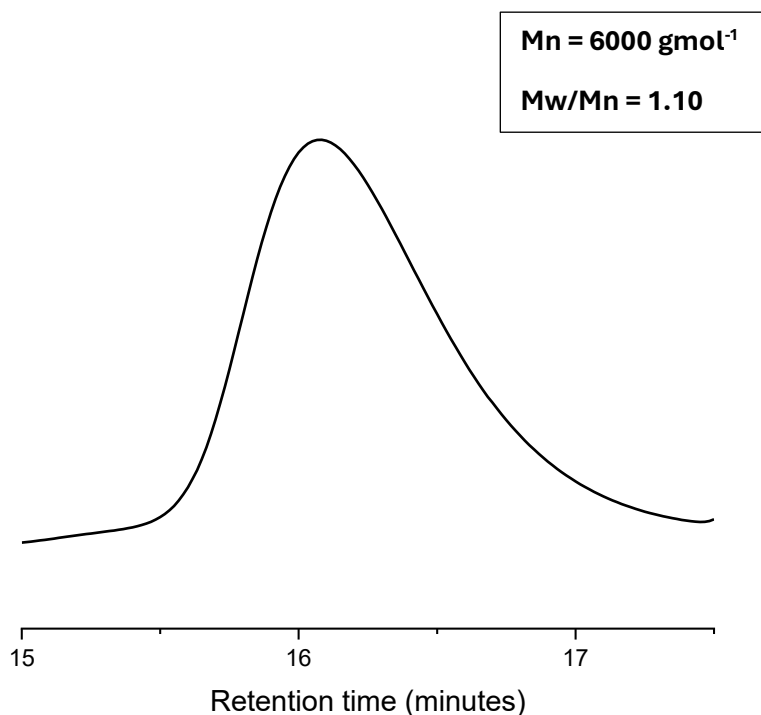


Figure 3.3. Chemical structure of CTPP RAFT agent [full chemical name 3-((((1-carboxyethyl)thio)carbonothioyl)thio)propanoic acid]

The conversion of the DMAm monomer was calculated using ^1H NMR. This was achieved by comparing the integrated residual monomer vinyl signals between 5.5-6.8 ppm to the combined monomer and polymer signals between 2.4-3.3 ppm. After 15 minutes of polymerisation, the conversion was found to be >99%.



Once the polymerisation was completed, GPC was used to characterise the m-CTAs' size and polydispersity, which also indicated polymer growth.

Figure 3.4. GPC chromatogram of PDMAm₅₀ m-CTA

The GPC chromatogram shows a clear monomodal peak for the targeted PDMAm₅₀ m-CTA. The polydispersity of the m-CTA is low, indicative of a highly controlled RAFT polymerisation. Also, the m-CTA's polydispersity is within the range expected (1.09-1.12) in current literature for PDMAm_x systems with DPs ranging from 40-77.³ A theoretical total ($M_{n,th}$), which is to $254.35 \text{ g mol}^{-1}$, of the m-CTA can be calculated by comparing the NMR conversions to the concentration of monomer and RAFT agent before polymerisation, as follows. Because,

$$\text{Equation 3.1 } M_{n,th} = \left(\left(\frac{[DMAm]_0}{[CTTP]_0} \right) \times DMAm \text{ conv.} \times M_{DMAm} \right) + M_{CTTP}$$

$$\text{Equation 3.2 } M_{n,th} = \left(\left(\frac{[101 \text{ mmol}]_0}{[2.019]_0} \right) \times 0.998 \times 99.13 \text{ g mol}^{-1} \right) + 254.35 \text{ g mol}^{-1}$$

Theoretical $M_n = 5203.38 \text{ g mol}^{-1}$

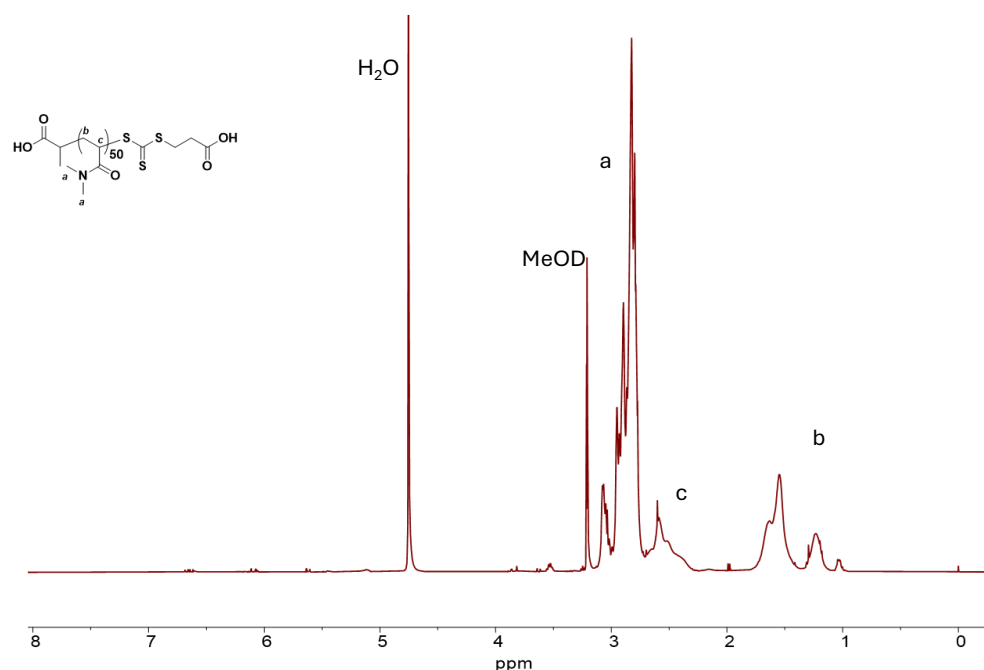


Figure 3.5. NMR spectrum for PDMAm50 m-CTA with peak assignments

The GPC recorded M_n of 6000 g mol^{-1} is significantly different from the theoretical M_n , possibly due to using PMMA standards in the GPC calibration. This may suggest that the PDMAm₅₀ m-CTA is of a higher degree of polymerisation than initially anticipated. It is not uncommon in RAFT polymerisation that targeted DPs are exceeded through a polymerisation reaction, but as the m-CTA synthesised is in the range expected for a DP of ~50-60, this m-CTA was used for the formation of di-block copolymers by chain extension with DAAM.

A relevant example comes from a study by Canning *et al.*, which found that DPs could be exceeded during PISA under specific conditions. The authors

observed that when using poly(glycerol monomethacrylate) as a macro-chain transfer agent for the RAFT aqueous dispersion polymerisation of 2-hydroxypropyl methacrylate, the core-forming block could grow beyond the targeted degree of polymerisation. This was attributed to the gradual loss of RAFT end-group fidelity during the polymerisation process.¹⁵

The macro-CTA was used without further purification, which is an advantage of this technique as it avoids cost and time expensive purification steps to achieve self-assembly.

3.1.4. RAFT-mediated PISA Synthesis and characterisation of PDMAm₅₀-b-PDAAm_y block-copolymer nano-objects in acidic conditions

Chain extension of the PDMAm₅₀ m-CTA with DAAM was conducted via RAFT aqueous dispersion polymerisation at 20% w/w solids following a similar scheme proposed by Byard and co-workers.¹⁰

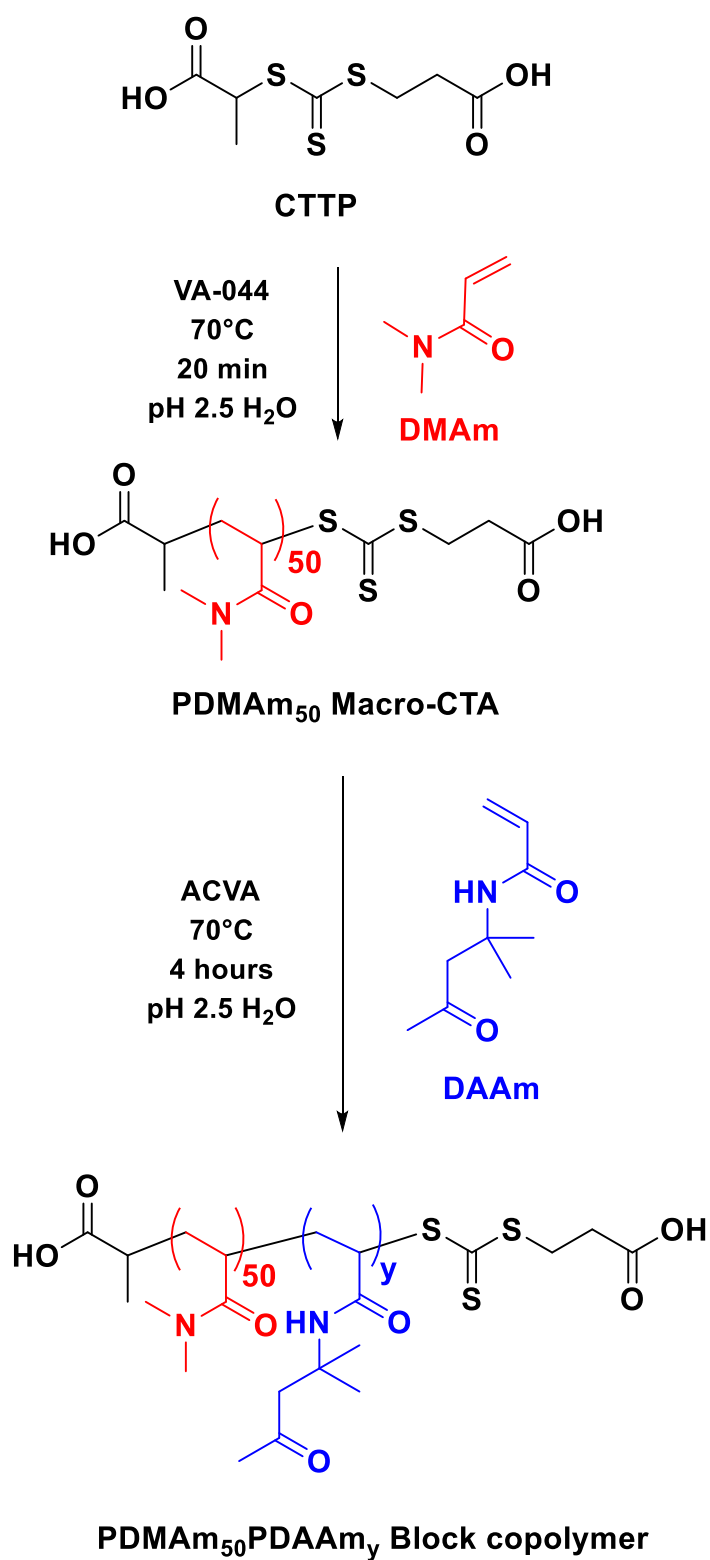


Figure 3.6. Chemical reaction scheme illustrating the two-step process in making PDMAm₅₀-b-PDAAm_y block co-polymers used in this work, adapted from Byard and co-workers.¹⁰

When designing this synthesis, AVCA was chosen as the radical initiator over VA-044. Due to the nature of the self-assembly of the nano-objects, a slower radical polymerisation was conducted with ACVA to allow for a more controlled growth of the larger structures. Byard and co-workers produced a kinetic study of the chain extension of a PDMAm₅₈ m-CTA with a targeted DAAM block length of 120 and found that 99% monomer conversion was achieved after 90 min. This, coupled with other kinetic studies of this system performed in the Warren group, convinced us to keep the polymerisation time to 4 hours to achieve maximum conversion of the DAAM monomer. For consistency, the PISA formulation was adjusted to pH 2.5 with HCl to ensure protonation of terminal carboxylic groups on the CTPP raft agent. A range of DPs of DAAM were targeted in order to assess and quantify expected changes in morphology of the diblock nano-objects, as a function of increasing length of the hydrophobic block. Byard and co-workers recently established a phase diagram for different PDMAm_x m-CTAs with DPs ranging from 40 to 70. These were chain-extended systematically with DAAM, targeting a range of DPs. This phase diagram was used as a rough guide to predict the morphologies of the PDMAm₅₀-b-PDAAm_y nano-objects produced in this work. A similar phase study was constructed by Wang and co-workers using a fixed m-CTA and varying the targeted DP of PDAAm at various solid content percentages, both phase diagrams are presented in figure 3.6.¹⁰

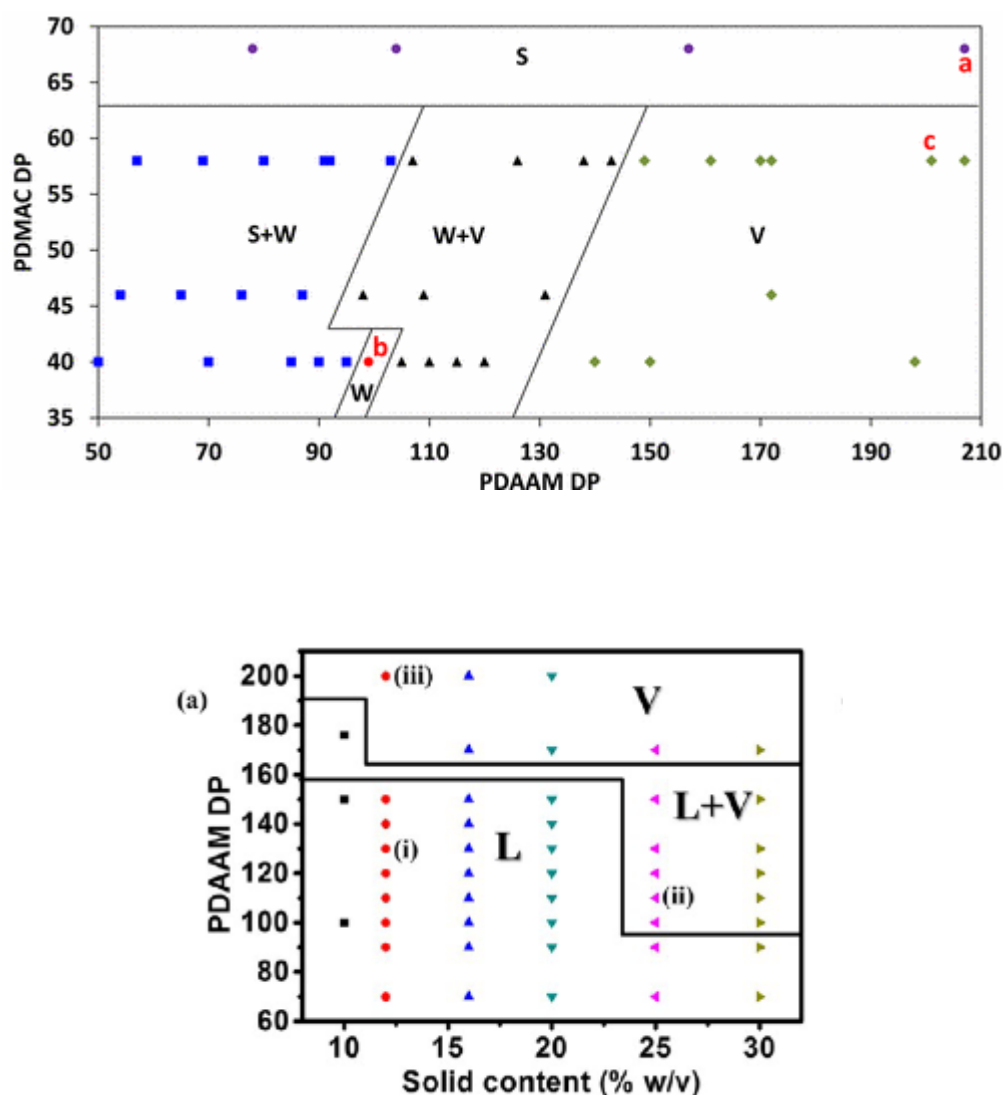


Figure 3.7. Phase diagrams were constructed for a series of PDMA_m-b-PDAA_m diblock copolymer nano-objects made via raft-mediated PISA. Data points represent individual PISA syntheses characterised by GPC, NMR, DLS, and TEM. *TOP* Byard and coworkers used a range of different sized m-CTAs to target a range of PDAAM DPs at a fixed solids content of 20% *BOTTOM* Wang and coworkers used a single m-CTA to target a range of PDAAM DPs at varying solid contents from 10-30%. S = spheres, S + W = mixed spheres and worms, W = worms, W + V = mixed worms and vesicles and V = vesicles L= Lamella.^{10,11}

However, there seems to be a gap in TEM images in their study at the boundaries of their hypothetical phase diagram. To exploit and understand this further, a PDMA₅₀-b-PDAA₁₀₀ block length was targeted, which is directly on the border between spheres, worms, and vesicles. Following on from the phase diagram created by Byard and co-workers, chain extending a PDMA₅₀

m-CTA with targeted blocks of DAAm > 150 would yield pure vesicular morphologies. In order to assess any physical differences in vesicle size, structure, and polydispersity, target DAAm DPs of 200, 300, 400, 600, 900, and 1200 were also synthesised. A summary of the characterisation data is presented below, Table 3.3.

Table 3.3: Summary of PISA nano-object characterisation; DAAm monomer conversion, molecular weight data and dynamic light scattering data for PDMAm₅₀-*b*-PDAAm_y di-block copolymer nano-objects at pH 2.5 Standard deviations are from triplicate individual PISA syntheses

Targeted DP of Sample	Average DAAm conversion (%) Via ¹ H NMR	Average (Kg mol ⁻¹) Via GPC	M _n Average Via GPC	M _w /M _n Average	Average Z-average diameter (nm)	Average PDI Via DLS
PDMAm50- <i>b</i> -PDAAm ₁₀₀	>98	23.5 ± 1.50	1.14 ± 0.03		127 ± 36	0.108 ± 0.007
PDMAm50- <i>b</i> -PDAAm ₂₀₀	>98	36.3 ± 2.50	1.24 ± 0.04		309 ± 17	0.170 ± 0.007
PDMAm50- <i>b</i> -PDAAm ₃₀₀	>98	61.3 ± 1.70	1.14 ± 0.01		369 ± 16	0.124 ± 0.012
PDMAm50- <i>b</i> -PDAAm ₄₀₀	>98	81.1 ± 2.80	1.22 ± 0.02		284 ± 10	0.098 ± 0.028
PDMAm50- <i>b</i> -PDAAm ₆₀₀	>98	90.4 ± 4.32	1.41 ± 0.03		322 ± 16	0.135 ± 0.085
PDMAm50- <i>b</i> -PDAAm ₉₀₀	>98	150.7 ± 9.67	1.46 ± 0.03		316 ± 18	0.140 ± 0.104
PDMAm50- <i>b</i> -PDAAm ₁₂₀₀	>98	194.7 ± 11.64	1.50 ± 0.04		367 ± 15	0.091 ± 0.023

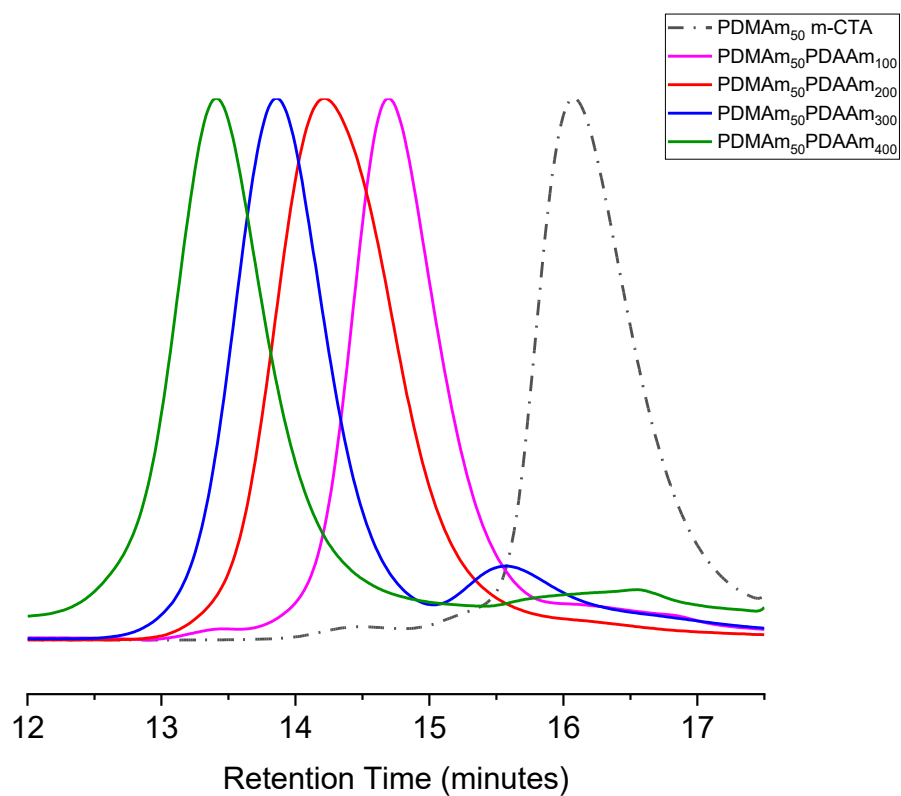


Figure 3.8. GPC chromatograms of PDMAm₅₀ m-CTA and of di-block copolymers extended with DAAm

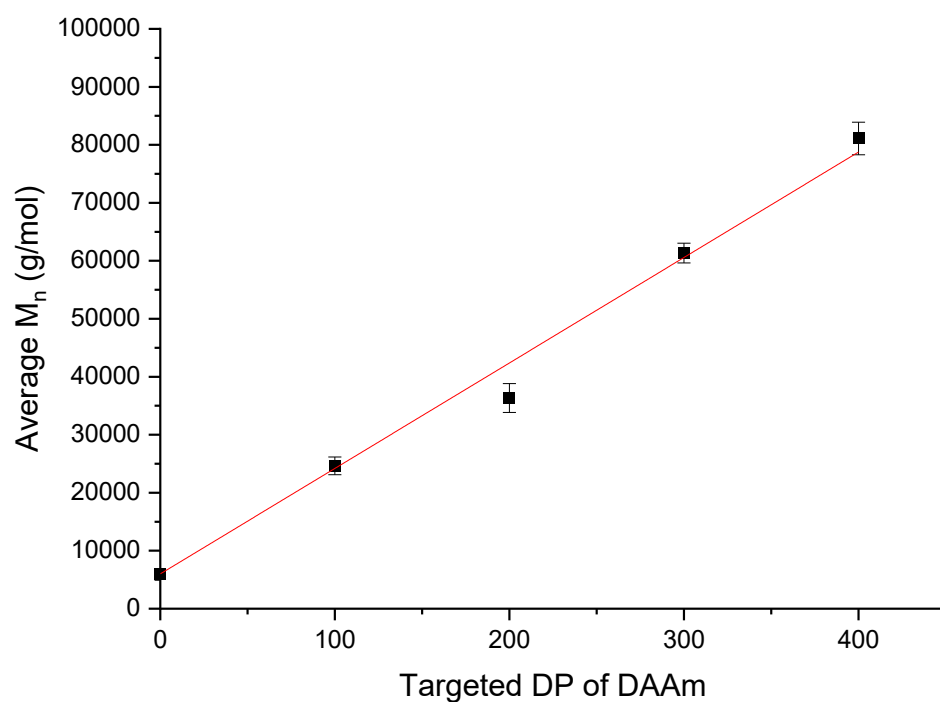


Figure 3.9. Change in the average M_n of $\text{PDMAm}_{50}\text{-b-PDAAm}_y$ Samples as a function of targeted DP of DAAM. Error bars represent the standard deviation of three independent block-copolymer samples.

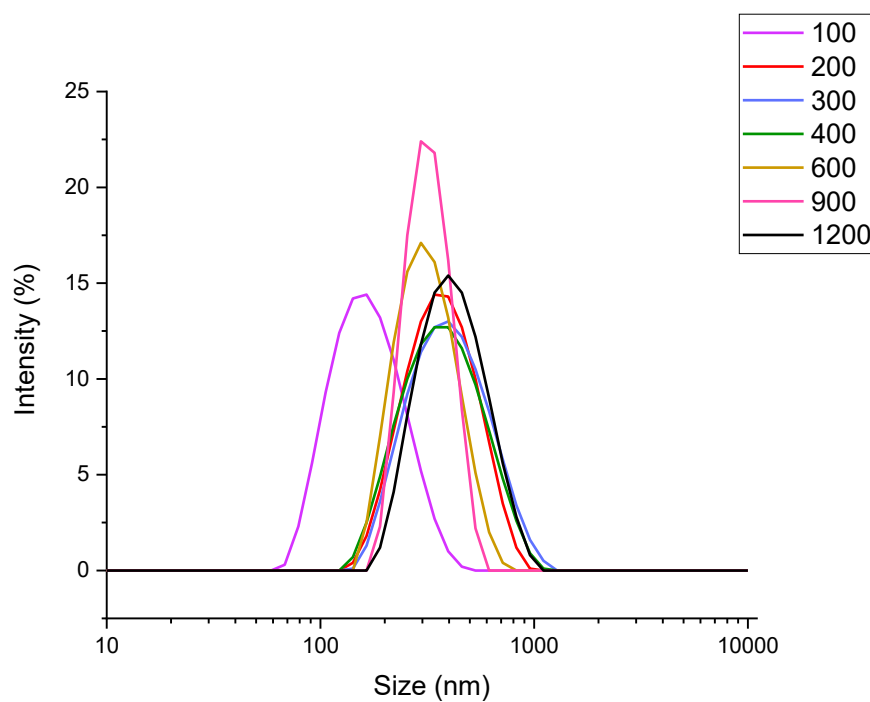


Figure 3.10. Nano-particle size distributions of $\text{PDMAm}_{50}\text{-b-PDAAm}_y$ block copolymers. Legend represents the targeted length of the DAAM block.

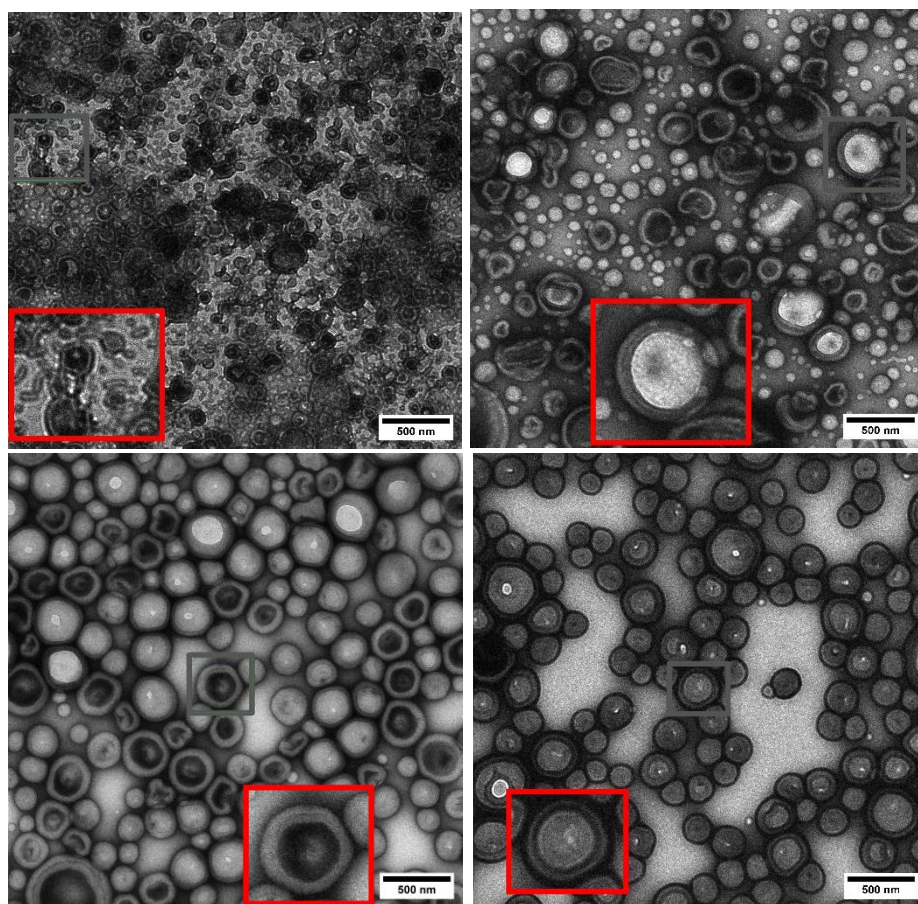


Figure 3.11. TEM micrographs for PDMA₅₀-b-PDAAm_y, di-block copolymer nano-objects at pH 2.5. Top left; PDMA₅₀-b-PDAAm₁₀₀, Top right; PDMA₅₀-b-PDAAm₂₀₀, Bottom left; PDMA₅₀-b-PDAAm₃₀₀ Bottom right; PDMA₅₀-b-PDAAm₄₀₀, Additional images are presented in the appendix (7.1-7.8)

The conversion of the DAAM monomer was determined by comparison of the residual vinyl signals at 5.45 – 6.18 ppm to the terminal ketone groups in the polymer backbone at 1.99-2.07 ppm as follows,

$$\text{Equation 3.3 } \text{Monomer conversion (\%)} = \left(1 - \frac{H_{\text{vinyl}}}{H_{\text{ketone}}}\right) \times 100$$

An example of a high-field NMR spectrum is shown below, highlighting the peak assignments for the PDMA₅₀-b-PDAAm₃₀₀ block copolymer.

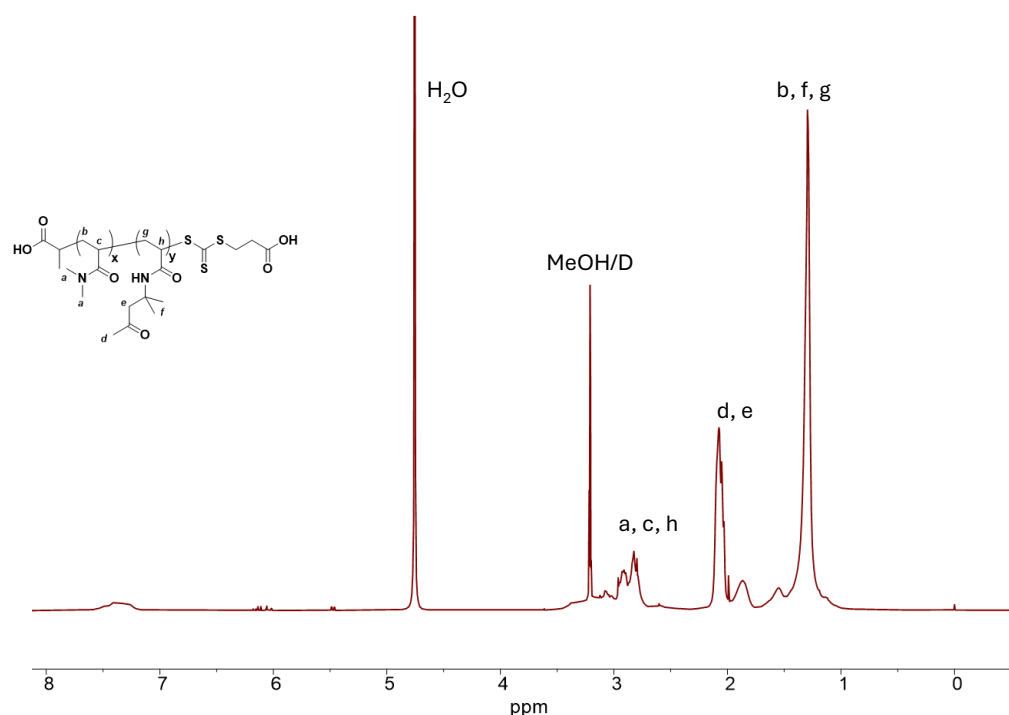


Figure 3.12. NMR spectrum for PDMAm₅₀-b-PDAAm₃₀₀ block copolymer with peak assignments

The integral of the residual monomer vinyl peaks was normalised to 3 and then compared to the total integral of the polymer and monomer peaks due to terminal methyl protons adjacent to the ketone at 1.99 – 2.07 ppm. Upon polymerisation of DAAM, the expected chemical shift of the terminal ketone groups broadens and shifts to a slightly higher ppm, coupled with peak broadening. In retrospect, the overlap of other alkyl signals in the polymer backbone may make it harder to identify the absolute integral of the residual monomeric shoulder at 1.99 ppm, as small discrepancies in the baseline correction of the residual monomer ketone peaks could cause errors in the calculation of the monomer conversion. Monomer consumption is widely accepted as a more facile method of determining the kinetics and conversion of a polymerisation reaction. Nonetheless, in all cases, after 4 hours, the residual monomer peaks were small in integrals compared to the polymer backbone and in some cases absent completely, qualitatively indicating the polymerisation was near 100% completion, supported by the quantitative determination by comparison of the terminal ketone groups in DAAM.

From Figure 3.9, the *M_n* of the polymers was also determined, which showed a linear increase as the targeted length of the DAAM block increased. The change

in M_n was plotted as a function of increasing targeted DAAM DP, which showed a linear increase when fitted, figure 7. This data suggests that the chain extension of the PDMAm₅₀ m-CTA was successful within the range of target DPs selected. This was also confirmed by an increase in turbidity of the solution as the polymerisation reaction proceeded. The intercept of the average M_n of the di-block copolymers was set as 6000 g mol⁻¹, the M_n of the PDMAm₅₀ m-CTA. The R^2 value of 0.997 was indicative of a good linear fit of the data. The theoretical M_n (5203 g mol⁻¹) was also calculated, which again showed some deviation from the expected M_n of the di-block copolymer systems, most notably in the case of PDMAm₅₀-b-PDAAm₂₀₀ samples. This could suggest that the actual DP of DAAM is slightly lower than expected.

From Figure 3.8, GPC chromatograms of the PDMAm₅₀-b-PDAAm₃₀₀ samples show a clear bimodal nature with a smaller shoulder appearing at 15-16 min. This was determined to be unreacted m-CTA, suggesting the chain extension of the PDMAm₅₀ m-CTA did not go to near 100% completion. This is also highlighted in the provisional NMR conversions, where there was a larger vinyl integral at 5.45 – 6.18 ppm.

The M_w/M_n values reported were lower in most cases than those previously reported by Byard and co-workers. Overall, the GPC dispersities were between 1.12 and 1.29, indicative of a well-controlled RAFT chain extension of the PDMAm₅₀ m-CTA. The highest reported M_w/M_n was that of the PDMAm₅₀-b-PDAAm₂₀₀ di-block copolymers. Assuming the DP of the m-CTA is between 50 and 60, this diblock copolymer corresponds to a phase boundary between spheres and vesicles in the phase diagram published by Byard and co-workers..

Before any size measurements of the di-block copolymer solutions could be conducted, the effect of pH and dilution on size measurements needed to be explored. Using DLS and electrophoresis studies, Byard and co-workers reported a pH tolerance of this diblock co-polymer system in most cases. However, this group reported a tendency for PDMAm₄₀PDAAm₉₉ 'worms' to undergo dissociation into shorter worms and spherical nanoparticles. It is common practice that when using the DLS apparatus, the solutions' turbidity should be reduced to avoid any multiple scattering effects. Therefore, it was key to explore the effect of dilution on the size of the nano-objects, as dilution would correspond to an increase in the solvent. It was shown that an increase in water

could cause swelling and changes in the permeability of vesicles, leading to variations in size measurements obtained by DLS.

Research by Miller et al. published in 2022 discusses this phenomenon. Specifically, they state that water transport into polymersomes can cause swelling, which can be measured as an increase in size using light scattering techniques like DLS. The authors note that this method "overcomes some of the limitations of microscopy" for observing these changes.¹⁶

During this experiment, the largest polymer with targeted hydrophobic DAAM blocks of 400 was used to study any changes in Z-average size and PDI.

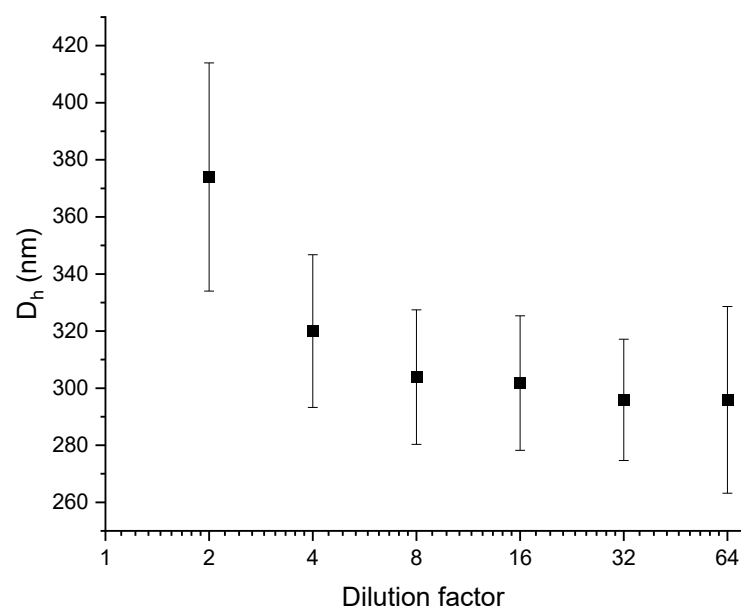
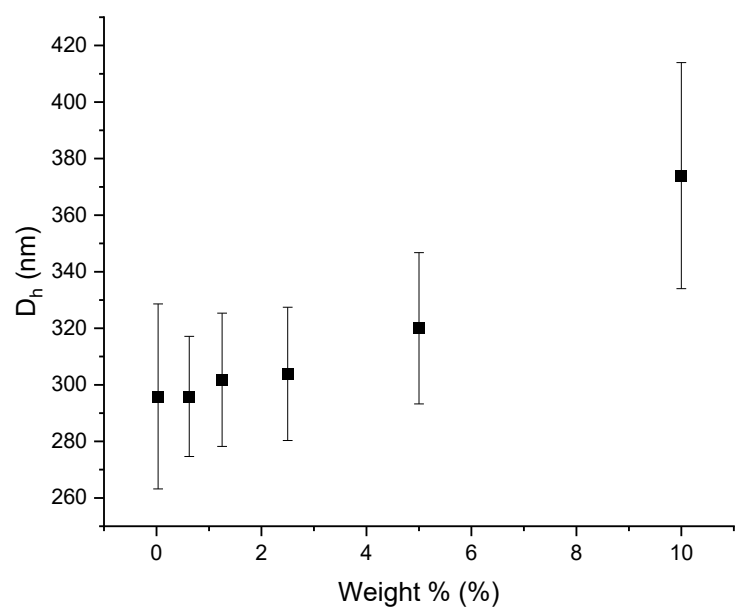


Figure 3.13: Change in Z-average hydrodynamic diameter and Polydispersity Index (PDI) measured via DLS as a function of serial dilutions from a PDMAm₅₀-b-PDAAm₄₀₀ block copolymer dispersion starting at 10 wt.% solids, reduced down by a factor of 0.5 per each subsequent dilution (Dilution factor 2) to a final concentration of 0.3125 wt.%

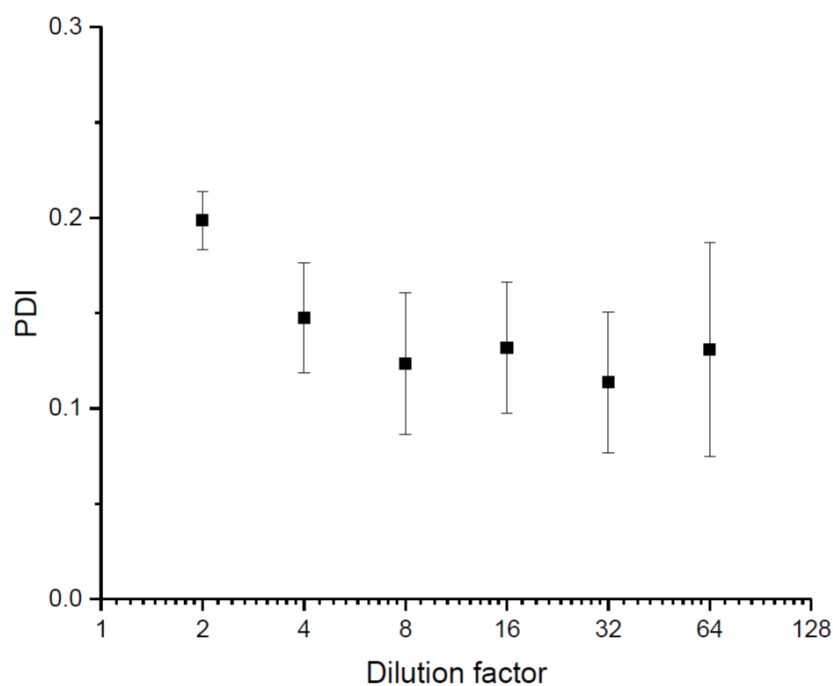


Figure 3.14. Change in Polydispersity Index (PDI) as a function of serial dilutions.

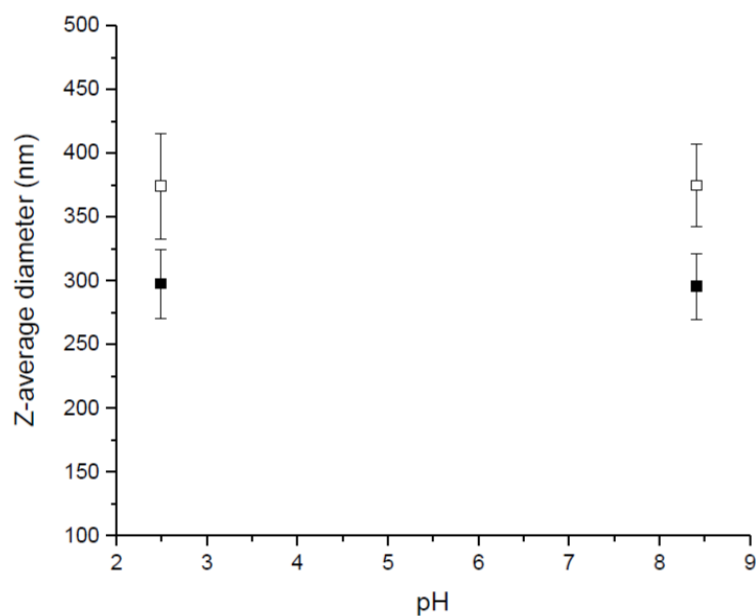


Figure 3.15. Change in Z-average hydrodynamic diameter of PDMA₅₀-b-PDAAm₄₀₀ Nano-objects as a function of pH. White squares represent a DF of 2 (10 wt%), black squares represent a DF of 16 (1.25 wt %). Error bars represent the standard deviation between triplicate samples from triplicate PISA syntheses.

The data shows an overall decrease in the average Z-average diameter of PDMAm₅₀-b-PDAAm₄₀₀ nano-objects with increasing dilution factor. The change in Z-average diameter of the di-block copolymer is most evident between initial dilutions, from DFs 2-8 (20 wt%-2.5 wt% %), before levelling off at around 300 nm. This trend is also observable in the recorded PDI, which reaches a plateau beyond DF 8. For the sake of the measurements, it can be concluded that a DF of 16 reduces the turbidity of the solution enough to allow for accurate size measurements, without considerable multiple scattering effects. When diluting copolymer solutions for DLS measurements, the effect of varying the pH of the diluent was also explored. As previously mentioned, during PISA syntheses, the pH was maintained at ~2.5 to ensure protonation of terminal carboxylic acid groups on the CTPP RAFT agent. To explore how pH may affect size measurements from DLS, the Z-average sizes at two distinct DFs, 2 and 16, were obtained in MilliQ water, before and after adjustment to pH ~2.5 after synthesis, with HCl. Figure 3.15

From the data, there seems to be little variation in the Z-average diameter of the nano-objects with increasing pH, in agreement with Byard and co-workers. Di-block copolymers samples at a DF 2 had an average Z-average diameter of around 374 nm, regardless of significant changes in the ionic strength of the solution. The same conclusion was found at the key operating DF of 16, as the di-block copolymers had an average Z-average diameter of ~296 nm. From the data discussed in this section, it was concluded that the effect of dilution provides more variation on size measurements than the ionic strength of the di-block copolymer solution. Henceforth, all DLS size studies for all di-block copolymer samples were conducted at DF 16 at pH ~2.5.

The DLS data in Table 3.1 shows there is an overall increase in the size of the Nano-objects as the targeted DP DAAM increases. This is also supported by TEM images, Figure 3.13, which show an overall increase in the sizes of nano-objects. From the TEM images in Figure 3.13, when targeting a DP of 100, it is evident that there is a mixed phase of smaller nano-objects. The study observes a wide range of morphologies for PDMAm₅₀-b-PDAAm_y block-copolymer nano-objects including micelles, worms and even vesicles for the PDMAm₅₀-b-PDAAm_{100A} sample. This was expected from the phase diagram produced by Byard and co-workers, where the targeted PDMAm₅₀-b-PDAAm₁₀₀ di-block copolymer is on the border between spheres, worms, and vesicles. This correlated to an overall smaller size observed with DLS, also observed by Byard

and co-workers, who observed a mixed phase at lower DPs of DAAm.⁶ Lower DPs of DAAm correlate to an overall smaller volume fraction of the hydrophobic block, which would favour micelle and worm morphologies.

When the targeted DAAm DP is increased to 200, there is a sharp increase in the size of the Nano-objects to ~300 nm. From TEM images of the PDMAm₅₀-b-PDAAm_{200A} sample, there is a change in morphologies to predominantly a mixture of micelles and vesicle-like structures. The research shows less worm-like morphologies as there is an increase in the targeted DAAm DP, which agrees with the literature. However, it is noted that based on the interfacial curvature model of self-assembly, it is unclear as to why worms disappear if spheres still coexist with vesicles. Vesicles produced from PDMAm₅₀-b-PDAAm_{200A} seem to be dispersed in diameter and membrane characteristics. Perhaps the study is observing “jellyfish” like structures in some cases where bilayers wrap up to form jellyfish structures before enclosing to form vesicles, as seen by Blanazs and coworkers who investigated the mechanism by which worms form vesicles.¹⁷

Increasing the targeted DP of DAAm to 300 yielded an increase in the number of vesicles, which coincided with a decrease in the number of kinetically trapped micelles. This was also supported by the DLS data, which showed an increase in the Z-average size of di-block copolymer Nano-objects and a decrease in the recorded PDI. There is a higher-than-expected Z-average size of Nano-objects under DLS, which may be since the intermediary “jellyfish” nano-objects between worms and vesicles would assume a larger hydrodynamic radius under DLS, where the Stokes-Einstein equation assumes spherical non-interacting particles. Interestingly, Byard and co-workers have reported pure vesicle morphologies in this region, which perhaps alludes to a higher PDMAm DP in the m-CTA than expected, going by their phase diagram. This agrees with the higher M_n of 6000 g mol⁻¹ observed in the m-CTA. A larger m-CTA would reduce the overall volume fraction of the hydrophobic DAAm block, which would encourage a mix of vesicles and spheres.

For the PDMAm₅₀-b-PDAAm₄₀₀ sample, it is observed that there is a clear shift in the number of vesicles, with almost no micelles observed. However, there is a drop in the Z-average size of the nanoparticles. A potential reason for this may be that, as there is an increase in the DP of the hydrophobic block, there may be fewer intermediary structures and more vesicles. This hypothesis is

supported by a lower PDI from DLS, suggesting most of the nano-objects are similar in hydrodynamic diameter.

From work by Warren and co-workers, we see a similar phenomenon when it comes to the reduction in size of polymersomes as the hydrophobic block grows from 200 to 400. In this research, the growth mechanism of $G_{55}-H_x$ diblock copolymer vesicles involves an increase in membrane thickness as the degree of polymerisation (DP) of the membrane-forming PHPMA block increases from 200 to 1000, while the overall vesicle diameter remains relatively constant. This process led to a reduction in the vesicle lumen size and total interfacial area, minimising the free energy of the system.¹⁸

3.1.5. Effect of pH on RAFT-mediated PISA of PDMAm₅₀-b-PDAAm_y Nano-objects.

Up until this point, all RAFT-mediated PISA syntheses of the PDMAm/PDAAm nano-objects have been conducted in pH 2.5 water following the work of Byard and co-workers. It was thought that acidic conditions would ensure protonation of terminal carboxylic acid groups, leaving no net charge across the individual block-copolymer at pH 2.5. It could be theorised that increasing the pH would lead to deprotonation of the carboxylic acid groups on the RAFT agent, leaving two anionic charges per polymer chain (Figure 3.16).

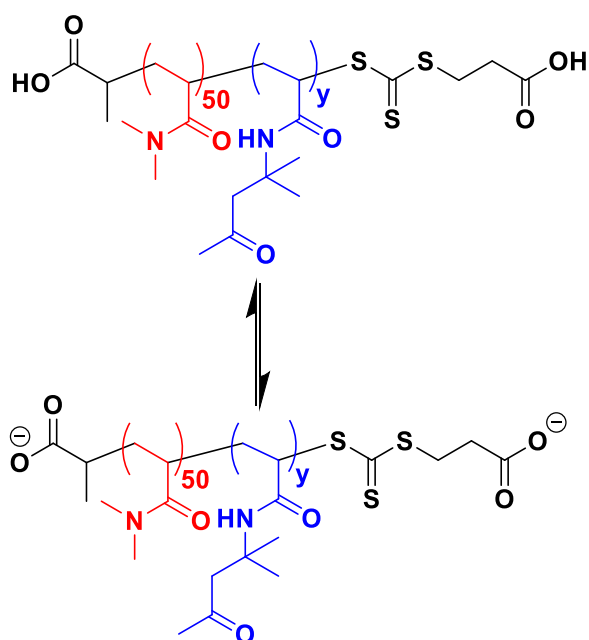


Figure 3.16 Chemical reaction scheme of PDMA_{m50}-b-PDAA_{m_y} block copolymer displaying reversible protonation as pH is increased from pH 2.5 to pH 7.5 with aqueous KOH.

Lovett *et al.* have shown that ionisation of the terminal carboxylic groups of the block copolymer led to morphological transitions in nano-objects prepared.¹³ This group theorised that the ionisation of the terminal carboxylic group on the polymer leads to increased hydration of the core forming GMA block, thus reducing the theoretical packing parameter. This group also observed an irreversible vesicle-to-worm morphological transition upon increasing the pH from 2.5 to physiological pHs around 7.

In the current PDMA_{m50}-b-PDAA_{m300} block copolymer conditions, it is routinely observed that pure vesicular morphologies via PISA at pH 2.5. To assess if the vesicular morphology persists at pH 7.5, analogous PISA syntheses were conducted in pH 7.5 water. A key application of polymeric nano-objects, mainly vesicles, is encapsulation. Further to the work in this thesis, studies on the encapsulation potential of the PDMA_{m50}-b-PDAA_{m300} vesicles made in this work will need to be concluded to maximise potential applications in biotechnology. Currently, the research is unable to encapsulate any dyes or dextrans due to the pH limitation of the system under acidic conditions. For encapsulation studies with the PDMA_{m50}-b-PDAA_{m300} polymer vesicles, the pH must be altered towards neutrality to be able to observe fluorescence of the encapsulated moieties. For example, 6-carboxyfluorecin, a simple organic dye,

has pH-dependent fluorescence and is not suitable for encapsulation at pH 2.5. Without the fluorescent properties of these encapsulated moieties, it would be nearly impossible to quantitatively assess the encapsulation efficiency of the PDMAm₅₀-b-PDAAm₃₀₀ polymer vesicles. Additionally, performing the RAFT-mediated PISA syntheses at neutral pHs widens the scope of potential encapsulants to proteins and biomolecules, which may be damaged or denatured under highly acidic conditions as opposed to in vivo intracellular pH (most commonly between 7.0 and 7.4, though there is variability between tissues).⁴

Increasing the pH to 7.5 may be problematic with the terminal carboxylic acid groups on the block copolymer. These groups are easily deprotonated to leave a negative charge on the terminal ends of the block copolymer, which could influence the self-assembly and resultant properties of the PDMAm₅₀-b-PDAAm₃₀₀ block copolymer vesicles by causing repulsion in the corona/bilayer. Considering this, it was chosen to explore the effect of increasing the pH with NaOH to pH 7.5 on the size and morphology of the vesicles previously prepared via RAFT-mediated PISA at pH 2.5. The pH was altered before the PISA syntheses occurred, i.e., a separate PISA synthesis was conducted at pH 7.5. The characterisation of the RAFT-mediated PISA self-assembly of PDMAm₅₀-b-PDAAm₃₀₀ block polymer at pH 7.5 is presented in Table 2 and Figure 10.

Table 3.4: Summary of PISA nano-object characterisation; DAAm monomer conversion, molecular weight data, and dynamic light scattering data for PDMAm₅₀-b-PDAAm_y di-block copolymer nano-objects at varying pHs.

pH of Syntheses	of PDMAm ₅₀ -b-PDAAm ₃₀₀	PISA of DAAm conversion (%) Via ¹ H NMR	M _n (Kg mol ⁻¹) Via GPC	M _w /M _n GPC	Via Z-average diameter (nm)	PDI Via DLS
pH 2.5		>98	61.3 ± 1.70	1.14 ± 0.01	369 ± 16	0.124 ± 0.012
pH 7.5		>983	76.0	1.26	299 ± 10	0.140 ± 0.036

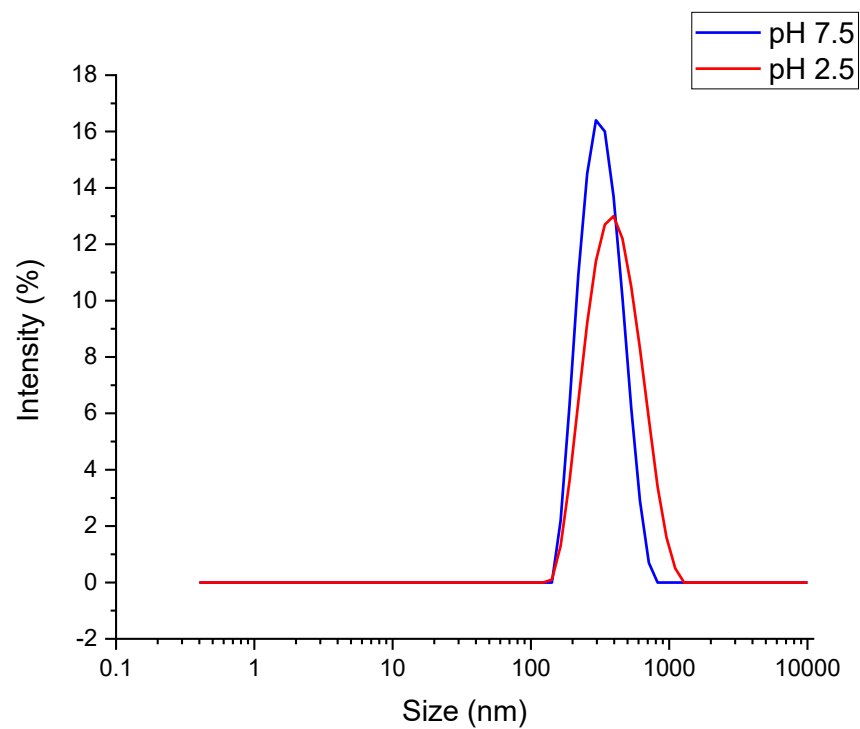


Figure 3.17. Nano-particle size distributions of PDMAm₅₀-b-PDAAm₃₀₀ block copolymers at pH 2.5 and 7.5, respectively.

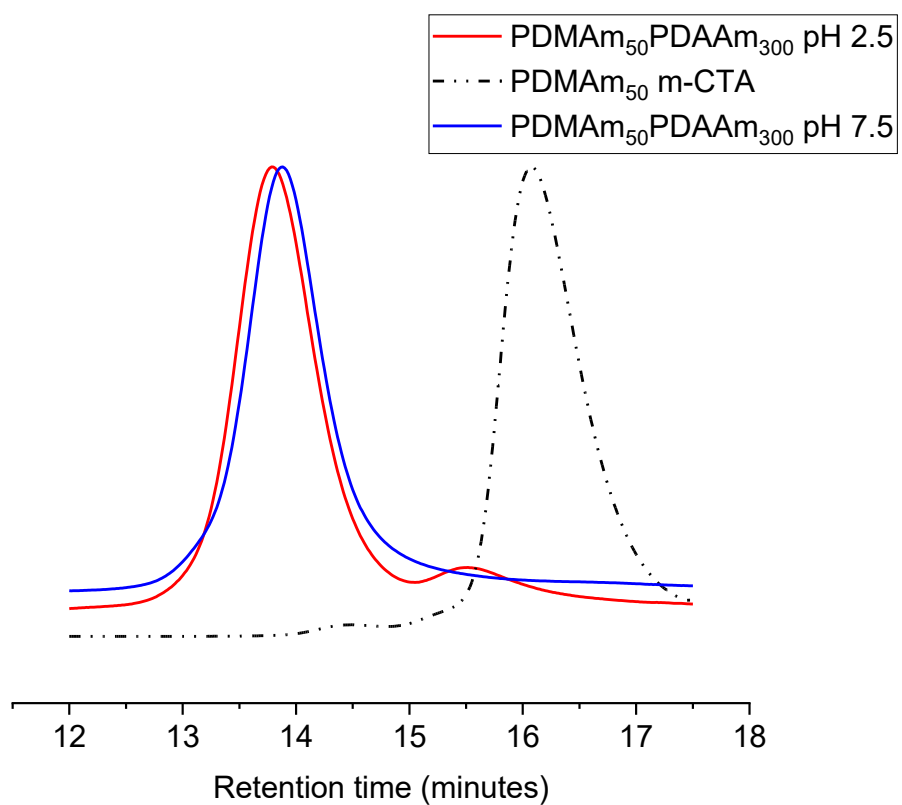


Figure 3.18. GPC chromatograms of PDMAm₅₀ m-CTA and of PDMAm₅₀-b-PDAAm₃₀₀ synthesised at pH 2.5 and 7.5, respectively.

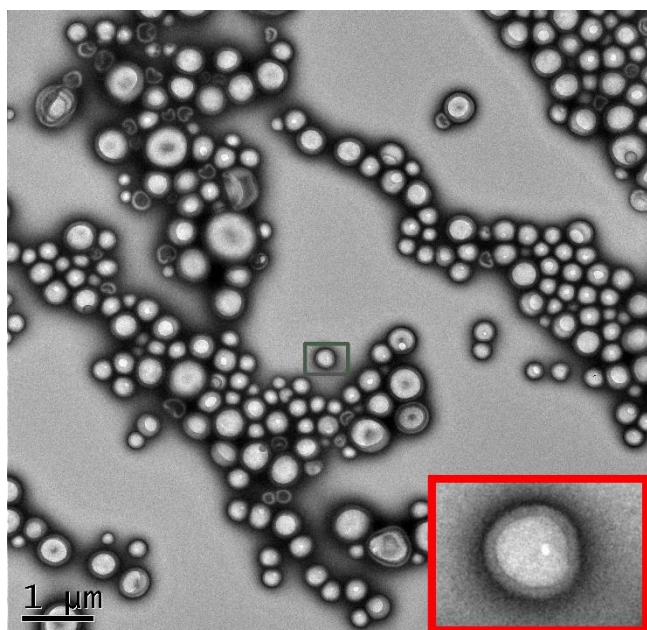


Figure 3.19. TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ di-block copolymer nano-objects at pH 7.5 via RAFT-mediated PISA. Additional images are presented in the appendix (Figures 7.9-7.13)

In Figure 3.17, overlapping size distributions obtained by DLS indicated a similar size distribution for the nanoparticles prepared at both pH 7.5 and 2.5. Both distributions are monomodal, with the pH 7.5 sample being slightly more shifted to smaller sizes and slightly less polydisperse. A small variation of c.a. 70 nm in z-average hydrodynamic radius of the nano-objects was observed when increasing the pH of the PISA solvent to 7.5. The PDI from DLS is very similar for both PISA syntheses, regardless of pH.

According to GPC measurements, figure 3.18, a shift in the M_n from 6000 to 49000 g mol⁻¹ was observed, alongside a near absence of a peak from the macro-CTA, indicating successful chain extension of the PDMAm₅₀ m-CTA under neutral conditions. There was some significant variation in polymer M_n when the PISA synthesis was conducted at pH 7.5. pH 7.5 samples were around 10 kg mol⁻¹ lower than the pH 2.5 PISA syntheses, although there is significant variation in M_n for the pH 7.5 sample compared to the pH 2.5 sample. This is reflected in the raw GPC chromatograms where the pH 7.5 sample has a slightly longer retention time distribution, hence corresponding to a smaller polymer with a smaller radius of gyration. Both peaks are monomodal in the

GPC chromatograms, with a slight shoulder on the pH 2.5 sample; this could be attributed to some unreacted m-CTA present in the sample. Most notably, there was a significant shift in the polydispersity from GPC. Initially, at pH 2.5, there is a PD of 1.14, but upon changing the pH of the synthesis to pH 7.5, the PD from GPC shifts to 1.43. This suggests that the polymer sizes during the RAFT polymerisation increase in dispersity with increasing pH. From the TEM micrograph of the sample it is evident that the main morphology of the nano-objects made via PISA at pH 7.5 is vesicles with very little evidence of spherical micelle morphologies. This is similar to the morphologies observed during the analogous RAFT-mediated PISA syntheses at pH 2.5. The importance of this result is that it allows for polymer vesicles at neutral pH via RAFT-mediated PISA for the block-copolymer system, potentially opening up doors leading to encapsulation of pH sensitive dyes, dextrans and other biomolecules in situ with the PISA process.

3.1.6. Effect of Salt and ionic strength on RAFT-mediated PISA of PDMAm₅₀-b-PDAAm₃₀₀ Nano-objects.

In order to see how salt and ionic strength effects the RAFT-mediated PISA syntheses the PDMAm₅₀-b-PDAAm₃₀₀ block copolymer, the salt concentration was varied in a series of analogous PISA syntheses. Sodium Chloride (NaCl) was chosen as an initial background salt as well as Phosphate Buffered Saline (PBS), which has the advantage of buffering the system at pH 7.4-7.5 without the need for acid-base alterations of the pH. To the best of the knowledge, this is the first instance where the electrolyte concentration and hence ionic strength of the PISA solvent has been altered for the synthesis and self-assembly of PDMAm₅₀-b-PDAAm_y based block copolymers. It is hoped that these experiments will provide valuable insight into how background salt effects the RAFT-mediated PISA process for the block-copolymer and also investigate the permeability of the polymer vesicles with respect to small ions. These experiments were conducted via chain-extension of the original PDMAm₅₀ m-CTA with DAAM in solutions varying in pH and electrolyte concentration. A summary of the polymer characterisation under these conditions is presented in table 3.4 and figure 3.18.

Table 3.5. Summary of PISA nano-object characterisation; DAAm monomer conversion, molecular weight data, and dynamic light scattering data for PDMAm₅₀-b-PDAAm_y di-block copolymer nano-objects at varying pHs with varying salt concentrations.

pH and ionic strength of PISA Syntheses of PDMAm ₅₀ -b-PDAAm ₃₀₀	DAAm conversion (%) Via ¹ H NMR	M _n (Kg mol ⁻¹) ¹) Via GPC	M _w /M _n GPC	Via Z-average hydrodynamic diameter (nm)	PDI Via DLS
pH 2.5	>98	61.3 ± 1.70	1.14 ± 0.01	369 ± 16	0.124 ± 0.012
pH 7.5	>98	76.0	1.26	299	0.140
pH 2.5 w/ 140mM NaCl	>98	43.3	1.42	337	0.139
pH 7.5 w/ 140mM NaCl	>98	25.9	2.34	348	0.128
pH 7.5 in PBS	>98	102.4	1.37	256	0.141

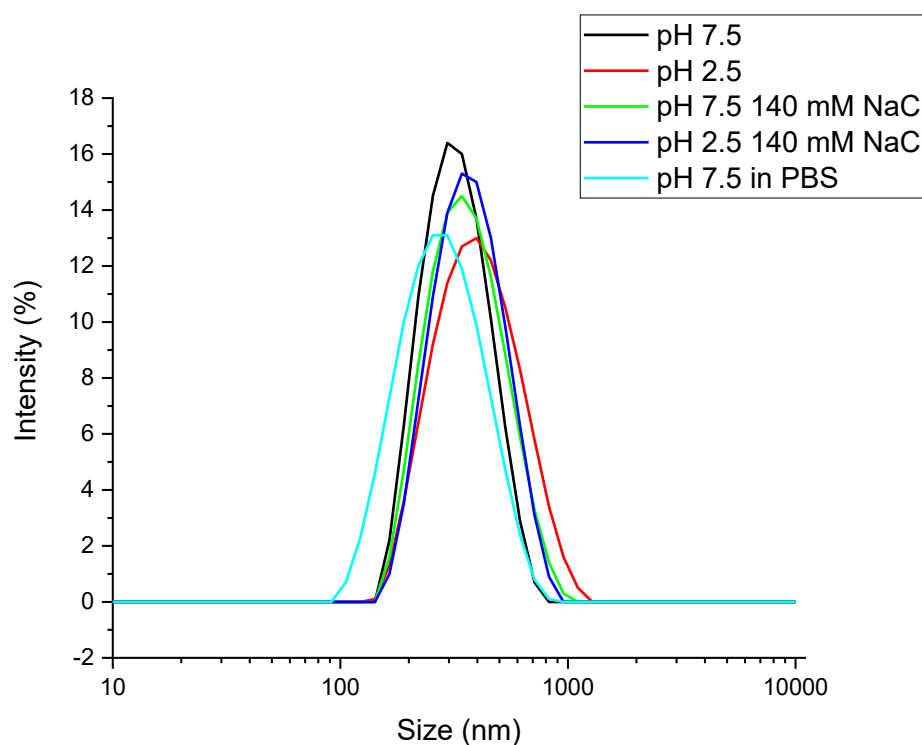


Figure 3.20 Nano-particle size distributions of PDMAm₅₀-b-PDAAm₃₀₀ block copolymers synthesised at pH 2.5 and 7.5, respectively, with varying salt concentrations (140 mM NaCl and PBS, respectively)

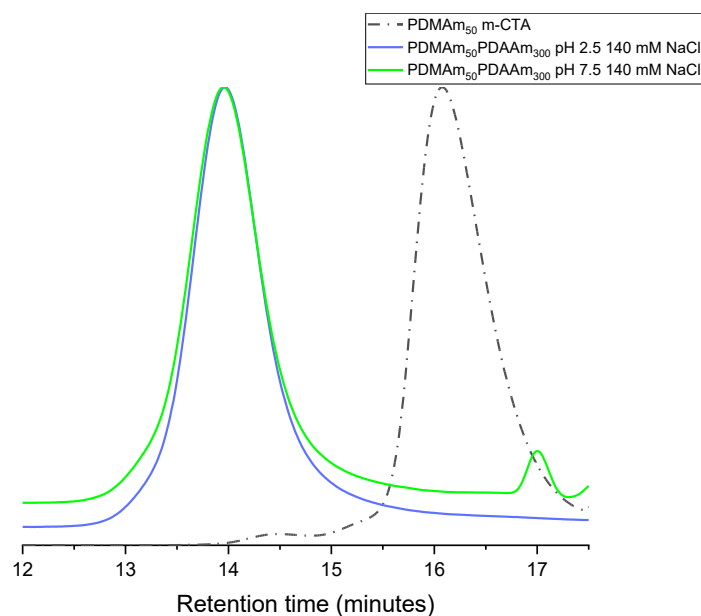


Figure 3.21. GPC chromatograms of PDMAm₅₀ m-CTA and of PDMAm₅₀-b-PDAAm₃₀₀ synthesised at pH 2.5 and 7.5 with 140 mM NaCl

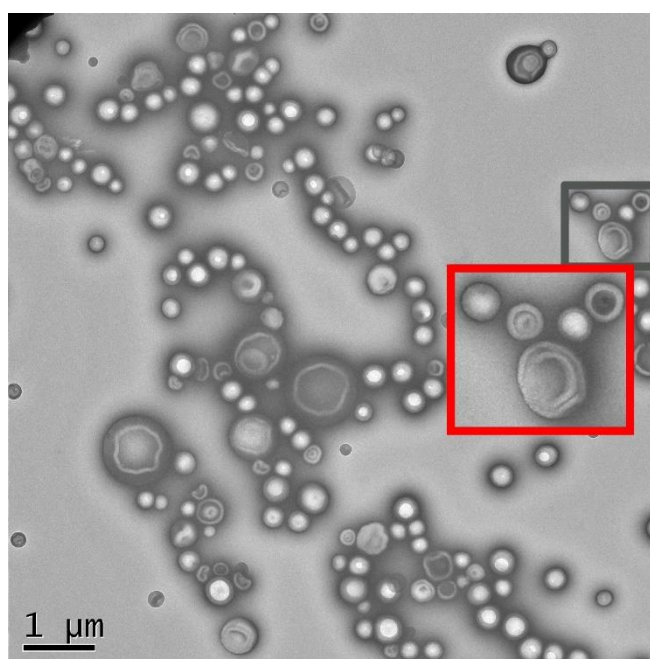


Figure 3.22. TEM micrographs for PDMAm₅₀-b-PDAAm₃₀₀ di-block copolymer nano-objects at pH 7.5 with 140 mM NaCl via RAFT-mediated PISA. Additional images are presented in the appendix (Figures 7.14-7.22)

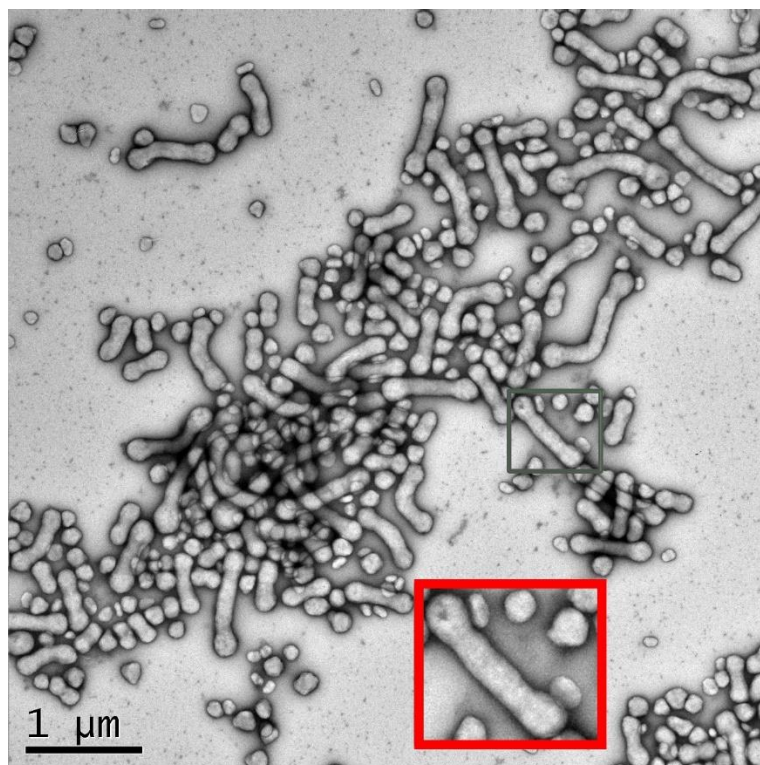


Figure 3.23. TEM micrographs for PDMAm₅₀-b-PDAAm₃₀₀ di-block copolymer nano-objects at pH 7.5 buffered in PBS via RAFT-mediated PISA. Additional images are presented in the appendix (Figures 7.23-7.25)

From the data in Table 3.5, a small variation in the Z-average hydrodynamic diameter of nano-objects was observed as the background salt concentration of 140 mM NaCl was introduced across both pH 2.5 and pH 7. The addition of 140 mM NaCl increases the Z-average hydrodynamic diameter in pH 7.5 samples by an average of 47 nm. Addition of NaCl in pH 2.5 PISA syntheses causes a small decrease of 32 nm compared to the analogous experiment with no salt added during the PISA syntheses. This is a small increase and decrease with the particle size distribution all being monomodal and overlapping heavily around the 100-1000 nm range. Intensity max values of the particle size distributions are all near the 200-300 nm range, suggesting very similar size distributions of PISA nano-objects regardless of ionic strength or pH of the solution during synthesis. The PISA sample made at pH 7.5 in PBS does, however, show the biggest shift in size with the lowest intensity max of all the size distributions. This sample also corresponds to the smallest Z-average hydrodynamic radius. From the GPC data in Table 3.5, it is evident that the addition of salt alters both the Mn and polydispersities of the PDMAm₅₀-b-PDAAm₃₀₀ block copolymers made via RAFT-mediated PISA syntheses. With

the addition of NaCl, PISA syntheses conducted at pH 2.5 varied in M_n from 61 to 43 kg mol⁻¹, which is similar to the change seen upon changing pH from 2.5 to 7.5, respectively.

When conducting the PISA syntheses at pH 2.5, the polydispersities of the block-copolymer also increase with the addition of salt, from 1.14 to 1.43, respectively. This increase suggests that the presence of background salt influences the RAFT-mediated PISA process somewhat, leading to more polydisperse polymer sizes under acidic conditions. This trend was exacerbated at pH 7.5, where the addition of 140 mM NaCl caused a dramatic shift in polydispersities from 1.43 to 2.34, respectively. This was also coupled with a reduction in the M_n of the polymer, decreasing from 49.8 to 25.9 kg mol⁻¹. From this data, the addition of 140 mM background NaCl affects both the M_n and polydispersities of the block-copolymers made via PISA, regardless of the pH of the syntheses. Surprisingly, PISA syntheses conducted in PBS buffer solutions were difficult to analyse via GPC. The higher salt concentration and more diverse salt compositions were problematic for the GPC instrument, as high salt could be detrimental to the GPC column and cause issues. Considering this, the GPC chromatogram will need to be repeated with the salt removed via drying for more accurate GPC characterisation of the RAFT-mediated PISA syntheses under PBS buffered conditions. The data from the initial GPC of the PDMAm₅₀-b-PDAAm₃₀₀ sample in PBS showed a large variation from other samples also prepared at pH 7.5. From the TEM micrograph in Figure 3.22, when background NaCl is added to the PISA syntheses at pH 7.5, vesicular morphology persists. Vesicles observed under these conditions are very monodisperse in size and highly symmetrical, similar to the vesicles observed at the same pH in the absence of NaCl at pH 7.5. From this result, adding 140 mM NaCl as a background salt does not alter the morphology or size of the PDMAm₅₀-b-PDAAm₃₀₀ nano-objects prepared via RAFT-mediated PISA processes. When the analogous experiment was carried out in PBS buffer, a change in size and morphology of the nano-objects was needed. From the TEM micrograph in Figure 3.25, the emergence of high aspect ratio tubular membrane-bound nano-objects is seen, as well as large numbers of kinetically trapped spheres and what appears to be dimers of spherical micelles. DLS data for this sample may not be accurate, as this size characterisation method assumes spherical, non-interacting particles. Nonetheless, the Z-average diameter of polymer nano-objects, 250 nm, is

slightly smaller when the RAFT-mediated PISA syntheses are buffered in PBS. This is also coupled by a size distribution shifted towards smaller sizes with a smaller intensity max of the nanoparticle size distribution. This could be explained by a larger proportion of smaller kinetically trapped micelles (and dimers of micelles), observed in the TEM micrograph in Figure 3.22. The change in morphology from pure vesicles to higher aspect ratio ‘rods’ can be characterised as an elongation of spherical polymersomes due to the higher concentration and presence of salt. The PBS buffer used is comprised of 140 mM NaCl, similar to the other PISA samples, but PBS buffer also contains significant amounts of potassium chloride (KCl) and sodium phosphates. It is believed that this increase in both concentration and species diversity of the salt has an effect on both the polymer syntheses and self-assembly of the PDMAm₅₀-b-PDAAm₃₀₀ block copolymer nano-objects. This shape transformation may be due to the outflow of water molecules from the core of the polymersomes to hydrate excess salt ions present in the PBS-buffered PISA synthesis solution. This is concurrent with recent literature from Abdelmohsen and co-workers based on biodegradable polymersomes comprising poly(ethylene glycol)-b-poly(D, L-lactide).¹⁹ In this study, this group demonstrated the osmotically induced shape transformation of spherical polymersomes into high aspect ratio tubular rods upon increasing the ionic strength of the solution to 50 mM of NaCl. It is theorised that a similar phenomenon may occur in the PDMAm₅₀-b-PDAAm₃₀₀ block copolymer vesicles synthesised in PBS buffer.

3.1.7 Comparative Analysis of Transmission Electron Microscopy and Dynamic Light Scattering for Characterisation of Self-Assembled Nano-Objects

The characterisation of self-assembled nano-objects represents a critical challenge in nanoscience and nanotechnology. Accurate assessment of morphological features, size distributions, and structural properties is essential for understanding structure-function relationships and optimising nano-object design for various applications. In this context, multiple complementary analytical techniques are typically employed to provide a comprehensive characterisation profile.

Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS) stand as two fundamental techniques widely used in the field of nano-object characterisation, each offering distinct advantages and limitations. TEM provides direct visualisation of individual nano-objects with nanometre-scale resolution, allowing detailed examination of morphology, size, and structural features. However, the sample preparation process involving drying and staining may introduce artifacts that could affect the native structure of the nano-objects. In contrast, DLS measures the hydrodynamic radius of particles in solution, providing a representation of the entire population under more realistic environmental conditions, but lacks the ability to distinguish morphological details or provide information about individual particles.

The combination of these techniques offers a more comprehensive understanding of nano-object properties than either method alone. TEM allows researchers to directly observe and measure specific structural features, including membrane thickness and contour length, while DLS provides valuable information about the size distribution and behaviour of nanoparticles in solution. However, the correlation between measurements obtained via these different techniques requires careful consideration of their respective limitations and the potential effects of sample preparation.

This chapter aims to establish a systematic comparison between TEM and DLS characterisation methods for self-assembled nano-objects, with particular focus on polymeric vesicles, worms, and micelles. The investigation seeks to determine the level of similarity between size measurements obtained through these techniques, develop robust methodologies for membrane characterisation using TEM image analysis, and establish the limitations and complementary aspects of each approach. Furthermore, the study aims to position these techniques within the broader context of nano-object characterisation methods, including fluorescence-based techniques, cryo-electron microscopy, and scattering techniques such as small-angle X-ray scattering (SAXS).

Morphological Characterisation of Self-Assembled Nano-objects

Transmission electron microscopy revealed a diverse range of morphologies formed by the self-assembly of PDMAm₅₀-b-PDAAm₃₀₀ block copolymers, with specific structures strongly dependent on both polymer composition and

assembly conditions. The high-resolution TEM micrographs provided clear visualisation of the different morphological features, enabling detailed classification and analysis of the self-assembled morphology into spherical micelles, worms, or vesicles.

At pH 2.5, where the PDMAm block exhibits maximum hydrophilicity due to protonation of the tertiary amine groups, the PDMAm₅₀-b-PDAAm₃₀₀ system predominantly formed well-defined vesicular structures. These polymersomes appeared in TEM as circular objects with distinct membrane boundaries, exhibiting diameters ranging from approximately 80 to 200 nm. The negative staining protocol employed resulted in the polymer membrane appearing as a lighter ring against the darker background and interior, providing good contrast for structural analysis. The uniformity of the membrane thickness around the vesicle perimeter confirmed the formation of consistent bilayer structures, a characteristic feature of polymersomes.

In contrast, at pH 7.5 buffered with PBS, the same polymer system displayed a morphological transition toward higher-aspect-ratio structures. TEM images revealed predominantly worm-like micelles characterised by their elongated cylindrical geometry. These worms exhibited relatively uniform diameters of approximately 20-30 nm with contour lengths extending from several hundred nanometres to micrometres. The flexibility of these structures was evident from their various conformations on the TEM grid, ranging from nearly straight segments to highly curved and occasionally branched architectures.

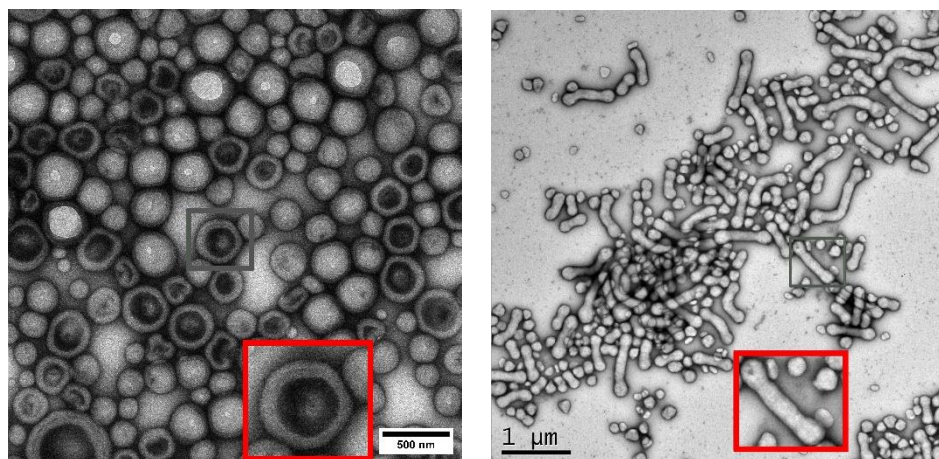


Figure 3.24: Direct comparison of PDMA₅₀-b-PDAAm₃₀₀ nano-objects made via PISA in various solvents. Left: pH 2.5 water, Right: pH 7.5 buffered with PBS.

The detailed morphological characterisation enabled by TEM provides critical information that cannot be obtained through scattering techniques alone. In particular, the ability to directly visualise transitional and complex morphologies offers unique insights into the self-assembly mechanisms and structural diversity of these polymer systems. However, the potential influence of sample preparation artifacts, particularly those related to drying and staining processes, necessitates careful interpretation and comparison with solution-state measurements.

Size Analysis and Comparison Between TEM and DLS

Histograms were made by taking manual images of all sphere/vesicle morphologies seen under TEM for the PISA synthesis of PDMA₅₀-b-PDAAm₃₀₀ at pH 2.5 and 7.5, buffered with PBS. The maximum diameter of the nano-object through the centre was recorded for 641 nano-objects.

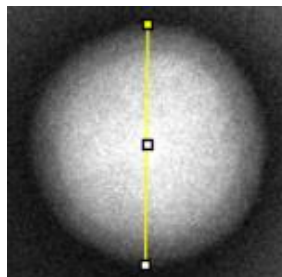


Figure 3.25: A visual example of the longest cross-sectional area recorded for a spherical polymer nanoobject.

Quantitative size analysis was performed on the TEM micrographs using ImageJ software, generating statistically significant distributions of key dimensional parameters for each morphology type. For vesicular structures synthesised via PISA at pH 2.5, the outer diameter was measured across multiple particles ($n = 287$ across multiple images), yielding an average diameter of 276 ± 96 nm.

During the PISA chain extension in PBS, there were three populations identified: high aspect ratio tubes, spherical micelles, and dimers of micelles, as seen in Figure 3.26 and the appendix chapter. The populations were split during ImageJ analysis, with the contour length used to characterise the high aspect ratio tubes and dimers. In the representative images studied, there were 333 spherical micelles, 104 dimers, and 90 high aspect ratio tubes. The histograms are presented in Figure 3.26, compared against the pure polymersome morphologies seen in the analogues PISA self-assembly at pH 2.5, along with the DLS traces for the samples previously shown in the chapter. A supplementary pH 5.5 PISA synthesis was run to show any incremental effect on the self-assembly due to a slight variation in pH and protonation of various groups on the polymer backbone, and is included in the figure.

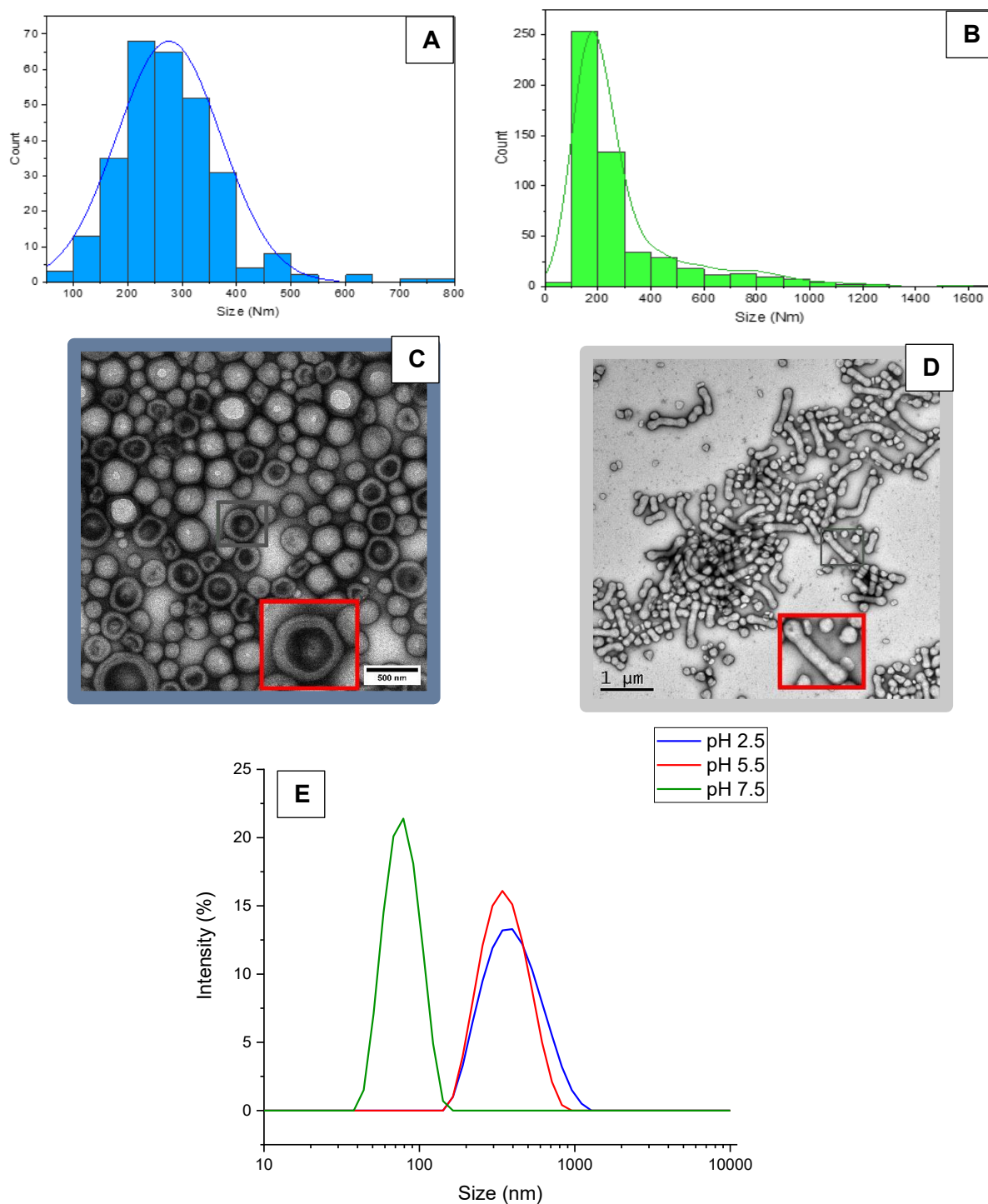


Figure 3.26: Comparison between DLS and TEM as size determination techniques for a variety of PISA synthesised PDMA₅₀-b-PDAA₃₀₀ morphologies in varying pH and ionic conditions. A) TEM Histogram and trend for PISA at pH 2.5, B) TEM Histogram and trend for PISA at pH 7.5 buffered with PBS, C) TEM image for vesicles pH 2.5, D) TEM image for vesicles pH 7.5 buffered with PBS, and E) DLS traces for each PISA synthesis.

Here, contour length measurements revealed greater variation, with an average of 703 ± 256 nm, reflecting the inherent flexibility in the assembly process of these high-aspect-ratio structures. The histogram of contour lengths approximated a log-normal distribution, consistent with theoretical models of worm-like micelle formation. The highest peak in the Histogram data for the PISA sample prepared in PBS was shifted to the left when compared to the vesicles sample, which can be attributed to larger number of spherical micelles as opposed to worms, which is in agreement with the DLS data as both the z-average and intensity max for the PBS sample is lower than the analogous sample at pH 2,5 which is predominantly vesicles.

Parallel DLS measurements provided complementary size information based on the hydrodynamic behaviour of the nano-objects in solution. For the vesicular samples, DLS analysis yielded a z-average hydrodynamic diameter of 369 ± 16 nm with a polydispersity index (PDI) of 0.124, indicating a relatively monodisperse population.

First, DLS measures the hydrodynamic diameter, which includes the hydration layer surrounding the nano-objects in solution. This hydration shell contributes to the effective size detected by DLS but is absent in the dried TEM samples. Additionally, the potential flattening or slight collapse of vesicles during TEM sample preparation may reduce their apparent size relative to their solution-state dimensions.

For worm-like micelles, the interpretation of DLS results requires careful consideration due to the non-spherical geometry of these structures. The z-average diameter from DLS (256 nm) represents neither the cross-sectional diameter nor the contour length directly, but rather an effective hydrodynamic diameter of a sphere with equivalent diffusion behaviour. The significantly larger hydrodynamic size compared to the TEM-measured diameter reflects the extended geometry of these structures, with their restricted rotational diffusion substantially impacting the apparent hydrodynamic diameter.

To facilitate more direct comparison between DLS and TEM results for non-spherical particles, theoretical models relating the hydrodynamic behaviour of cylinders to their physical dimensions were applied. Using the worm diameter and contour length from TEM analysis, the calculated theoretical hydrodynamic diameter for high aspect ratio tubes approximated the DLS-measured values

reasonably well, with deviations attributable to the flexibility of the worms and potential interparticle interactions in solution.

The comparative analysis between TEM and DLS measurements highlighted the complementary nature of these techniques. While TEM provided detailed information about morphology and precise dimensional measurements of individual nano-objects, DLS offered valuable insights into the solution-state behaviour and overall population characteristics. The observed discrepancies between the techniques underscore the importance of multi-method approaches for comprehensive nano-object characterisation.

Membrane Thickness Analysis of Vesicular Structures

The bilayer membrane represents a critical structural feature of polymersomes, with its thickness directly influencing properties such as permeability, mechanical stability, and interaction with biological systems. TEM analysis coupled with image processing enabled detailed characterisation of membrane properties across different polymer compositions.

Two methods were employed to study a range of polymersomes ($n > 10$) from various images using ImageJ: Line profile and Radial Integration. The methodology is visualised below in Figure 3.27.

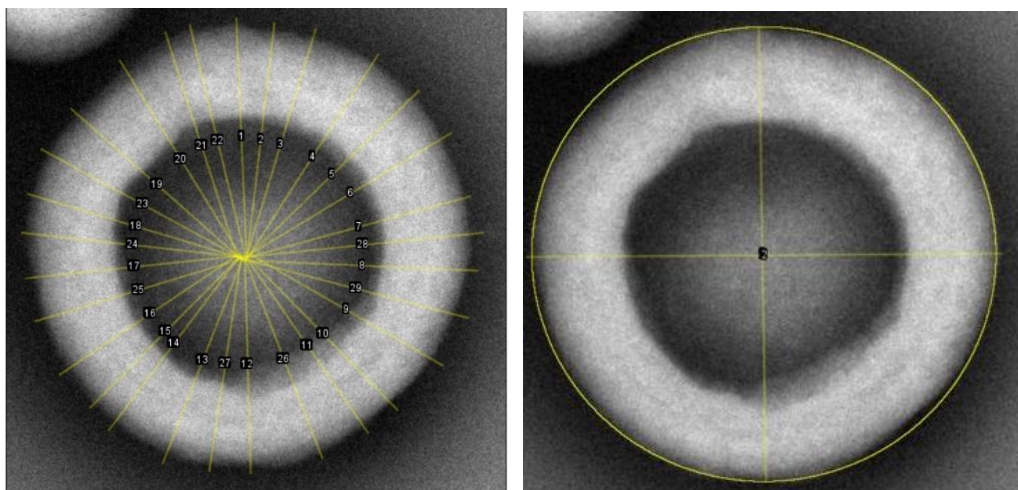


Figure 3.27: Line profile method and radial Integration method for a model symmetrical polymersome.

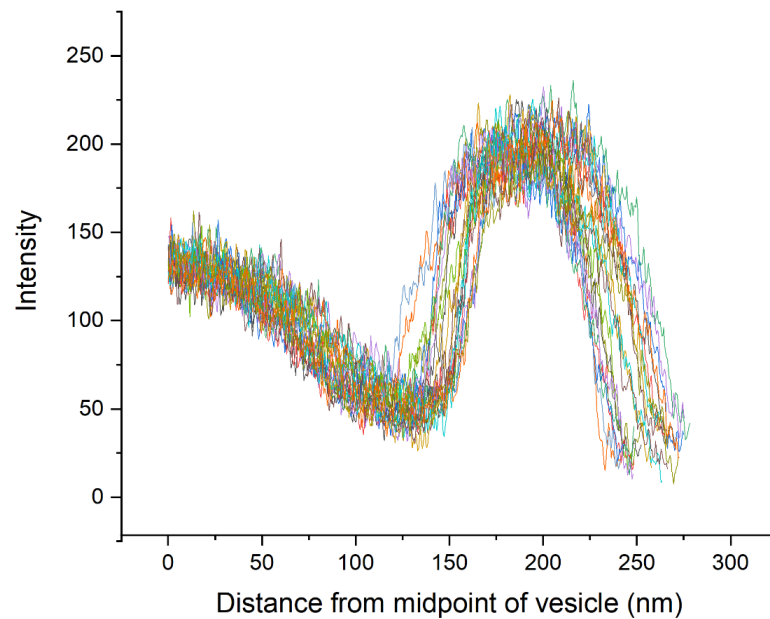


Figure 3.28: Line profiles for a model symmetrical polymersome. Intensity represents the amount of negative stain captured on the polymersome during TEM.

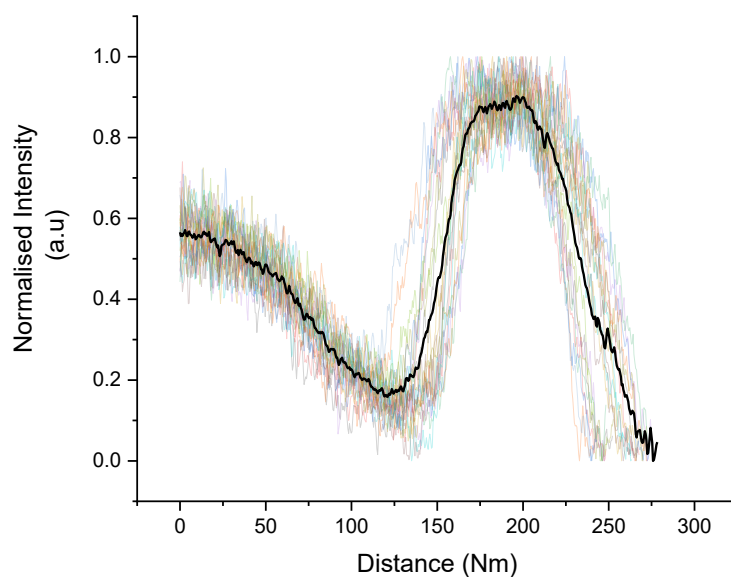


Figure 3.29: Normalised and averaged Line profiles for a model symmetrical polymersome. Intensity represents the amount of negative stain captured on the polymersome during TEM.

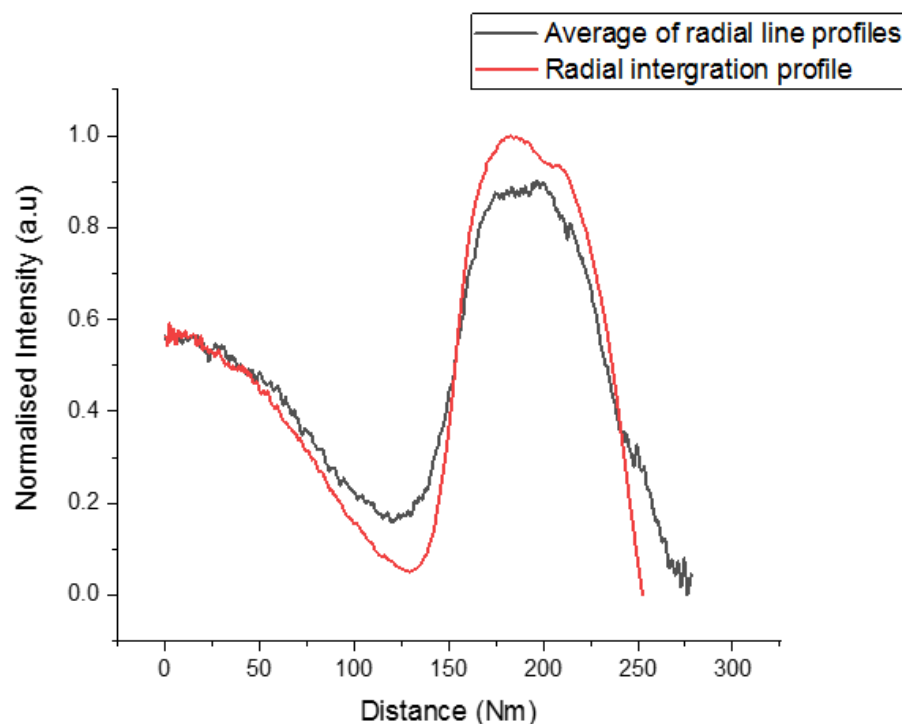


Figure 3.30: Line profile method and radial Integration method for a model symmetrical polymersome. Intensity represents the amount of negative stain captured on the polymersome during TEM.

For the PDMAm₅₀-b-PDAAm₃₀₀ system, membrane thickness analysis was performed using both the line profile and radial integration methods. The line profile approach, involving intensity measurements perpendicular to the membrane, and the radial integration method, applicable to more spherical vesicles, provided complementary data in comparison to the line profile method data. The small discrepancy between the methods can be attributed to the averaging effect of the radial approach, which reduces the impact of local irregularities but potentially obscures genuine structural variations. For non-spherical or deformed vesicles, the line profile method proved more adaptable, allowing targeted measurement at specific membrane regions.

To validate the TEM-derived membrane measurements, theoretical calculations were performed based on polymer chain dimensions. For PDMAm₅₀-b-PDAAm₃₀₀, the expected end-to-end distance of a fully extended PDAAm₃₀₀ chain was estimated using standard bond lengths and angles. The calculation considered the backbone configuration:

$$2(1.43 \text{ \AA}) + 355(1.54 \text{ \AA}) + 4(1.82 \text{ \AA}) = \mathbf{557 \text{ \AA} \text{ or } 55.7 \text{ nm}}$$

However, the measured membrane thickness (approximately 12 nm) suggests significant chain folding within the hydrophobic layer, consistent with the formation of a more compact, entangled polymer arrangement rather than a fully extended chain configuration. Assuming the membrane thickness represents twice the effective hydrophobic block length (for a bilayer structure), each PDAAm₃₀₀ chain occupies approximately 6 nm in the radial direction, indicating substantial deviation from the fully extended conformation.

This discrepancy between theoretical full extension and measured dimensions provides valuable insights into the molecular organisation within the polymersome membrane. The ratio between the fully extended chain length and the effective membrane contribution (approximately 9:1) suggests extensive chain folding and potentially interdigitation between the opposing leaflets of the bilayer. These structural characteristics have significant implications for membrane properties, including permeability, stability, and response to environmental stimuli.

The membrane analysis results demonstrated the capability of negative-stain TEM, coupled with appropriate image analysis methods, to provide detailed information about nanoscale structural features. However, the potential influence of staining artifacts and dehydration effects during TEM sample preparation must be considered when interpreting these results. A method to potentially circumvent this issue is the incorporation of complementary techniques, such as cryo-TEM or small-angle neutron scattering, which could provide additional validation of these structural parameters under more native conditions.

Limitations and Complementarity of TEM and DLS Techniques

The comprehensive characterisation of self-assembled nano-objects revealed distinct advantages and limitations of both TEM and DLS techniques, highlighting their complementary nature in nanomaterial analysis.

TEM provides unparalleled capabilities for direct visualisation of morphology and detailed structural features at the nanoscale. The technique clearly distinguished between different morphologies (spheres, worms, and vesicles) and enabled precise measurement of dimensional parameters such as diameter, contour length, and membrane thickness. These capabilities proved

particularly valuable for identifying complex and transitional structures that would be indistinguishable by scattering techniques alone.

However, TEM analysis exhibited several important limitations. Sample preparation involving drying and staining introduced potential artifacts, including structure flattening, aggregation, and selective adsorption to the TEM grid. These effects may alter the apparent morphology and dimensions relative to the solution state. Additionally, TEM provides a static snapshot of selected regions, potentially leading to sampling bias, especially for heterogeneous systems. The technique also offers limited statistical power compared to ensemble methods, typically analysing hundreds rather than millions of particles. In contrast, DLS excels in providing population-based size information under native solution conditions. The technique offers exceptional statistical robustness, analysing large particle populations to generate representative size distributions. The non-invasive nature of DLS measurements preserves the native state of the nano-objects, avoiding preparation artifacts. Furthermore, the ability to perform real-time measurements enables monitoring of dynamic processes such as morphological transitions or aggregation phenomena.

The limitations of DLS include its inability to distinguish between different morphologies with similar hydrodynamic behaviour and its disproportionate sensitivity to larger particles due to the intensity-weighted nature of the measurements. For polydisperse or multimodal samples, interpretation of DLS results requires careful consideration of these biases. Additionally, the technique provides limited structural information beyond the effective hydrodynamic size, necessitating complementary methods for detailed characterisation. The comparison between TEM and DLS measurements for various nano-object systems revealed consistent trends in their relationship. For non-spherical structures such as worms, the relationship between TEM dimensions and DLS hydrodynamic size requires more complex interpretation based on theoretical models of the hydrodynamic behaviour of anisotropic particles.

The complementary strengths of these techniques highlight the importance of multi-method approaches for comprehensive nano-object characterisation. TEM provides detailed morphological and structural information that contextualises the interpretation of DLS results, while DLS offers insights into

solution-state behaviour and population characteristics that complement the more detailed but statistically limited TEM analysis.

For the comprehensive size characterization of PDMAm₅₀-b-PDAAm₃₀₀ polymer nano-objects, DLS measurements demonstrated good agreement with TEM-ImageJ size analysis, providing consistent hydrodynamic diameter values that validate both techniques, as seen as a characterisation tool like in literature. This correlation between DLS and TEM data is particularly significant as it confirms the reliability of the size measurements across different methodological approaches. TEM analysis further revealed critical information regarding the polymer membrane thickness, which is consistent with literature values for similar polymer systems. Byard and co-workers found that the average membrane thickness for a PDMAm-b-PDAAm block copolymer with a comparable hydrophilic: hydrophobic ratio was X, which highlights the accuracy of both the line profile and the radial integration methods for gaining insights into membrane thickness in the absence of Cryo-TEM and SAXS.¹⁰ Although it should be stated that these techniques would give more representative data on the membrane characteristics of the PDMAm₅₀-b-PDAAm₃₀₀ block copolymer vesicles. Such membrane thickness measurements are essential for understanding the structural properties of the nano-objects and their potential applications.

3.1.8. Conclusions

This chapter has aimed to fully characterise PDMAm₅₀-b-PDAAm_y block copolymer nano-objects made via RAFT-mediated PISA in aqueous conditions, specifically at neutral pHs and physiological salt conditions. A PDMAm₅₀ m-CTA was synthesised successfully, showing high monomer conversion and polymer growth via GPC. Subsequently, this m-CTA was successfully chain extended with DAAM at pH 2.5, targeting a range of different hydrophobic block lengths ranging from 100 to 1200. As the length of the hydrophobic block increases, it is hypothesised that there is an increase in the packing parameter of the overall block copolymer, leading to changes in morphology as the hydrophobic DAAM block increases. High monomer conversion was observed, as well as a linear increase in the Mn of the block-copolymer as the targeted block length of DAAM

increased. This, coupled with relatively low dispersities, demonstrated successful RAFT-mediated PISA syntheses under acidic conditions. DAAM block lengths of 100 yielded a mixed morphology of kinetically trapped micelles, worms, and a few polymer vesicles. Increasing the hydrophobic block length to 200 increases the number of vesicles seen with fewer kinetically trapped micelles. DAAM block lengths equal to or greater than 300 yielded pure vesicular morphologies with similar sizes of 200-300 nm as determined by TEM and DLS.

The RAFT-mediated PISA syntheses were also extended to neutral pH conditions. PDMA_{m50}-b-PDAA_{m300} block copolymer vesicles were synthesised and self-assembled in pH 7.5 water with little variation in the polymer characteristics from GPC. The vesicular morphology persisted at neutral pH, which allows for the encapsulation of pH-sensitive cargos across a range of pH values between 2.5 and 7.5. This chapter also explored the effect of ionic strength on the RAFT-mediated PISA synthesis of PDMA_{m50}-b-PDAA_{m300}. Polymeric vesicles at pHs 2.5 and 7.5 in the presence of background NaCl, similar to the ionic strength and electrolyte concentration in intercellular conditions, were successfully synthesised. Polymer characterisation data from GPC varied with increased ionic strength, but the sizes and morphologies of the polymersomes prepared were almost identical when NaCl was introduced as a background electrolyte. PBS buffered PISA syntheses were difficult to characterise via GPC, differing greatly in both M_n and PD when compared to analogous PISA syntheses with no background salt. PBS buffered samples but showed slightly smaller Z-average hydrodynamic diameters. This was accounted for by the presence of smaller micelles and dimers seen via TEM and also the shape transformation of spherical polymersomes to high aspect ratio tubes, caused by osmotic effects due to the presence of phosphate and potassium ions during the synthesis.

The combination of DLS for ensemble measurement of hydrodynamic properties and TEM for direct visualisation of morphological features offers the most comprehensive approach to characterising both the size distribution and structural properties of PDMA_{m50}-b-PDAA_{m300} polymer nano-objects. This dual-technique methodology enables allowing for the correlation of bulk properties with individual particle characteristics, yielding a more complete understanding of these complex nanomaterials and their potential applications in drug delivery and other biomedical fields.

3.1.9. References

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Chapter 4

4.1. Investigation of alternate self-assembly pathways, including Solvent exchange parameters to obtain nanoparticles from PDMAm₅₀-b-PDAAm₃₀₀ block-copolymer.

1.1.1. Introduction

The efficiency and/or yield of polymersome production can be strongly affected by the conditions and procedures used, and thus, many protocols have been developed. Typically, post-polymerisation self-assembly of block copolymers can be achieved using a wide variety of techniques, including thin film rehydration ¹, Electroformation ², Solvent exchange ³, microfluidics ⁴, and emulsion phase transfer ⁵. The nature of the self-assembly technique can have a significant impact on the polymersome. Comparing these methods can give valuable insights into the differences between two or more self-assembly protocols.⁶ Additionally, having more than one self-assembly method in place for the block copolymer could potentially offer multiple avenues towards encapsulation. The polymerisation conditions required for PISA present one example where syntheses at 70°C could denature any potential enzymatic cargo during encapsulation. Alternative self-assembly avenues provide a solution to this problem for designing polymersomes with encapsulation of temperature-sensitive soft matter in mind.⁷

Herein, self-assembly of PDMAm₅₀-b-PDAAm_y into polymersomes is attempted via two different protocols, thin film rehydration (TFR) and solvent exchange (SE). TFR was chosen due to its facile nature and potential ability to self-assemble polymersomes at ambient temperatures. The methodology of the TFR for PDMAm₅₀-b-PDAAm_y polymers was adapted from Rideau and co-workers.¹ This feature is crucial for the encapsulation of temperature-sensitive soft matter within the polymersome and provides a potential avenue to do so.⁸ As well as this, film rehydration techniques are seen as cleaner alternatives to solvent methods, as there is no trapping of undesirable organic solvents in their membrane, as well as other potentially undesired additives (surfactants, polyelectrolytes, etc.)¹. Differential Scanning Calorimetry (DSC) was used in this chapter to analyse any thermal events (exothermic events; heat released to surroundings or endothermic events; heat adsorbed from surroundings) during the heating of the polymer, as heating of the polymer could be explored as a way to aid the TFR process. This chapter also explores the solvent exchange protocol towards PDMAm₅₀-b-PDAAm₃₀₀ block copolymer. Solvent exchange is a generally facile method for the preparation of a range of nano-objects, which include polymersomes. In doing so, this optimisation looks at

several factors and alters those to see their effect on the size and morphology of nano-objects produced via Solvent exchange.

1.1.2. Materials and Methods

Thin film rehydration of PDMAm₅₀-b-PDAAm_y block copolymers

A 25 μ L aliquot of each diblock copolymer solution made previously via PISA was lyophilised to remove water. The entirety of the dried polymer was dissolved in an excess of methanol (500 μ L), which was removed under vacuum to leave an invisible thin film of the neat polymer on a glass substrate. The polymer was rehydrated in 2 mL of pH 2.5 water and left to stir at 150 rpm for 2 weeks. Aliquots were taken from the supernatant solution for DLS analysis.

Differential Scanning Calorimetry of PDMAm₅₀-b-PDAAm_y block copolymers

The PDMAm₅₀ m-CTA, PDMAm₅₀-b-PDAAm₁₀₀, and PDMAm₅₀-b-PDAAm₁₂₀₀ block copolymers were lyophilised to remove water. Each dried polymer (10 mg) was added to a crucible. The crucible was heated from 25 °C to 350 °C with a heating rate of 10 °C/min under a nitrogen environment.

Solvent exchange of PDMAm₅₀-b-PDAAm_y block copolymers under aqueous conditions

The PDMAm₅₀-b-PDAAm₃₀₀ block copolymer solution made previously via PISA was lyophilized to remove water. Lyophilised PDMAm₅₀-b-PDAAm₃₀₀ (2 mg) was added to methanol (0.05, 0.1, 0.15, and 1 mL) and stirred with a magnetic stirrer for 10 min or until it formed a clear solution. The methanol-polymer solution was added to an aqueous environment (1 mL) either rapidly by hand or with a syringe pump at varying flow rates (33 and 333 μ L/min) and allowed to stir at 1000 RPM for 5 min. The solvent was removed under reduced pressure by rotary evaporation or slow evaporation to the atmosphere by stirring in an open vial at 150 RPM for 3 days at 20°C $\pm$ 2°C.

1.1.3. Thin film rehydration of PDMAm₅₀-b-PDAAm₃₀₀ block copolymer

Thin film rehydration (TFR) was selected as a relatively facile method to form colloidally stable nano-objects. It was hoped that the nano-objects yielded by this technique could initially be compared against the size and morphology of nano-objects made via PISA. The PDMAm₅₀-b-PDAAm₃₀₀ block-copolymers, made via PISA, were lyophilized to leave a dry block-copolymer powder. The block-copolymers were then dissolved in methanol, which was found to be a good solvent for both DMAm and DAAm blocks. Upon removal of methanol, a thin film of polymer remained on the inside of the glass vial. Attempted rehydration in pH 2.5 water resulted in a change of colour of the polymer from colourless and clear to white and opaque, roughly after 1 day of stirring for all samples. It was hoped that the hydration effects of the water would encourage the re-assembly of the block-copolymers in solution into bilayers, which could form vesicles. This methodology follows on from previous work published by Rideau and co-workers, who have shown that this film-rehydration method (and its alterations) is suitable for a wide range of amphiphiles, including block-copolymers.¹

Due to the initial observation by eye of the inability of the polymer film to be re-dispersed in solution, Differential Scanning Calorimetry (DSC) was used to determine any thermal transitions of the polymer in the solid phase. To explore this, DSC was used to systematically heat the sample and observe any endothermic or exothermic events as a result of temperature. The PDMAm₅₀ macro-CTA, and a PDMAm₅₀-b-PDAAm₄₀₀ and a larger polymer, PDMAm₅₀-b-PDAAm₁₂₀₀, were heated from 10 °C to 350 °C at 10°C/min, as shown in Figure 4.1.

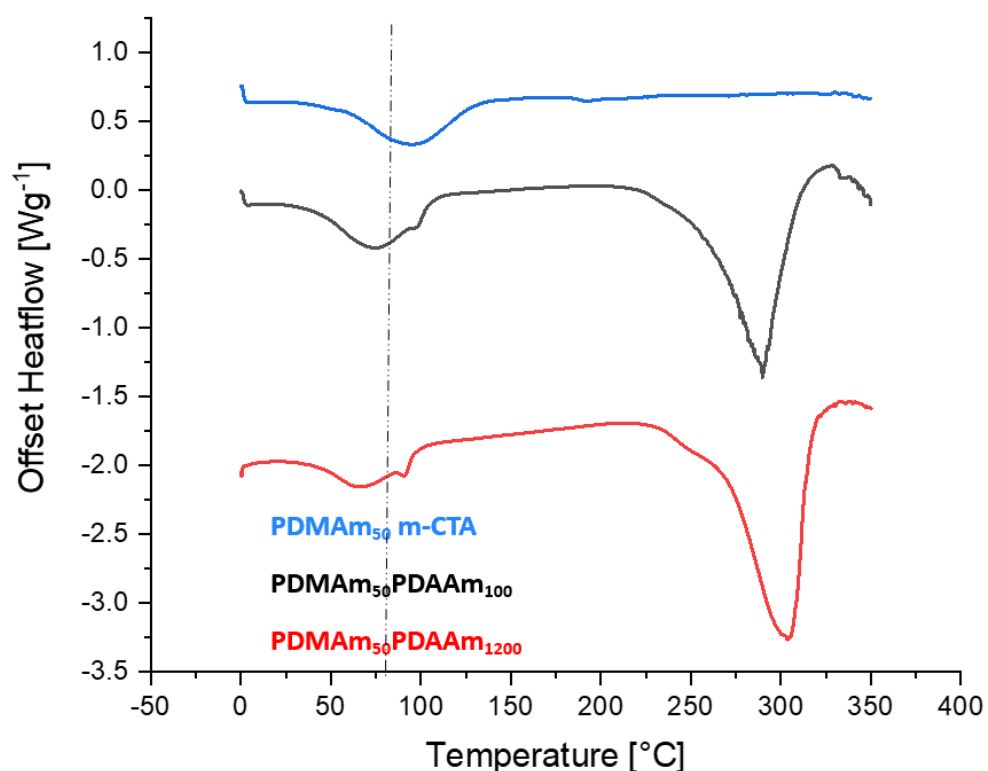


Figure 4.1. DSC curves for the PDMA₅₀ m-CTA prior to chain extension with DAAM (blue), PDMA₅₀-b-PDAAm₁₀₀ (black), and PDMA₅₀-b-PDAAm_{1200B} (red). DSC curves are reported as heat flow changes as a function of increasing temperature.

This experiment determines the glass transition temperature (T_g) of the polymers. During TFR, it is expected that a state of decreased mobility of the polymer chains, thus temperature could influence the rate at which the TFR process occurs. A typical T_g for a polymer should correspond to a baseline shift in the recorded heat flow of a DSC curve. From the DSC curves, it is seen that some melting of the block-copolymers occurs in the region of ~40-100°C. Interestingly, a small shoulder on these melting peaks at 80-100°C is observed, which is in the range expected for decomposition of the pure PDMA₅₀ m-CTA. Heating beyond 240°C yields large exothermic peaks, which can be attributed to oxidation of the ketone groups and/or total decomposition of the polymer itself. This was supported by the fact that the solid block-copolymers changed colour to black upon extreme heating beyond 200°C. In hindsight, a limitation of this experiment was that thermal decomposition occurred during the first run cycle. If this experiment were to be redesigned, TGA should have been done to determine the polymer decomposition temperatures. From this experiment, it

was concluded that the use of a sonication bath for the TFR experiments would be feasible up to 85 °C, where water begins to boil and evaporate. A preliminary TFR experiment was conducted at room temperature to compare the sizes of polymersomes formed. The samples were left to stir for two weeks before size studies were completed. It was noted that during the TFR, most of the polymer remained stuck to the bottom of the glass vial after two weeks or simply became suspended as macroscopic precipitate in the vial. It was hypothesised that the PDMAm₅₀-b-PDAAm_y block copolymers used were too large and too hydrophobic and preferentially remained in the solid phase when introduced to an aqueous environment. It follows that perhaps the current TFR conditions are not rigorous enough to promote dissolution of the polymer in the aqueous phase. It was noted that after the experiment, sonication was employed to promote dissolution of the polymer film, but to no avail, as the polymer became a macroscopic precipitate in the vial without any evidence of dissolution or self-assembly when introduced to the aqueous environment for two weeks. Literature for TFR from Rideau and co-workers show that both the chemical nature and size of the polymer used were different from the PDMAm₅₀-b-PDAAm_y polymers used, with this group self-assembling their largest polymer with a Mn of 9000 g mol⁻¹ compared to 23000 g mol⁻¹ for the smallest polymer used here, PDMAm₅₀-b-PDAAm₁₀₀.¹ Despite this, DLS studies were conducted to determine the presence of nano-sized particles in the supernatant solution due to partial self-assembly.

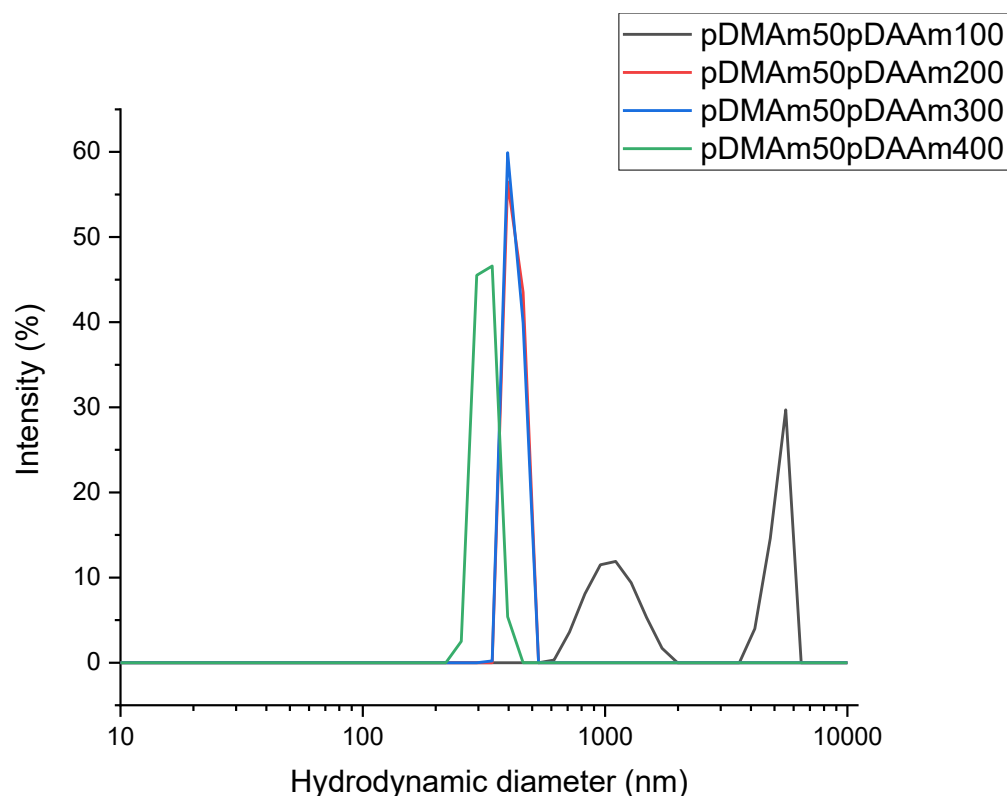


Figure 4.2. Particle size distributions of supernatants of PDMAm₅₀-b-PDAAm_y Nano-objects formed via slow TFR over 2 weeks at 150 rpm.

DLS studies conducted on the supernatant solution collected from the TFR studies produced relatively poor quality data (Figure 4.2), with a low derived count rate indicating minimal presence of nanoparticles. From these data, the shortest polymer, PDMAm₅₀-b-PDAAm₁₀₀, results in the most reliable distribution, where a defined peak corresponding to micron-sized particles is observed. The average size reported is, however, much larger than that reported from the parent PISA sample. Nevertheless, it is likely that the lower molecular weight of this sample facilitates better mobility on hydration, similar to observations by Bartenstein and co-workers.⁹ Ultimately, it was not possible to produce a homogeneous dispersion of polymersomes using TFR.

1.1.4. Direct rehydration of lyophilised PDMAm₅₀-b-PDAAm₃₀₀

Solvent exchange (SE) was explored as an alternative route to self-assembly of the PDMAm₅₀-b-PDAAm_y block copolymer. To explore the process, a PDMAm₅₀-b-PDAAm₃₀₀ block copolymer was synthesised, which routinely

yielded polymersomes via PISA. This polymer was lyophilized and rehydrated in water at 1 mg/ml, which was the concentration selected for the SE optimisation experiments, which is on a similar scale to that used for amphiphile self-assembly in recent literature^{6,7}. This concentration is thought to be the critical micellar concentration (CMC) while still being in a dilute regime to dissuade agglomeration of block copolymer nano-objects in principle. As well as this, concentrations of this magnitude have been routinely used for amphiphile self-assembly in recent and historic literature. This concentration falls within a typical range that allows for effective vesicle formation while being economical with materials.^{10,11}

The aim of this experiment was to find out if the vesicular morphologies of the self-assembled polymer can persist upon the simple polymer processing steps needed for the SE technique. The size distribution of the polymer, both under native PISA conditions and after lyophilisation and rehydration, and resultant TEM micrographs are presented in Figure 4.4. From the DLS data, it is known that the PDMAm₅₀-b-PDAAm₃₀₀ block copolymer produced via PISA has an intensity max around 300 nm, as mentioned in chapter 1, this block copolymer yields vesicular morphologies. The particle size distribution for the lyophilised and rehydrated polymer at 1 mg/ml is similar in monomodal nature to the PISA conditions. Additionally, both peaks occupy a similar size distribution from 100-1000 nm with similar Z-average sizes and Intensity max values. One notable discrepancy in the particle size distribution of the lyophilised block-copolymer is that there is a small shoulder peak at around 100 nm. This shoulder is unique to the lyophilised and rehydrated sample at 1 mg/ml and does not appear in the PISA sample. This indicates a second, smaller population of nano-objects being

present in the sample, expected to consist purely of polymersome morphologies.

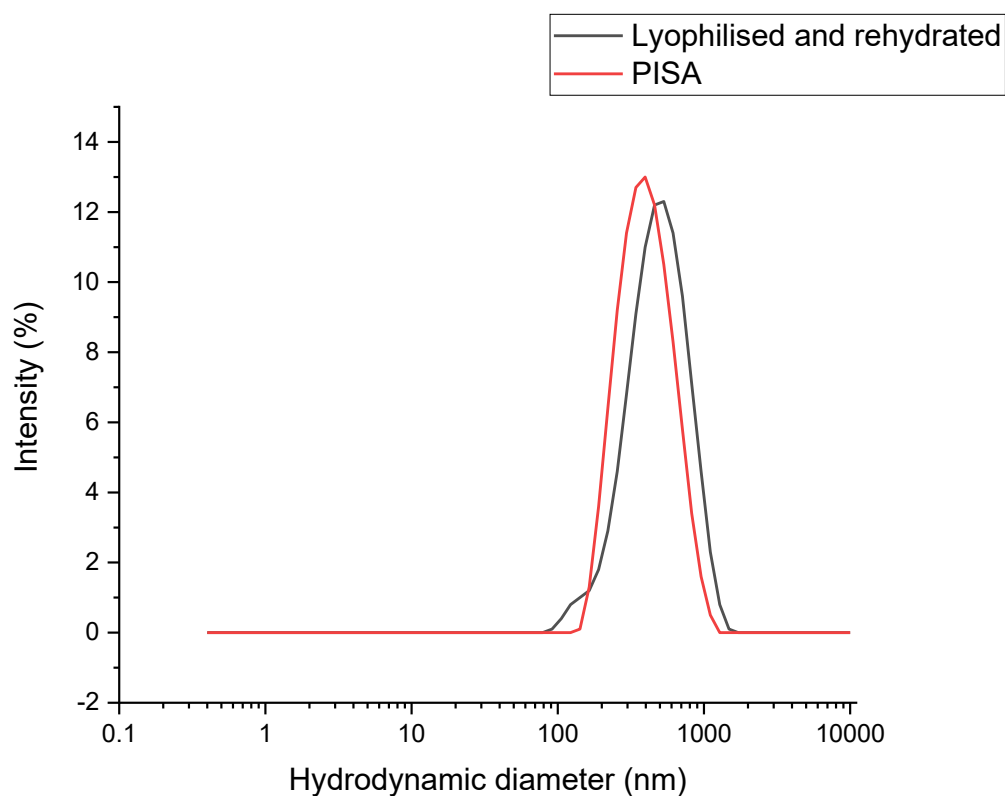


Figure 4.3. Particle size distribution for PDMA₅₀-b-PDAAm₃₀₀ block-copolymer nano-objects made via PISA (red) and rehydration of lyophilised polymer from PISA (black)

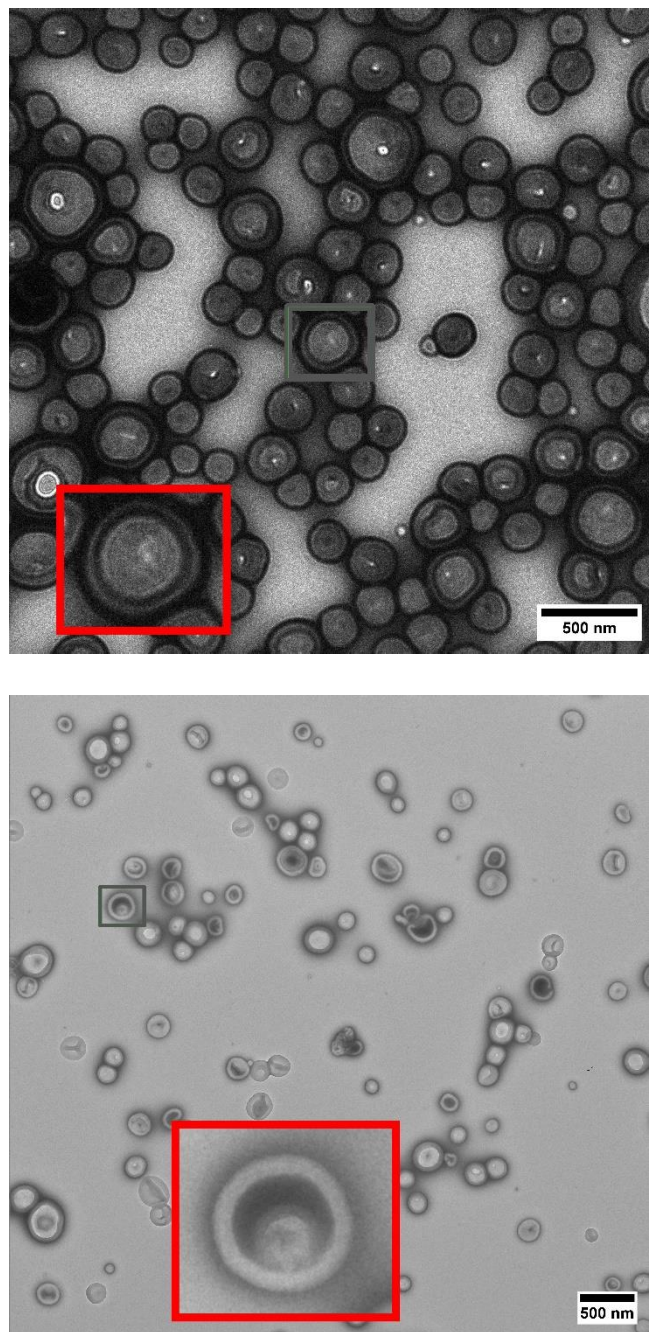


Figure 4.4. TEM micrographs of native PDMAm₅₀-b-PDAAm₃₀₀ from PISA (top) vs lyophilised and rehydrated PDMAm₅₀-b-PDAAm₃₀₀ block copolymer nano-objects (bottom) previously made via PISA. Additional images presented in the appendix (Figures 7.26-7.33)

TEM imaging (Figure 4.4) confirmed that the vesicular morphology was dominant in this case, with a larger population of 200-300 nm particles with a clearly defined membrane or a collapsed/deflated structure. Individual polymersomes are highly symmetrical and very similar in size before and after lyophilisation and rehydration (Figure 4.4). A population of smaller nano-objects is also observed, in Figure 4.4 (bottom), which has no features characteristic of a vesicle membrane. It is likely that these spheres account for the shoulder in the particle size distribution at lower diameters. Based on the above observations, it is likely that lyophilisation of the PISA dispersion does not affect the overall structure of the vesicles, meaning they can simply be re-suspended on hydration. In this context, there is no self-assembly process occurring, and therefore, it would not be possible to encapsulate a payload. It should be noted that freeze-drying and rehydration are an impressive feature for these vesicles, as this proves that the vesicles can be packaged and processed in an industrial setting.

4.1.5. Evaluating parameters affecting self-assembly of PDMAm₅₀-b-PDAAm_y via solvent exchange from methanol to water

Solvent selection

Solvent exchange requires the polymer to be primarily dissolved in a water-miscible “good solvent” for both blocks. Within this solution, the polymer needs to be present as molecularly dissolved chains with little or no interaction between individual chains caused by solubility. Furthermore, there must be a convenient method for removing the solvent following self-assembly. This is often achieved via dialysis¹²⁻¹⁹, or evaporation²⁰. For solvent exchange studies, methanol²¹, tetrahydrofuran²²⁻²⁴, and acetonitrile²⁵ are typical examples of solvents used.

In the context of this study, methanol was selected since it is widely available, low-cost, relatively low-hazard, and volatile. In principle, the latter feature enables facile removal by evaporation, again a low-cost method which does not rely on expensive membranes for dialysis. A further benefit of methanol is that

it is less problematic (although not fully) than other solvents with respect to the potential encapsulation of biomolecules.

For the self-assembly process, the effect of addition rates (of the organic phase/polymer to the aqueous phase) on morphologies was studied for three different final compositions of methanol solution. The rates chosen were $33 \mu\text{L min}^{-1}$, $333 \mu\text{L min}^{-1}$, and 'rapid addition' corresponding to an approximate addition time of 30 min, 3 min, and 1-2 seconds for adding 1 mL solution to the aqueous phase. It was anticipated that these were within a range that would be realistic for routine encapsulation. Initial methanol concentrations of 5, 10, and 15 % were selected. Lower methanol concentrations were selected to reduce the amount of solvent that needed to be removed. The resulting samples were characterised by DLS (Figure 4.5, 4.7, 4.9) and stained TEM (Figure 4.6, 4.8, 4.10).

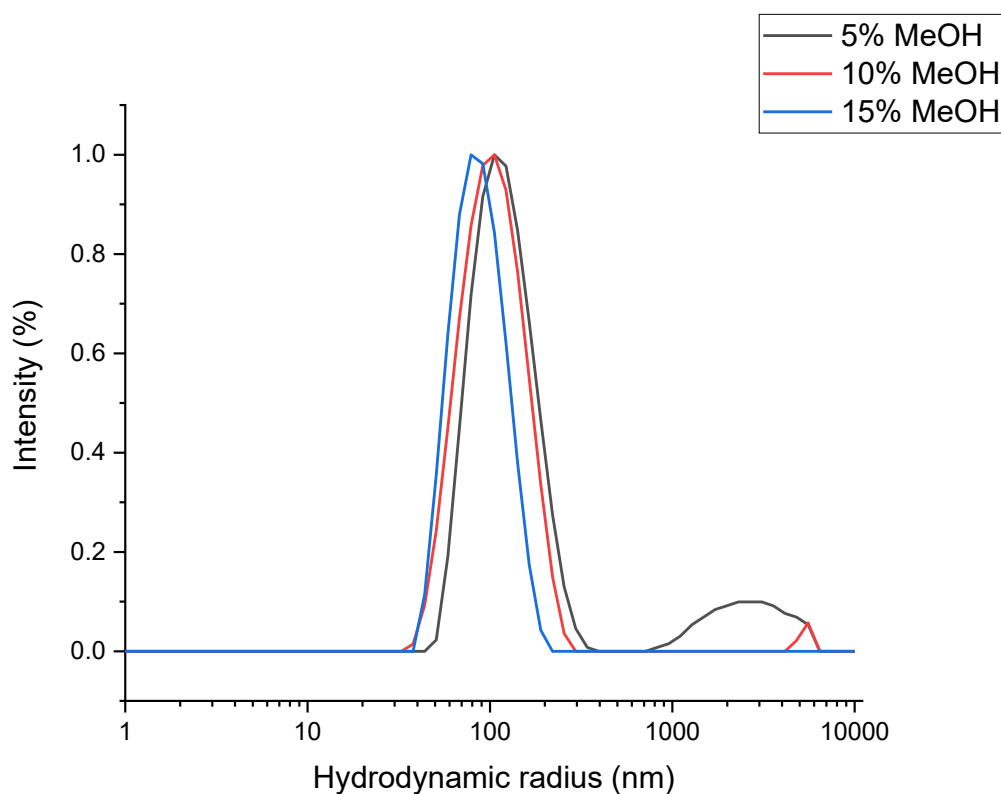


Figure 4.5. Particle size distribution for PDMA₅₀-b-PDAAm₃₀₀ block-copolymer nano-objects made via SE at 33 $\mu\text{L/min}$ at 5-15% initial MeOH volume.

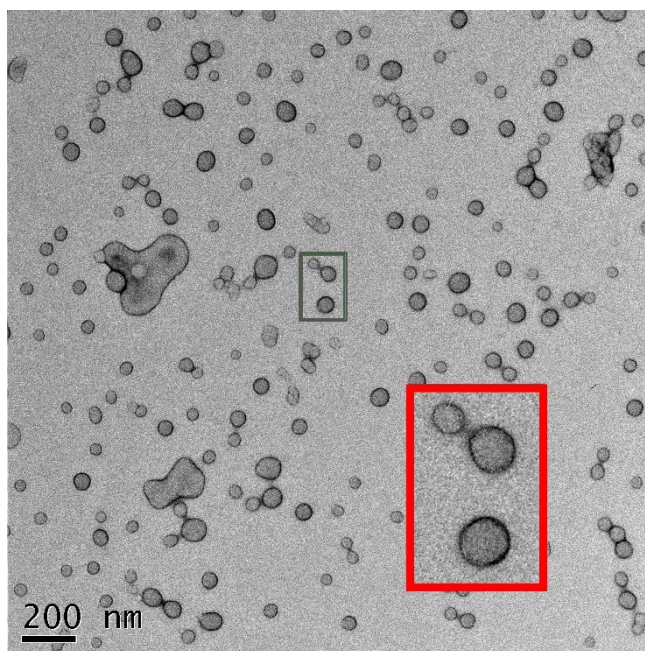


Figure 4.6. TEM micrographs of PDMAm₅₀-b-PDAAm₃₀₀ block-copolymer nano-objects made via SE at 33 μ L/min at 10% initial MeOH volume. Additional images presented in the appendix (Figures 7.34-7.36)

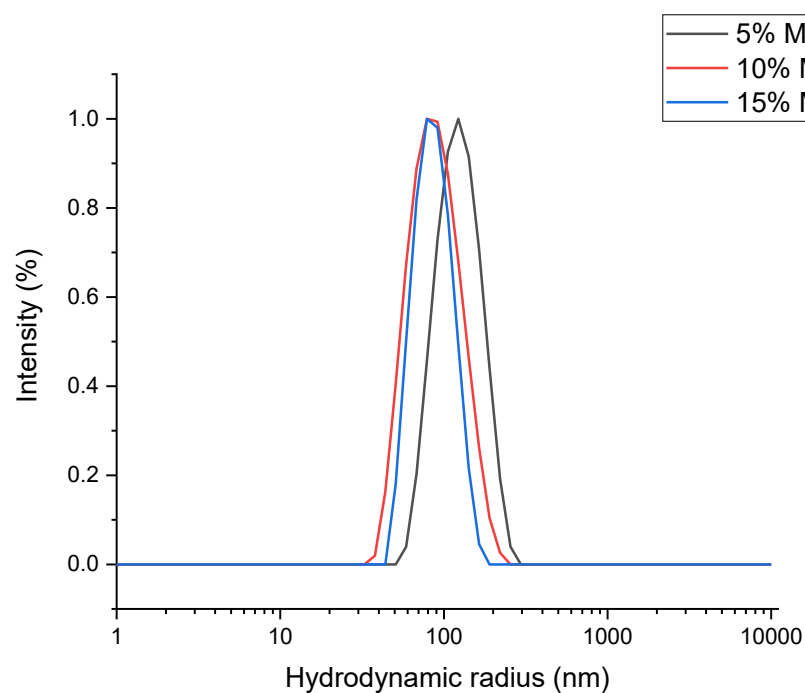


Figure 4.7. Particle size distribution for PDMAm₅₀-b-PDAAm₃₀₀ block-copolymer nano-objects made via SE at 333 μ L/min at 5-15% initial MeOH volume.

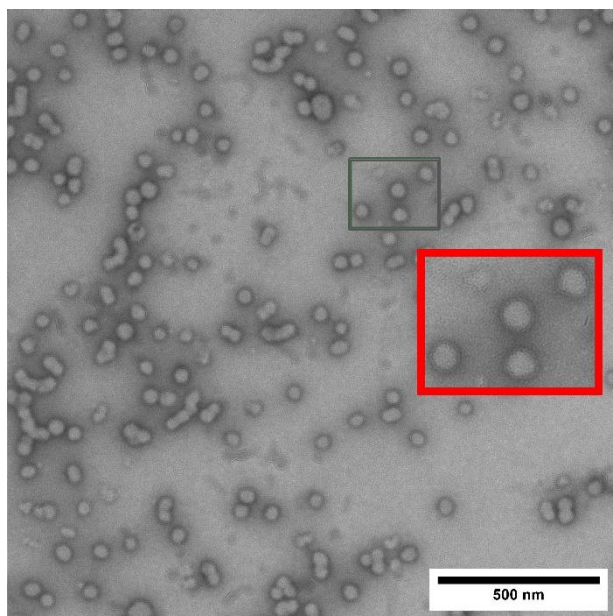


Figure 4.8. TEM micrographs of PDMA₅₀-b-PDAAm₃₀₀ block-copolymer nano-objects made via SE at 333 μ L/min at 10% initial MeOH volume. Additional images presented in the appendix (Figures 7.37-7.45)

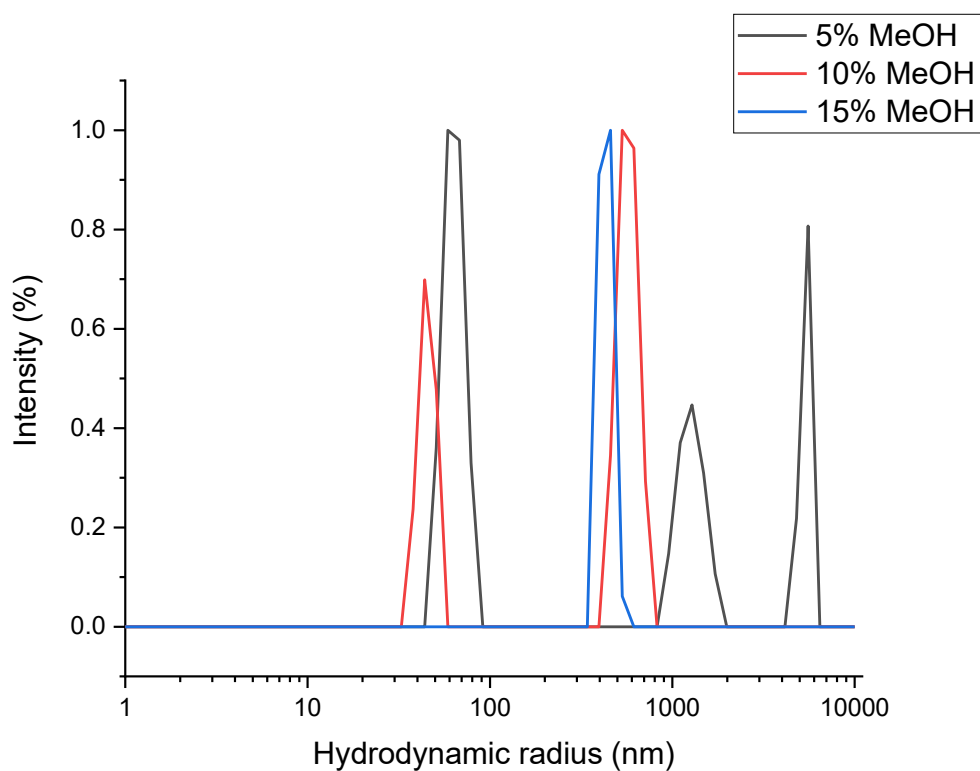


Figure 4.9. Particle size distribution for PDMA₅₀-b-PDAAm₃₀₀ block-copolymer nano-objects made via SE at rapid addition at 5-15% initial MeOH volume.

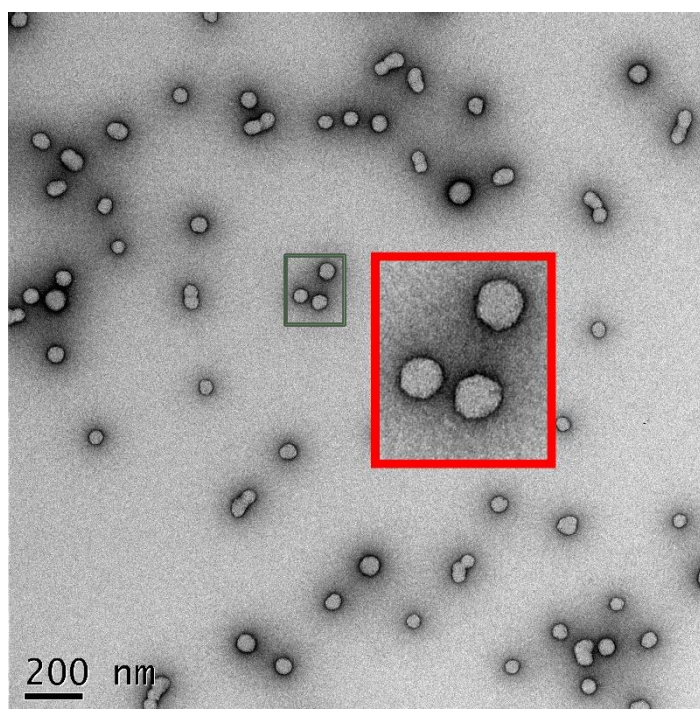


Figure 4.10. TEM micrographs of PDMA₅₀-b-PDAAm₃₀₀ block-copolymer nano-objects made via SE at rapid addition at 10% initial MeOH volume. Additional images presented in the appendix (Figures 7.46-7.50)

4.1.6. 30-minute addition of Polymer Solution

DLS data for the SE at 33 $\mu\text{L}/\text{min}$ (Figure 4.5) shows single-peaked particle size distributions, with methanol concentration (5-15%). In some cases, a small peak near the micron range was observed, which can be attributed to dust particles or macroscopic aggregated polymer being present during the sample characterisation. In light of this, all the nano-objects formed via this slow addition of the polymer-MeOH solution are between 50 and 250 nm, with the intensity max of all three particle size distributions being around 100 nm. The particle size distribution for the 5% MeOH SE sample does have a small shoulder near 1000 nm. A problem that occurred during this SE addition was that some of the block-copolymer nano-objects were aggregating at the needle, which was inside the aqueous phase, due to the slow rate of addition of the

polymer-MeOH mixtures. This could cause the polymer to aggregate into larger particles and may have reduced the number of polymer chains present in the aqueous phase, potentially leading to fewer nano-objects being present in the aqueous phase. For these slow addition samples, the intensity max of the particle size distributions seems to decrease slightly with increasing MeOH from 5-15%.

To better understand the morphology of the nano-objects observed using slow addition during the SE process, the 10% MeOH sample was taken forward for TEM analysis to shed some light on the size, morphology, and shapes of the nano-objects. From the TEM micrographs (Figure 4.6), the dominant morphology observed is spherical micelles. The size of these dominant spheres is quite uniform, with a size of 75 nm. In most cases, these spherical micelles are highly symmetrical, but there seems to be evidence of non-spherical entities being present on the TEM Grid. These nano-objects are more exotic in shape, forming flatter, more deformed polymer nano-objects. These non-spherical nano-objects are also, at times, bigger than the spherical micelles, which dominate the representative morphology of this SE sample at slow rates of addition. This sample still has around 10% MeOH, and it is thought that the MeOH co-solvent may be acting as a plasticiser for the PDMAm₅₀-b-PDAAm₃₀₀ block co-polymer, which in turn allows for swelling of spherical nano-objects to form larger, less symmetrical polymer nano-objects. As well as these nano-objects observed, there also seems to be dimer formation. Dimers can be thought of as two individual spherical micelles coming into contact and coalescing to form a larger block copolymer nano-object. From the TEM (Figure 4.6), it is clear to see the presence of such dimers and even in some cases trimers and higher order clustering of micellar nano-objects. The TEM micrographs seem to be in good agreement with the polymer nano-object size distributions observed via DLS, with no nano-objects observed with a radius larger than 250 nm. Due to the similarity in the particle size distributions as a function of MeOH concentration, it was assumed that the 5% and 15% samples would be of similar size, shape, and morphology to the median SE sample of 10% MeOH. From these experiments, it can be concluded that for low concentrations of MeOH, a slow rate of addition of 33 μ L/min leads to mainly spherical micelles with a few higher-order clusters and methanol-swollen nano-objects.

4.1.7. 3-minute Addition of Polymer Solution

In the case of the intermediate rate of 333 $\mu\text{L}/\text{min}$, the total addition of the polymer-MeOH solution into the aqueous phase took no longer than 3 min in all cases. From the particle size distributions observed via DLS (Figure 4.7), monomodal peaks are observed for the SE of PDMAm₅₀-b-PDAAm₃₀₀ at 333 $\mu\text{L}/\text{min}$ between 50 and 250 nm. Unlike the size distributions seen at 33 $\mu\text{L}/\text{min}$ (Figure 4.5), these distributions are purely monomodal with no evidence of dust or other contaminants in the micron size range of the distribution. Similarly to the optimisation experiments seen at 33 $\mu\text{L}/\text{min}$, the intensity max of the size distributions seems to decrease slightly with increasing MeOH. Nonetheless, all three size distributions at 333 $\mu\text{L}/\text{min}$, regardless of methanol concentration, have intensity max values centred on 100 nm. This size range is indicative of the self-assembly of spherical micelles, which were seen previously to be characteristically sized between 50 and 150 nm. This trend in the particle size distributions was confirmed in the TEM (Figure 4.8), where the dominant morphology of the nano-objects was spherical micelles with radii close to 100 nm for the 10% MeOH sample prepared at 333 $\mu\text{L}/\text{min}$. Typically, in most cases, the internal symmetry of the spheres is high, with very few nano-objects observed to be in a prolate-style shape with slightly higher aspect ratios than the dominant symmetrical spheres. As well as these prolate nano-objects, dimerization and higher-order clustering are present, especially on the TEM Micrograph (Figure 4.8). Here, two, three, and sometimes four or more spherical micelles have coalesced together to form dimers and worm-like clusters with similar internal radii to a sphere but elongated as more spheres combine and coalesce. There are a few nano-objects that are similar in size to vesicles, but evidence of a dominant vesicular morphology is yet to be seen via SE of this PDMAm₅₀-b-PDAAm₃₀₀ block copolymer at the current MeOH concentrations and injection speeds.

4.1.8. Rapid addition of Polymer Solution

The rapid addition of the polymer-MeOH mixture to the aqueous phase was explored by instantaneous injection of the entire organic phase. During this addition, small amounts of polymer-MeOH solution were splashed on the side

of the 2 mL vial, leading to small regions of high polymer density and potentially poorer mixing of the organic and aqueous phases. Upon addition of the entire 100 μ L of the polymer-MeOH solution, there was evidence of larger particles suspended in the aqueous phase. From the particle size distributions (Figure 4.9) for the SE of PDMAm₅₀-b-PDAAm₃₀₀ block copolymer at rapid additions, the distributions were more varied than the previous data at 33 and 333 μ L/min. When using 5% MeOH for the rapid addition SE, a bimodal particle size distribution was observed with maximum intensity values centred on 70 nm and 1000 nm, respectively, with potential dust contaminants or aggregated polymer being observed at 5000 nm. In the case of the rapid addition at 10% MeOH, the particle size distribution is bimodal with one peak centred on 50 nm and a larger, more intense peak centred around 500 nm. Finally, the 15% MeOH sample displayed a monomodal peak centred at 500 nm. Unlike at slower rates of addition, the rapid addition led to the most polydisperse of the nano-objects prepared via SE. There is some similarity with other experiments (Figures 4.5, 4.7, and 4.9) in that the region between 50-100 nm, where spherical micelles are expected, is populated well by both the 5% and 10% MeOH samples. This suggests, based on previous data, that it may be forming spherical micelles under the rapid addition regime also. To confirm this, TEM was deployed on the intermediary 10% MeOH sample to gain insights on the size, shape, and morphology of the nano-objects prepared during these experiments (Figure 4.10). To gain a representative sample, the vial containing the nano-objects was shaken and allowed to stand for 5 min. It was hoped that any micron-sized polymer aggregates would not be present on the grid, and the focus would be on the nanoscale polymer nano-objects prepared. Interesting aggregates may be colloiddally unstable enough to settle to be imaged via TEM. From the TEM micrographs of the 10% MeOH Rapid addition sample (Figure 4.10), clear evidence of spherical micelle formation during the addition sequence is observed. Spherical micelles are the dominant morphology and are individually highly symmetrical. As previously seen, there seems to be evidence of dimerisation of individual micelles to combine and form dimers and higher-order clusters, which resemble worm-like or cylindrical micelles. The rate of addition does not influence the size, shape, and morphology of the nano-objects self-assembled via this SE approach. Moving forward, it was decided that a rapid rate would be taken forward for all SE experiments. This is because there was no obvious benefit observed from slower/medium addition in terms of morphology of the PDMAm₅₀-b-PDAAm₃₀₀ nanoparticles. In terms of mixing,

quicker addition ensures that the good solvent for the polymer, methanol, is at the lowest concentration as quickly as possible. In slower additions, it was observed that sometimes precipitation at the needle occurred when the needle was immersed in the solution. Conversely, splashing and bubbles were observed at slower rates of addition when the needle was not immersed. Faster additions may remedy this issue as the polymer/organic mixture enters directly into the bulk, avoiding interfacial issues that may arise. It is also noted that although slower addition speeds may be better for kinetic processes, no advantage was seen. Additionally, Faster, more time-efficient processes are more advantageous in industrial settings.

4.1.9. Effect of co-solvent removal on solvent exchange nano-object size and morphology

Addition rate and solvent concentration studies were conducted, with post assembly solvent removal conducted via evaporation of methanol by stirring (open to air) for 3 days. Ultimately, this is potentially a key step in the SE process since the mobility of the chains can be aided by the presence of a cosolvent.²² In order to assess how the nano-objects evolve as the MeOH is removed, a SE sample made at 10% MeOH rapid addition was left stirring, open to air over 3 days. After 3 days, there was a clear increase in the turbidity of the sample.

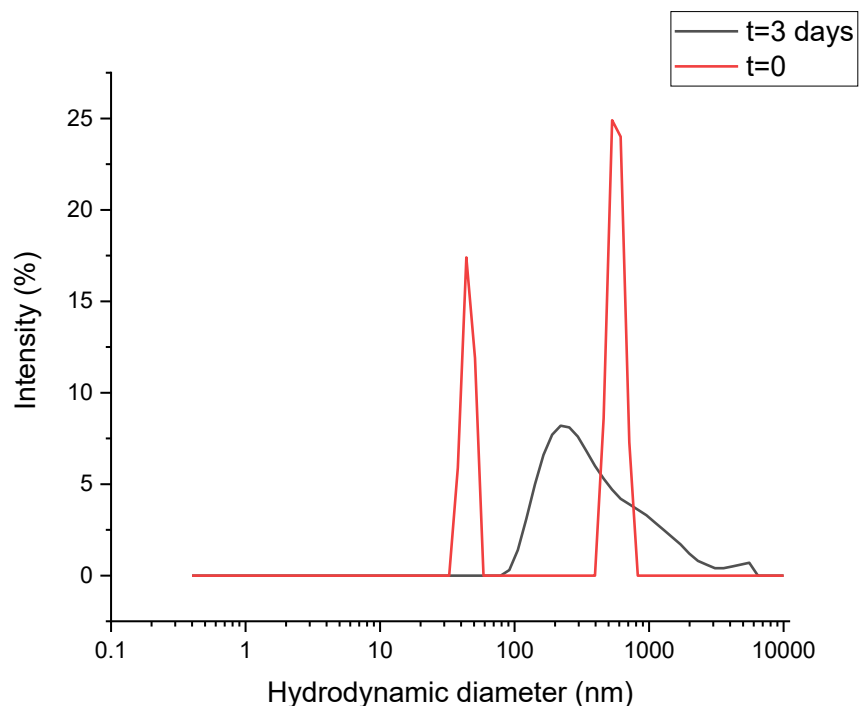


Figure 4.11. Particle size distribution for PDMA₅₀-b-PDAA₃₀₀ block-copolymer nano-objects made via SE at rapid addition at 10% initial MeOH volume, t=0 min after addition (red) and after 3 days of stirring open to air (black).

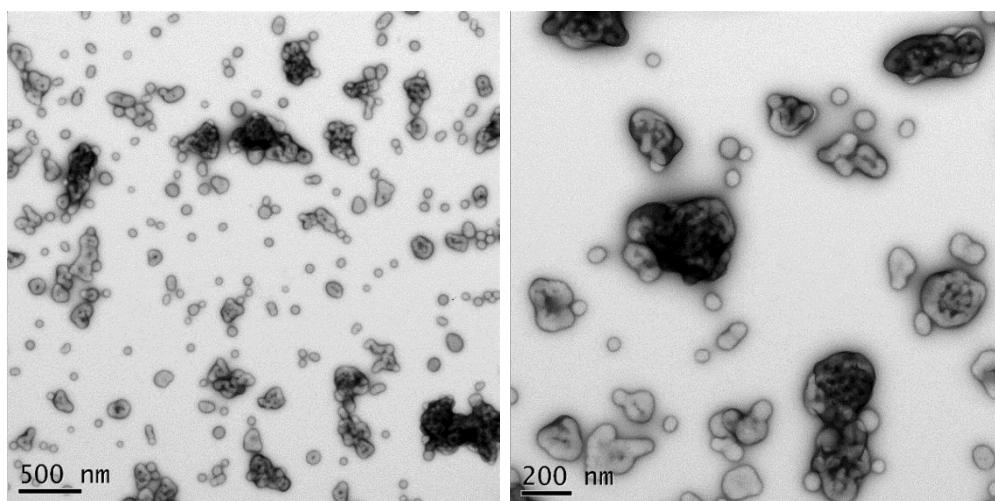


Figure 4.12. TEM micrographs of PDMA₅₀-b-PDAA₃₀₀ block-copolymer nano-objects made via SE at rapid addition at 10% initial MeOH volume after 3 days of stirring open to air. Additional images presented in the appendix (Figures 7.51-7.61)

A clear change in the particle size distribution is observed, where the $t=0$ sample after rapid addition and 5 min of stirring comprises two peaks with relatively high intensity, indicating the presence of ill-defined objects.

From Figure 4.11, the size distribution was similar to that shown in the previous experiments with this 10% MeOH rapid sample. Upon removal of MeOH, there is a transition in the DLS of the sample to a broad, lower intensity peak, which occurs at approximately 200 nm with an associated shoulder extending into the micron range. DLS diameters of 200 nm are often observed for vesicular samples synthesised by PISA²⁸ and specifically those based on the PDAAm₃₀₀-based vesicles reported here and by others^{29,30}. However, TEM micrographs reveal a more exotic mixture of nano-objects of varying sizes between 200 and 400 nm, with an absence of any obvious vesicular structures.

Although based on the apparent volume of polymer, the non-spherical objects dominate, there is still a significant population of spherical particles with some evidence of dimerisation and coalescence into worm-like micelles, highlighted in Figure 4.11. It should be noted that DLS, by design, is intended to measure spherical non-interacting particles, and the presence of non-spherical polymeric nanoparticles could affect the measurements made. The rest of the nano-objects observed on the grid are more complex. There seem to be a few nano-objects sized around which appear to be clusters of spheres fused together into a larger nano-object. These larger entities are non-symmetrical and vary greatly in size between 100 and 600 nm. It is believed that this is due to the removal of MeOH from the individual spherical micelles, causing coalescence of multiple spheres into larger entities, where the size depends on N_{sphere} , the total number of individual spherical micelles coalesced together. Unlike previous clustering and dimerisation from spheres to worms, there seems to be three-dimensional structuring to the larger nano-objects. This, along with the entangled nature of the nano-objects, suggests that these may have formed from the convoluting of worm-like micelles, which could be an intermediate between spheres and these clustered nano-objects.

In all SE experiments, the PDMAm₅₀-b-PDAAm₃₀₀ block copolymer yielded spherical micelles, which, upon evaporation, yielded clustered nano-objects. In order to explore all of the experimental space in the variables selected, it was chosen to look at the total MeOH concentration during mixing. In the previous experiments, MeOH concentrations of 5-15% were utilised. However, it is

hypothesised that this volume of MeOH may be insufficient to allow the block copolymer to form higher-order nano-objects, resulting in spherical micelles which may be kinetically trapped into this morphology due to the low mobility of the polymer chains. Increasing the MeOH concentration could hypothetically plasticise the block copolymer more, allowing for more chain mobility, and allowing for morphological transitions beyond spherical micelles.²²

In order to explore this hypothesis, an analogous SE experiment was performed with 50% MeOH by volume under rapid addition. This accounted for a larger total synthesis, increasing from 1.15 to 2.00 mL.

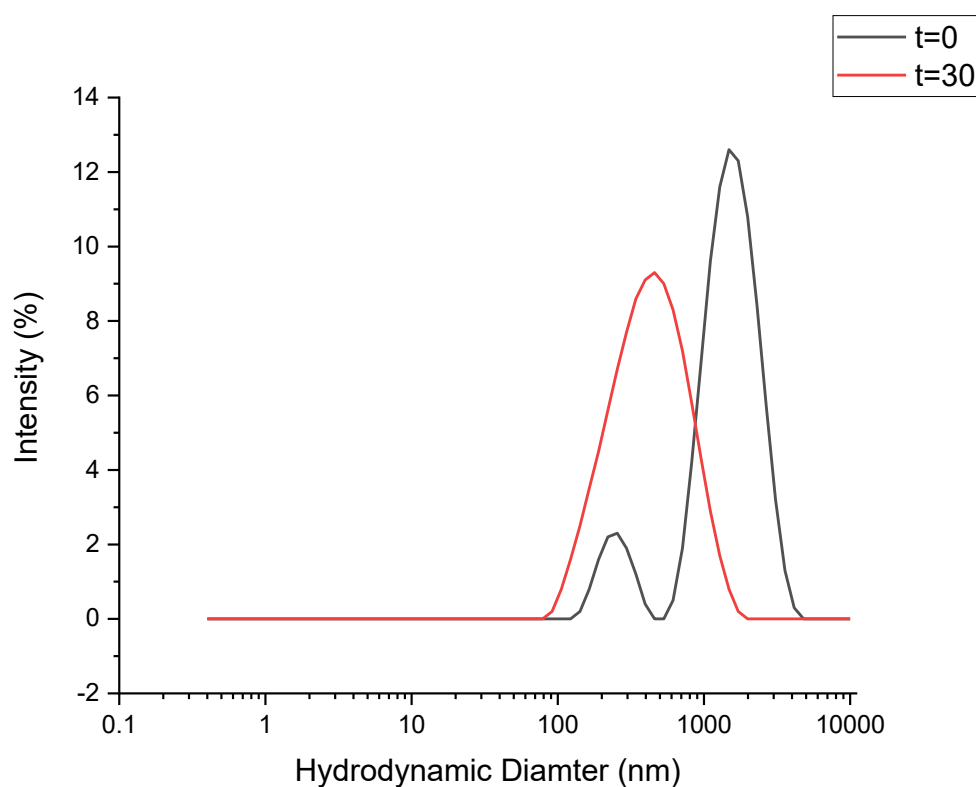


Figure 4.13. Particle size distribution for PDMA₅₀-b-PDAAm₃₀₀ block-copolymer nano-objects made via SE at rapid addition, at 50% initial MeOH volume, t=0 min after addition (black) and after 30 min under rotary evaporation (red).

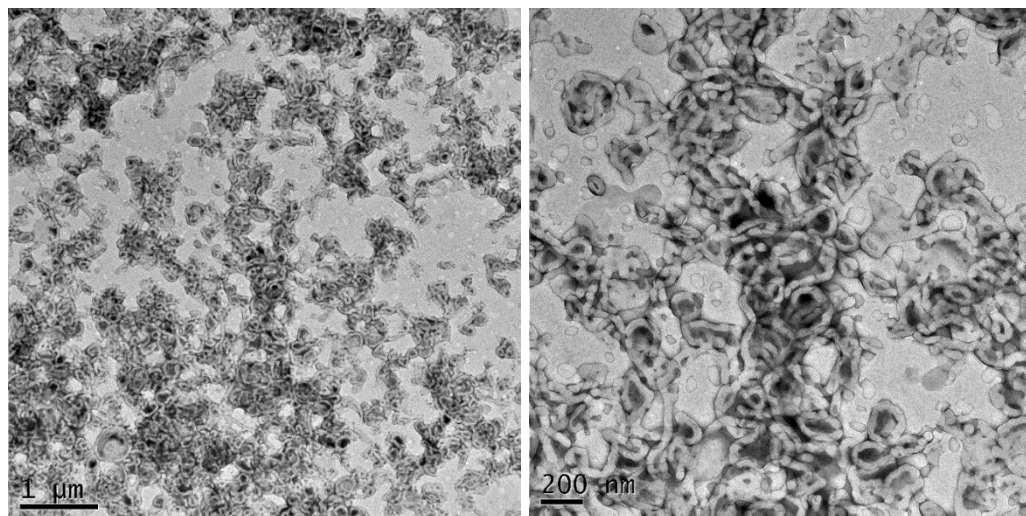


Figure 4.14. TEM micrographs of PDMAm₅₀-b-PDAAm₃₀₀ block-copolymer nano-objects made via SE at rapid addition at 50% initial MeOH volume after 5 min stirring (t=0). Additional images presented in the appendix (Figures 7.62-7.71)

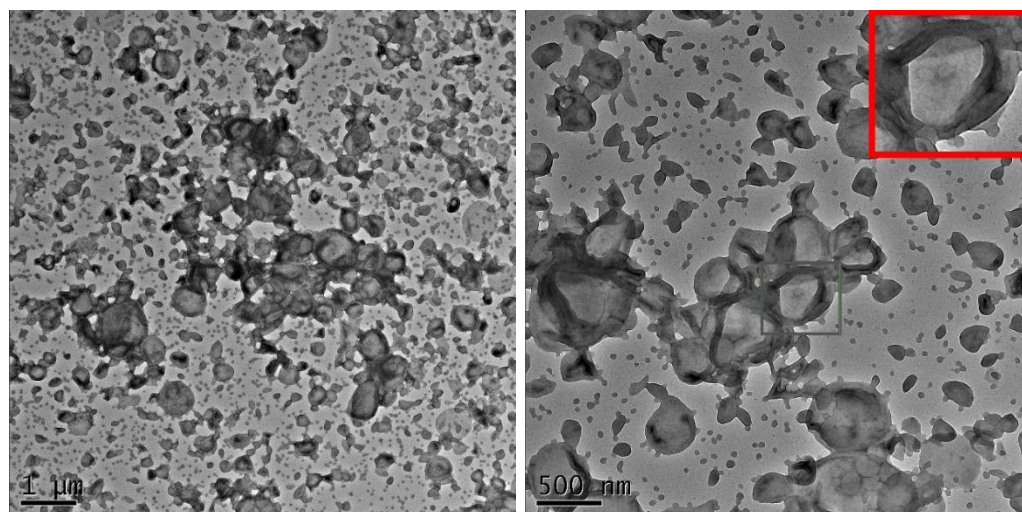


Figure 4.15. TEM micrographs of PDMAm₅₀-b-PDAAm₃₀₀ block-copolymer nano-objects made via SE at rapid addition at 50% initial MeOH volume after rotary evaporation for 30 min (t=30). Additional images presented in the appendix (Figures 7.72-7.84)

From the DLS data, it is apparent that increasing the MeOH concentration to 50% has influenced the particle size distribution of the nano-objects obtained. Initially, after 5 min of mixing, there is a bimodal peak with the larger peak centred at 2000 nm with a smaller peak, roughly 20% in intensity, centred on 250 nm. This is a clear difference from the analogous 5-15% SE optimisation experiments, which yielded spherical micelles with intensity max values around 50-100 nm. This, coupled with the fact that the 50-200 nm range is not populated, suggests that the polymer may no longer be forming spherical micelles upon increasing the MeOH concentration to 50%. Upon removal of the MeOH, the particle size distribution changes to a more dispersed monomodal peak with an intensity max of 300 nm. The particle size distribution is a lot more dispersed upon MeOH removal, with sizes ranging from 100-1000 nm, suggesting a mixed morphology of various-sized nano-objects. In Figure 3.14, the TEM micrograph of the SE optimisation at 50% MeOH at $t=0$, it is seen that there is a more populated grid with a high number of nano-objects than previously seen for SE optimisations. This was also seen when inspecting the sample visually, where there was an increase in turbidity of the SE mixture when the total MeOH concentration was increased to 50%. From this TEM Micrograph in figure 3.15, a morphological transition is observed away from kinetically trapped spherical micelles. The TEM grid is populated with worm-like cylindrical micelles, which are the dominant morphology observed under these optimisation conditions. The micelles are very polydisperse, and this is due to methanol being present in the cores and swelling them to different sizes and shapes. This is a hypothesis, and there is no data to suggest that the methanol is not uniformly distributed. The worms have a similar thickness to the diameter of a spherical micelle, which is between 50 and 100 nm in diameter. These worms have seemed to undergo aggregation and convolution with other worm-like micelles to form a so-called "spaghetti" microstructure. High levels of entanglement and clustering of these worms have led to large domains of clustered polymer nano-objects spanning microns in size and populating most of the TEM Grid. The average size of clusters of worms varies but is around the micron range for each cluster. It is useful to wonder if this is the preferential conformation of these worm like micelles in solution, or whether the process of staining and drying these nano-objects onto a 2D solid surface may have induced some sort of drying artefacts and clustering. These vesicles appear to

be somewhat spherical, with evidence of areas of high uranyl acetate stain on the outer edges of the spherical vesicles, which may be indicative of a bilayer membrane formation which provides a favourable environment for the hydrophobic uranyl acetate dye. The DLS data can be useful for this as it suggests that at $t=0$ the nano-objects are populating the 1-4 micron range. This is in agreement with the TEM micrograph of these worm-like micelles, which suggests these larger micron-sized clusters with internal nano-structures may be the native conformation for this block copolymer under these SE optimisation conditions.

4.1.10. Conclusions

TFR was tried as a viable option to form polymer vesicles, but ultimately yielded no self-assembly of any morphology of nanoparticles. SE self-assembly protocol was more fruitful and showed that PDMAm₅₀-b-PDAAm₃₀₀ block copolymers tend to form spherical micelles at low methanol concentrations of 5-15%. The rate of addition was not found to be an important factor in the self-assembly of these spherical micelles. There is a change in nano-object morphology from low MeOH samples, which previously showed kinetically trapped micelles across all speeds of additions and MeOH concentrations (5-15%). Interestingly, at 50% initial MeOH, when MeOH is removed, the morphology changes from clustered worm-like micelles to a vesicle-dominant mixture of morphologies. From Figure 15, a diverse mixture of polymer nano-objects with a few identifiable morphologies is observed. The largest aggregates, roughly around 300-500 nm, appear to resemble asymmetrical vesicle structures. These vesicles appear to be somewhat spherical, with evidence of areas of high uranyl acetate stain on the outer edges of the spherical vesicles, which may be indicative of a bilayer membrane formation which provides a favourable environment for the hydrophobic uranyl acetate dye.

4.1.11. References

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Chapter 5

5.1. Understanding morphological transitions during co-solvent evaporation

5.1.1. Introduction

In chapter 4, it was established that during the SE of PDMAm₅₀-b-PDAAm₃₀₀, the initial methanol concentration was a key factor in unlocking higher order morphologies as opposed to kinetically trapped spherical micelles. This was conducted at pH 2.5, which is highly acidic conditions to ensure protonation of carboxylic acid end-groups. However, this was not favourable for the potential encapsulation of pH-sensitive payloads. Herein, the SE of PDMAm₅₀-b-PDAAm₃₀₀ under neutral pH conditions (pH 7.5) is explored to understand the effect. Furthermore, this chapter investigates morphological evolution during the evaporation of the 'good' solvent (a solvent which dissolves both the blocks of the block copolymer). Previous results in chapter 4 show that at pH 2.5, the SE of PDMAm₅₀-b-PDAAm₃₀₀ transforms from cylindrical micelles or worms to a mixed vesicle morphology. It is hoped that by scaling up the SE self-assembly process, aliquots can be removed and characterised by DLS and TEM. This will also aid in understanding how residual methanol contributes to the process.

By isolating potential intermediates, it was hoped that these experiments could provide a mechanistic insight into how the removal of methanol from these polymer nano-objects allows them to form higher-order morphology nano-objects. This will allow for a deeper understanding of how methanol facilitates morphological transitions during block-copolymer self-assembly. This will be done using ¹H NMR on the aliquots taken to accurately determine the residual methanol remaining during the evaporation of methanol, which, combined with DLS and TEM, can paint a broad picture of what is happening to the nano-objects when methanol is being removed. This approach, using ¹H NMR to monitor solvent evaporation during SE, has yet to be explored in the literature.

Also, this chapter concludes with an experiment into adding a second smaller lipid amphiphile, which is membrane directing, to the SE mixture to promote a morphology change into pure vesicles. A similar study was done by LoPresti and co-workers, who mixed two block-copolymers of differing sizes and chemistries to form patchy polymersomes, which are polymersomes with differing surface domains.¹ However, this chapter proposes a much more radical difference in size and chemistry between the two amphiphiles mixed, namely POPC and PDMAm₅₀-b-PDAAm₃₀₀. This chapter seeks to develop a solvent evaporation technique to utilise solvent-antisolvent interactions to

induce polymer-lipid self-assembly. Literature on the use of SE to self-assemble hybrid nanoparticles is scarce. Current literature. However, a study by Di Leone explored the preparation of poly(lactide-co-glycolide) (PLGA)- phospholipid nanoparticles using nanoprecipitation techniques, focusing on the use of binary solvent systems like methylene chloride-acetone to optimise particle size and encapsulation efficiency. They employed SPG membrane emulsification to achieve uniform nanoparticle formation, with process parameters such as stabiliser type, phase ratio, and membrane pore size playing critical roles.

5.1.2 Methodology

Small-Scale solvent exchange of PDMAm₅₀-b-PDAAm₃₀₀ block copolymers under various aqueous conditions

Lyophilized PDMAm₅₀-b-PDAAm₃₀₀ block copolymer (1 mg) was dissolved in 1 mL of methanol. This was subsequently added to 1 mL of aqueous solution and allowed to stir at 1000 RPM for 5 min before characterisation via DLS and TEM. The methanol was then removed under reduced pressure by rotary evaporation for 30 min at 150 RPM. The resultant polymer solution was again characterised via DLS and TEM.

A series of aqueous solutions was evaluated, comprising either no NaCl or 140 mM NaCl; the aqueous solution was varied before the solvent exchange process began. The pH was also altered to 7.5 or 2.5 using HCl and KOH stock solutions.

Solvent-exchange & evaporation over three hours for PDMAm₅₀-b-PDAAm₃₀₀ block copolymer under various aqueous conditions

The PDMAm₅₀-b-PDAAm₃₀₀ block copolymer, synthesised previously via PISA, was lyophilised to remove water. The polymer used was either in its singly or doubly charged state, as previously made via PISA. Lyophilised PDMAm₅₀-b-PDAAm₃₀₀ (10 mg) was dissolved in 10 mL of methanol to give a polymer-methanol solution at 1 mg/mL. This was rapidly injected into an aqueous solution, which was stirred at 1000 rpm. The mixture was then stirred in a sealed

100 mL round-bottom flask (1000 rpm). After 5 minutes, an aliquot was extracted for analysis by DLS and TEM. The solvent was then removed under reduced pressure for 3 hours using a rotary evaporator operating at 40 RPM. 1 mL Aliquots were taken at regular intervals during the evaporation and replaced with 1 mL of the aqueous buffer to prevent evaporation of the aqueous solution, leading to an increase in concentration of polymer. Aliquots were characterised via DLS, TEM, and benchtop ^1H NMR.

Determination of methanol composition using low-field NMR spectroscopy

Initially, a calibration curve was obtained for a range of water and methanol mixtures ranging from 50% MeOH to 0.02% MeOH. Each sample was transferred into a standard NMR tube and characterised via low-field ^1H NMR Spectroscopy using a 60 MHz benchtop NMR (Magritek). The absolute integral of the peak due to the methanol protons observed at 3.18 ppm was determined for each sample and plotted as a function of the solution concentration.

A water-methanol mixture was prepared in a round-bottom flask. 10 mL of pH 7.5 water was added with 10 mL of methanol to give a 50% Methanol 50% water mixture by volume. The mixture was allowed to evaporate for 3 hours under reduced pressure at 150 RPM via rotary evaporation. 1 mL aliquots were taken at regular intervals during the evaporation and replaced with 1 mL of the aqueous buffer. Aliquots were characterised via benchtop ^1H NMR.

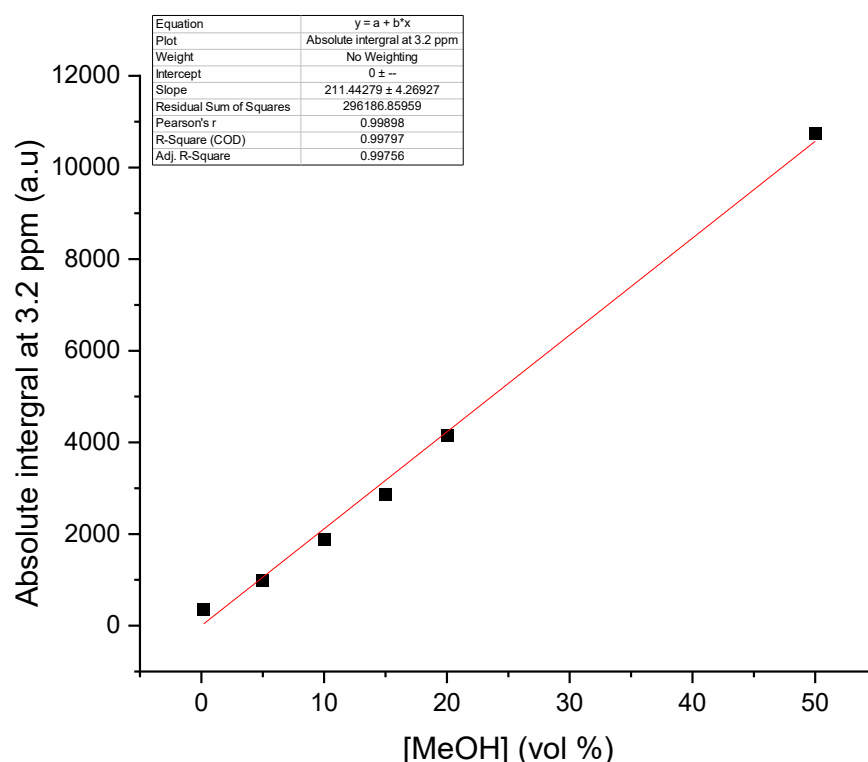


Figure 5.1. MeOH volume % vs absolute integral calibration curve

From Figure 5.1, it is evident that there is a clear linear trend in the data, with the absolute integral at 3.2 ppm increasing linearly with MeOH vol % used for the benchtop NMR experiments. A trendline was fitted using OriginLab, with an R^2 value of 0.997 suggesting a good fit of the data to the trendline. The intercept was set as 0. From this data an equation to relate absolute integrals at 3.2 ppm to MeOH volume % was formed as follows,

Equation 1:
$$\text{Absolute integral at 3.2 ppm} = 21143 \times \text{MeOH volume \%}$$

This equation can easily be rearranged to give MeOH volume % as follows

Equation 2:
$$\text{MeOH volume \%} = \frac{\text{Absolute integral at 3.2 ppm}}{21143}$$

Small-Scale Solvent exchange of PDMAm₅₀-b-PDAAm₃₀₀ block copolymers and POPC lipid under physiological salt and pH

The PDMAm₅₀-b-PDAAm₃₀₀ block copolymers synthesised previously via PISA were lyophilised to remove water. The dried block copolymers (1 mg) was dissolved in 1 mL of methanol. POPC lipid was dried under vacuum to remove

chloroform. The POPC lipid was dissolved in Methanol to give a stock solution with the concentration of 1 mg/ml. The POPC and PDMAm₅₀-b-PDAAm₃₀₀ block copolymer stock solutions were combined in various ratios (25:75, 50:50 and 75:25) and allowed to mix for 5 min. The aqueous phase was prepared by adjusting the pH to 7.5 using KOH and HCL. Depending on the aqueous conditions employed, Sodium Chloride (140 mM) was added to the aqueous environment before the solvent exchange process began. A water: methanol volume ratio of 50:50 was used throughout all the solvent exchange experiments during this chapter. The block copolymer-POPC-methanol solutions were added to the aqueous environment and allowed to stir at 1000 RPM for 5 min before characterisation via DLS and TEM. The methanol was then removed from the mixture by rotary evaporation for 30 min at 150 RPM. The resultant polymer-lipid solution was then characterised via DLS and TEM.

5.1.3. Solvent exchange (SE) of PDMAm₅₀-b-PDAAm₃₀₀ under physiological salt and pH conditions

Solvent exchange was tried under the same conditions as in chapter 4, but the pH of the aqueous phase was increased to pH 7.5 before self-assembly. Before evaporation, after 5 min of mixing, there seems to be self-assembly as the solution became slightly turbid under light. DLS data for the solvent exchange of PDMAm₅₀-b-PDAAm₃₀₀ with 50% methanol with rapid addition at pH 7.5 is presented in Figure 5.2. Prior to evaporation of the solvent, (t_0), DLS indicated the presence of a single population of particles with an average hydrodynamic diameter of 104 nm. The size increased to 280 nm after 30 minutes of evaporation, but remained as a single population, as judged by the single unimodal peak. TEM micrographs (Figure 5.3) obtained for these samples indicate the presence of short worms/ cylindrical micelles. This is contrary to experiments conducted at pH 2.5, where polymersomes were observed. Such behaviour can be explained by the fact that at pH 7.5, the carboxylic acid groups are ionised, with the resulting charge repulsion favouring a higher curvature at the aggregate interface. An extreme demonstration of this was reported by Lovett *et al*, where only spheres could be produced by PISA for an ionised macro-CTA.²

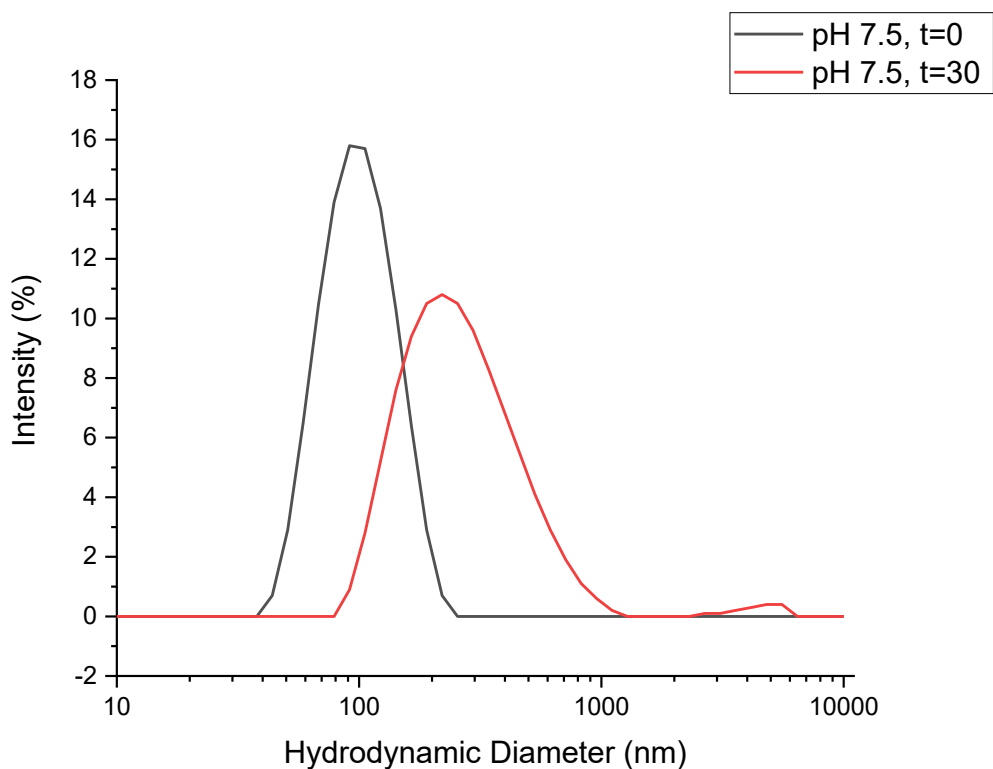


Figure 5.2. Particle size distribution for SE of PDMA₅₀-b-PDAAm₃₀₀ block copolymers at pH 7.5 before and after evaporation for 30 min.

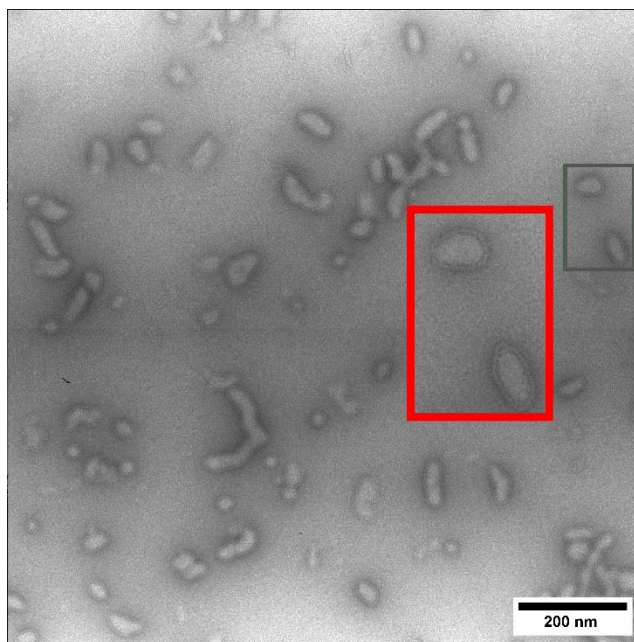


Figure 5.3. TEM micrographs for SE of PDMA₅₀-b-PDAAm₃₀₀ block copolymers at pH 7.5 after evaporation for 30 min. Additional images presented in the appendix (Figures 7.85-7.91)

To remedy this, and potentially unlock higher-order morphologies at pH 7.5, an analogous solvent exchange experiment was conducted at pH 7.5 in the presence of 140 mM NaCl. The salt is capable of screening the charge on the polymer, preventing repulsion.

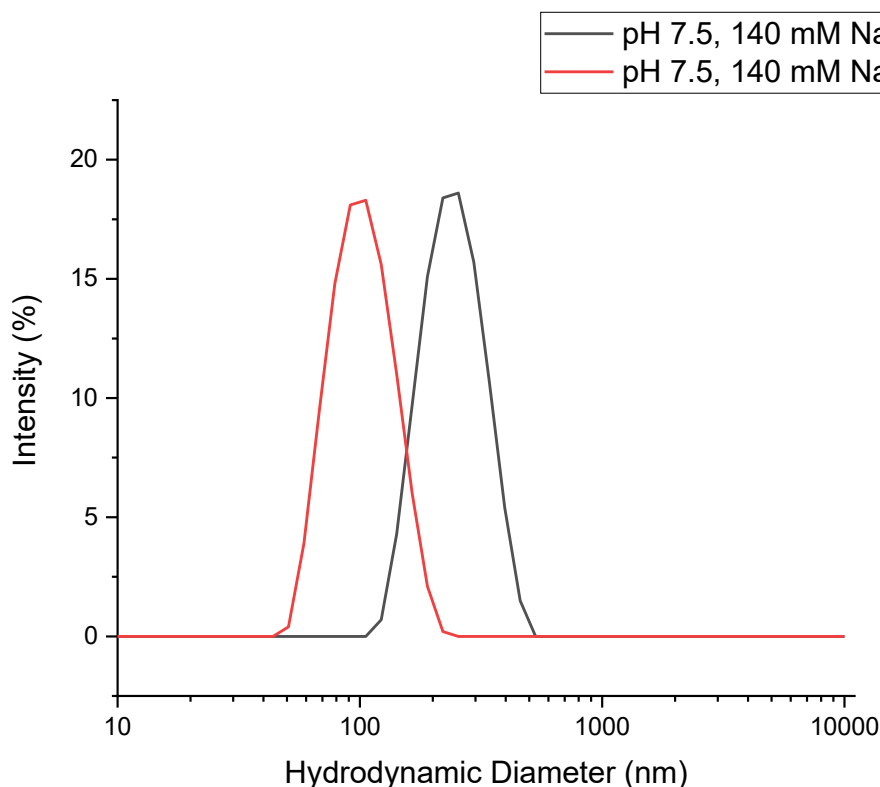


Figure 5.4. Particle size distribution for SE of PDMAm₅₀-b-PDAAm₃₀₀ block copolymers at pH 7.5 with 140 mM NaCl before and after evaporation for 30 min.

A monomodal particle size distribution with a corresponding hydrodynamic diameter of 261 nm was obtained by DLS for the sample before evaporation (Figure 5.4), which is larger than that observed in the absence of salt. After 30 min of evaporation, there is a shift to smaller-sized nano-objects, with a single monomodal peak with an intensity max of 100 nm. This is the opposite trend being seen previously without electrolytes added. To gain more insight into how the polymer nano-objects change morphology during methanol removal, TEM was employed on the post-evaporation sample (Figure 5.5).

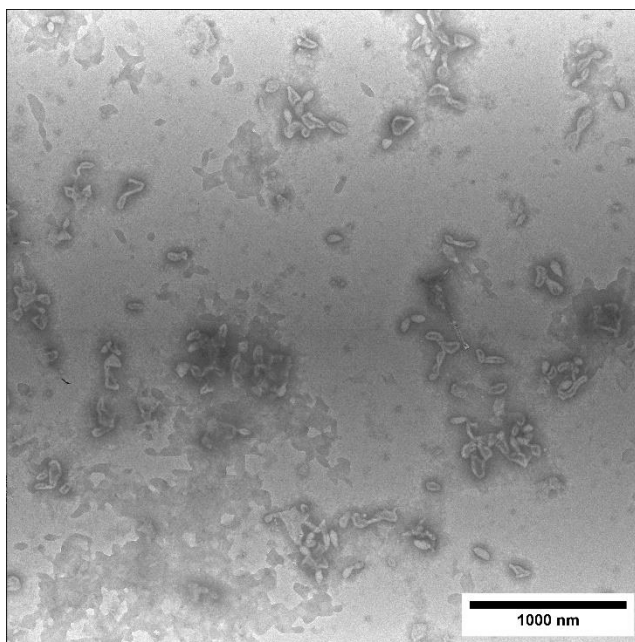


Figure 5.5. TEM micrographs for SE of PDMAm₅₀-b-PDAAm₃₀₀ block copolymers at pH 7.5 with 140 mM NaCl after evaporation for 30 min. Additional images presented in the appendix (Figures 7.92-7.99)

From the TEM micrographs for the SE of PDMAm₅₀-b-PDAAm₃₀₀ at pH 7.5 with added 140 mM NaCl, a range of ill-defined nano-objects with sizes between 100-200 nm is observed. Interestingly, there seems to be evidence of membranes suggesting a polymersome-like morphology, which undergo deformation during TEM sample preparation as seen by Dorsman and co-workers.³ This may be due to water rushing out of the core of the polymersome to solvate the electrolytes on the outer membranes. These particles are considerably different from the short worms obtained at pH 7.4 in the absence of NaCl. This does indeed suggest the salt plays a role in changing the final morphology from worms to membrane-bound nano-objects/polymersomes.

5.1.4. Time-resolved morphological evolution during methanol evaporation.

To better understand the phenomena occurring during methanol evaporation throughout this thesis, aliquots were taken from a scaled-up batch of self-

assembled particles during methanol evaporation. These were analysed by DLS and TEM to determine particle characteristics.

The overall SE process was increased from 2 mL to 10 mL to facilitate aliquot removal during evaporation. To facilitate the removal of the additional methanol, the evaporation time was increased from 30 min to 3 hours. Aliquots were taken at 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, and 180 min. to understand the effects of charge and ionic strength on self-assembly. The ionic strength of the solution and pH were altered towards biological conditions (pH 7.5, 500 mM NaCl).

5.1.5. Synthesis of New Singly Charged (SC) PDMAm₅₀-b-PDAAm₃₀₀ via RAFT-PISA

Also, a new block copolymer was synthesised with one terminal carboxylic acid instead of two on the RAFT agent, Figure 5.6

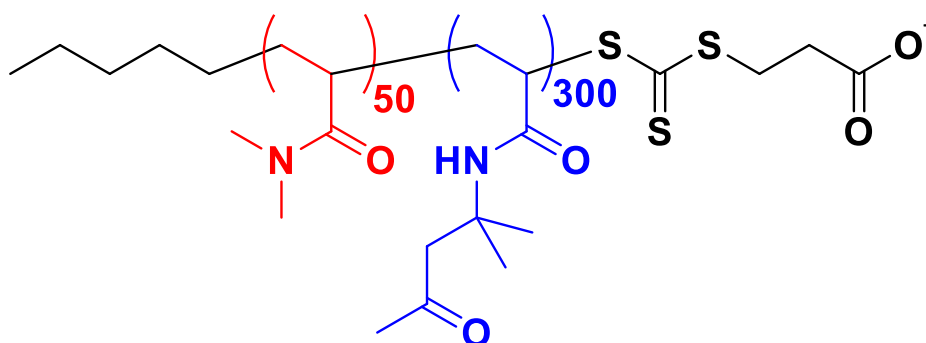


Figure 5.6: Chemical structure of singly charged PDMAm₅₀-b-PDAAm₃₀₀

Table 5.1. Summary of characterisation data (¹H NMR, GPC, DLS) for PISA nano-objects made at pH 2.5, SC Singly Charged, DC=Doubly charged

Sample	Monomer conversion via ¹ H NMR (%)	Mn (mol ⁻¹) via GPC	(Kg via	Mw/Mn Via GPC	Z-average diameter (nm) via DLS	PDI DLS	via
SC PDMAm ₅₀ -b-PDAAm ₃₀₀	>98%	47.7		1.17	324	0.130	
DC PDMAm ₅₀ -b-PDAAm ₃₀₀	>98%	61.3		1.14	369	0.124	

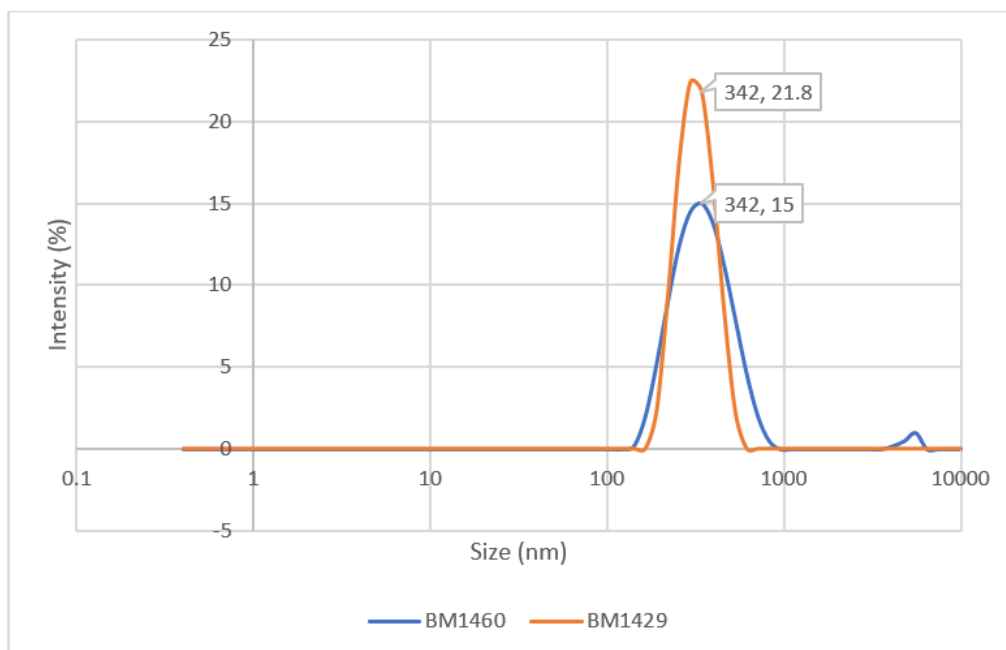


Figure 5.7: DLS particle size distributions for Singly Charged (BM1460) and Doubly Charged (BM1429) PDMAM₅₀-b-PDAAm₃₀₀ block copolymers made via RAFT-mediated PISA at pH 2.5.

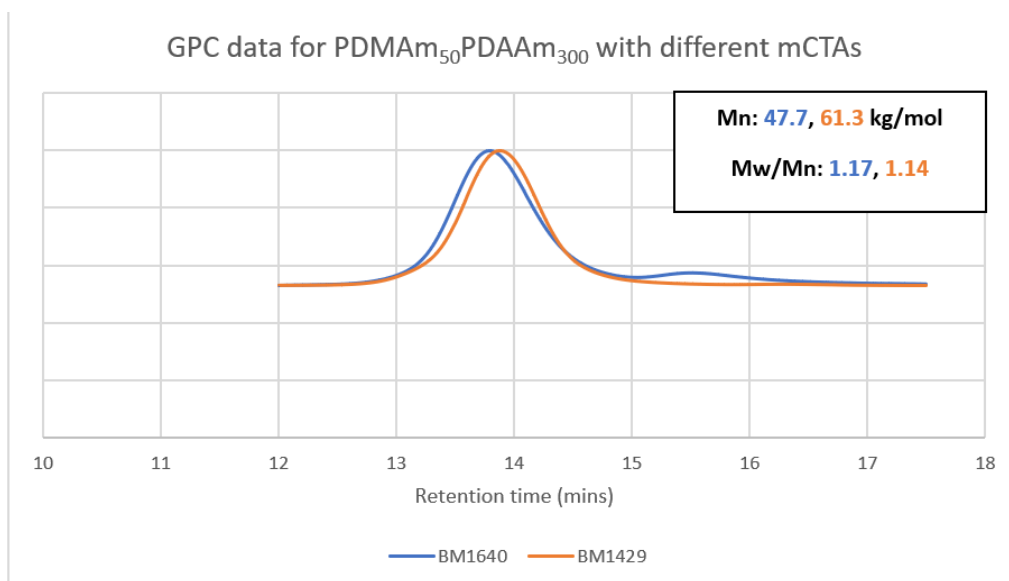


Figure 5.8. GPC Chromatograms for Singly Charged (BM1640) and Doubly Charged (BM1429) PDMAM₅₀-b-PDAAm₃₀₀ block copolymers made via RAFT-mediated PISA at pH 2.5.

This singly charged PDMAM₅₀-b-PDAAm₃₀₀ block copolymer was synthesised by RAFT-mediated PISA at pH 2.5, similar to the protocol used to make the doubly charged polymer. The monomer conversion during the polymerisation was greater than 98% but GPC indicates a discrepancy in molecular weight

between the doubly and singly charged polymer M_n , with the latter being slightly lower at 47.7 kg mol^{-1} as opposed to 61.3 kg mol^{-1} for the doubly charged (DC) polymer. The dispersity of the single COOH is comparable at 1.17. The GPC chromatograms in Figure 7 show clear overlap in the retention times for both the DC polymer and its SC polymer, with the doubly charged polymer being slightly larger, as confirmed by the longer retention times. The DLS particle size reported for both polymer nano-object dispersions was 342 nm, with broadly overlapping size distributions. On the basis of these observations, it was assumed that the SC polymer displays similar self-assembly behaviour to the DC polymer, producing pure vesicular morphologies at pH 2.5

In this study, zeta potential measurements were attempted in order to assess how the number of terminal carboxylic acid groups and hence charge on the polymer is affected across a range of pH values. In doing so, problems with the experimental procedure were experienced. Upon measurement of the PDMAm₅₀-b-PDAAm_y block copolymer solutions, there was a black aggregate that accumulated on the electrodes of the zeta potential cell. This study would be recommended for future work in this field as it would provide key information about the block copolymers and how the charge changes in response to varying pH.

5.1.6. NMR Methanol concentration study

During the SE process, the absolute volume of MeOH remaining is a factor to be monitored. This is rarely reported in the literature, but using a benchtop NMR machine, the MeOH concentration can be monitored during the 3-hour SE process. This is done by integrating and using the absolute integrals at 3.2 ppm, which is a singlet peak corresponding to MeOH protons. Using this method, a MeOH volume% % vs Absolute integral at 3.2 ppm calibration curve was developed, Figure 5.1.

Using the calibration curve, it is now possible to monitor the MeOH concentration during SE evaporations. This is useful to allow determination of residual MeOH remaining after the SE process and to help understand the effect that MeOH evaporation has on the size, shape, and morphology of the nano-objects prepared via SE. The NMR study of residual MeOH during the SE process is presented below in Figure 9, along with a blank evaporation study with no block copolymer involved during the evaporation.

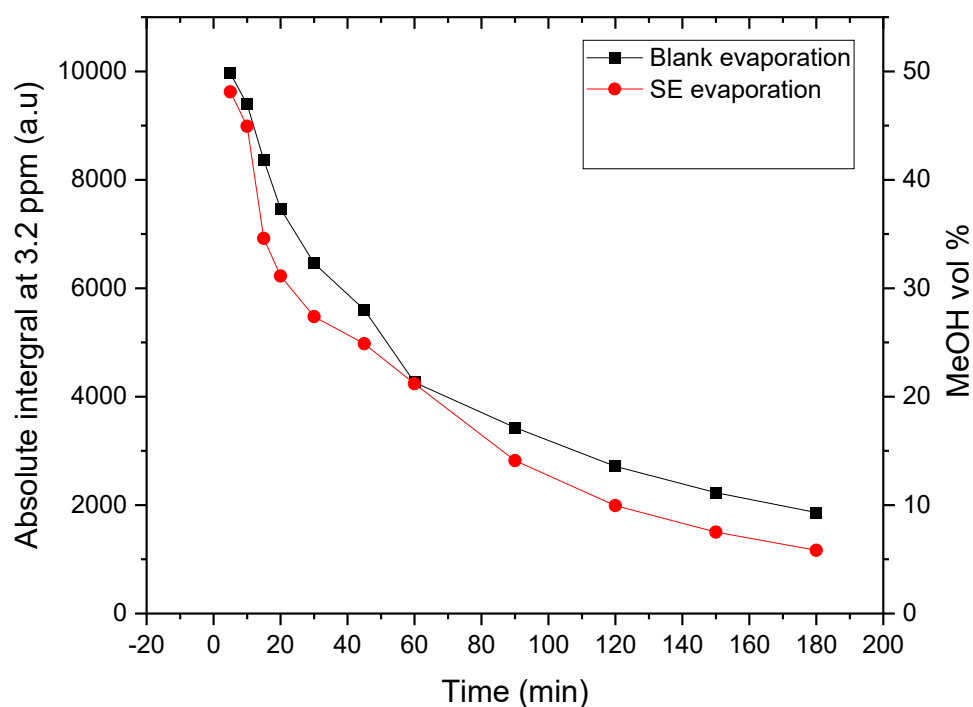


Figure 5.9: Absolute integral at 3.2 ppm and corresponding MeOH vol %s as a function of time spent on the rotary evaporator.

From the NMR data in Figure 5.9, it is evident that with increased time spent on the rotary evaporator, the total MeOH volume% decreases. This decrease is most evident between 0 and 20 min, with a very steep gradient across these time points. From 60 min onwards, there is a less steep gradient with MeOH evaporating more slowly across these time points. Interestingly, the final volume% of MeOH in both the blank study and the SE evaporation does not reach zero after 3 hours. This suggests that not all the MeOH has been removed after 3 hours of evaporation. After 3 hours, the residual methanol left in the polymer SE mixture is 5.5 vol% and 8.8 vol% in the blank MeOH evaporation study. Since methanol and water do not form an azeotropic mixture, it follows that methanol may be held in the core of the nano-objects, preventing it from fully evaporating after 180 min. Interestingly, there is still 8.8 vol% of MeOH left after the blank evaporation without the block copolymer. This suggests that perhaps longer times of evaporation are needed to fully evaporate all of the methanol in the SE system.

5.1.7. 3-hour SE evaporation of PDMAm₅₀-b-PDAAm₃₀₀ under various pH and ionic strengths

The size of the nano-objects in each aliquot was measured via DLS for the SE of PDMAm₅₀-b-PDAAm₃₀₀ with a final methanol composition of 50% and presented in figure 5.10, TEM micrographs of key aliquots were taken and presented in figure 5.11, 5.12 and 5.13 for the different conditions employed (DC at pH 2.5, DC at pH 7.5 and SC at pH 7.5 respectively).

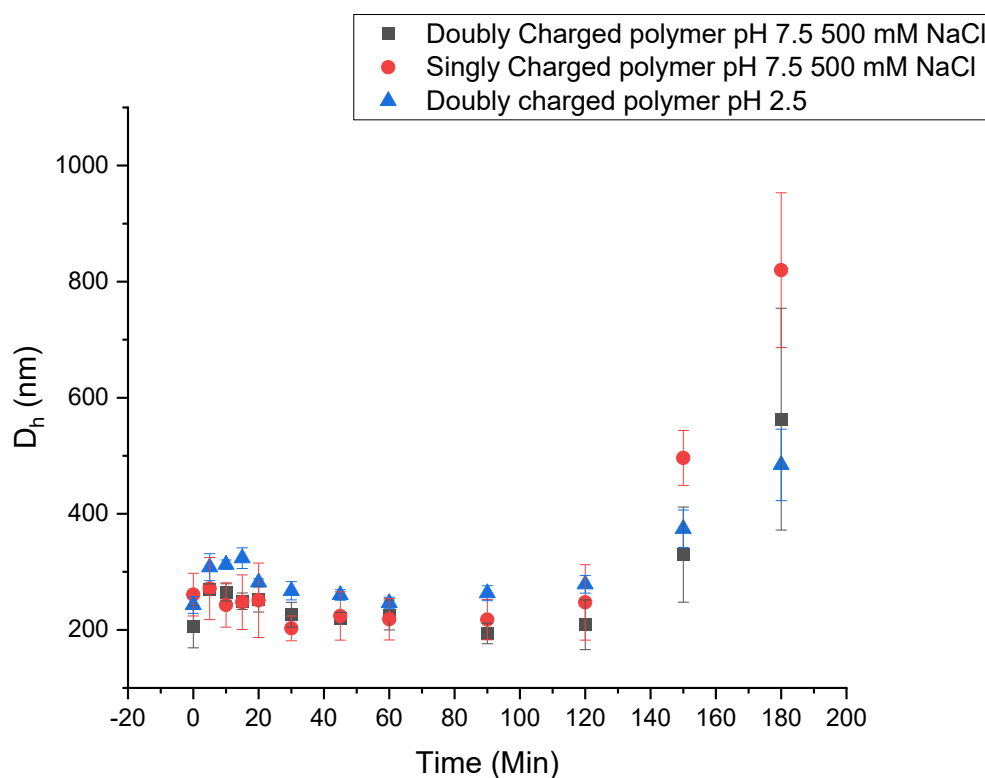


Figure 5.10. Change in hydrodynamic radius of nano-objects made via SE as a function of time spent on the rotary evaporator.

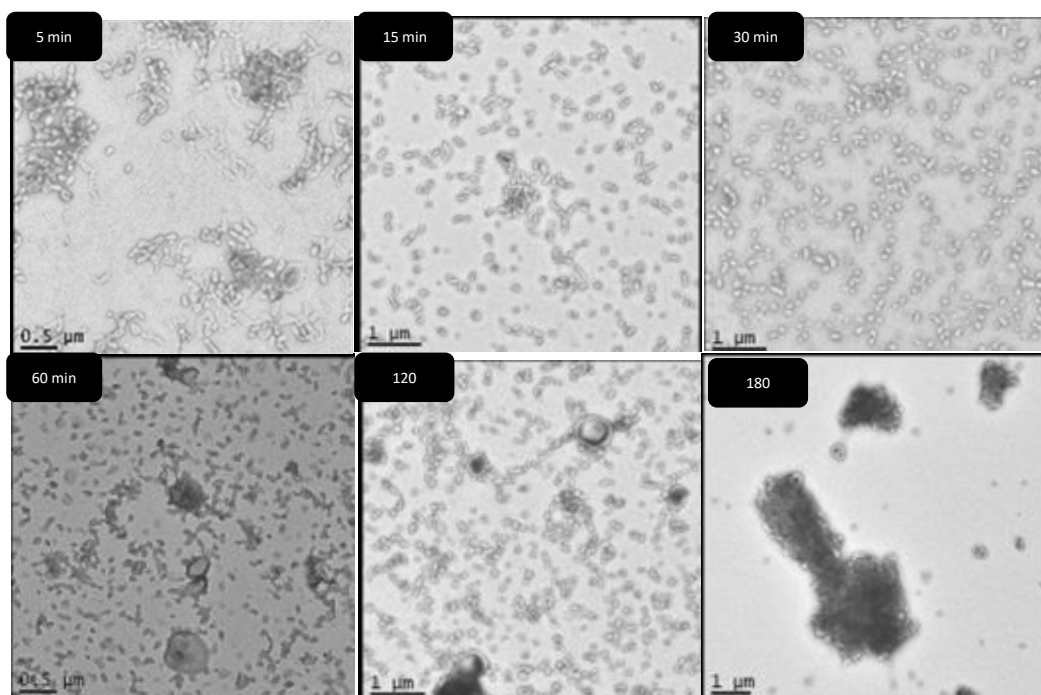


Figure 5.11. TEM micrographs for the SE of PDMA₅₀-b-PDAA₃₀₀ at 1 mg/ml, 50% methanol, pH 2.5, after evaporation under reduced pressure for x min. Additional images presented in the appendix (Figures 7.100-7.147)

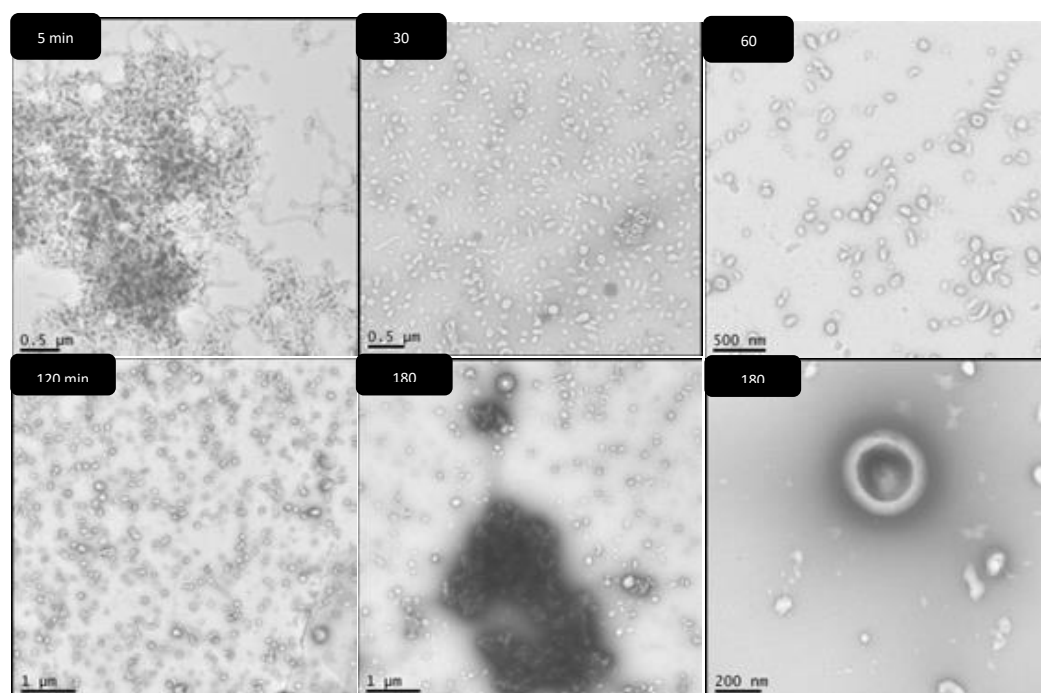


Figure 5.12. TEM micrographs for the SE of Doubly charged PDMA₅₀-b-PDAA₃₀₀ at 1 mg/ml, 50% methanol, pH 7.5, with 500 mM NaCl after evaporation under reduced pressure for x min. Additional images presented in the appendix (Figures 7.148-7.197)

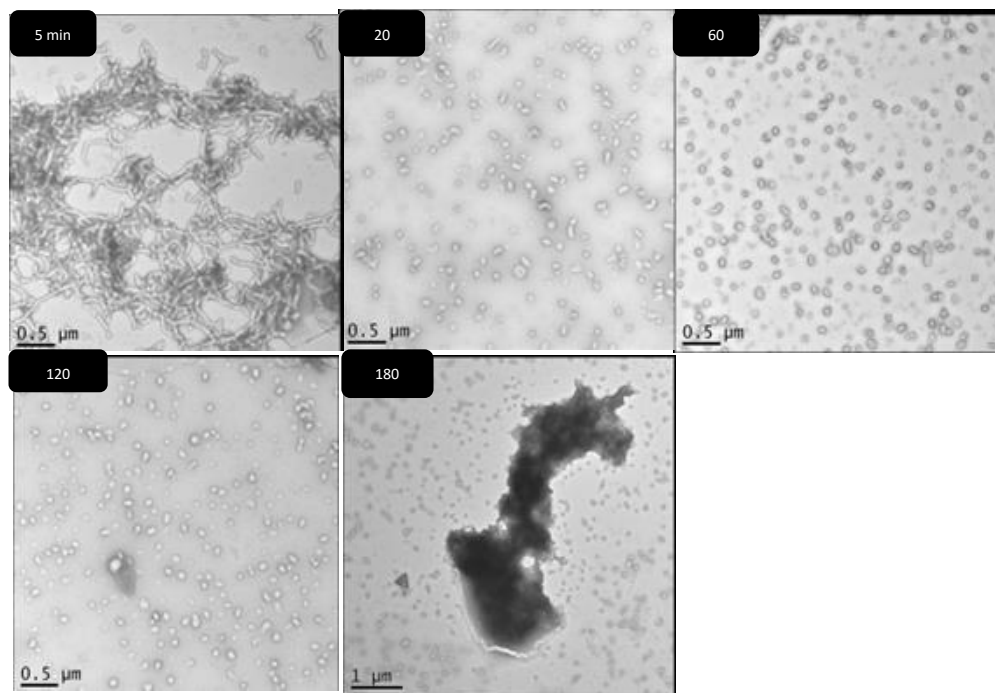


Figure 5.13. TEM micrographs for the SE of singly charged PDMA₅₀-b-PDAAm₃₀₀ at 1 mg/ml, 50% methanol, pH 7.5, with 500 mM NaCl after evaporation under reduced pressure for x min. Additional images presented in the appendix (Figures 7.198 -7.237)

From the DLS data in Figure 5.10, it is clear that there is a common trend in the data. Initially, from time points 0 to 20 min, there is a small increase and decrease in the size of the polymer nano-objects as methanol is removed. This may be due to the nano-objects swelling as the methanol is removed from the core of the polymer nano-objects. As methanol continues to be removed, this causes the nano-objects to swell to sizes that plateau at around 30 min. Sizes remain constant up until 120 min, where there is a sharp increase in the size of the polymer nano-objects, which could suggest that there is aggregation occurring at these time points. This was theorised as there was physical evidence of the polymer crashing out of solution visible to the naked eye during the evaporation. The mechanism of aggregation may be a concatenation of spherically trapped micelles.

From the DLS particle size distributions in Figure 5.10, for the DC polymer at pH 2.5, initially, at t=0, the nano-objects have an average size of 240 nm. After 5 min of evaporation under reduced pressure, there is a sharp increase in the size of the nano-objects to 310 nm, TEM, in Figure 5.11, indicates the presence of short worms or cylindrical micelles. It seems that the short worms have clustered, forming a spaghetti-like structure seen previously in chapter 2, for the SE of PDMA₅₀-b-PDAAm₃₀₀ after 5 min of mixing. Both other conditions,

including the SC and DC polymer at pH 7.5 with 500 mM NaCl, show similar behaviour at $t=5$ min of evaporation. Both conditions from figures 12 and 13 show a highly clustered worm structure after 5 min on the rotary evaporator.

After 5 min of evaporation, an increase in the size of the nano-objects is observed by DLS for all three conditions. For the DC polymer at pH 2.5, there is a local maximum at $t=15$ min, with this sample containing nano-objects with an average size of 325 nm. From the corresponding TEM micrograph in Figure 9, it is evident that the dominant morphology of the nano-objects is spherical micelles. These micelles are not completely symmetrical and often combine with other micelles to form dimers and short worms. The DC and SC polymer at pH 7.5 with 500 mM NaCl at $t=15$ min has an average size of 250 nm, which suggests, based on the DC polymer, that these conditions also favour micelles, as the average size of the particles is smaller.

This is confirmed by looking at the next TEM time point, $t=20$ min, for the SC polymer at pH 7.5 with 500 mM NaCl. This time point from Figure 5.13 shows clear evidence of spherical micelles being the dominant morphology. These spherical micelles are mostly symmetrical but often cluster together to form dimers and trimers (short worms).

The next time point studied via TEM was $t=30$ min, which corresponded with a decrease in the average size of the nano-objects to 270 nm for the DC polymer at pH 2.5. The TEM for this time point showed that most of the nano-objects form spherical micelles. These micelles are oval-shaped with a higher aspect ratio, possibly due to methanol swelling and dimerisation. For the DC polymer at pH 7.5 with 500 mM NaCl, the TEM in Figure 10 shows that for this time point, the dominant morphology is spherical micelles. These micelles are also prone to dimerisation and formation of short worms, which is evident from the TEM micrograph in Figure 10. The DLS size data in Figure 10 shows that the average size of the particles at $t=30$ is fairly consistent between 230-270 nm for all conditions employed, suggesting these nano-particles are of similar size 30 min after evaporation, regardless of charge or pH of the polymer employed. The SC polymer also behaves similarly at pH 7.5 with 500 mM NaCl. From the DLS in Figure 5.10, the average size of the nano-objects made via SE for the SC polymer at pH 7.5 is 200 nm. This is slightly smaller than the other conditions employed with the DC polymer at pH 2.5 and pH 7.5 with 500 mM NaCl. Based on this, TEM was not employed as it was assumed that these polymer nano-

objects remained as spherical micelles, consistent with the other conditions employed.

From Figure 5.10, the average size of the nano-objects decreased linearly from $t=30$ to $t=60$ min, where there is a local minimum in the size of the PDMAm₅₀-b-PDAAm₃₀₀ block copolymer nano-objects during SE solvent removal.

After 60 min of rotary evaporation, the TEM micrograph for the DC polymer at pH 2.5 in Figure 5.12 shows a more exotic range of nano-objects. The dominant morphology still seems to be spherical micelles; however, there seems to be evidence of very few polymersomes being formed on the grid. There seem to be key areas where there is a membrane-like darkness around the outside of the polymersome. Compared to the other conditions, the DC polymer at pH 2.5 is similar in average size of nano-object made via SE at $t=60$ min. The average size of the DC polymer at pH 7.5 with 500 mM NaCl and the SC polymer under the same conditions remains consistent between 215-225 nm, suggesting the polymer nano-objects are of similar size at this time point, regardless of charge or ionic strength. From the TEM micrographs for the DC polymer at pH 7.5 with 500 mM NaCl, a dominant morphology of spherical micelles is observed. These spherical micelles are oval-shaped with high aspect ratios, and there is some evidence of intermediate structures, such as dimers and membrane-bound nano-objects, with central darkening due to the uranyl acetate stain. A similar case is experienced for the SC polymer at pH 7.5 with 500 mM NaCl. From figure 5.13, at $t=60$ min, the dominant morphology is spherical micelles, with evidence of methanol swelling and asymmetrical tendencies due to dimerisation.

The next time point studied was $t=120$ min, which corresponded with an average size of 275 nm, larger than the $t=60$ min sample with an average size of 250 nm for the DC polymer at pH 2.5, Figure 5.10. The average size of the SC polymer at pH 7.5 with 500 mM NaCl also increases to 250 nm, suggesting there is an increase in the size of nano-objects as evaporation continues from 60 to 120 min. The DC polymer at pH 7.5 with 500 mM NaCl also shows a decrease from 225 to 210 nm when the evaporation is continued from 60 to 120 min. More light can be shed by looking at the corresponding TEM images for this time point under differing charge and ionic strengths during this SE evaporation. From Figure 5.11, it is evident that the dominant morphology for the DC polymer at pH 2.5 is spherical micelles. However, there are a few

examples of vesicle intermediates on the grid. These intermediates are larger than the general micelle population, with sizes approximately 500 nm-1000nm, suggesting vesicle formation. However, the grid is still dominated by spherical micelles, suggesting that vesicle formation is a rarity at this time point. The TEM micrographs in Figure 5.12 show that for the DC polymer at pH 7.5 with 500 mM NaCl, most of the polymer nano-objects remain as spherical micelles, with some evidence of larger spherical vesicle-sized objects occupying some of the grid. This result shows similarity to the previous conditions at pH 2.5, suggesting that pH and ionic strength of the buffer solution play no role in the size and morphology of the nano-objects produced at this time point. The same can be said for the SC polymer at pH 7.5 with 500 mM NaCl, with the TEM micrograph in Figure 5.13 showing that spherical micelles are the dominant morphology, with evidence of one vesicle-like intermediate on the grid, highlighted by the darker membrane due to the uranyl acetate stain.

For pH 2.5 conditions, the TEM micrograph for this time point, $t=120$ min, in Figure 5.10 shows close similarity to the time point $t=60$ min. The dominant morphology is spherical micelles. These micelles seem to be more clustered than those presented in the $t=30$ and $t=60$ samples. Amidst the spherical micelles, there are a few polymersomes on the grid, with clear membranes highlighted by the additional outer dark stain from the uranyl acetate dye.

The last time point studied was $t=180$ min, which, for the DC polymer at pH 2.5, corresponded to the largest sized particles with an average size of 480 nm, Figure 5.10. During this experiment, signs of polymer aggregation were visible in the sample by eye. Nonetheless, TEM was employed on this sample to see what the larger-sized particles looked like in terms of morphology and to confirm the size of the particles. From the TEM micrograph in Figure 5.11, a large body of aggregated polymer nano-objects in the micron size range is observed. The largest aggregate spans a size range of around 10-15 μm . The uranyl acetate stain seems to be incorporated in the aggregate, as the aggregate looks dark to the eye. Also, on the grid, there are a couple of smaller membrane-bound polymersomes. These polymersomes are somewhat symmetrical with a clear indication of membranes, suggesting vesicle formation is competing with the aggregation of spherical nano-objects.

Aggregation is also present in both the DC polymer and the SC polymer at pH 7.5 with 500 mM NaCl. From the DLS data in Figure 5.10, the average sizes of

the nano-objects are considerably larger than previous time points studied, with averages of 560 and 820 nm for the DC and SC polymers at $t=180$ min, respectively. For the DC polymer at pH 7.5 with 500 mM NaCl, the TEM micrographs in Figure 5.12 show that aggregation is present, as there is a large micron-sized aggregate on the grid. As well as this, there are some spherical micelles still present on the grid. Also, there is evidence of vesicles on the grid; these vesicles are highly symmetrical, with thick membranes of around 40 nm in thickness. This confirms that aggregation of spherical nano-objects into large micron-sized aggregates is occurring at the same time as polymersome formation, similar to the previous condition for this DC polymer at pH 2.5. For the final condition, the SC polymer at pH 7.5 with 500 mM NaCl, the TEM micrograph in Figure 5.13 shows a very large, aggregated structure on the grid. This aggregate takes up most of the grid and is many microns in size, a clear indication of the aggregation of spherical micelles on the grid. Also on the grid is a population of spherical micelles, which are somewhat more symmetrical than those seen previously. This result, as well as the previous TEM micrograph in Figure 5.12 for the same time point, shows that after evaporation for 180 min, the SE evaporation leaves a mixed morphology dominated by spherical micelles and their micron-sized aggregates and a few rare vesicle morphologies.

The changes seen here can be attributed to a change in the amount of residual methanol, which in turn changes the amount of methanol-induced swelling of the nanoparticles. Swelling and dimerisation can be linked to the reason cylindrical micelles are observed at high methanol contents, but as this methanol is reduced, this causes a breakup of worms into kinetically trapped micelles, which aggregate at low methanol volumes.

5.1.8. Solvent Exchange of PDMAm₅₀-b-PDAAm₃₀₀ using POPC as a membrane directing agent

The SE experiment was altered to incorporate a smaller amphiphile, namely 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), Figure 5.14. This lipid routinely forms membranes, and its incorporation into the PDMAm₅₀-b-PDAAm₃₀₀ SE experiments might promote self-assembly of the block copolymer into membrane-bound nano-objects. POPC lipids generally form

liposomes easily as it is a diacylglycerol and phospholipid with a polar hydrophilic phosphate head group and hydrophobic fatty acid. The POPC lipid is a lot smaller than the PDMAm₅₀-b-PDAAm₃₀₀ block copolymer, with an average molecular weight of 760.1 g·mol⁻¹ compared to 62000 g mol⁻¹ for the PDMAm₅₀-b-PDAAm₃₀₀ block copolymer.

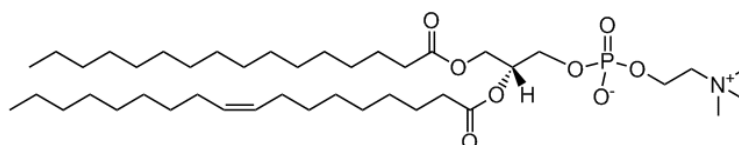


Figure 5.14: Chemical structure of the POPC lipid used in the SE experiments

In the following section of this chapter, the SE of PDMAm₅₀-b-PDAAm₃₀₀ with varying amounts of POPC lipid at pH 7.5 with 140 mM NaCl will be discussed. This will be discussed for both the DC and SC polymer, exploring the effect, if any, of the charge on the block copolymer during SE. Before doing so, the SE of neat POPC was studied, with DLS particle size distributions presented in Figure 5.15, alongside TEM micrographs presented in Figure 5.16.

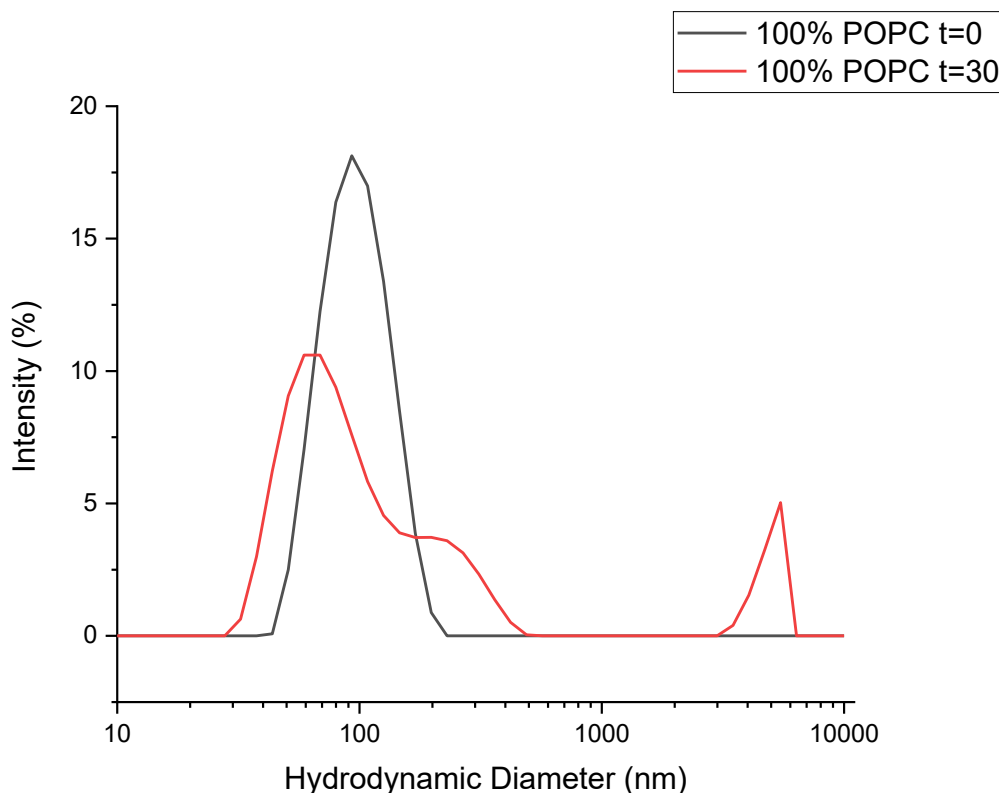


Figure 5.15: Particle size distributions for SE of POPC lipid. POPC lipid/methanol mixture added to water, measurements taken before and after Methanol evaporation for 30 min under rotary evaporation.

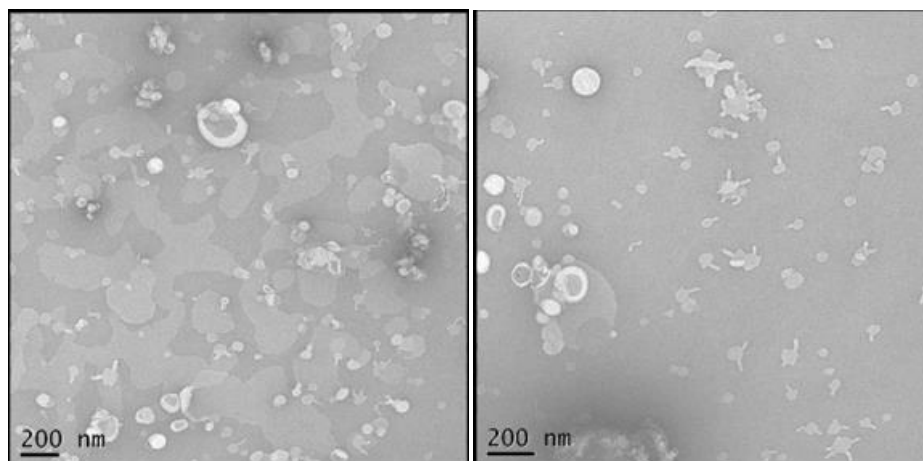


Figure 5.16: TEM micrographs for SE of POPC lipid after evaporation for 30 min. POPC lipid/methanol mixture added to water, measurements taken before and after Methanol evaporation for 30 min under rotary evaporation. Additional images presented in the appendix (Figures 7.238-7.252)

From Figure 5.15, the DLS particle size distribution for the SE of neat POPC at $t=0$ shows a clear monomodal peak centred at 100 nm. After evaporation for 30 min, there is a change in the size of the nano-objects. The particle size distribution becomes bimodal, with the main peak centred at 70 nm and a broad shoulder peak centred at 200 nm. This suggests two populations of nano-objects present when the POPC SE is evaporated for 30 min. More light can be shed by looking at the TEM micrographs in Figure 5.16 for the $t=30$ time point, which shows a mixture of morphologies. There seem to be liposomes present on the grid, characterised by their highly symmetrical spherical nature and membranes. These liposomes are typically smaller than the PDMA₅₀-b-PDAA₃₀₀ polymersomes, with sizes between 150-200 nm in diameter. Additionally, the stain seems to be lighter in colour than previous TEMs using PDMA₅₀-b-PDAA₃₀₀, suggesting the uranyl acetate staining TEM is slightly different in brightness/exposure than when using pure polymer samples. The second population on the TEM grid is a much smaller spherical particle with exotic shapes (potentially membrane tubes) protruding from the centres of the spheres. From this data, it can be concluded that POPC lipid on its own forms vesicle-dominant morphologies using SE protocols in this chapter.

To understand how these two different amphiphiles behave during SE, various mixtures of POPC and PDMA₅₀-b-PDAA₃₀₀ (DC and SC) were combined pre-SE and allowed to mix before evaporation for 30 min. The conditions

employed were pH 7.5 with 500 mM NaCl to allow for milder conditions of self-assembly in biological conditions. The following ratios (weight percentages)

were studied: 75% POPC 25% PDMAm₅₀-b-PDAAm₃₀₀, 50% POPC 50% PDMAm₅₀-b-PDAAm₃₀₀ and 25% POPC 75% PDMAm₅₀-b-PDAAm₃₀₀. The DLS particle size distributions and TEM micrographs for each ratio studied are presented below, Figures 6.17-6.20.

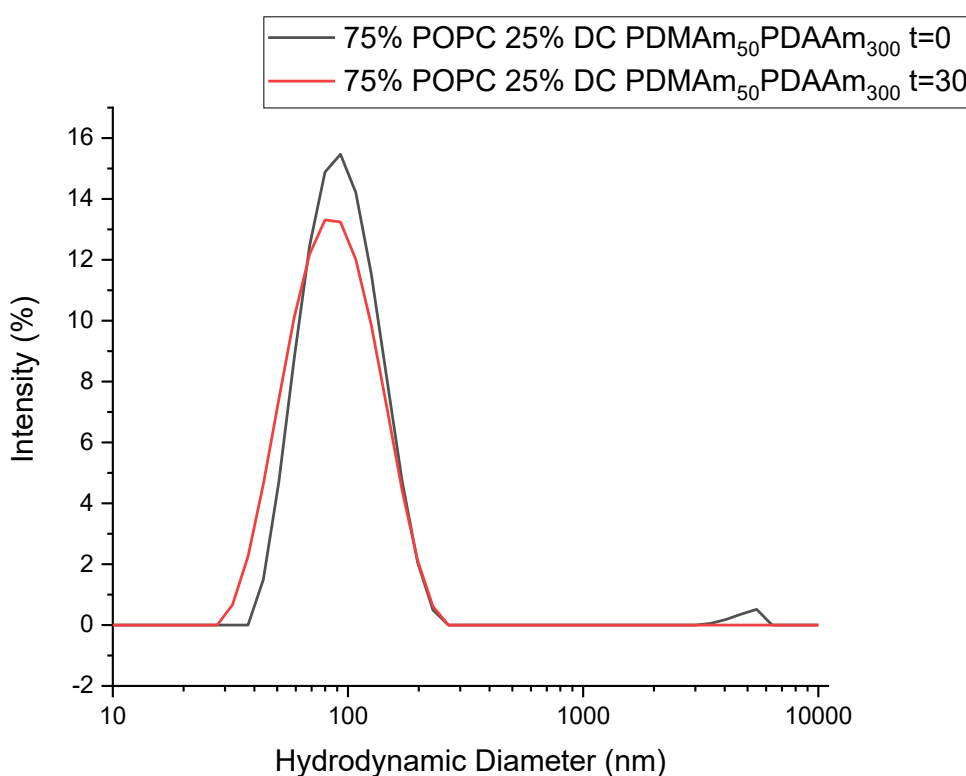


Figure 5.17. Particle size distributions for SE of POPC lipid- DC PDMAm₅₀-b-PDAAm₃₀₀ (75:25%) before and after evaporation for 30 min.

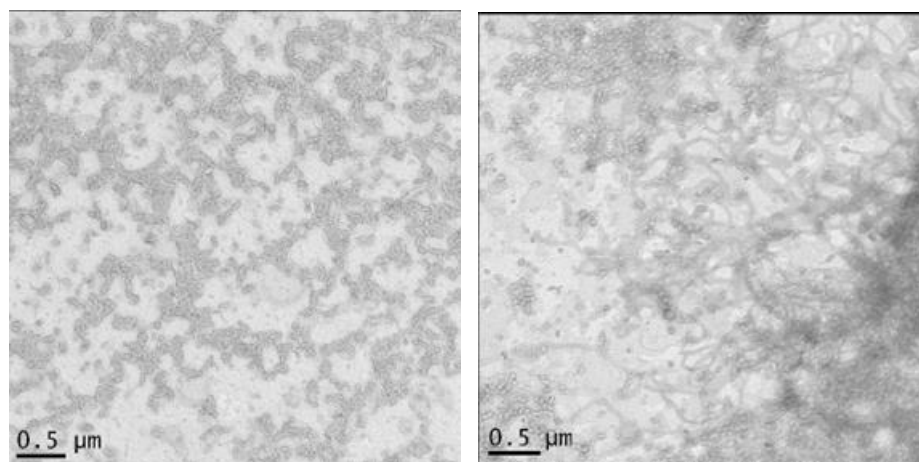


Figure 5.18. TEM micrographs for SE of POPC lipid- DC PDMAm₅₀-b-PDAAm₃₀₀ (75:25%) before (left) and after (right) evaporation for 30 min. Additional images presented in the appendix (Figures 7.253-7.267)

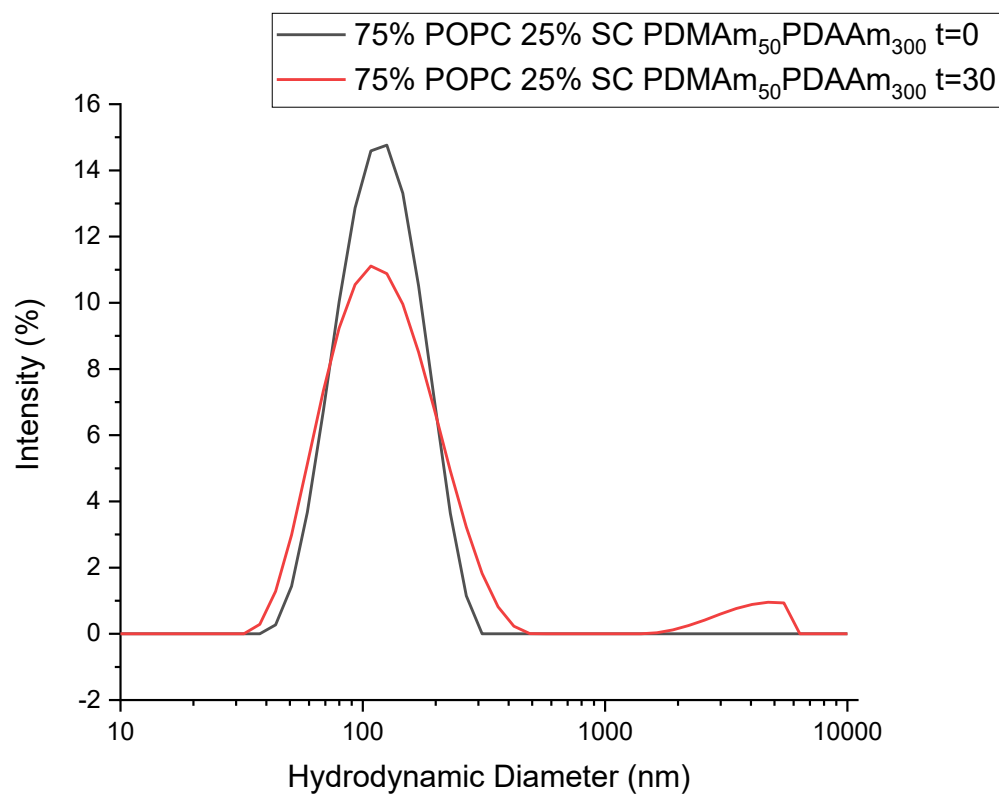


Figure 5.19. Particle size distributions for SE of POPC lipid- SC PDMAm₅₀-b-PDAAm₃₀₀ (75:25%) before and after evaporation for 30 min.

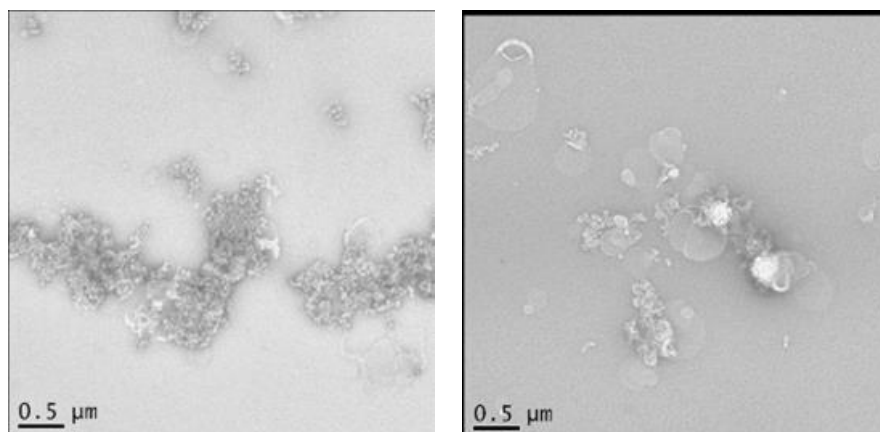


Figure 5.20. TEM micrographs for SE of POPC lipid- SC PDMAm₅₀-b-PDAAm₃₀₀ (75:25%) before (left) and after (right) evaporation for 30 min. Additional images presented in the appendix (Figures 7.268-7.279)

The DLS data in Figure 5.17 suggests that for the SE of 75% POPC 25% DC PDMAm₅₀-b-PDAAm₃₀₀ from nanosized morphologies. Both the t=0 and t=30 min samples have similar DLS particle size distributions with intensity max values centred on 100 nm. After 30 minutes of evaporation to remove methanol, the size of the nano-objects reduces slightly with a small shift to the left in the particle size distribution. More information can be found by looking at the corresponding TEM micrographs in Figure 5.18, which show that the majority of the nano-objects form short worms at t=0. These short worms and spherical micelles appear to be aggregated together in a larger matrix of nano-objects. However, after 30 minutes of evaporation, there is a slight change in the morphology of the nano-objects prepared with 75% POPC and 25% DC PDMAm₅₀-b-PDAAm₃₀₀, with most of the nano-objects forming spherical micelles exclusively. These spherical micelles cluster together to form a “grape-like” complex of spherical micelles bundled up together. There appear to be large areas on the bottom right of the TEM grid which show darker staining and aggregation of spherical micelles.

The second condition study was the analogous experiment with the SC PDMAm₅₀-b-PDAAm₃₀₀ block copolymer. From the DLS data in Figure 5.19, it is evident that there are clear monomodal peaks centred around 100 nm, similar to what was seen in the previous analogous experiment with the DC PDMAm₅₀-b-PDAAm₃₀₀ block copolymer under the same conditions. At t=0, when the block copolymer and the POPC lipid are mixing for 5 min prior to any evaporation, it can be seen that there is a clean monomodal peak with an

intensity max value of 100 nm. This is similar to the previous conditions with the SC PDMAm₅₀-b-PDAAm₃₀₀ polymer, which suggests the same phenomena of self-assembly are occurring at this time point. After 30 minutes of evaporation under reduced pressure, there is a slight change in the particle size distribution of the nano-objects prepared via SE. The t=30 min peak is slightly broader than the t=0 peak, suggesting that there is a wider size range of the nano-objects upon evaporation of methanol. There is also a second peak in the micron range, which suggests that perhaps there may have been dust contamination or aggregation during the DLS size experiment. More light can be shed by looking at the corresponding TEM micrographs for t=0 and t=30 min of the SE of this mixture. At t=0, the left image suggests that the nano-objects form a cluster of spherical nano-objects aggregated together on the grid. After 30 min of evaporation, there is a mixed population of nano-objects with lighter POPC regions forming vesicle-like intermediates clustered together with darker spherical aggregates of PDMAm₅₀-b-PDAAm₃₀₀ regions. This result suggests that there may be some separation between the POPC and PDMAm₅₀-b-PDAAm₃₀₀ regions on the grid, with the POPC lipid forming membrane-bound nano-objects.

5.1.9. 50% POPC 50% PDMAm₅₀-b-PDAAm₃₀₀

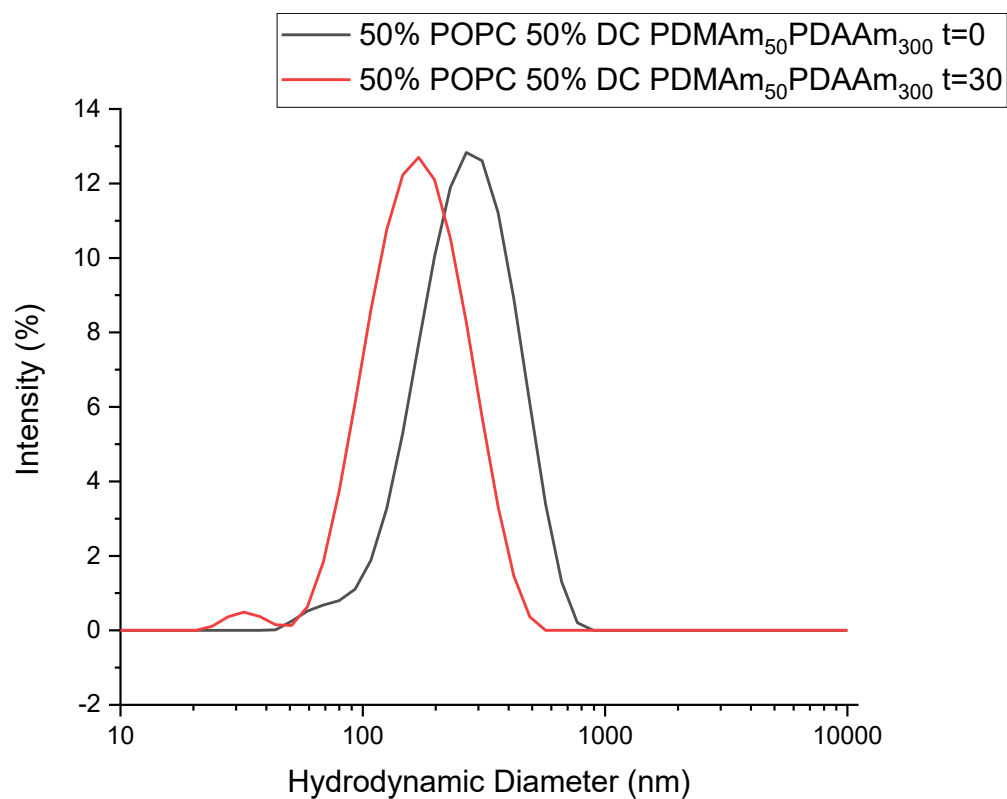


Figure 5.21. Particle size distributions for SE of POPC lipid- DC PDMAm₅₀-b-PDAAm₃₀₀ (50:50%) before and after evaporation for 30 min.

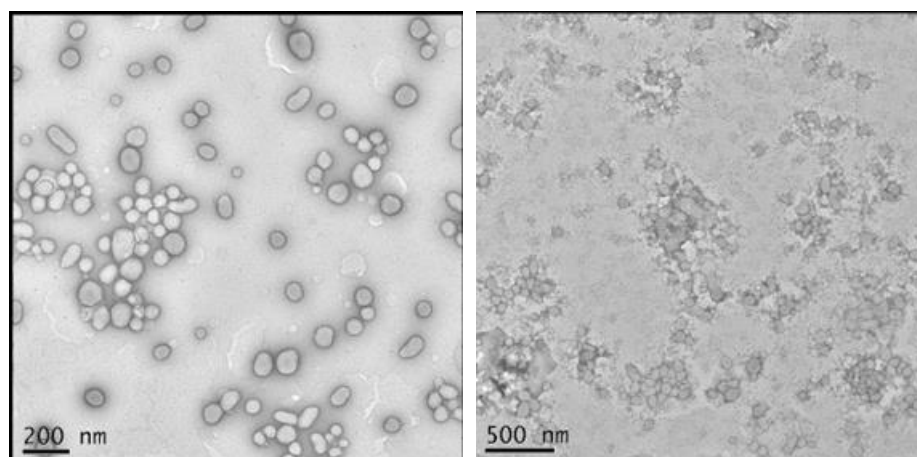


Figure 5.22. TEM micrographs for SE of POPC lipid- DC PDMAm₅₀-b-PDAAm₃₀₀ (50:50%) before (left) and after (right) evaporation for 30 min. Additional images presented in the appendix (Figures 7.280-7.294)

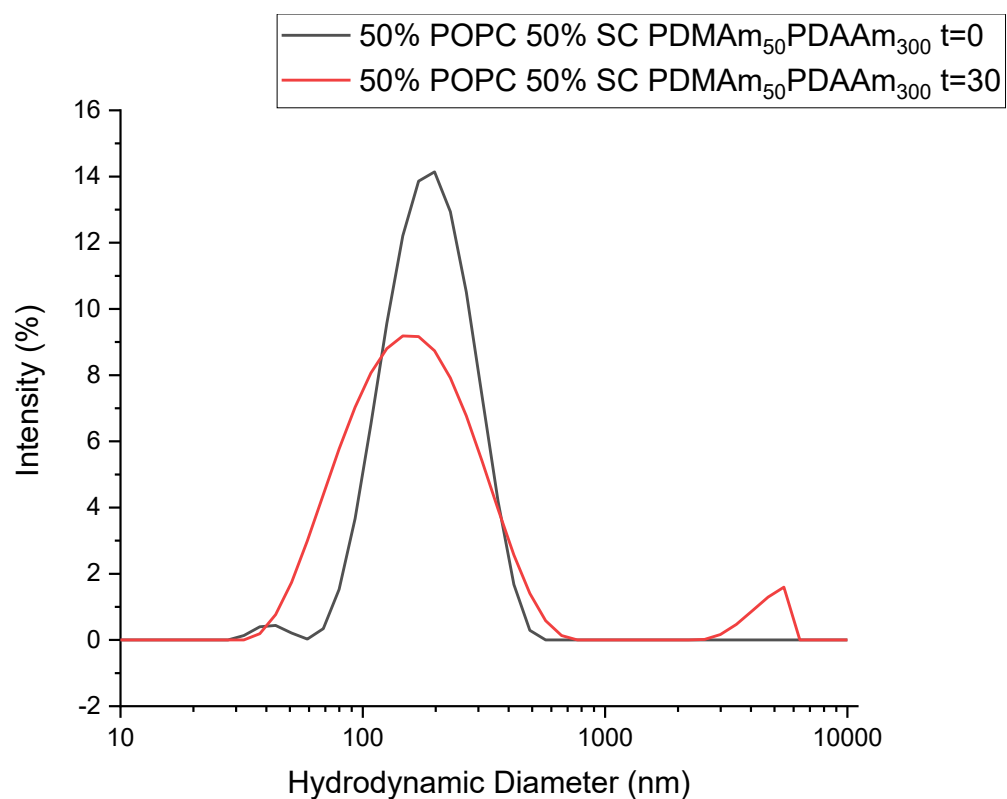


Figure 5.23. Particle size distributions for SE of POPC lipid- SC PDMA₅₀-b-PDAA₃₀₀ (50:50%) before and after evaporation for 30 min.

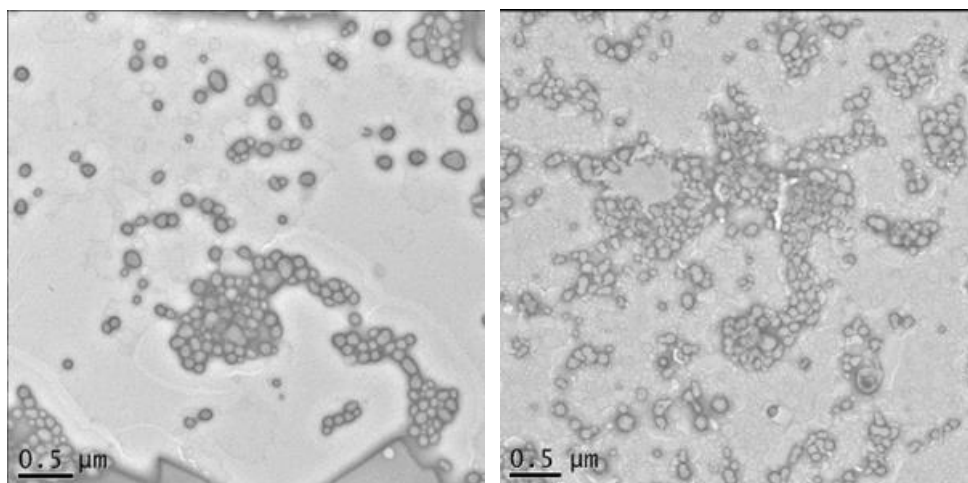


Figure 5.24. TEM micrographs for SE of POPC lipid- SC PDMA₅₀-b-PDAA₃₀₀ (50:50%) before (left) and after (right) evaporation for 30 min. Additional images presented in the appendix (Figures 7.295-7.309)

From the DLS data in Figure 5.21, it is evident that the SE under these 1:1 polymer lipid ratios produces clear monomodal peaks for the most part for the SE of DC PDMAm₅₀-b-PDAAm₃₀₀. At t=0, after 5 min of mixing the POPC lipid and DC PDMAm₅₀-b-PDAAm₃₀₀ block copolymer in methanol and water, the DLS shows a clear monomodal peak with an intensity max value of 300 nm. This is significantly larger than previous POPC-PDMAm₅₀-b-PDAAm₃₀₀ SE experiments, which suggests that larger nano-objects are being formed under these ratio conditions. After 30 min of evaporation at reduced pressure, there is a shift in the nanoparticle size distribution from the DLS. It is evident that once methanol is reduced, these particles become smaller in size according to the DLS data. The DLS shows a peak with an intensity max value of 150 nm, also there is a small shoulder peak centred at 30 nm, which may suggest a second smaller population of nano-objects. By looking at the TEM micrographs in Figure 5.22, it is evident that at t=0, there is a pure morphology of spherical nano-objects visualised on the grid. These spherical micelles are highly symmetrical and cluster together on the grid to form a 'grape-like' structure of clustered spherical micelles. After 30 min of evaporation at reduced pressure, the right-hand side image in Figure 5.22 shows that there is little to no change in the size or morphology of the spherical micelles seen. Highly symmetrical spherical micelles can be seen after 30 min, which also shows evidence of clustering on the grid.

The second condition studied at this ratio was the analogous SE experiment with the SC PDMAm₅₀-b-PDAAm₃₀₀ block copolymer. The DLS data in Figure 5.23 suggests that after 5 min of mixing at t=0, there is a clear monomodal peak in the nanoparticle size distribution with an intensity max value of 200 nm, slightly smaller than the previous SE experiment with the DC polymer. After 30 minutes of evaporation at reduced pressure, there is a shift to a smaller particle size distribution for the nano-objects prepared. At t=30 min, there is a clear monomodal peak with an intensity max value of 160nm. This reduction in size after methanol is removed agrees with previous data with the DC polymer. More light can be shed on the nature of the polymer nano-objects by looking at the corresponding TEM micrographs in Figure 24. The left-hand side image shows that after 5 min of mixing, the t=0 sample shows that there is a pure morphology of spherical micelles, in agreement with previous data with the DC block copolymer. These spherical micelles cluster together to form a 'grape-like' structure and are highly symmetrical. After evaporation, the t=30 min sample

shows a similar morphology dominated by spherical micelles. These micelles also cluster up to form the 'grape-like' structure and show more evidence of clustering together. Also, these spherical micelles are slightly less uniform, with larger spherical micelles being present on the grid.

5.1.10 25% POPC 75% PDMAm₅₀-b-PDAAm₃₀₀

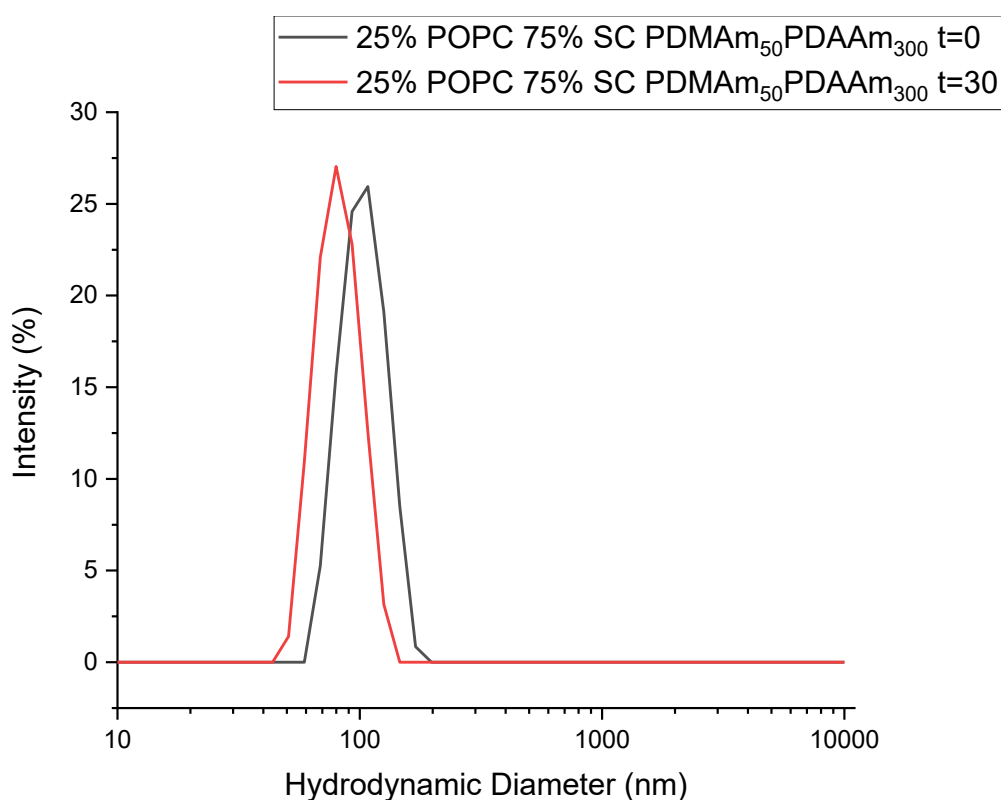


Figure 5.25. Particle size distributions for SE of POPC lipid- SC PDMAm₅₀-b-PDAAm₃₀₀ (25:75%) before and after evaporation for 30 min.

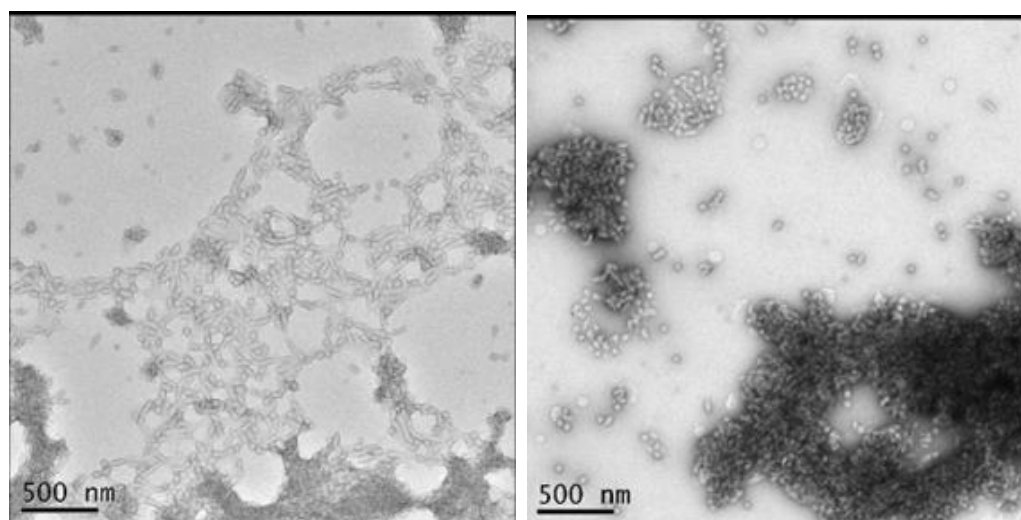


Figure 5.26. TEM micrographs for SE of POPC lipid- SC PDMAm₅₀-b-PDAAm₃₀₀ (25:75%) before (left) and after (right) evaporation for 30 min. Additional images presented in the appendix (Figures 7.310-7.324)

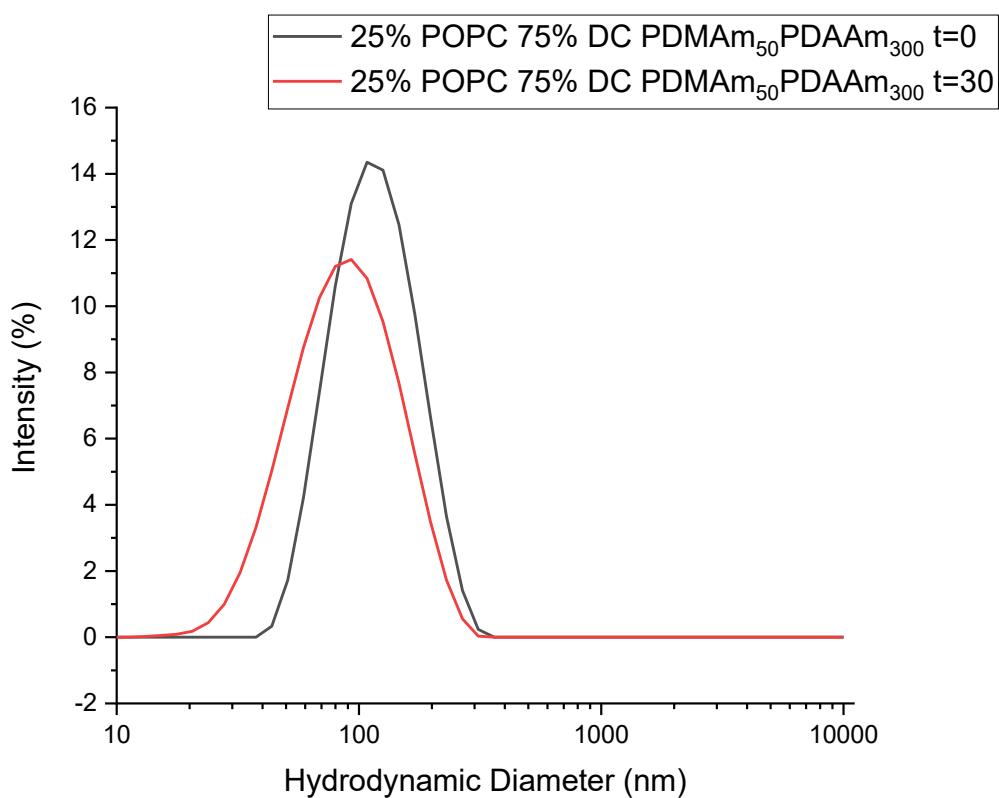


Figure 5.27. Particle size distributions for SE of POPC lipid- DC PDMAm₅₀-b-PDAAm₃₀₀ (25:75%) before and after evaporation for 30 min.

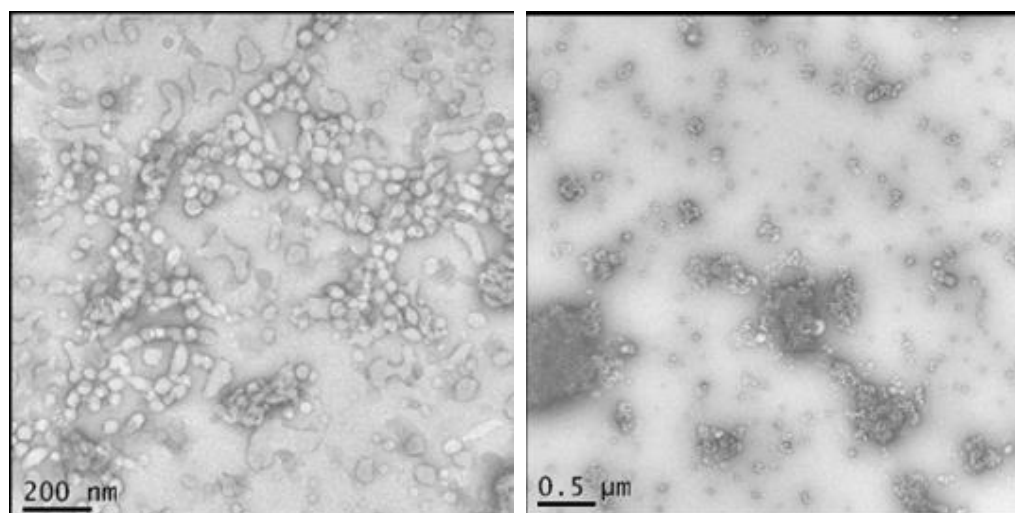


Figure 5.28. TEM micrographs for SE of POPC lipid- DC PDMAm₅₀-b-PDAAm₃₀₀ (25:75%) before (left) and after (right) evaporation for 30 min. Additional images presented in the appendix (Figures 7.25-350)

The DLS data in Figure 5.25 shows the particle size distributions for the SE of 25% POPC and 75% SC PDMAm₅₀-b-PDAAm₃₀₀ block copolymer. From the particle size distributions, it is evident that after 5 min of mixing, the $t=0$ sample shows a narrow monomodal peak centred at 100 nm. After evaporation for 30 min under reduced pressure, there is a slight shift to the left, with the $t=30$ sample having a slightly smaller intensity max value of 80 nm. This result suggests that after methanol is removed from the system, there is a shift towards smaller-sized nanoparticles. This could be due to the methanol solvent swelling the nanoparticles to larger sizes. The TEM micrographs for this study in Figure 5.26 show that the $t=0$ sample, left, forms a network of short worms. This is similar to the neat PDMAm₅₀-b-PDAAm₃₀₀ SE conditions without any polymer. However, the worms appear to be shorter compared to the above-mentioned conditions. After 30 min of evaporation at reduced pressure, the TEM micrograph in Figure 5.26 (left) shows that the majority of the nano-objects form spherical micelles, which are highly clustered. These spherical nano-objects cluster up to form large structures over a micron in size. Also on the grid are faint spherical liposomes, which can be seen to be free from clustering on the grid. These attributed liposomes are around 100 nm in size and appear as faint spheres on the grid. This result suggests that there is some phase separation between the POPC lipid and the SC PDMAm₅₀-b-PDAAm₃₀₀ block copolymer.

The final condition looked at was the SE experiment of 25% POPC and 75% DC PDMAm₅₀-b-PDAAm₃₀₀. The DLS data in Figure 27 shows that for t=0, after 5 min of mixing, the nano-objects record a clear monomodal peak with an intensity max of 120 nm. After 30 min of evaporation at reduced pressure, there is a shift to a smaller particle size distribution, with the t=30 min sample recording an intensity max value of 90 nm. Also, this peak is broader than the t=0 peak, suggesting that the nano-objects become more polydisperse in size after the evaporation for 30 min. More light can be shed by looking at the TEM micrographs for these nanoparticles, Figure 5.28. From the TEM micrographs for t=0 (left), there is an exotic mix of nano-object morphologies dominated by spherical micelle-like entities. The spherical nano-objects are highly clustered together to form a 'grape-like' structure. There is also evidence of dimerization and the formation of short worms. After 30 minutes of evaporation under reduced pressure, there is a change in the morphology of the nano-objects seen in Figure 28 (right). There is a mixed population, with spherical micelles being littered around the grid. These spherical micelles also come together and cluster up to form larger, more exotic clusters of nano-objects, often near the micron size range. There are also smaller clusters around 100 nm in size with two or more spherical micelles combining in these aggregates.

In summary, a wide plethora of nano-objects can be prepared with SE, adding POPC lipid inside. Evidence of vesicle morphologies is yet to be apparent with the POPC lipid mixtures, suggesting that these membrane-directing lipids do not allow for the formation of polymer vesicles when mixed in the SE mixture. The sizes of the polymer-lipid nano-objects have been plotted against the POPC ratio and are presented in Figure 5.29.

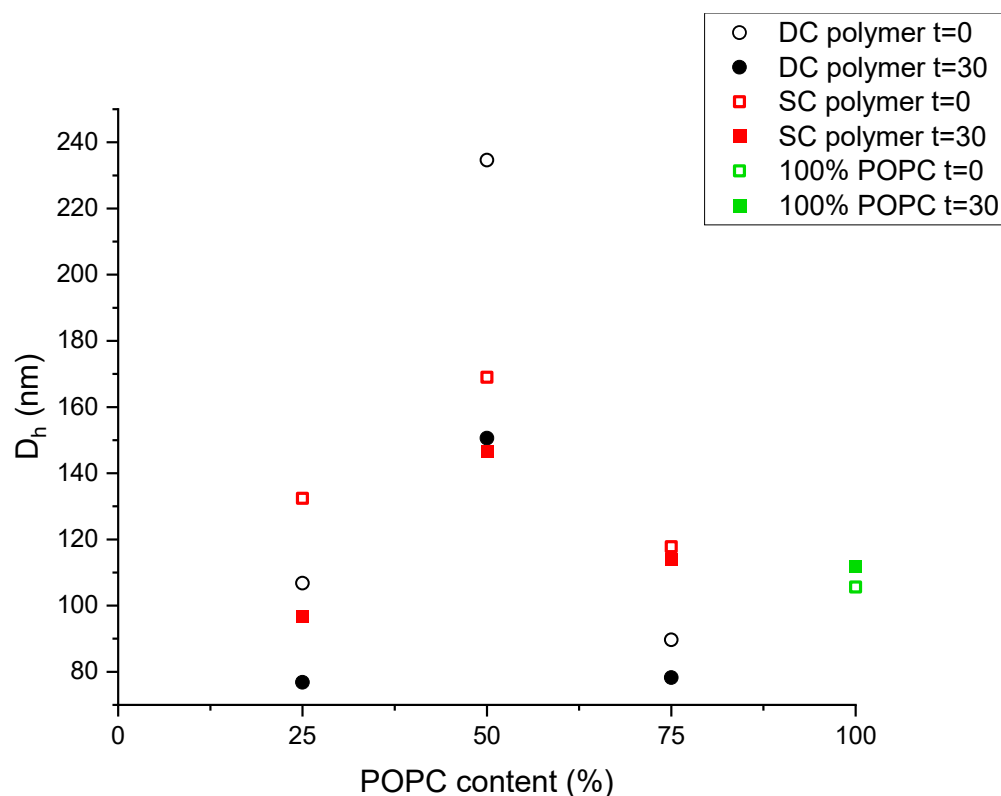


Figure 5.29: Change in hydrodynamic radius of nano-objects prepared via SE of POPC lipid- PDMA₅₀-b-PDAA₃₀₀ mixtures as a function of POPC content.

From the data in Figure 5.29, it is apparent that for all the polymer-POPC lipids mixtures, the t=0 samples are larger in size than the t=30 min samples when methanol has been removed. This may suggest that methanol is causing these nano-objects to swell up to larger sizes. This phenomenon is not present in the pure POPC-lipid sample, which may suggest that the polymer is more prone to swelling due to methanol than the POPC-lipid. Additionally, the data also suggest that the 50% POPC 50% PDMA₅₀-b-PDAA₃₀₀ nano-objects behave differently during SE than the 25% or 75% POPC ratio mixtures. These 50% POPC nano-objects form larger intermediates compared to the rest of the data, with sizes ranging from 150-235 nm, respectively. On average, there is no correlation between the sizes of the SC and DC polymers, suggesting that the charge on the polymer plays no part in the differences observed.

Summary of small-scale SE experiments varying pH and ionic strength of the solution

The main findings of all the small-scale SE experiments are presented below in Table 5.1. This was done for all samples using the doubly charged PDMA₅₀-

b-PDAAm₃₀₀ block copolymer with data from chapters 4 and 5, with the exclusion of POPC lipid experiments.

Table 5.1. Summary of SE small-scale experiments for DC PDMAm₅₀-b-PDAAm₃₀₀ with characterisation data summarised.

pH	Ionic strength	Initial [MeOH] (vol%)	Final [MeOH] (vol%)	Addition speed	MeOH removal speed	Size (nm)	Morphology (via TEM)
2.5	0	5	5	Fast	-	68	Spherical micelles
2.5	0	10	10	Fast	-	44	Spherical micelles
2.5	0	15	15	Fast	-	459	Spherical micelles
2.5	0	5	5	333 μ L/min	-	122	Spherical micelles
2.5	0	10	10	333 μ L/min	-	79	Spherical micelles
2.5	0	15	15	333 μ L/min	-	79	Spherical micelles
2.5	0	5	5	33 μ L/min	-	106	Spherical micelles
2.5	0	10	10	33 μ L/min	-	106	Spherical micelles
2.5	0	15	15	33 μ L/min	-	106	Spherical micelles
2.5	0	50	50	Fast	-	1484	worms
2.5	0	50	8	Fast	Fast	459	Vesicles and Spherical micelles
7.5	0	50	50	Fast	-	106	Worms
7.5	0	50	8	Fast	Fast	220	Worms and Spherical micelles
7.5	140 mM NaCl	50	50	Fast	-	255	Worms
7.5	140 mM NaCl	50	8	Fast	Fast	106	Distorted vesicles

For low methanol content SE experiments (5-15% vol), the predominant morphology observed via TEM is spherical micelles. This is regardless of the speed of addition of the organic phase into the aqueous phase. It is only when the methanol concentration is increased to 50% that there is a change in the morphology of the polymer nanoparticles made via SE. Methanol concentrations of 50% routinely provide worms or cylindrical micelles being formed via SE. This is common regardless of pH and salt concentration. This result suggests that at low methanol concentrations, the block copolymer is not adequately plasticised enough by the methanol to allow it to form higher order morphologies and is becoming kinetically trapped as spherical micelles. At pH 2.5, when the 50% methanol sample is evaporated to give an 8% methanol sample, a change in morphology as the methanol is reduced from the system is observed. The polymer nanoparticles change from worms into vesicles and spherical micelles, as confirmed via TEM in Chapter 4. In this case, at low pHs, the terminal carboxylic acid groups on the block copolymer remain in their

neutral protonated state, allowing for the formation of vesicles without any charge interference. This is not the case at pH 7.5 for the same polymer, where it is evident that the morphology changes from worms to spherical micelles as the methanol content is reduced in the system. At pH 7.5, the terminal carboxylic acid groups are deprotonated, leaving a negative charge on either end of the block copolymer. This could have detrimental effects on the self-assembly of the block copolymer, as repulsion could affect the curvature of the membrane whilst trying to form vesicles. Instead, due to this charge, lower-order morphologies such as worms and spherical micelles are seen via TEM.

For the pH 7.5 sample with 140 mM NaCl, when the methanol concentration is reduced, there is a change away from worms into distorted, non-symmetrical vesicles. This can be accounted for by discussing the charge on the polymer. As with the analogous experiment without salt concentration at pH 7.5, the block copolymers' terminal carboxylic acid groups become deprotonated, leaving a negative charge on each end of the block copolymer due to this relatively high pH. However, the presence of NaCl remedies this charge problem as the dissociated salt ions, especially the sodium cations, can screen the charge on the block copolymer, allowing it to form higher-order morphologies such as vesicles. The curvature of the membrane is not hindered by any repulsions due to charges, as the charge is adequately screened by the addition of NaCl. This is why vesicles are present when looking at the TEM for this sample. The findings from this study are summarised in Figure 5.30. The summary of the DLS size data for all SE experiments is also provided in Figure 5.31.

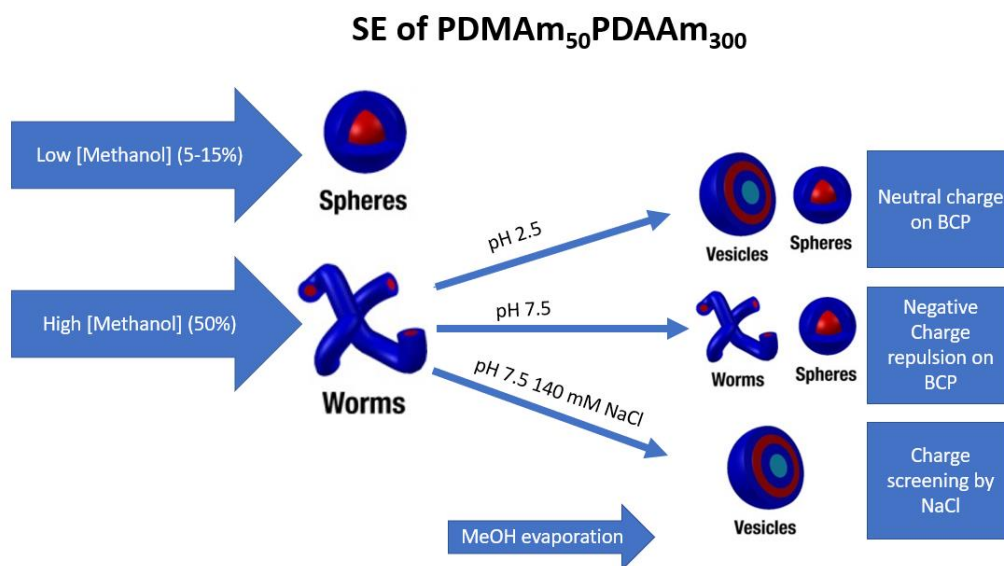


Figure 5.30. Summary of SE experiments for DC PDMAm₅₀-b-PDAAm₃₀₀ under different conditions explained in the main text.

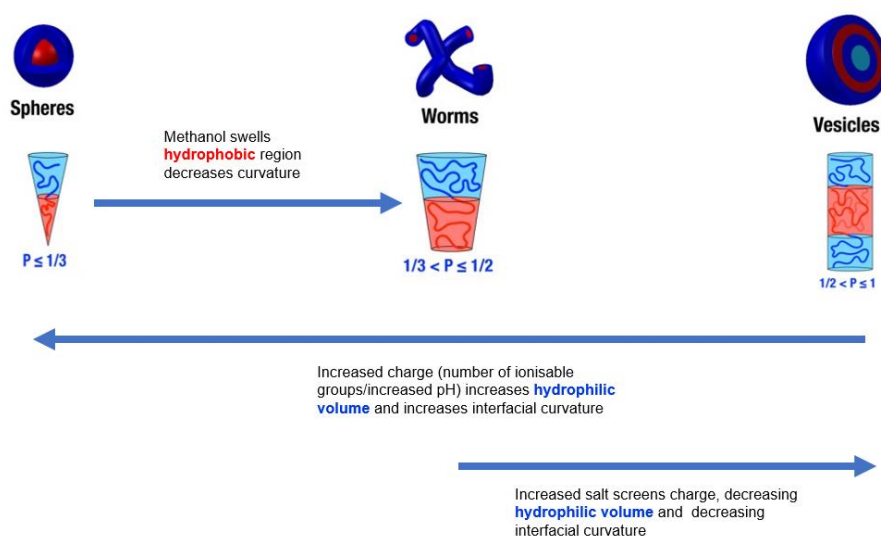


Figure 5.31. Mechanical insights into block copolymer morphological transformations during SE.

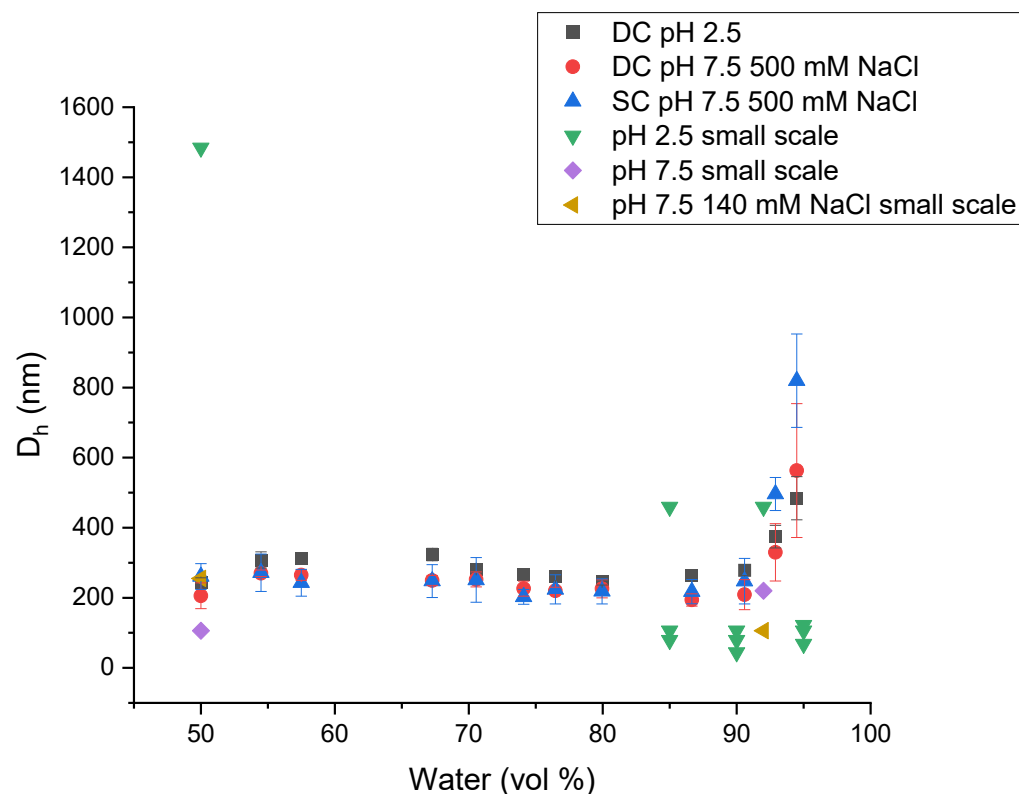


Figure 5.32. Summary of DLS size data vs Water vol% SE experiments for PDMAm₅₀-b-PDAAm₃₀₀

5.1.11. Conclusions

The SE experimental procedure from chapter 4 was extended towards new conditions and solvent environments. The aqueous environment was altered from pH 2.5 to pH 7.5. This resulted in a change in morphology from vesicles to spherical micelles when a pH of 7.5 was used for SE of PDMAm₅₀-b-PDAAm₃₀₀ (singly charged). However, when 140 mM of NaCl was added, the SE produced non-spherical vesicles. This result suggests that the NaCl screens the charge on the polymer enough for the polymer to form larger order nano-objects, such as membrane-bound vesicles.

To better understand the phenomenon happening during SE evaporation, the three-hour evaporation of methanol was studied. This resulted in a range of different nano-objects being isolated at various time points and methanol concentrations studied via ¹H NMR. This experiment showed that the methanol concentration decreases steadily with time spent on the rotary evaporator and

reaches a final concentration of ~5 vol % after 3 hours of evaporation. The morphology changes were mapped, and it was found that in all cases (DC/SC polymer, ionic strength/ pH), a similar pathway is observed. The nano-objects start as a highly intertwined 'spaghetti' like structure of cylindrical micelles or worms, at high methanol concentrations near 50% vol. between the time points of 15-60 min, corresponding to methanol concentrations of 35-20% vol, the worms break apart to form spherical micelles. There is evidence of a few isolated vesicles being formed at t=60 – t=120 min (20%-9% Methanol), which suggests that vesicle formation occurs at lower methanol concentrations. However, the majority of the nano-objects form spherical micelles which aggregate and crash out of solution as seen by the large clusters and aggregates present at t=180 min of evaporation (5% Methanol). This trend was seen for both the DC and SC PDMAm₅₀-b-PDAAm₃₀₀ polymers throughout pH 2.5-7.5 and with or without 500 mM NaCl, suggesting that the charge on the polymer plays no part in the final morphologies observed.

A membrane-directing lipid was also added to the SE mixture, hoping for plasticisation of the membrane enough to form vesicles after 30 min of evaporation. However, this resulted in some phase separation of the lipid and polymer nano-objects, with lighter regions on the TEM being attributed to the POPC lipid. The most notable change in morphology was the 50% POPC sample, which showed a change away from the highly intertwined worm structure to kinetically trapped spherical micelles, which persisted after 30 min of evaporation. The 25% and 75% POPC ratio SE experiments lead to spherical micelles that cluster up to form large aggregates and clusters.

5.1.12 References

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Chapter 6

6.1. Conclusions and future work

6.1.1 Conclusions

A detailed discussion of the data obtained for PISA vs solvent exchange is discussed in this section. This discussion aims to critique differences in approaches with regard to key variables, including temperature, time and plasticiser and their effects on the self-assembly pathways.

Temperature

PISA typically operates at elevated temperatures, at 70°C for all PISA experiments in this work, and the reaction took place in pure aqueous conditions in the absence of co-solvent. This temperature range is chosen, based on this polymer system's previous use in literature, to optimise the decomposition rate of the initiator and maintain suitable polymerisation kinetics.

¹.

In contrast, SE was performed at room temperature during this work, as this self-assembly pathway does not rely on thermal initiation of polymerisation, being a post-polymerisation technique. The temperature at which self-assembly occurs plays a crucial role in the self-assembly of block copolymers, affecting various aspects of the process through multiple mechanisms. For example, temperature significantly influences the mobility and dynamics of polymer chains, which in turn impacts self-assembly: Higher temperatures enhance the mobility of polymer chains, allowing them to reorganise more easily and reach thermodynamic equilibrium faster.² This increased mobility can facilitate the formation of more ordered structures, which is what was seen in chapter 3 of this thesis, as PISA at varying hydrophobic block lengths produced more uniform and ordered phases, spheres, worms and vesicles. In these cases, elevated temperatures provide the necessary energy for polymer chains to overcome local energy barriers, enabling them to explore different conformations and assemblies.³

Additionally, temperature directly affects the thermodynamic parameters governing the self-assembly process. Specifically, the self-assembly process is driven by the balance between entropy (ΔS) and enthalpy (ΔH), as previously mentioned in this work. As temperature increases, the entropic term ($T\Delta S$) becomes more significant in the Gibbs free energy equation ($\Delta G = \Delta H - T\Delta S$).

Hence, the PISA syntheses performed at 70 °C, compared to 25 °C where SE was conducted, led to a higher entropic contribution to the Gibbs free energy equation than SE conditions.

Another factor worth discussing is the Critical Micelle Temperature (CMT). Above a certain temperature, known as the CMT, the formation of micelles becomes thermodynamically favourable. This temperature-dependent behaviour is crucial for controlling the onset of self-assembly.² This supports the data, as it suggests that PISA self-assembly, at higher temperatures than SE self-assembly, led to more facile, thermodynamically favourable self-assembly. Furthermore, the Lower Critical Solution Temperature (LCST) is a factor. Some block copolymers exhibit LCST behaviour, where they become less soluble at higher temperatures, leading to enhanced self-assembly and aggregation.⁴

As well as having a direct effect on the polymer thermodynamics, the temperature of self-assembly also has an indirect effect on the self-assembly via solvent quality and interactions. Temperature changes can alter solvent quality and polymer-solvent interactions, specifically, for example, solvent selectivity. As the temperature at which self-assembly occurs increases, the solvent quality for one block may change relative to the other, affecting the driving force for microphase separation.²

This microphase separation can also be influenced by the Flory-Huggins interaction parameter (χ), which is temperature-dependent. This characteristic of the PDMAm₅₀-b-PDAAm_y block copolymers influences the compatibility between different blocks and the solvent, impacting the self-assembly process. In addition, the higher temperatures employed in PISA compared to SE can accelerate both the nucleation of self-assembled structures and their subsequent growth, which is often seen during this work as PISA formulations became turbid very quickly into the chain extension of the PDMAm₅₀ macro-CTA.

Additionally, temperature induces morphological changes in self-assembled structures post-nucleation. Increasing temperature can lead to transitions between different ordered morphologies (e.g., spheres to cylinders to lamellae) due to changes in the relative volume fractions of the blocks.^{4b} This may be why PISA, conducted at 70 °C, forms higher ordered morphologies such as vesicles more uniformly when compared to the SE experiments in this thesis.

Plasticiser effects

In the PISA experiments in this work, the DAAM monomer itself acts as a plasticiser during the polymerisation process. As the polymerisation progresses, the growing polymer chains become increasingly hydrophobic, leading to in-situ self-assembly.⁵

The concentration of DAAM monomer decreases over time as it is consumed in the polymerisation reaction. The SE experiments in this thesis, on the other hand, used a methanol co-solvent system. The lyophilised PDMAm₅₀-b-PDAAm₃₀₀ polymer was initially dissolved in methanol, which was then added to the aqueous conditions and removed via rotary evaporation.⁶ The co-solvent concentration, similar to the monomer concentration in the case of PISA, decreases over time, which induces self-assembly. The nature of the plasticiser plays a crucial role in block copolymer self-assembly, significantly influencing the mobility of polymer chains and the resulting nanoparticle characteristics. Comparing the plasticisers used in the PISA experiments (DAAM monomer) and SE (methanol co-solvent) reveals distinct differences in their effects on the overall self-assembly process.

In the PISA experiments in this work, the monomer provides high chain mobility throughout the polymerisation process. As the core-forming block grows, the unreacted monomer continues to plasticise the assembling structures, allowing for dynamic reorganisation.⁷ The continuous presence of monomer enables access to a wide range of morphologies, including spheres, worms, and vesicles. The evolving volume fractions of the hydrophilic and hydrophobic blocks during polymerisation drive these morphological transitions.⁷

Additionally, the gradual consumption of monomer during polymerisation allows for a continuous evolution of the self-assembled structures, resulting in more thermodynamically favoured morphologies, a theme through the differences between PISA and SE self-assembly pathways.

In solvent exchange, methanol (typically at 10-50% of the total formulation) acts as a cosolvent. Initially, the high methanol content provides good solvation for the polymer chains. However, as the solvent is removed, chain mobility decreases, potentially leading to kinetically trapped structures.²

In this thesis, Solvent exchange typically results in spherical micelles and in some instances vesicles, with less morphological diversity compared to PISA, as PISA experiments offered access to a wider range of morphologies due to the dynamic nature of the assembly process where as SE experiments in this work generally produced a more limited range of morphologies, primarily spheres and vesicles post methanol removal.²

Self-assembly time:

Throughout this thesis, a key difference between PISA and SE is the time window in which the self-assembly occurs. The PISA experiments took place in 4 hours, much longer than the 30-minute self-assembly of the SE pathway. This key difference may be a crucial factor in determining the changes seen in yield, size, uniformity, and morphology of the polymer nano-objects between these self-assembly pathways. Nanoparticle size in solvent exchange is influenced by polymer concentration and the rate of solvent removal. Slower solvent removal generally results in larger, more uniform particles.²

The gradual removal of methanol with water induces a stepwise self-assembly process, where the structures form as the solvent quality for the hydrophobic PDAAm block decreases. Perhaps a different methanol removal pathway, such as dialysis, would shed more information on the self-assembly potential of the SE pathway.

Statement of originality

This thesis makes significant original contributions by systematically investigating areas that have not yet been addressed in existing literature. Although the polymerisation of block copolymers and the technique of Polymerisation Induced Self-Assembly (PISA) have been extensively studied over the past fifteen years, this work presents the first consistent set of PISA syntheses comparing the effect of salt concentration and ionic strength on the PDMAm₅₀-b-PDAAm_y system. This systematic study fills a notable gap in our understanding of how ionic conditions affect this specific block copolymer system, which can also be used for insights into other analogous PISA systems.

Additionally, whilst Solvent Exchange (SE) and Thin Film Rehydration (TFR) are well-established techniques for the self-assembly of lipids, there has not previously been a comprehensive comparative study of these methods applied to block copolymers. This research provides new insights into how various self-assembly techniques influence the morphological outcomes in block copolymer systems.

Most notably, this thesis presents an original and systematic investigation into the interplay between solvent plasticisation, charge interactions, charge screening by ionic salts, and their effects on both the hydrophobic and hydrophilic segments of block copolymers. This work explores how these factors collectively influence interfacial tension and the resultant self-assembly behaviours & relationships, which have not been previously examined in such detail or in a systematic fashion.

Chapter conclusions

Chapter 3

The aims of this chapter were to use PISA to fabricate a range of block copolymers capable of self-assembly into nano-objects under aqueous conditions. Polymersomes or polymer vesicles were highly sought after as a potential encapsulation vehicle, and their favourable properties when compared to liposomes, hence fabricating vesicles via PISA was a clear aim of this chapter. The final aim of this chapter was to be able to synthesise and self-assemble these block copolymers under biologically relevant conditions. During the research in this chapter, the PISA system was explored for a block copolymer in aqueous conditions. PISA allowed for the fabrication of pure polymersome samples as well as mixed morphology samples, all dependent on the length of the hydrophobic block and thus the packing parameter of the block copolymer. The critical hydrophobic block length of DAAM was a DP of 200 for pure vesicle phases using PISA. It is thought that PISA is under equilibrium control, forming thermodynamically stable nano-objects. This Self-assembly pathway for PISA is under thermodynamic control, leading to equilibrium state nano-objects. During PISA, the monomer acts as a plasticiser, altering the physical and chemical properties of the block copolymer during synthesis and

self-assembly. Plasticisation of this polymer could lead to the formation of thermodynamically stable nano-objects. The PISA synthesis was altered towards biological conditions by the increase of pH to 7.5 and the addition of electrolyte via PBS or NaCl. This allowed for the fabrication of pure polymersome morphologies at biologically relevant conditions, opening the door for encapsulations of biologically relevant cargo such as enzymes and proteins.

Chapter 4

The aims of this chapter were to understand the alternative self-assembly pathways for the fabrication of polymer nano-objects under aqueous conditions. Two alternative self-assembly pathways were investigated, thin film rehydration (TFR) and solvent exchange (SE), both with differing self-assembly pathways. In this chapter, alternative self-assembly methods and pathways for the fabrication of polymer nano-objects under aqueous conditions were also explored. TFR proved to be difficult under current conditions, which suggests the polymer may be too hydrophobic and is not plasticised enough during the TFR to form nano-objects. Lack of plasticisation could be a key hindrance for this block copolymer in forming stable nano-objects using TFR. SE was studied as another viable method for the fabrication of polymer nano-objects. The two main variables looked at were the addition speed of the organic polymer phase to the aqueous phase and the initial methanol cosolvent concentration. All experiments with a cosolvent percentage of between 5% and 15% lead to kinetically trapped spherical micelles regardless of the addition speed. By increasing the cosolvent concentration to 50% higher order morphologies were observed with cylindrical micelles and some polymersomes fabricated using the SE method. This suggests that the plasticisation effect of the methanol is key to avoiding kinetically trapped micelles. As the methanol is increased to 50%, the amount of plasticisation of the block copolymer increases to allow for special rearrangement into higher order structures, including worms and some polymersomes. This theme of plasticisation is key as the PISA pathway is plasticised by monomer molecules and SE was plasticised by methanol, with both methods allowing for the fabrication of higher-order nano-objects, whereas with TFR, no plasticisation occurred, leading to little to no self-assembly of the block copolymer into nano-objects.

Chapter 5

The aims of this chapter were to understand the SE self-assembly pathway by scaling up and identifying any intermediate structures during this self-assembly. Also, during this chapter, the SE self-assembly pathway was altered towards biological conditions (salt and pH). Finally, a membrane-directing lipid was employed during the SE self-assembly pathway to try and unlock higher-order membrane-bound polymer vesicles, which were not routinely fabricated during the SE self-assembly method thus far. Mechanistic insights into SE were achieved by a time-resolved self-assembly, focusing on the amounts of residual methanol, and hence, plasticising agent left in the self-assembly during cosolvent evaporation. It was found that all conditions, irrespective of pH, salt conditions, or level of charge on the polymer, all behaved the same during the time-resolved 3-hour SE self-assembly. Initially, at high methanol concentrations, cylindrical micelles or worms are fabricated, which leads to a breakup into spherical micelles as the cosolvent is removed. These spherical micelles persist for up to 2 hours before aggregation, and after 3 hours. A few polymersomes are visible on the grid after 120 min, which suggests a very small fraction of the block copolymer self-assembles into polymersomes using this self-assembly pathway and conditions. The effect of salt and pH on the SE pathway suggests that polymersome-like particles are fabricated using the addition of NaCl at neutral pHs. This suggests that the charge of the polymer due to the terminal carboxylic acid groups at pH 7.5 was screened well by the addition of NaCl. Sometimes, charge repulsion in the membrane could be detrimental when fabricating polymersomes using this SE self-assembly pathway. These results suggest that the SE pathway is under kinetic control, unlike PISA, which forms thermodynamically stable nano-objects. SE self-assembly tends to favour kinetically trapped nano-objects, such as spherical micelles. This could explain why polymersomes, which are thought of as metastable, are few and far between under this self-assembly pathway.

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6.1.2. Future work

Throughout this study, new research questions have arisen that need to be addressed in the future. Several experiments and approaches have been planned during the PhD process, which could culminate in a new PhD entirely. These experiments aim to answer a few research questions highlighted in this PhD, but also open avenues towards new research questions and objectives.

6.1.3. Alternative self-assembly methods: Inverse-emulsion technique

Another self-assembly method, which was not discussed during this work, is the inverse-emulsion technique. Typically used for lipids, this method is a good way of forming giant unilamellar vesicles (GUVs). This method starts with the initial dissolution of a specific concentration of a chosen lipid or polymer mixture in oil that is later added on top of an aqueous solution to form an oil–water interface. The lower aqueous solution will eventually become the outer environment of the GUVs (typically a glucose solution). At the same stage, a specific volume of denser aqueous solution is added to the lipid/polymer–oil

phase (in a separate vial) to produce a water-in-oil emulsion. This aqueous phase will become the inner compartment of the GUV and will therefore contain the solutes to be encapsulated. Owing to their amphipathic nature, lipids/polymers present in the oil phase self-assemble at the interfaces (both the emulsion and the lower interfacial layer, see Figure 5 to form lipid or polymer monolayers. The emulsion is then added on top of the interface, and a centrifugal force is applied to assist the movement of denser water-in-oil emulsion droplets through the interfacial lipid monolayer. This process will result in the simultaneous wrapping of the second monolayer of lipids/ polymers around the droplets and the formation of GUVs in the lower aqueous phase.¹

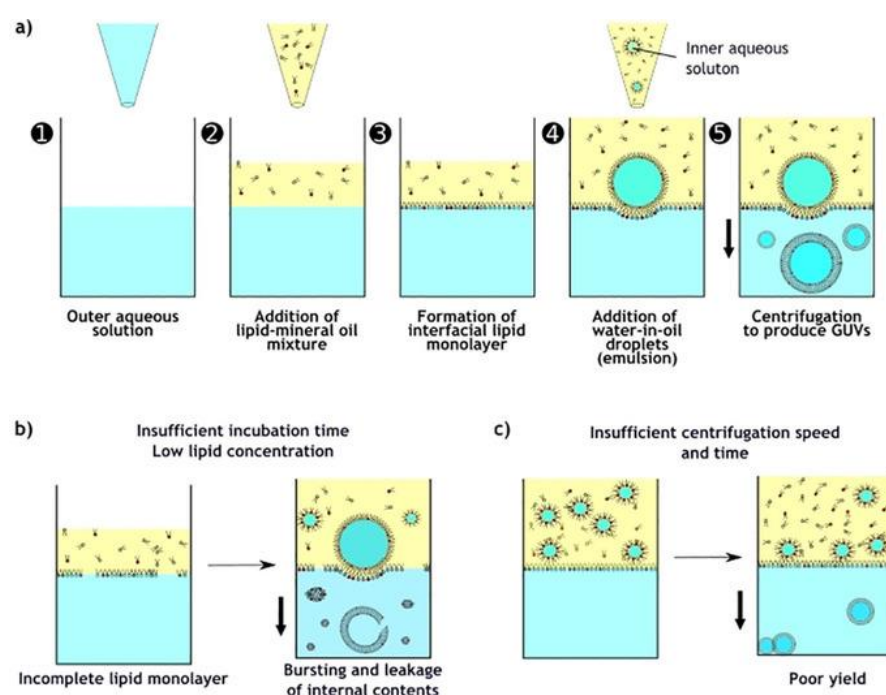


Figure 6.1. Overview and the pitfalls of the inverted emulsion method. a) Schematic representation of the five steps of the inverted emulsion method with the ideal GUV production. Parameters, such as b) low lipid concentrations and insufficient incubation times, may result in an incomplete interfacial lipid monolayer that can compromise overall GUV yield and size. c) Centrifugation speed and duration also have an impact on the morphology and yield of the GUVs, as the droplets may remain in the oil phase or at the interface.¹

A proposed plan of action for the inverse-emulsion of PDMAm₅₀-b-PDAAm_y block copolymers is presented below. This proposed plan compares two or more similar studies made by Moga *et al.* and Pautot *et al.*,^{1,2}

Proposed Method.

Part A – Formation of a stable inverse-emulsion.

1. Prepare an aqueous solution of 300 mM glucose, pH 2.5, with 0.1 wt. % polymer.
2. For emulsification, sonication of dodecanal and (aqueous solution) 0.5% vol: Dodecanal
3. DLS measurements to determine the size and stability of the inverse emulsion.

Part B – Stable monolayer of BCP at the interface of the Oil-Water boundary.

1. In a Falcon 50 mL, 3 mL pH 2.5 with 400 milliQ water, gently add 2mL dodecane containing amphiphiles starting at 500 μ M (comprisable)
2. Incubation for 5, 10, 15 min

Part B – Stable monolayer of BCP at the interface of the Oil-Water boundary.

1. Addition of 0.1-1 mL Inverse emulsion solution
2. Centrifugation for 200 g for 3 min

For optimisation and higher throughput. (After proof of concept)

A 96-well titration plate will help with encapsulation experiments

This method presents itself as another viable vesicle fabrication method, which would allow for comparison with PISA and SE methods, highlighting the differences in self-assembly pathways between the different methods utilised. The rest of the future work section will focus on the utilisation of polymersome morphologies for various applications, which have not yet been completed in this body of work.

6.1.4. Encapsulation studies

One of the most obvious applications for polymersomes is the encapsulation of target payloads. This is due to the polymersomes' structure, which has an aqueous core capable of trapping and encapsulating target molecules. The first encapsulation experiment planned for the future work is the encapsulation of 6-carboxyfluorescein, Figure 6.2, a small hydrophilic dye capable of being encapsulated in the core of the polymersomes made via PISA, namely PDMAm₅₀-b-PDAAm₃₀₀ polymersomes. The main reasoning behind this future work experiment is that dextran and small fluorescent molecules are a good model encapsulant reference. In recent literature, this has been tried as an initial model study with a further encapsulation study focusing on therapeutics. This could be due to the ease of analysis; as the smaller dextrans and fluorophores are easy to detect and analyse, as opposed to larger therapeutics in which you have to look in UV region, and also which may require High Performance Liquid Chromatography (HPLC) to separate, which is both time and cost expensive as an initial experiment. Additionally, smaller fluorophores allow the use of the visible region where the PDMAm-50-b-PDAAm polymer does not absorb.

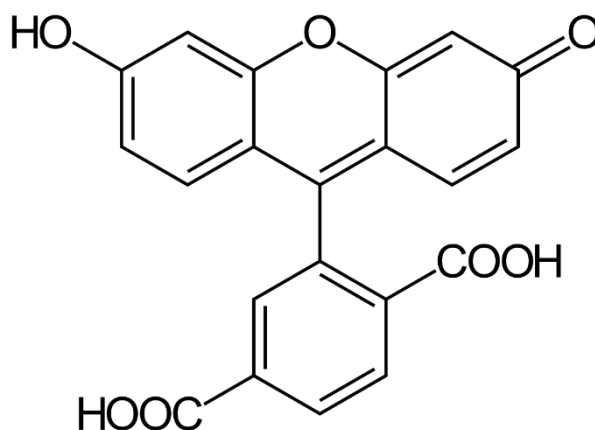


Figure 6.2. Chemical structure of 6-carboxyfluorescein.

6-carboxyfluorescein is a fluorescent dye with an absorption wavelength of 495 nm and an emission wavelength of 517 nm. This makes the dye easily visualisable via fluorescent imaging. The proposed experiment is a PISA chain extension of PDMAm₅₀ macro-CTA targeting a DAAM block length of 300 in pH 7.5 water. This is because carboxyfluorescein is insoluble at acidic pHs, ruling out encapsulation at pH 2.5. Also in the mixture will be a quantity of 6-

carboxyfluorecin. The polymerisation will be characterised via GPC, NMR, DLS, and TEM, to ensure polymersome morphology persists in the presence of the 6-carboxyfluorecin. The encapsulated dye will be separated from the non-encapsulated dye via column separation, then the concentration of the dye remaining in the polymersomes will be determined via fluorescent imaging and UV-Vis spectroscopy. This will help determine the encapsulation efficiency of the polymersomes towards 6-carboxyfluorescein.

Another encapsulation experiment would be to move on from 6-carboxyfluorecin to larger macromolecules such as fluorescently labelled dextrans, Figure 6.3.

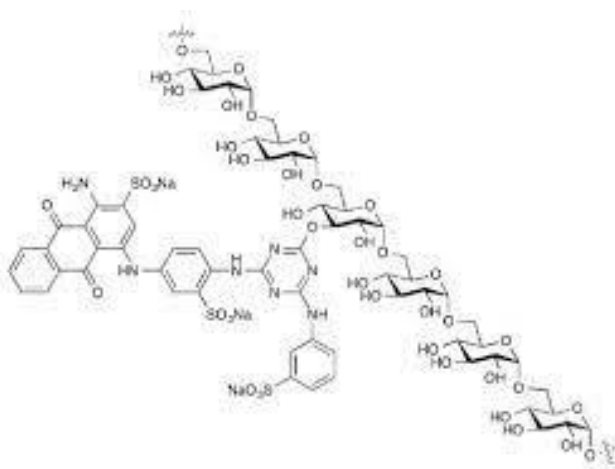


Figure 6.3. Chemical structure for blue dextran.

Dextran is a complex branched glucan (polysaccharide derived from the condensation of glucose). IUPAC defines dextrans as "Branched poly- α -d-glucosides of microbial origin having glycosidic bonds predominantly C-1 $\rightarrow$ C-6". Dextran chains are of varying lengths (from 3 to 2000 kilodaltons). Encapsulation of a dextran would highlight the ability of the PDMAm₅₀-b-PDAAm₃₀₀ polymersomes to encapsulate larger macromolecules. The proposed experiment is also a PISA chain extension of PDMAm₅₀ macro-CTA targeting a DAAm block length of 300 in pH 7.5 water, similar to the experiment planned with 6-carboxyfluorecin. Also in the mixture will be a quantity of fluorescent dextran. The polymerisation will also be characterised via GPC, NMR, DLS, and TEM, to ensure polymersome morphology persists in the presence of the dextran. The encapsulated dextran will be separated from the

non-encapsulated dextran also via column separation, then the concentration of the dye remaining in the polymersomes will be determined via fluorescent imaging and UV-Vis spectroscopy. This will help determine the encapsulation efficiency of the polymersomes towards the blue dextran. This determined encapsulation efficiency will be compared against that of the 6-carboxyfluorescein to see if there is a better encapsulation for one target compound over the other.

For dextran encapsulation during PISA, GPC conditions need to be considered carefully. During dextran encapsulation, the Mw of interest is the molecular loading of dextran. A GPC method may need to be developed, but the main effort would be to determine the encapsulation efficiency of the polymersomes towards the dextran. A possible way to achieve this may be to run a GPC measurement of the dextran alone to determine elution time and determine if the dextran GPC peaks overlap with the PDMAm₅₀-b-PDAAm₃₀₀ GPC peaks. If this is the case, it can be suggested that an HPLC column may be used to separate unencapsulated dextran and the polymer vesicles, which may involve more complex additional separation steps.

.6.1.5. Biological Nano-reactor encapsulation

Proposed experimental goals

The final encapsulation experiments would involve trying to create a biological nano-reactor based on the PDMAm₅₀-b-PDAAm₃₀₀ PISA system. The design of this experiment would depend on what size things can remain encapsulated and what types of substrates and products can cross these membranes in the PDMAm₅₀-b-PDAAm₃₀₀ PISA system. This experiment would involve encapsulating an enzyme, namely, horseradish peroxidase (HRP), into the system during PISA. To do this, the PISA process would need to be altered to lower temperatures, as 70 °C would denature the proteins in the enzyme and cause it to be non-reactive towards the substrates. However, there are thermophilic enzymes or highly stable enzymes that can resist high temperatures for a certain amount of time. To do this, the PISA process would need to be shifted towards a light-initiated system, using a UV-Vis Lamp and a photoreactive radical initiator. Photo initiators are compounds that produce

radicals when exposed to UV light. These then react with monomers to initiate polymer chain growth. The photo-initiator chosen for this PISA polymerisation is 2,2-dimethoxy-1,2-diphenyl-ethan-1-one, which breaks down under UV light to form a methoxybenzyl and benzoyl radical. The benzoyl radical initiates free radical polymerisation, whereas the methoxybenzyl radical decomposes to give the more stable methyl radical and methyl benzoate (Figure 5.4).

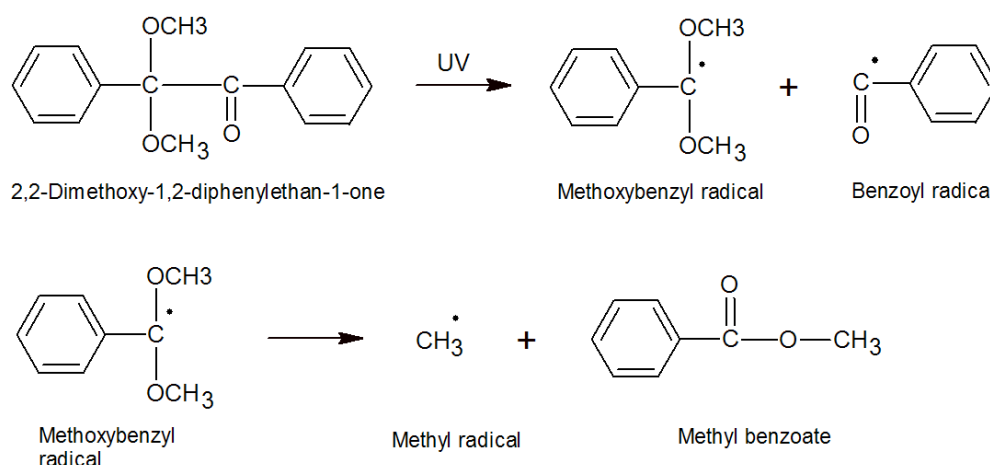


Figure 6.4. Chemical structure of 2,2-dimethoxy-1,2-diphenyl-ethan-1-one and its breakdown into radicals under UV light.

The photo-PISA chain extension of PDMAm₅₀ with DAAM targeting a block length of 300 would need to be done first without any encapsulants to verify that polymersome morphologies persist when the reaction is changed from thermally to light-mediated initiation. Verification of polymersome morphologies would be completed via GPC, DLS, NMR, and TEM, and would be compared against the thermally initiated PISA polymersomes. Assuming there is no difference between the thermally and light-initiated polymersomes, the light-initiated polymersome formulation will be loaded with HRP enzyme. A wide range of concentrations of the enzyme will also be studied to find the optimum concentration to be used. The formulation will also add luminol, which will be oxidised to 3-aminophthalate by peroxides and HRP enzyme. In doing so, there will be a built nano-reactor capable of oxidising a chromophore capable of being detected by UV-Vis spectroscopy. This will demonstrate the PDMAm₅₀-b-PDAAm₃₀₀ polymersomes' capability to act as a nano-reactor with selective permeability towards small molecules such as peroxides, meanwhile keeping the HRP enzyme trapped in the aqueous cavity of the polymersome. The next

step after this would be to design a polymersome with functionality that would bring about a change in permeability towards a given substrate for targeted delivery of certain payloads. This would need to involve inserting a pH-responsive group into the polymer backbone. pH can be considered as one of the most important parameters in biological systems. An example of this conceptual idea was carried out by Abd Al-Wali Mohammed M. Japir and coworkers. This group developed a series of membrane-cross-linked polymersomes with cross-linking density-mediated and tumor pH-tunable selective membrane permeability for stimuli-responsive drug delivery and controllable enzymatic reaction, Figure 5.5.¹

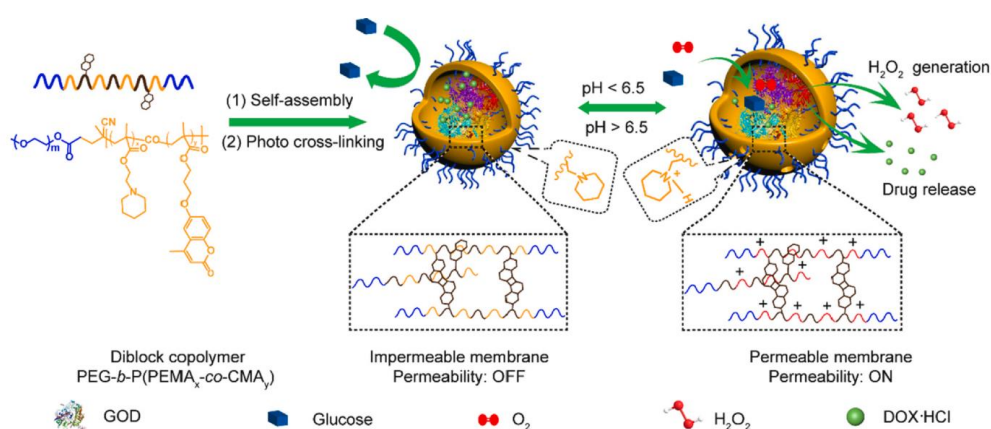


Figure 6.5. Schematic showing the preparation of DOX/GOD@MCL-PSs via self-assembly in aqueous solution (PBS, pH 7.4) with cross-linking density-mediated and pH-responsive selective membrane permeability for stimuli-responsive DOX release and controllable enzymatic reaction to in situ generate H_2O_2 under the catalysis of GOD.¹

The risk of enzyme denaturation in the presence of monomers and solvents, and during PISA polymerisation and SE.

Enzymes play vital roles as biocatalysts in numerous industrial and scientific applications, yet their functionality depends critically on maintaining their native three-dimensional structure. When enzymes encounter organic solvents, monomers, or participate in polymerisation processes, they face significant risks of denaturation.

Enzyme denaturation occurs when an enzyme loses its native conformation or three-dimensional structure, rendering it unable to bind effectively to its

substrate and catalyse product formation. The two primary causes of enzyme denaturation are deviations from optimal temperature and pH conditions.

When temperature increases beyond the optimal range, various intramolecular bonds holding the enzyme together—including hydrostatic bonds, van der Waals forces, and covalent bonds—break down, causing the enzyme to lose its structure. Similarly, both increases and decreases in pH can create environments that disrupt the three-dimensional structure of an enzyme.

At the molecular level, key degradative mechanisms include deamidation of asparagine and glutamine residues, and succinamide formation at aspartate and glutamate residues, leading to peptide bond hydrolysis.⁵ These reactions are not only temperature-dependent but also strongly influenced by the conformational freedom of the susceptible amino acid residues.⁵ Interestingly, evidence suggests that even at temperatures as high as 100°C, deamidation and succinamide formation proceed slowly or not at all in conformationally intact, native enzymes.⁵ This underscores the critical importance of the protein's tertiary structure in maintaining stability.

The degree of denaturation can vary, with mild cases affecting only the active site of the enzyme, reducing its ability to effectively bind to its substrate. In more severe cases, the entire structure of the enzyme breaks down, resulting in a complete loss of function. Because enzyme function is dependent upon the ability to bind to substrates, even partial denaturation can render enzymes ineffective, slowing biochemical reactions to rates that are not sustainable for biological processes.

Solvent-Induced Enzyme Denaturation

Water-miscible organic solvents are frequently required in enzymatic reactions to improve substrate solubility in aqueous media, yet these solvents can significantly impact enzyme stability.⁶ The traditional parameter for assessing enzyme stability—melting temperature (T_m)—does not reliably predict activity in the presence of organic solvents.⁶ Recent research by Sorgenfrei and colleagues proposed the concentration of co-solvent at 50% protein unfolding at a specific temperature (c_{U50}^T) as a more effective parameter for predicting enzyme performance in solvent environments.⁶

Studies of ene reductases in the presence of acetonitrile have demonstrated that even low concentrations (2.5%) of the solvent can negatively affect enzyme activity^[4]. In unbuffered solutions, a steady decrease in enzyme activity corresponds to increasing acetonitrile content.⁷ Buffer solutions, particularly ammonium acetate, can provide some protection against solvent-induced denaturation, with greater tolerance observed at pH 6.2 compared to pH 7.2.⁷

While PISA offers opportunities for enzyme encapsulation and protection, the polymerisation process itself presents risks for enzyme denaturation. PISA utilises controlled radical polymerisation techniques, which inherently involve reactive radical species that can interact with and potentially damage enzyme structures. The monomers employed in PISA, typically methacrylates or other vinyl compounds, can themselves act as organic solvents before polymerisation, potentially destabilising enzymes through the mechanisms previously discussed.

Ring-Opening Metathesis Polymerisation Induced Self-Assembly (ROMPISA) represents a specific variation of PISA using commercially available Ru-based catalysts.⁸ This technique, showcased by O'Reilly and coworkers, has revealed exceptional self-assembly behaviour with respect to morphology and morphological evolution, but the polymerisation conditions must be carefully controlled to maintain enzyme stability. The permeability of polymersome membranes synthesised by ROMPISA is particularly important for maintaining enzyme function while providing protection

Radical-mediated modifications of Horseradish Peroxidase

Horseradish peroxidase (HRP) serves as an excellent model for understanding enzyme denaturation during radical-mediated processes due to its well-characterised structure and function. HRP is a metalloenzyme containing protoporphyrin IX with an Fe³⁺ ion, found in numerous isoforms, with type C being the most studied. It catalyses the oxidation of various organic substrates by hydrogen peroxide through a cycle involving compound I, compound II, and a return to native HRP.¹⁰

The catalytic cycle of HRP can be represented as follows:

1. $\text{HRP} + \text{H}_2\text{O}_2 \rightarrow \text{Compound I} + \text{H}_2\text{O}$
2. $\text{Compound I} + \text{AH}_2 \rightarrow \text{Compound II} + \text{AH}^\cdot$

3. $\text{Compound II} + \text{AH}_2 \rightarrow \text{HRP} + \text{AH}^\cdot$

Where AH_2 is an electron donor substrate and $\text{AH}^\cdot$ is the radical reaction product.¹⁰

In the first stage, two electrons are removed from the enzyme because of its interaction with hydrogen peroxide, with one from the iron atom and one from the porphyrin ring.¹⁰ This process involves the heterolytic cleavage of the H_2O_2 oxygen-oxygen bond, controlled by key active site residues His42 and Arg38.¹¹ His42 acts initially as a proton acceptor (base catalyst) and then as a donor (acid catalyst), while Arg38 helps align H_2O_2 in the active site.¹¹

The radicals formed during this catalytic cycle can cause significant damage to the enzyme itself through multiple mechanisms. Heme-modifying radicals formed in the catalytic reaction can interact with the protein moiety, contributing to enzyme inactivation.¹² Chemical modification of the polypeptide chain has been experimentally confirmed by introducing radioactive labels to corresponding substrates, with the proportion between modifications in the protoporphyrin and protein moiety substantially depending on the substrate type.¹²

Native HRP contains six lysine residues, plus a buried N-terminal α -amino group (which is blocked by pyrrolidone carboxylic acid)^[10]. These lysine residues present potential targets for radical-mediated modifications. Studies have shown that specific lysine residues (174, 232, and 241) can be selectively modified, altering the hydrophobic profile and charge distribution at the protein surface.¹² Beyond hydrogen peroxide, HRP can catalyse oxidation reactions with various reactive species, including peroxynitrite.¹³ The mechanism involves one-electron oxidation followed by radical disproportionation.¹³ Various reactive oxygen and nitrogen species play roles in these processes, including superoxide, hydrogen peroxide, peroxynitrite, hydroxyl radical, nitrogen dioxide radical, and carbonate radical anion.¹³

The radical reactions with amino acids in HRP can proceed through multiple pathways. Free radicals can act as shuttles for transferring oxidising equivalents from peroxidase intermediates to amino acid residues¹⁴. In studies of diiodotyrosine (DIT)-stimulated coupling, free DIT radicals functioned as shuttles for transferring oxidising equivalents from peroxidase intermediates to DIT residues in thyroglobulin.¹⁴

Polymer nano-object advanced characterisation and analysis.

Throughout the project, there have been opportunities to investigate the structure of the PDMAm₅₀-b-PDAAm₃₀₀ polymer nano-objects in greater detail. Some of these more advanced characterisation techniques could help provide more information about the structure of these polymersomes in their native aqueous environments. This section of the future work will detail some techniques that could be used to further study these systems.

Small Angle X-ray Scattering (SAXS)

SAXS is a technique by which electron density differences in a sample can be quantified. This means that it can resolve the size and shape of (monodisperse) macromolecules, determine pore sizes, characteristic distances of partially ordered materials, and much more. This is achieved by analysing the elastic scattering behaviour of X-rays when travelling through the material, recording their scattering at small angles (typically $0.1 - 10^\circ$). It is typically done using hard X-rays with a wavelength of $0.07 - 0.2$ nm. Depending on the angular range in which a clear scattering signal can be recorded, SAXS can deliver structural information of dimensions between 1 and 100 nm, and of repeat distances in partially ordered systems of up to 150 nm.

In the context of the PDMAm₅₀-b-PDAAm₃₀₀ polymersomes, Small-angle X-ray scattering provides detailed information about the polymersome bilayer, but due to the need for access to large-scale radiation facilities, their use for routine measurements for this purpose is somewhat limited. With SAXS, particle shape and size information are collected by monitoring the elastic X-ray or neutron scattering at low angles ($0.1-10^\circ$). X-rays interact with the electron clouds of molecules or elements. These SAXS experiments typically provide information about the polymersome bilayer thickness, size, and morphology of the PDMAm₅₀-b-PDAAm₃₀₀ polymersomes.

A proposed experiment would be to make up solutions of each polymer sample and run the samples through a stopped-flow SAXS instrument. This will allow for the collection of scattering data, which will provide insight into the size, shape, and bilayer characteristics of these polymersomes in their native aqueous environments.

6.1.6. Cryo-TEM

Another technique that could provide detailed information about the PDMAm₅₀-b-PDAAm₃₀₀ polymersomes is cryo-TEM. Unlike negative staining TEM, cryo-TEM provides more detail in the images and can be used to look directly at the bilayer of these polymersomes. During cryo-TEM, samples are cooled to cryogenic temperatures. An aqueous polymersome sample solution is applied to a grid-mesh and plunge-frozen in liquid ethane or a mixture of liquid ethane and propane. The great drawback is that TEM only works under vacuum conditions; Cryo-TEM overcomes this obstacle. To overcome vacuum-induced sample deformation, the sample is quick-frozen in liquid alkanes in order to capture it in its original shape in liquid solution and observed at -180 °C. This provides a more accurate imaging of the PDMAm₅₀-b-PDAAm₃₀₀ polymersomes in their native aqueous environment, without drying artefacts.

A proposed experiment would be to take the polymersome samples generated via PISA and run them on cryo-TEM to look at the structure of the bilayer as well as the shape and size of these polymersomes in their native aqueous environment. Also, a few key samples from the SE would need to be looked at in more depth, namely the mixed polymersome/micelle sample made at pH 2.5, which will be proposed to run under cryo-TEM. Single-particle analysis cryo-EM is a powerful method to get high-resolution electron density profiles of these polymer nano-objects, as highlighted by Seneviratne and co-workers.⁴ This method could be ideal for application to the polymer nano-objects fabricated in this body of work.

6.1.7. EDX-TEM

Energy Dispersive X-Ray Analysis (EDX) is an X-ray technique used to identify the elemental composition of materials. EDX systems are attachments to Electron Microscopy instruments, such as transmission electron microscopy (TEM) instruments, where the imaging capability of the microscope identifies the specimen of interest. The data generated by EDX analysis consists of spectra showing peaks corresponding to the elements making up the true composition of the sample being analysed. Elemental mapping of a sample and image analysis are also possible.

During the POPC lipid SE of PDMAm₅₀-b-PDAAm₃₀₀, there were a few more exotic structures with light staining, which, from the difference compared to normally stained PDMAm₅₀-b-PDAAm₃₀₀ polymer, were suspected of being pure POPC regions. To clarify this further, a proposed EDX experiment should be conducted, which would highlight the phosphorus atoms uniquely belonging to the POPC lipid. This would help to provide information about how the POPC lipid affects the self-assembly of the PDMAm₅₀-b-PDAAm₃₀₀ block copolymer during SE experiments, and if there is a separation between the two different amphiphiles.

6.1.8. References

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7. Appendix

7.1 TEM supplementary information:

Chapter 3 Supplementary TEM images:

PISA PDMAm₅₀-b-PDAAm₁₀₀

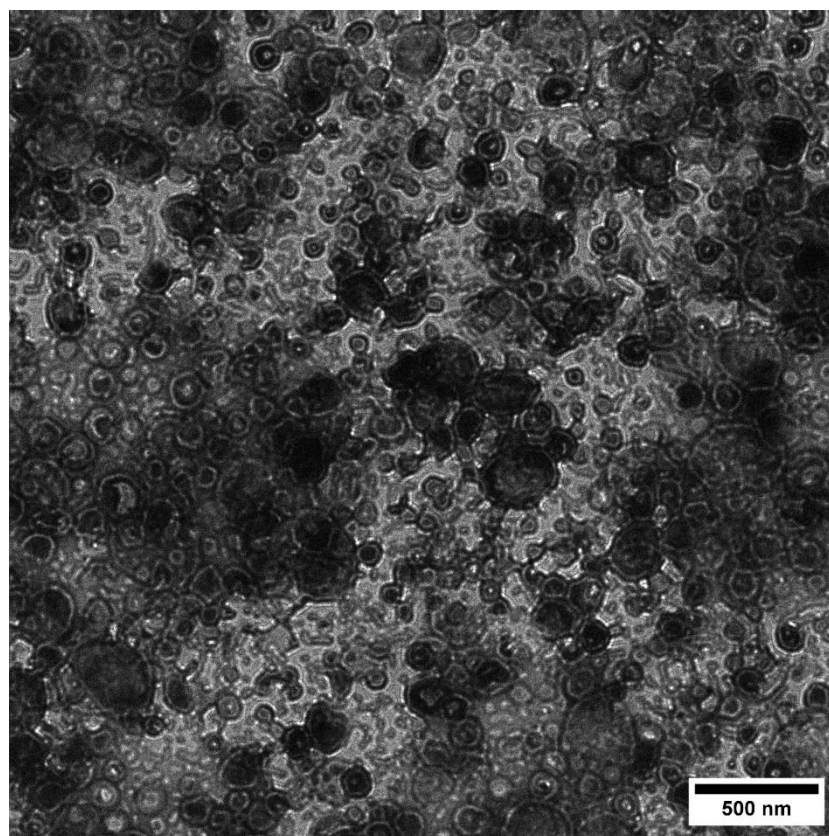


Figure 7.1: TEM micrograph for PDMAm₅₀-b-PDAAm₁₀₀ synthesised via PISA at pH 2.5

PISA PDMAm₅₀-b-PDAAm₂₀₀

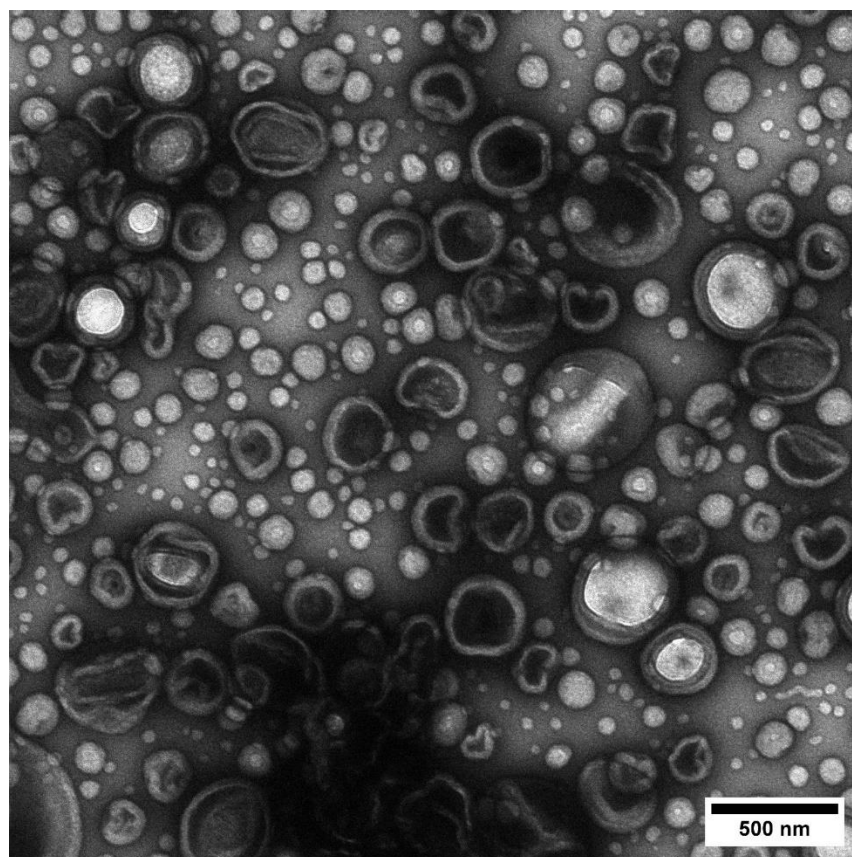


Figure 7.2. – TEM micrograph for PDMAm₅₀-b-PDAAm₂₀₀ synthesised via PISA at pH 2.5

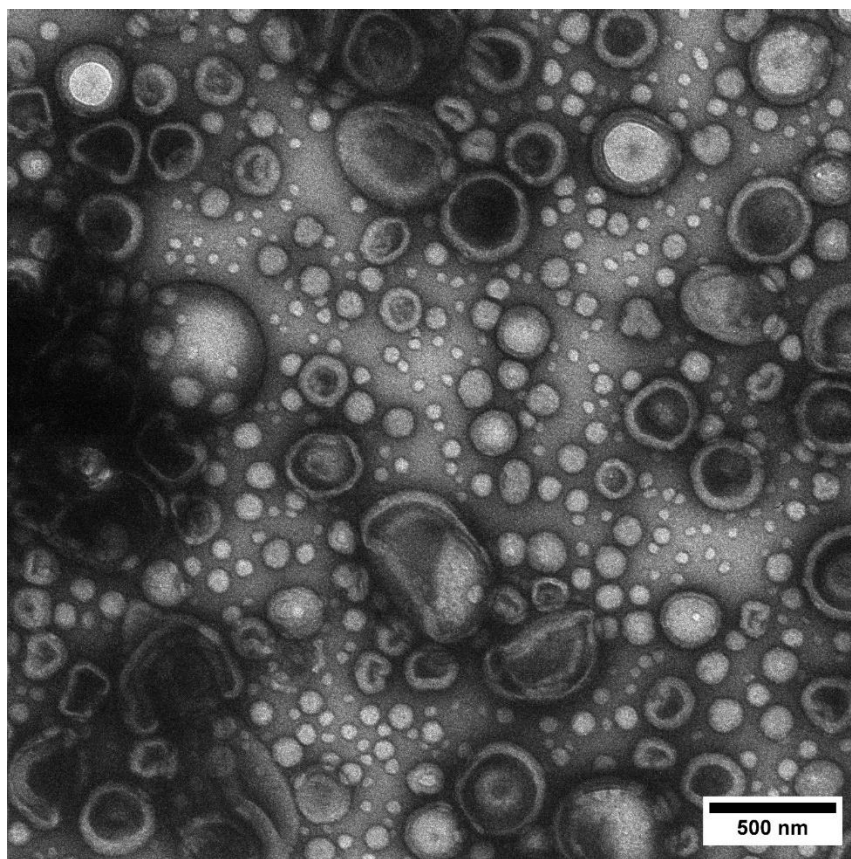


Figure 7.3.: – TEM micrograph for PDMA₅₀-b-PDAAm₂₀₀ synthesised via PISA at pH 2.5

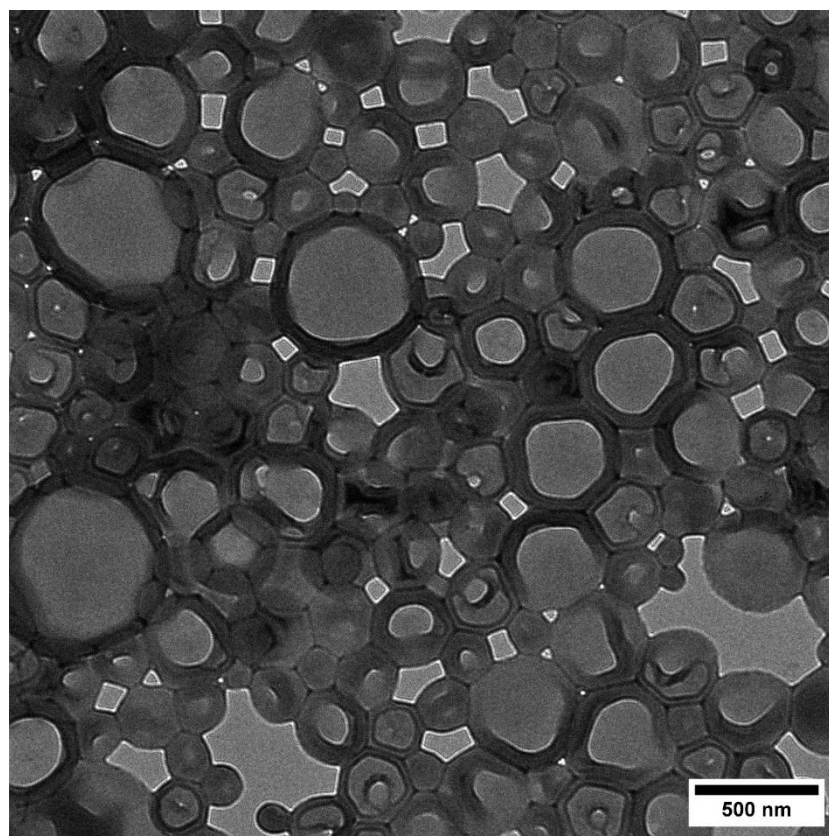


Figure 7.4: TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 2.5

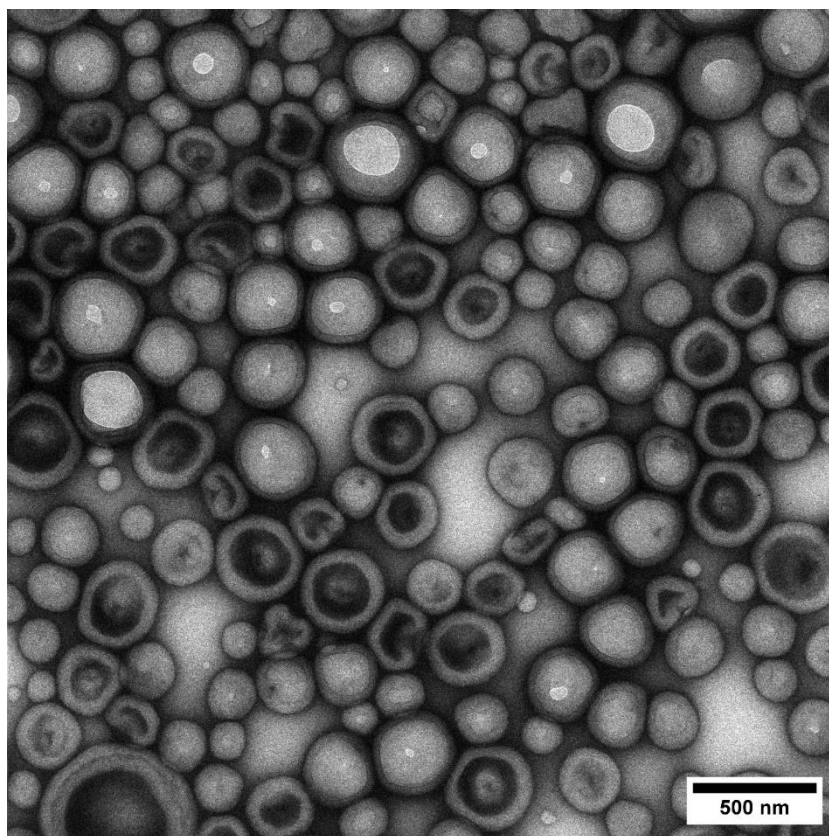


Figure 7.5: TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 2.5

PISA PDMAm₅₀-b-PDAAm₄₀₀

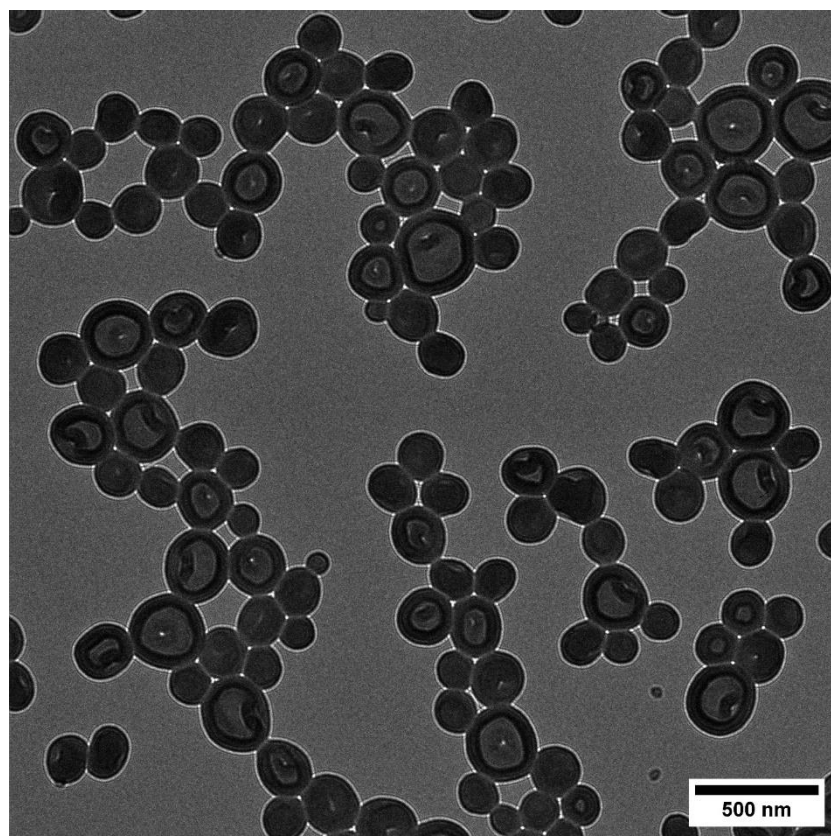


Figure 7.6: TEM micrograph for PDMAm₅₀-b-PDAAm₄₀₀ synthesised via PISA at pH 2.5

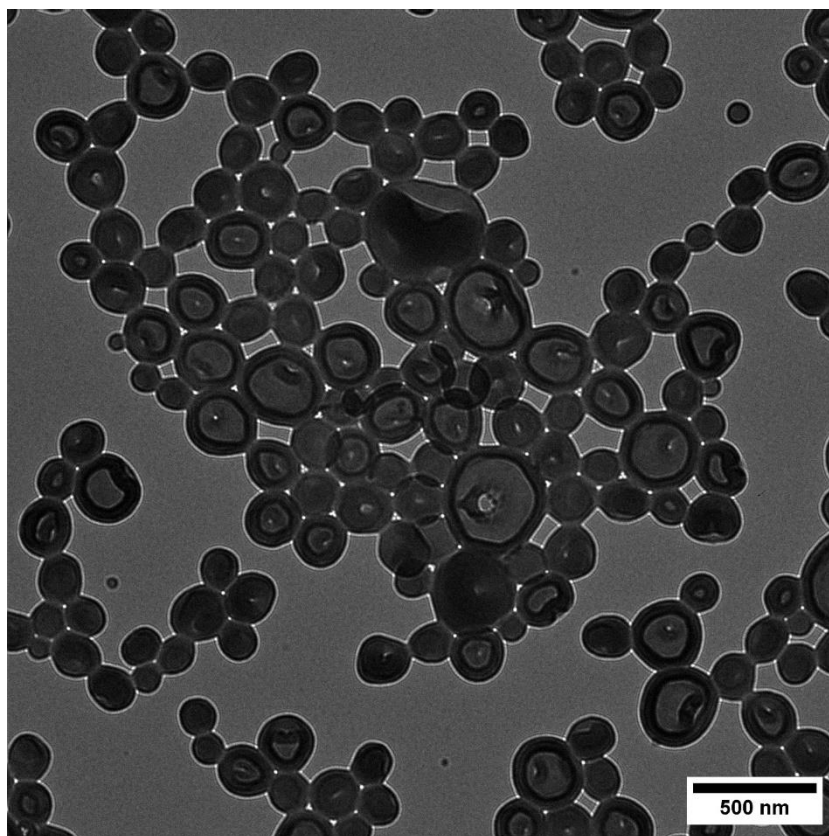


Figure 7.7: TEM micrograph for PDMAm₅₀-b-PDAAm₄₀₀ synthesised via PISA at pH 2.5

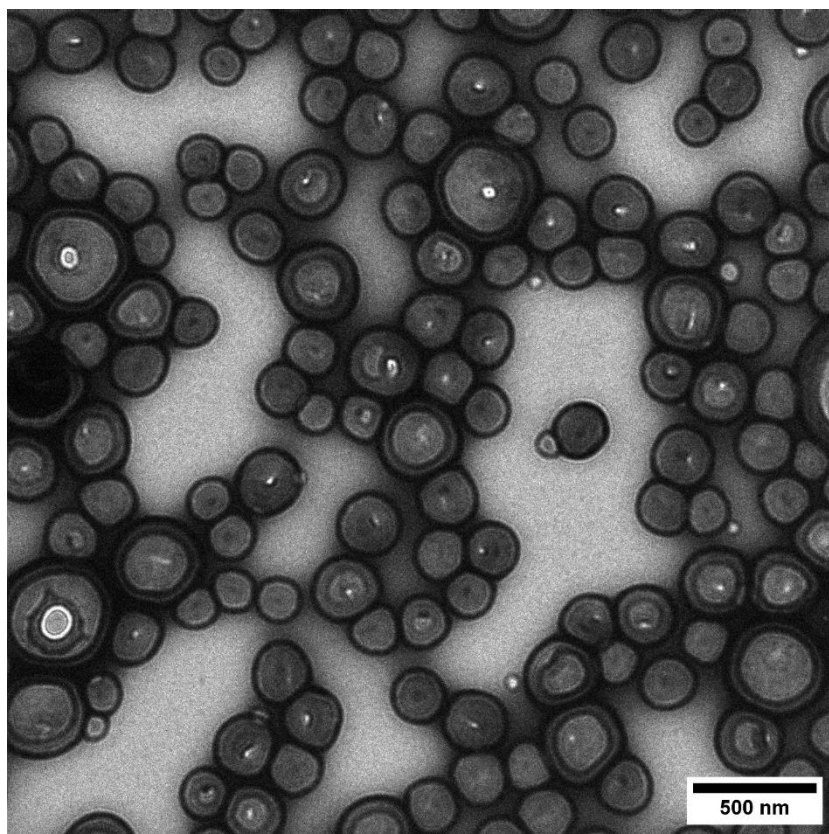


Figure 7.8: TEM micrograph for PDMA₅₀-b-PDAAm₄₀₀ synthesised via PISA at pH 2.5

PISA PDMAm₅₀-b-PDAAm₃₀₀ at pH 7.5

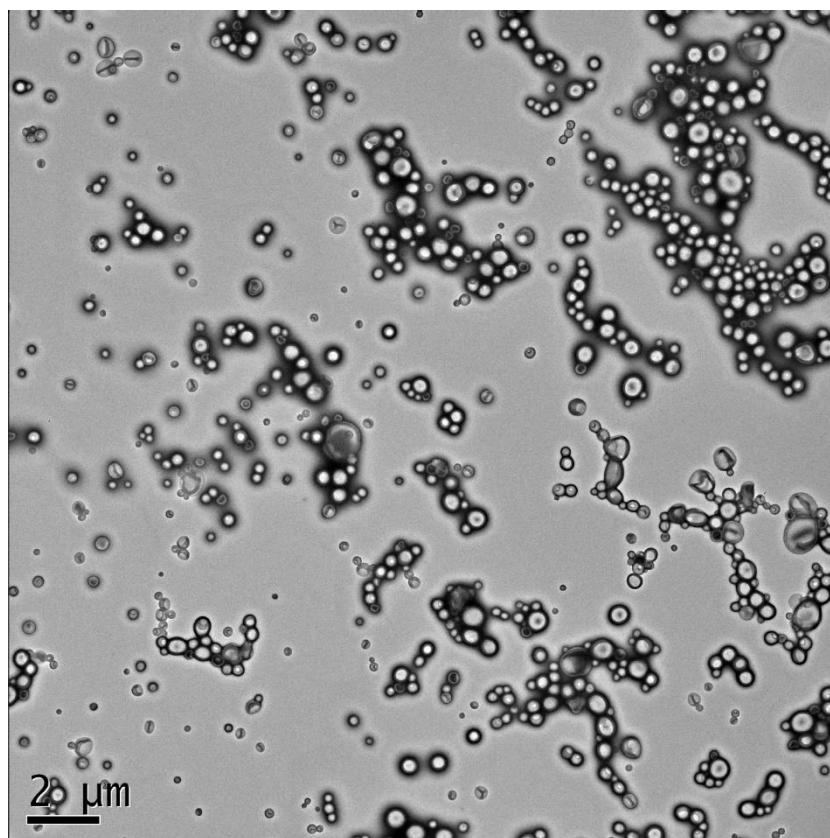


Figure 7.9: TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 7.5

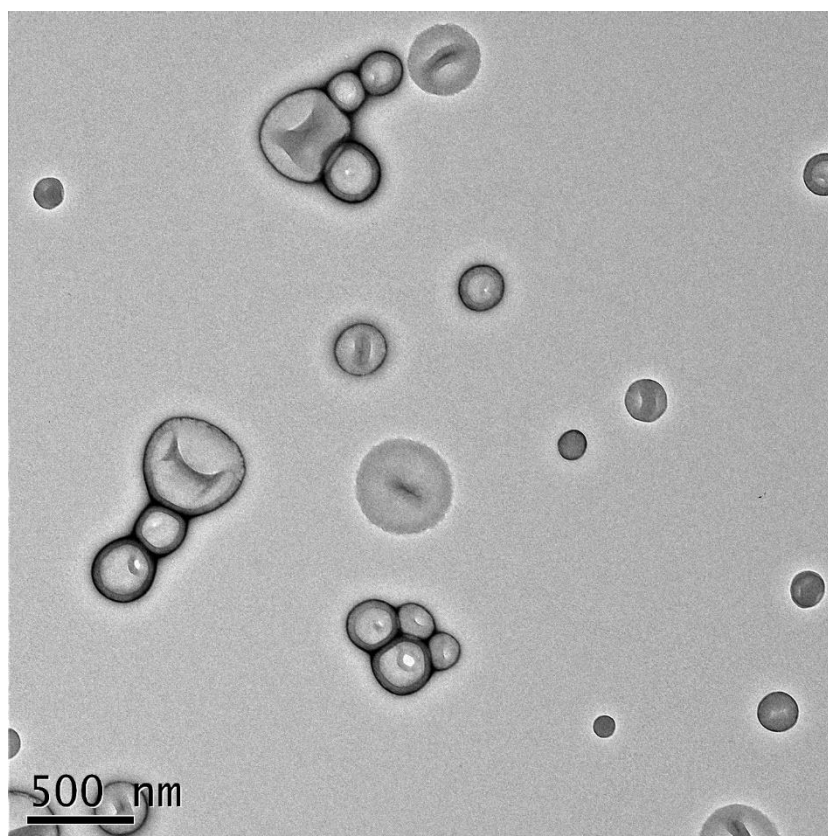


Figure 7.10: TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 7.5

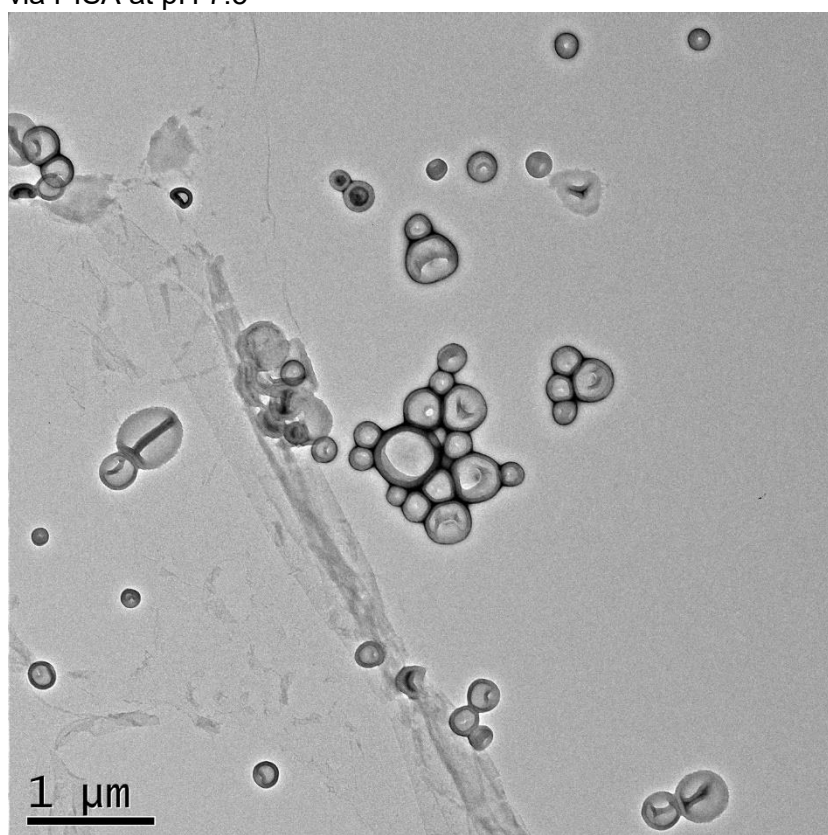


Figure 7.11: TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 7.5

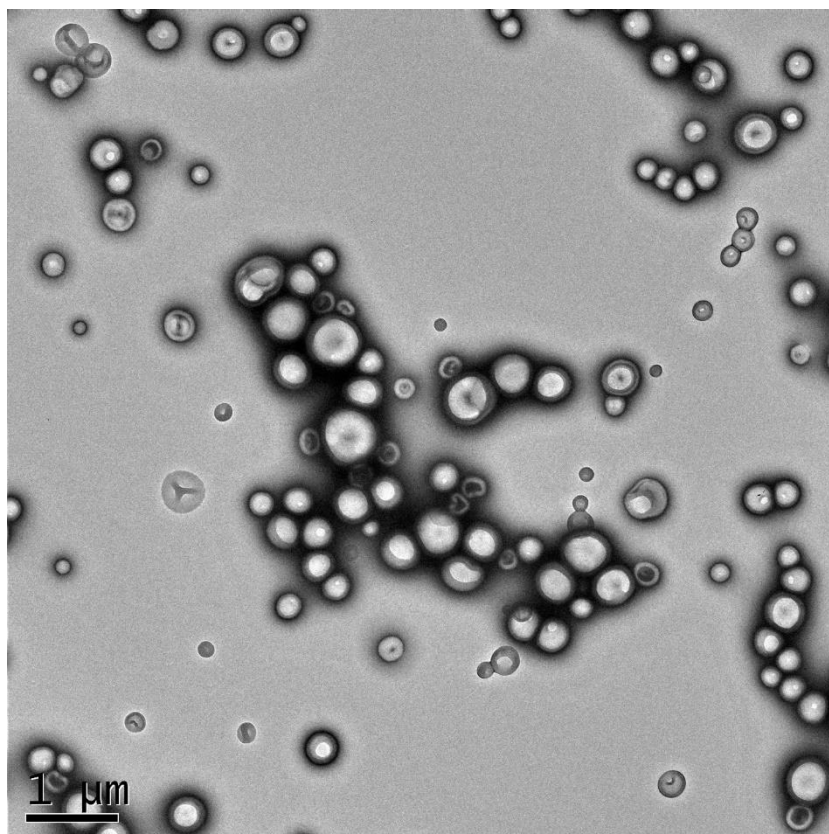


Figure 7.12 TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 7.5

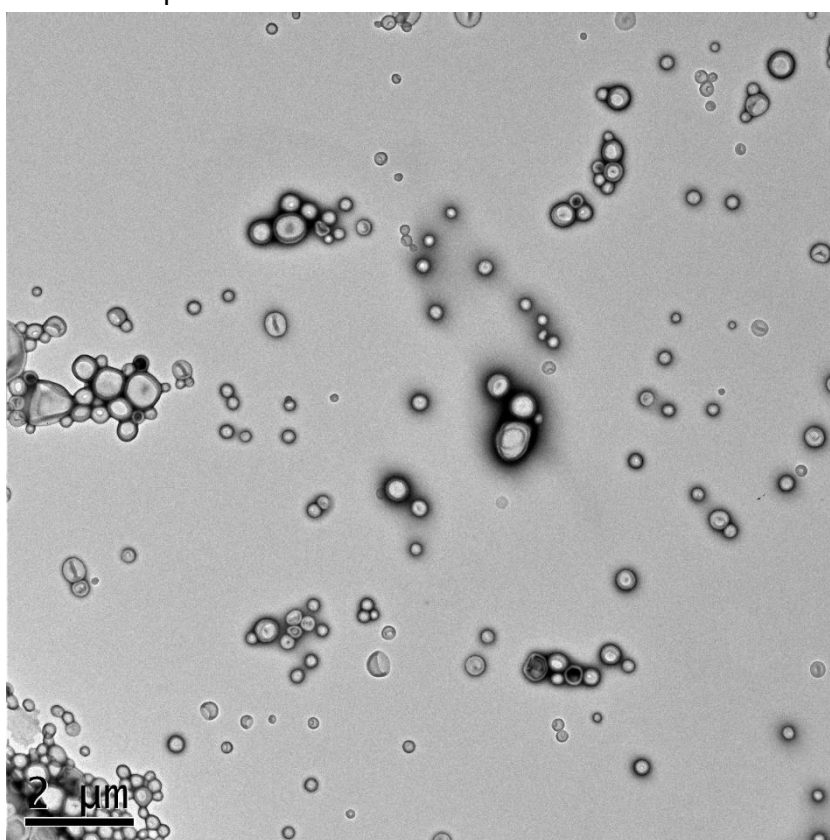


Figure 7.13: TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 7.5

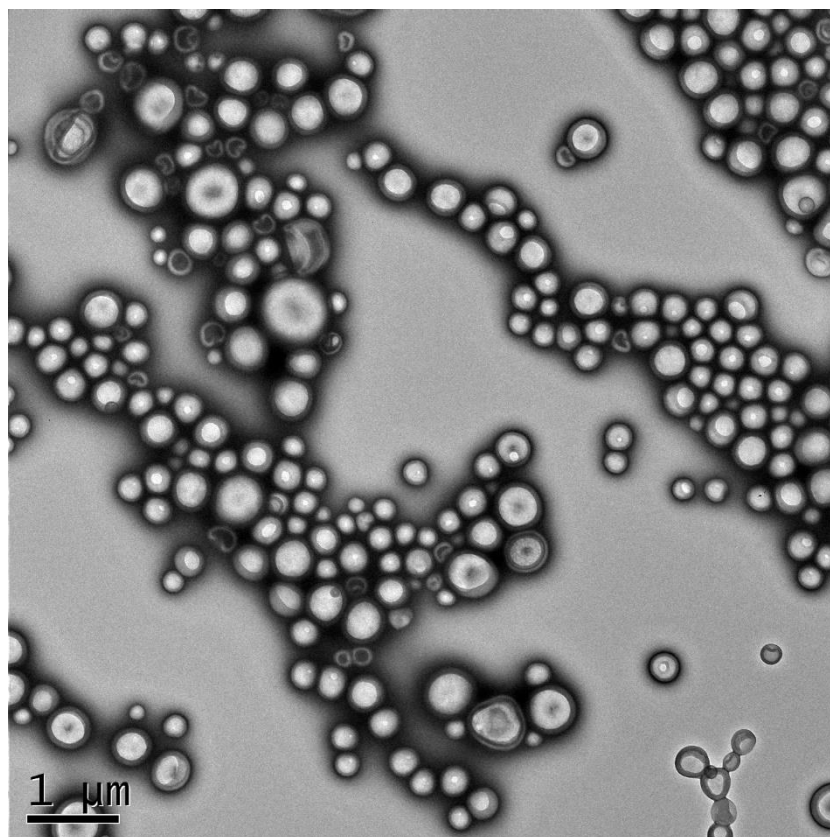


Figure 7.13: TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 7.5

PISA PDMAm₅₀-b-PDAAm₃₀₀ at pH 7.5 with 140 mM NaCl

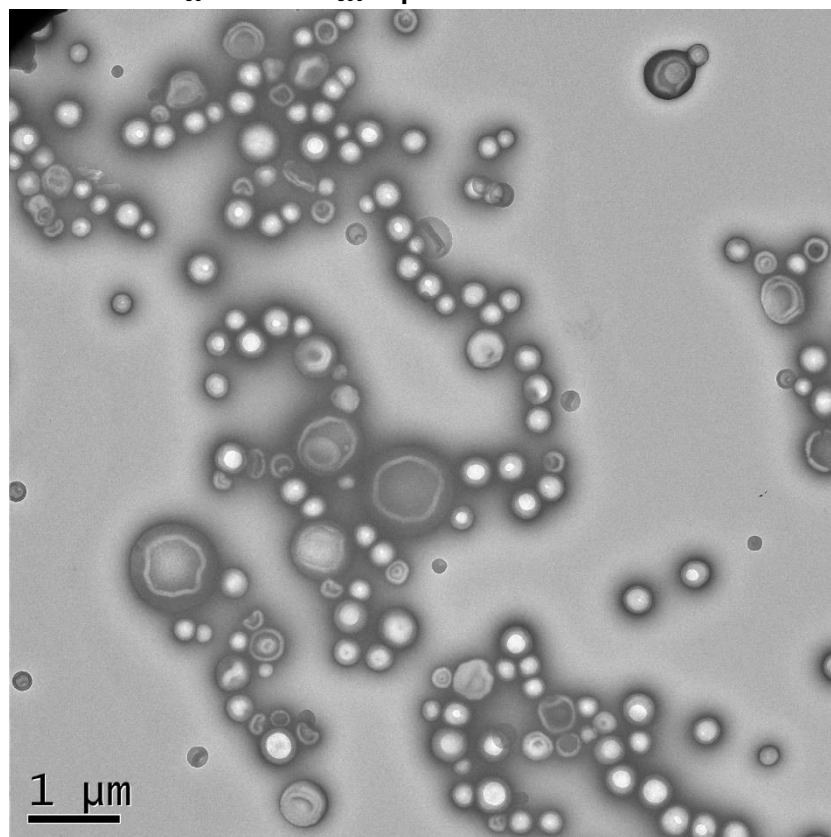


Figure 7.14: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 7.5 with 140 mM NaCl

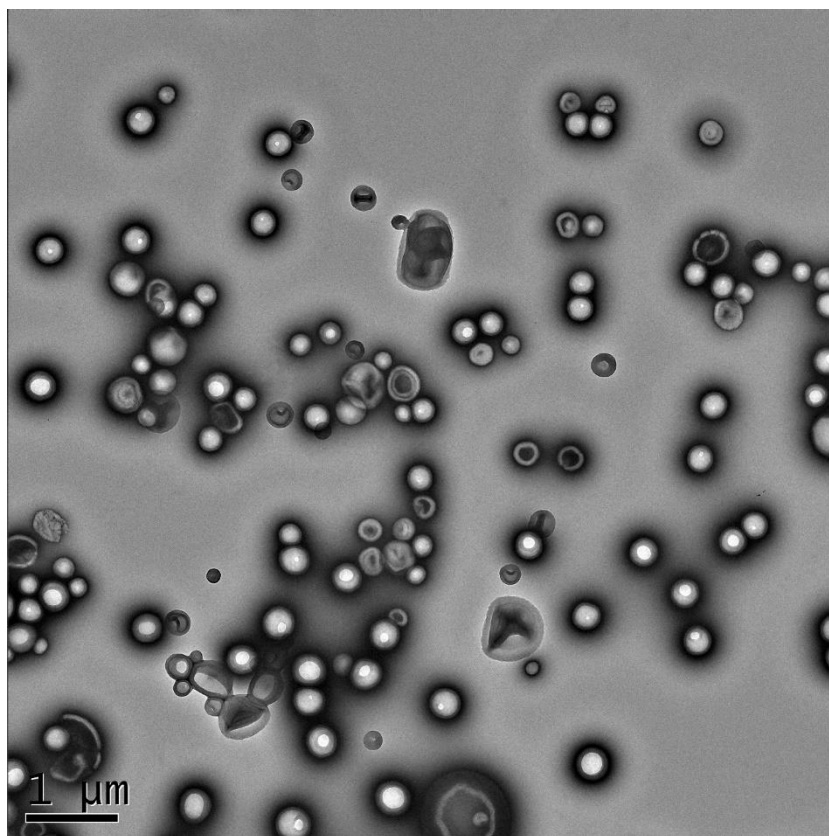


Figure 7.15: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 7.5 with 140 mM NaCl

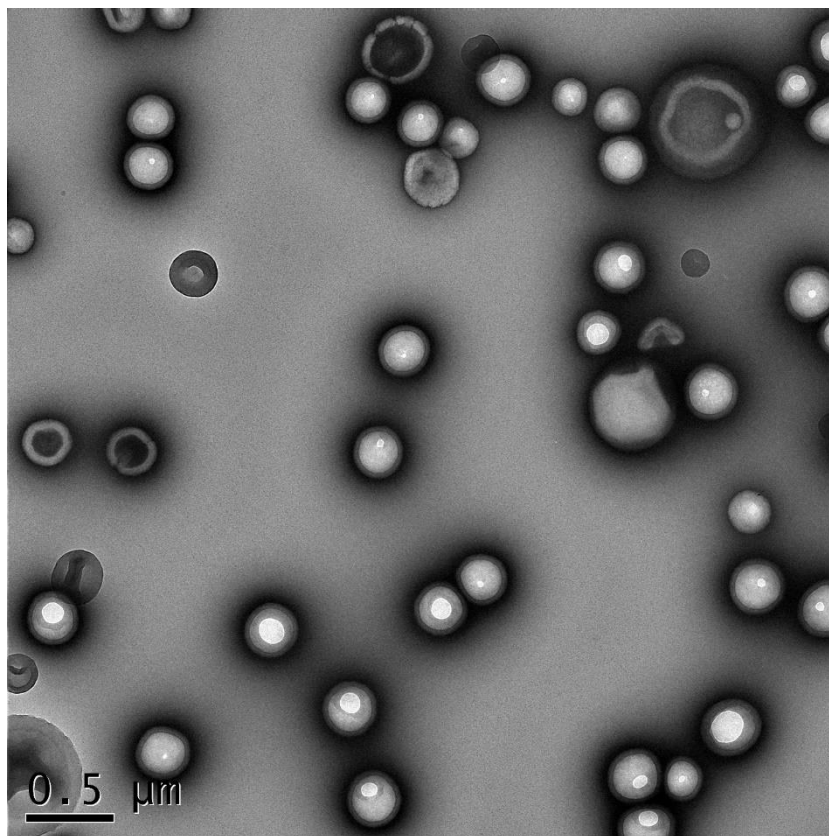


Figure 7.18: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 7.5 with 140 mM NaCl

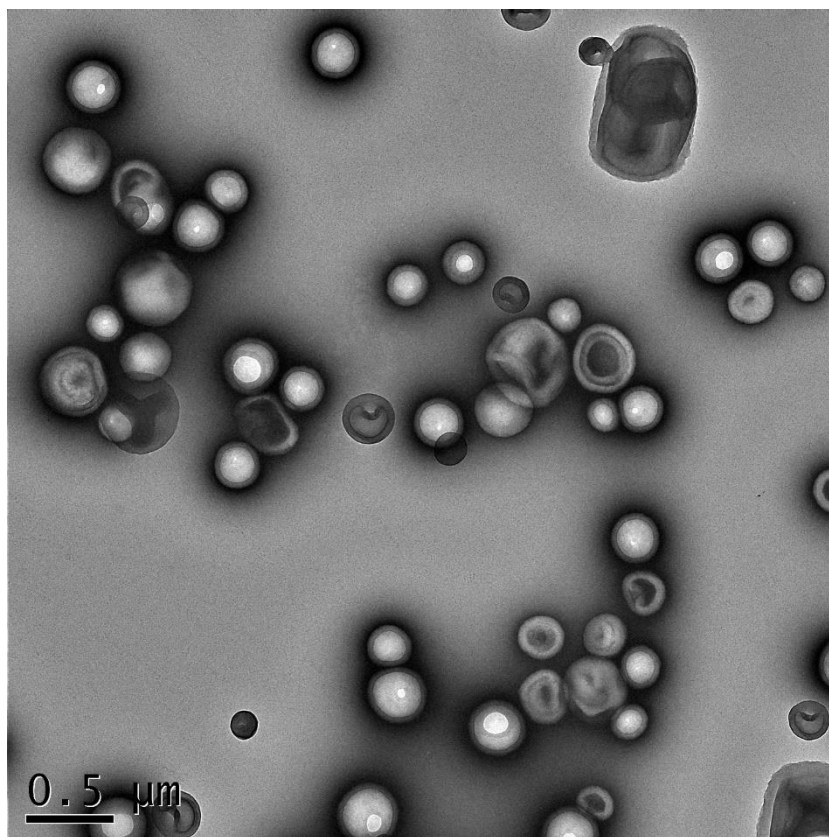


Figure 7.19: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 7.5 with 140 mM NaCl

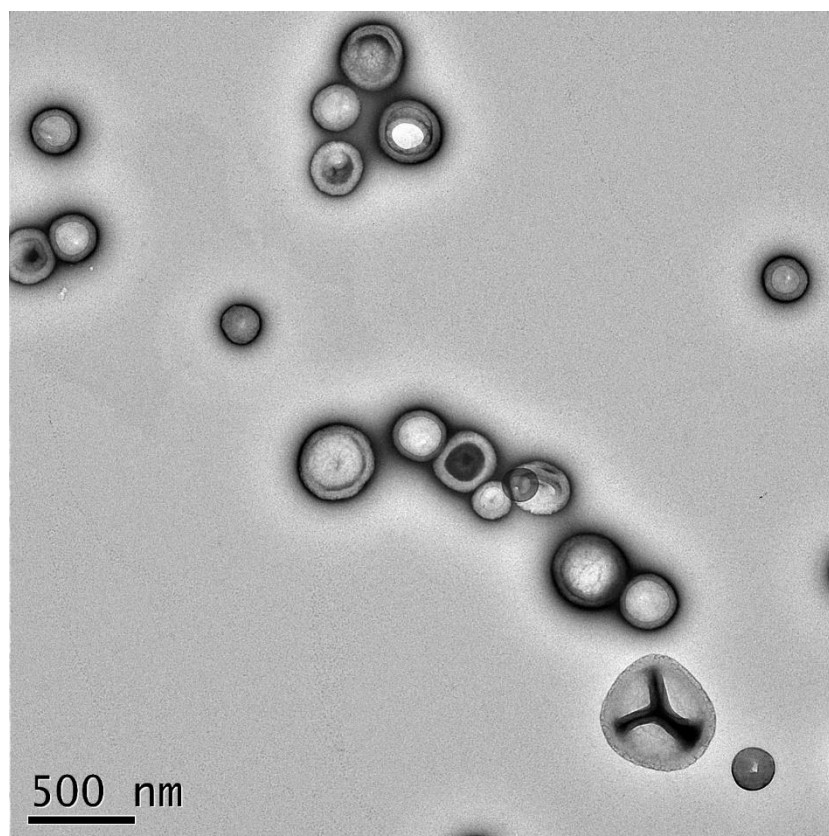


Figure 7.20: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 7.5 with 140 mM NaCl

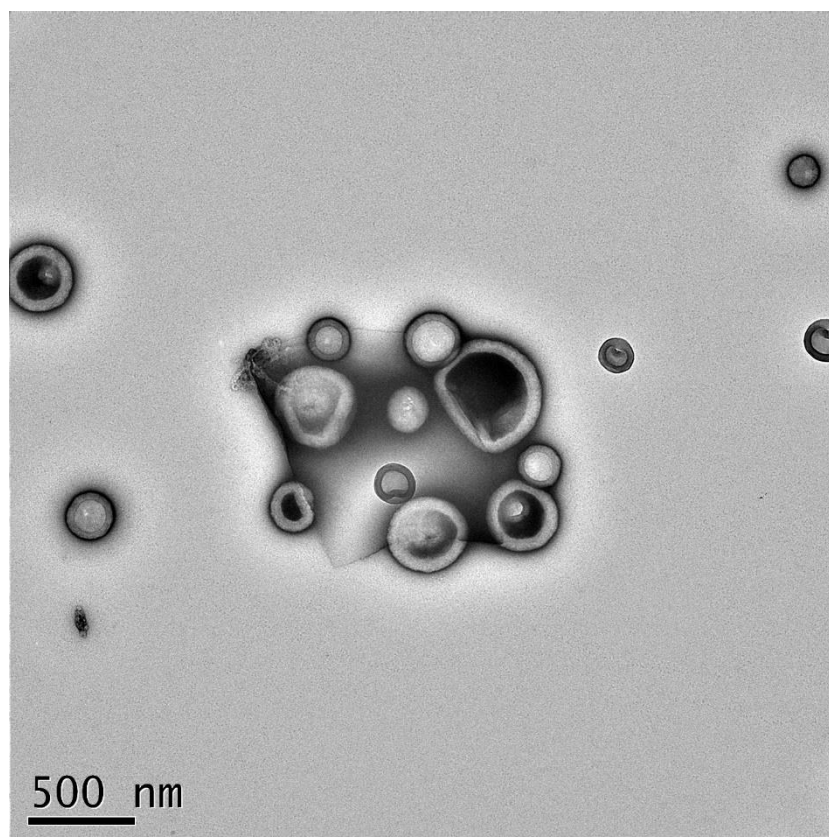


Figure 7.21: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 7.5 with 140 mM NaCl

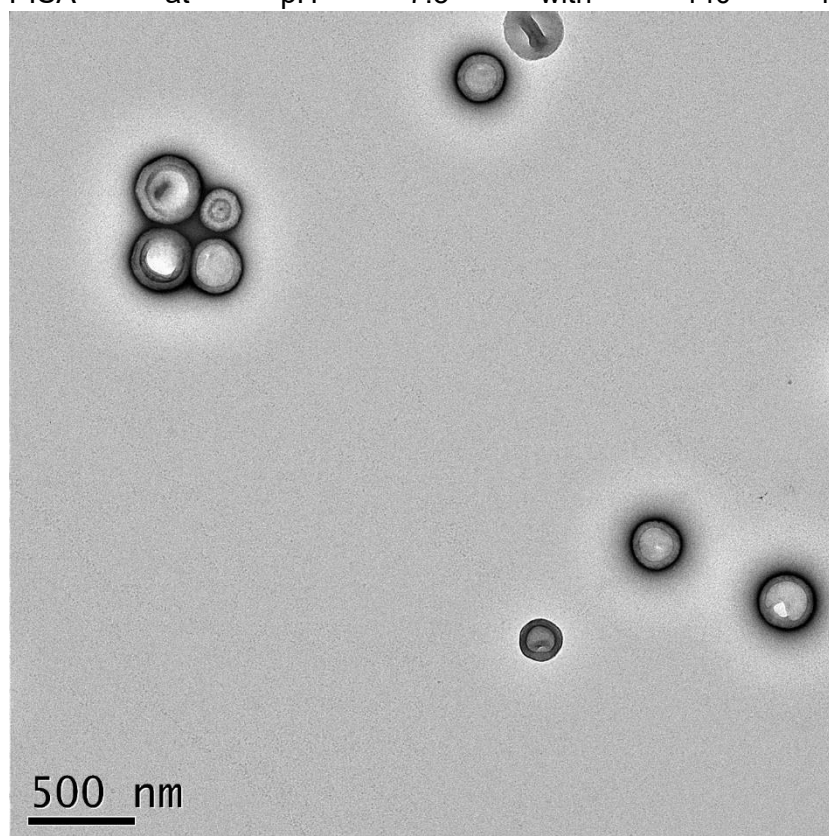


Figure 7.22: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 7.5 with 140 mM NaCl

PISA PDMAm₅₀-b-PDAAm₃₀₀ in PBS

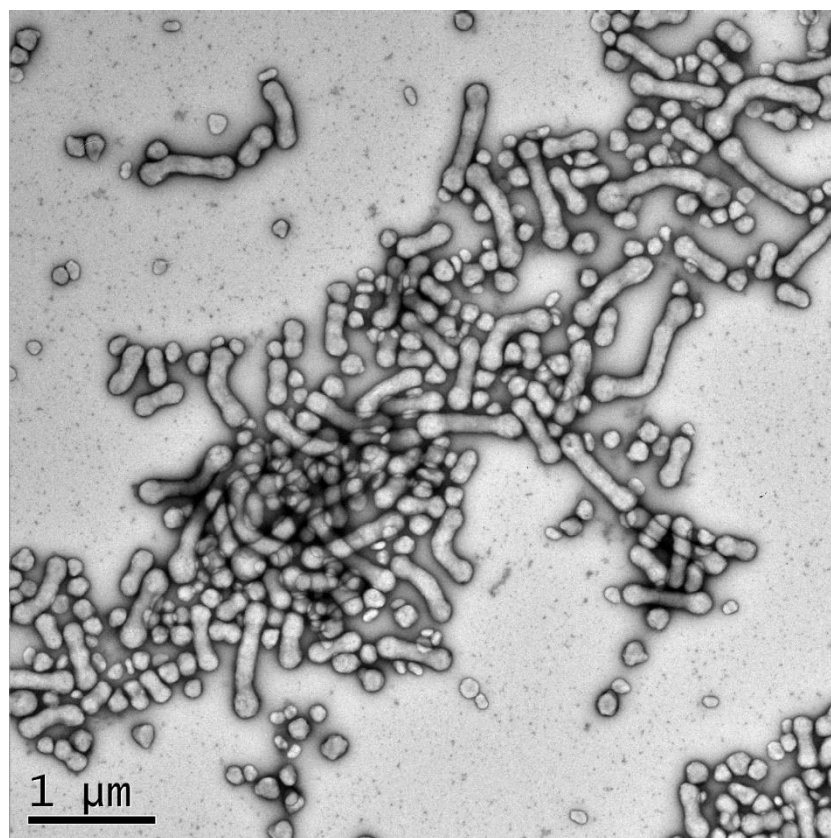


Figure 7.23: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 7.5 buffered with PBS

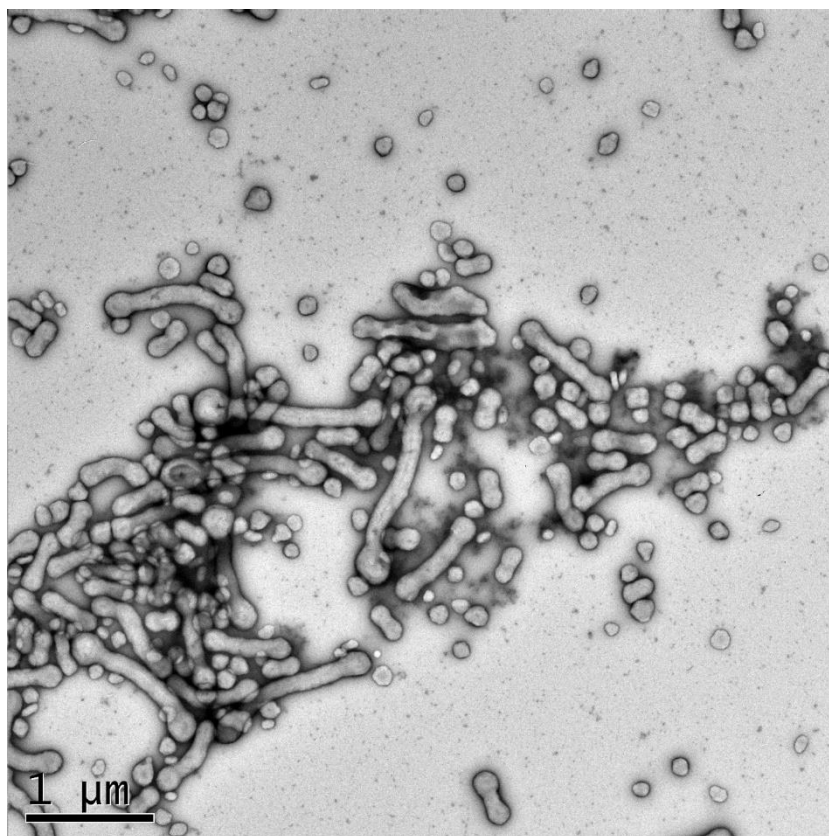


Figure 7.24: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 7.5 buffered with PBS

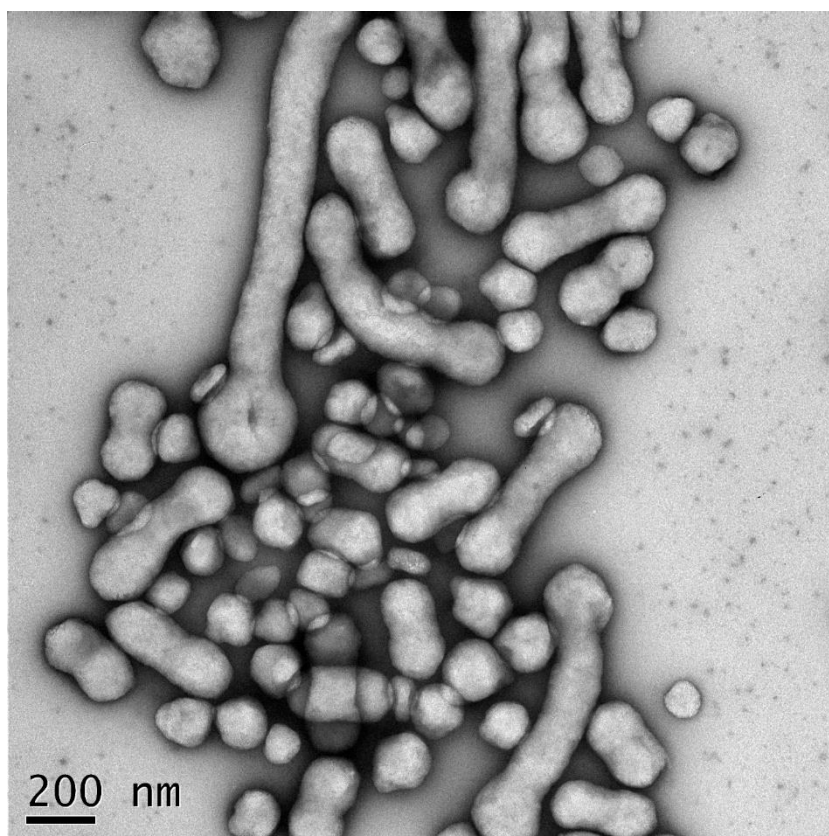


Figure 7.25: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 7.5 buffered with PBS

Chapter 4 Supplementary TEM images:

PISA PDMAm₅₀-b-PDAAm₃₀₀ rehydrated in pH 7.5 water

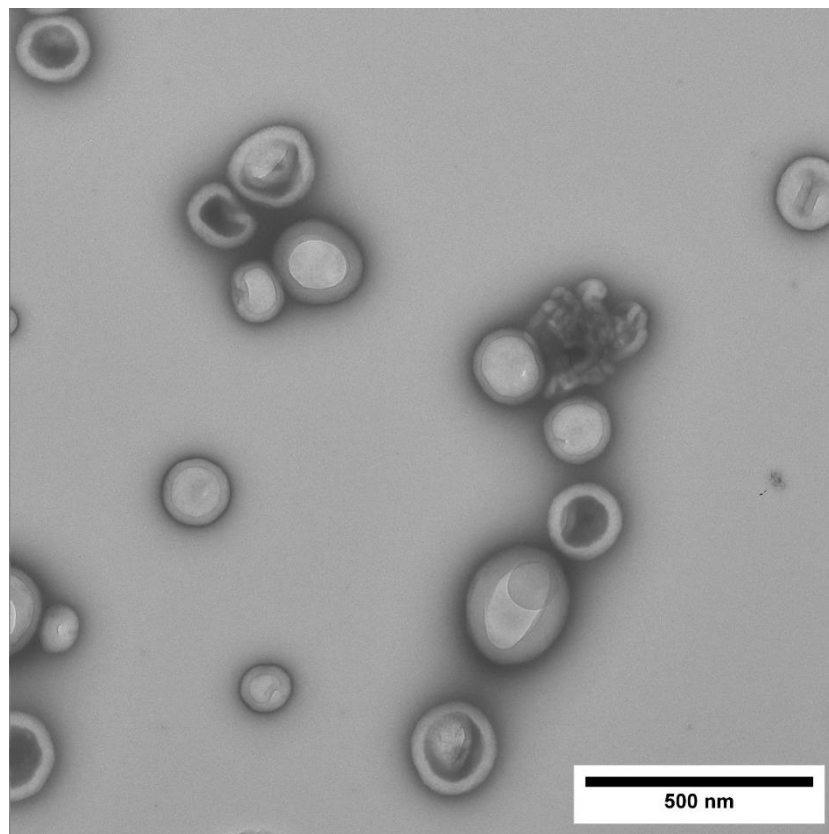


Figure 7.26: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 2.5, lyophilised and dehydrated at pH 7.5

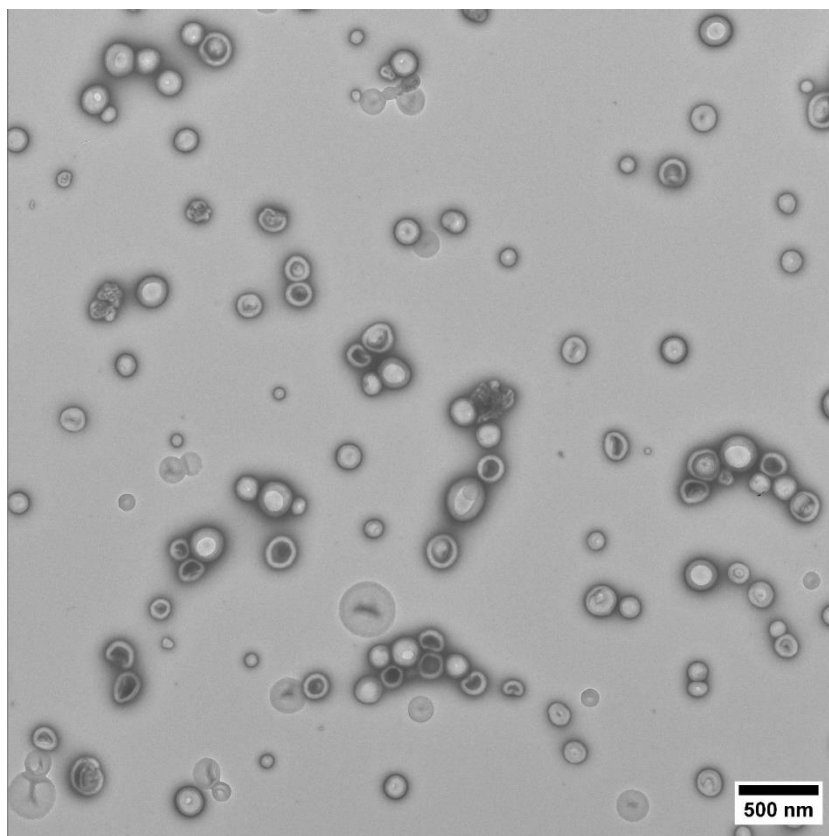


Figure 7.27: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 2.5, lyophilised and dehydrated at pH 7.5

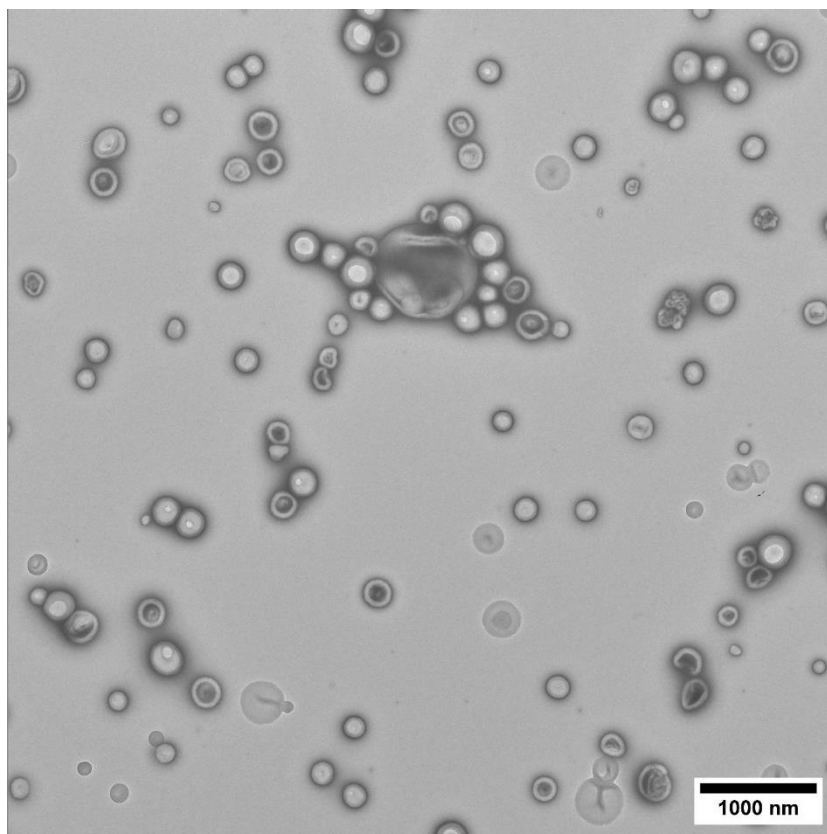


Figure 7.28: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 2.5, lyophilised and dehydrated at pH 7.5

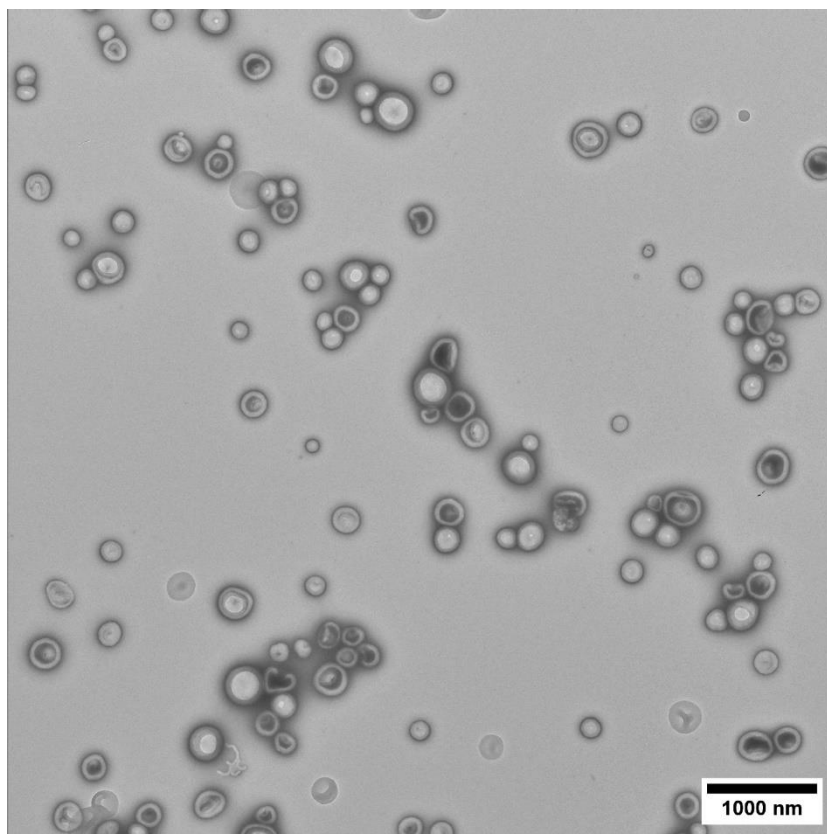


Figure 7.29: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 2.5, lyophilised and dehydrated at pH 7.5

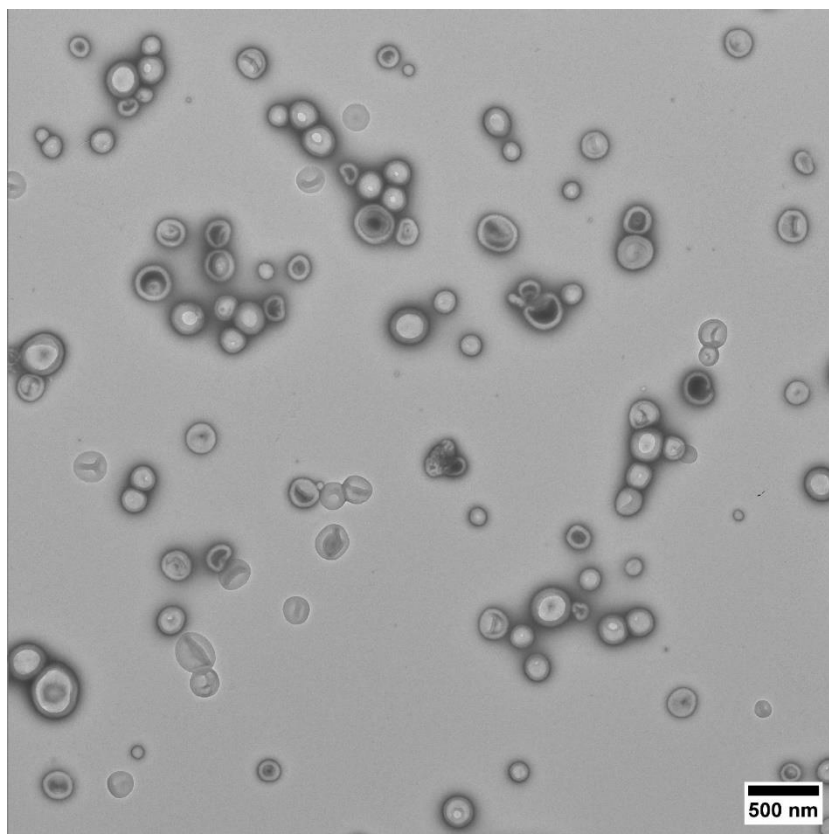


Figure 7.30: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 2.5, lyophilised and dehydrated at pH 7.5

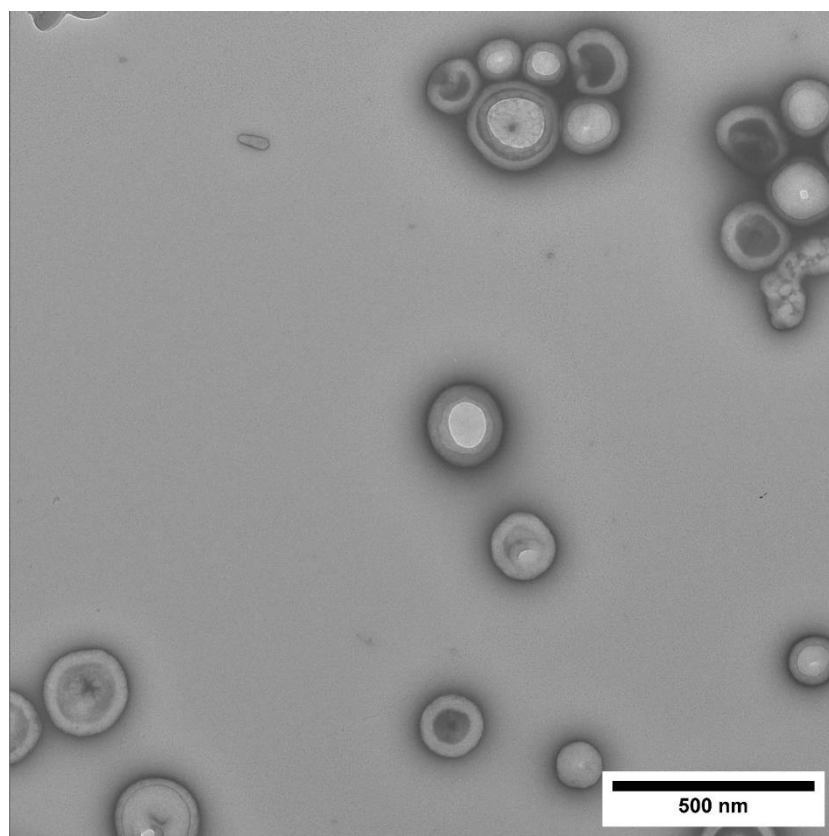


Figure 7.31 – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 2.5, lyophilised and dehydrated at pH 7.5

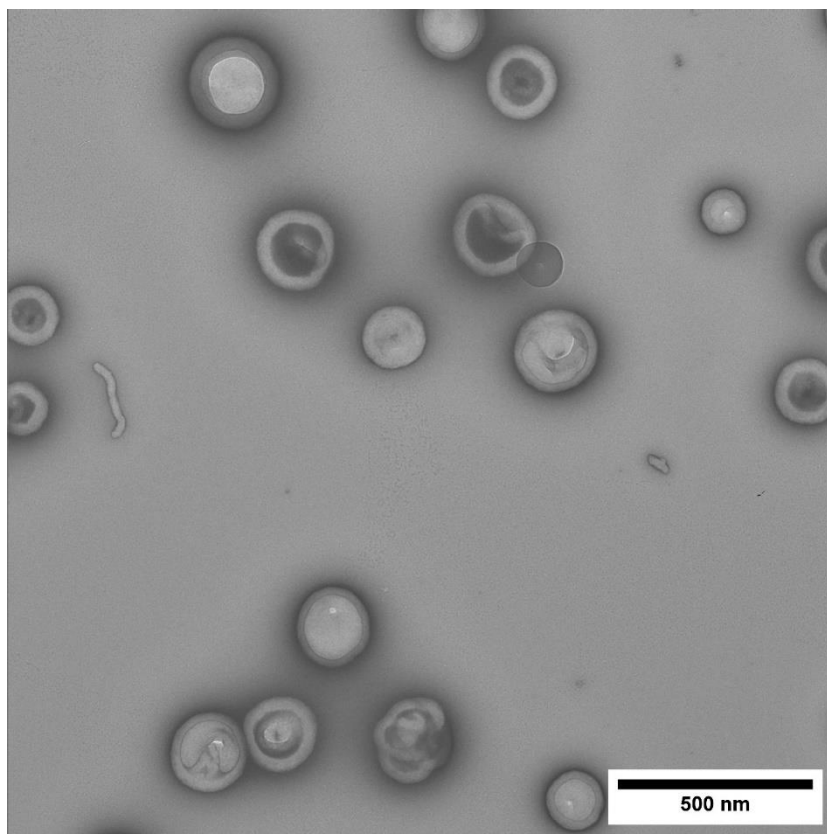


Figure 7.32: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 2.5, lyophilised and dehydrated at pH 7.5

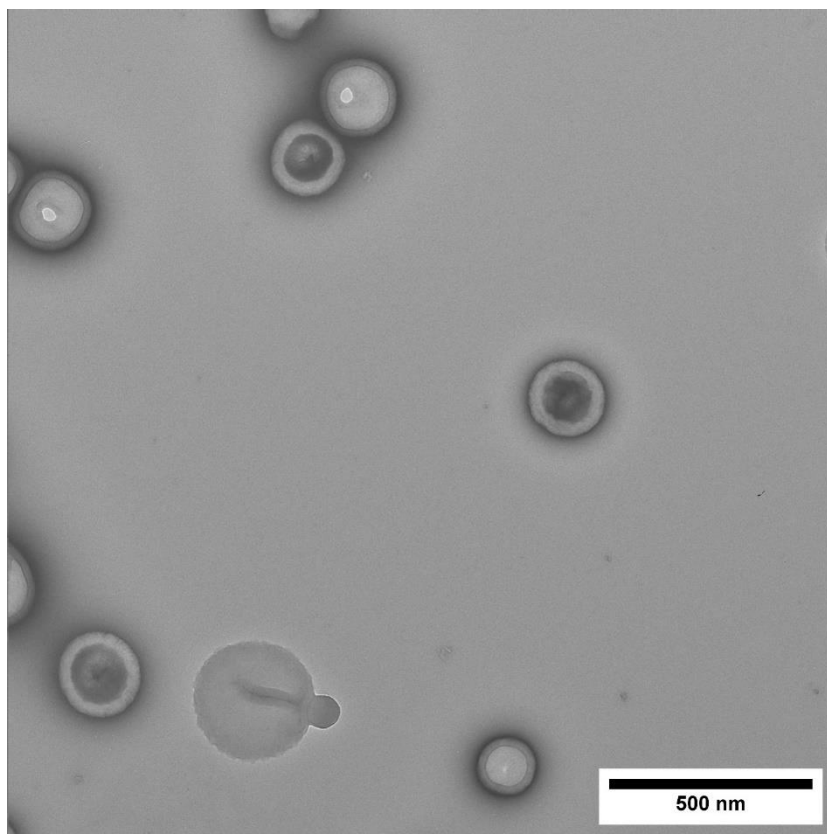


Figure 7.33: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ synthesised via PISA at pH 2.5, lyophilised and dehydrated at pH 7.5

SE PDMAm₅₀-b-PDAAm₃₀₀ 10% MeOH - 33 μ L/min

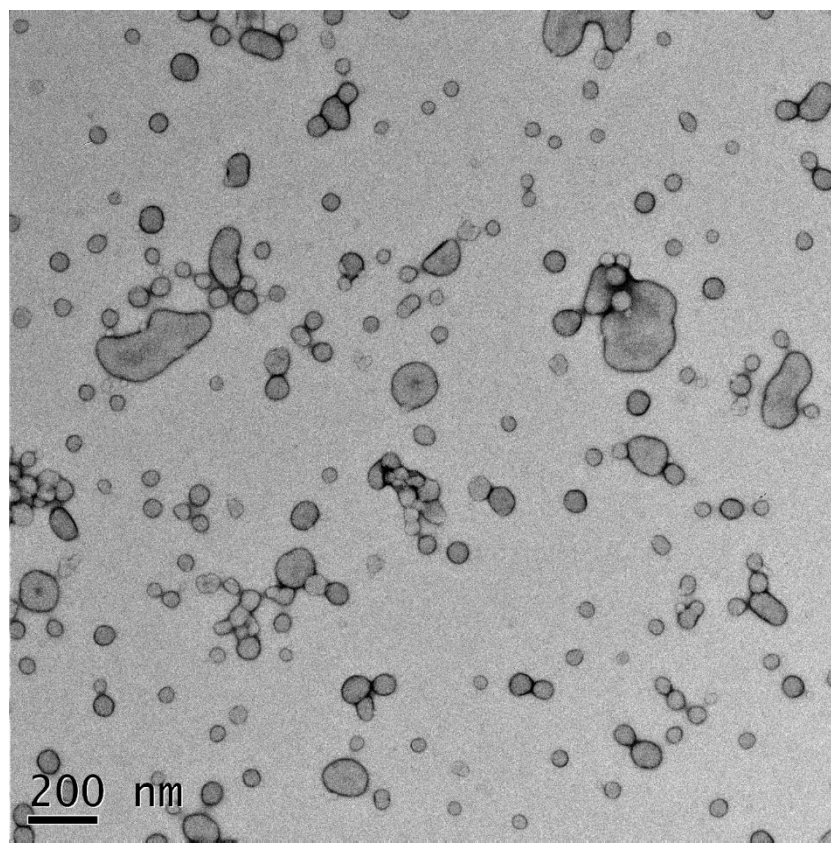


Figure 7.34: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH - 33 μ L/min)

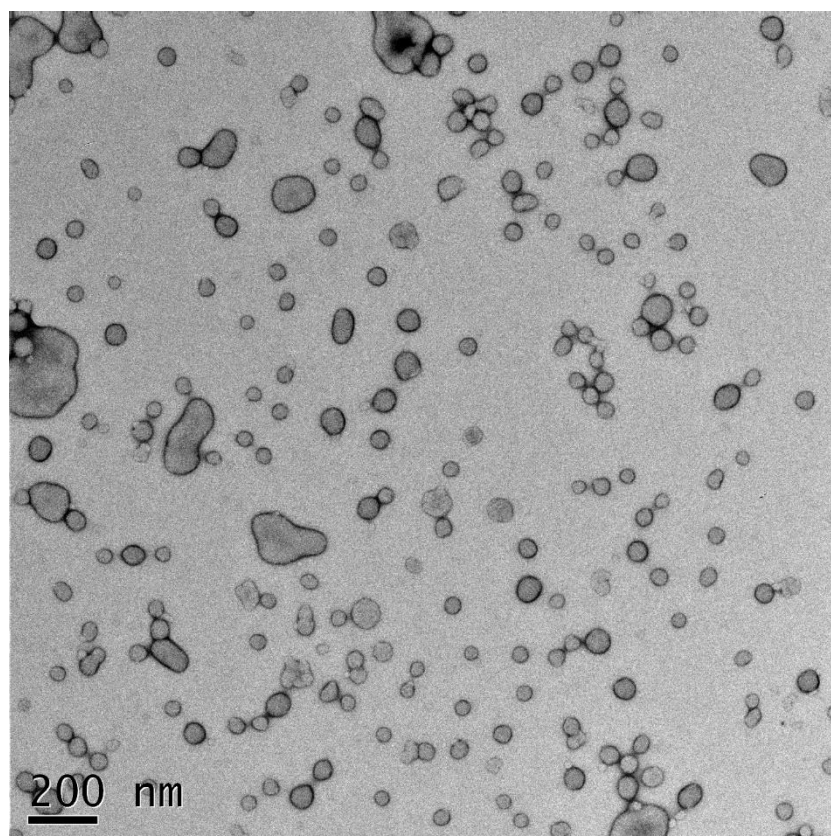


Figure 7.35: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH - 33 μ L/min)

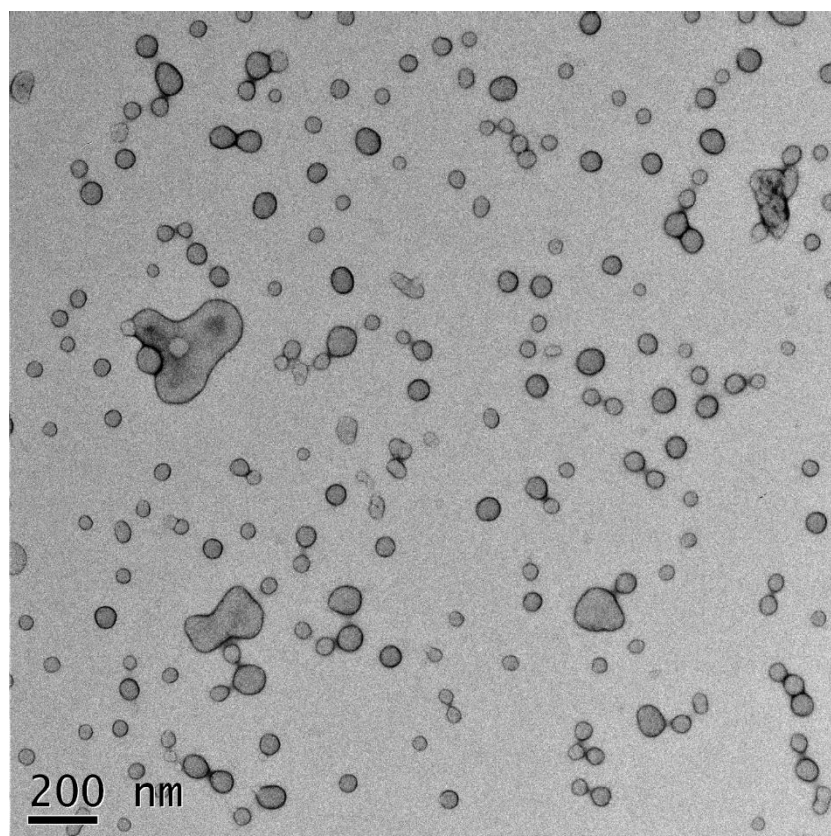


Figure 7.36: –TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH - 33 μ L/min)

SE PDMAm₅₀-b-PDAAm₃₀₀ 10% MeOH – 333 μ L/min

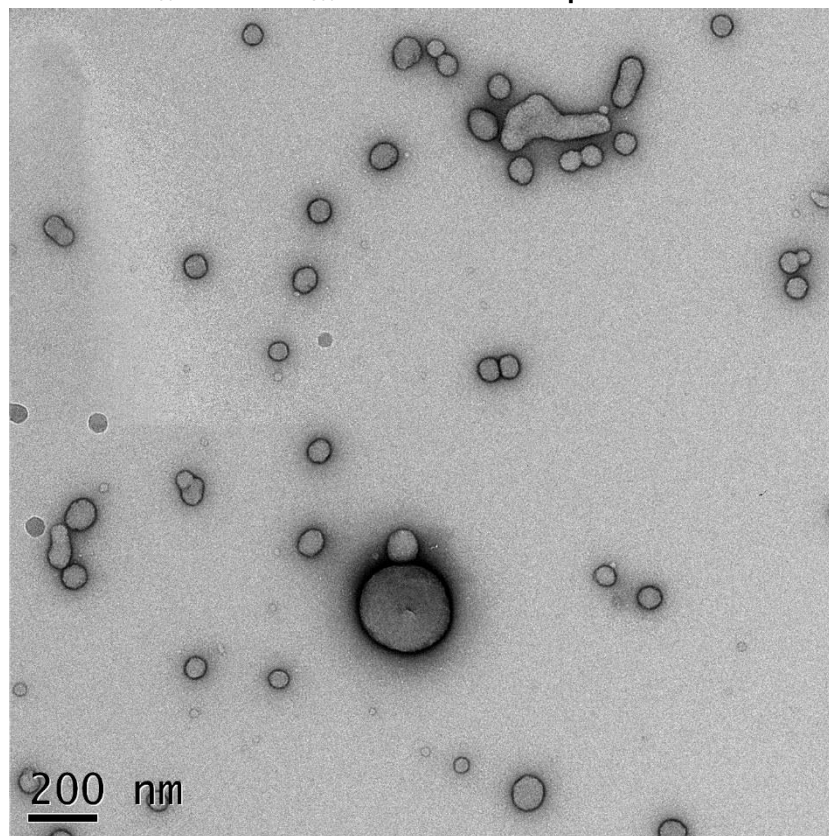


Figure 7.37: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH - 333 μ L/min)

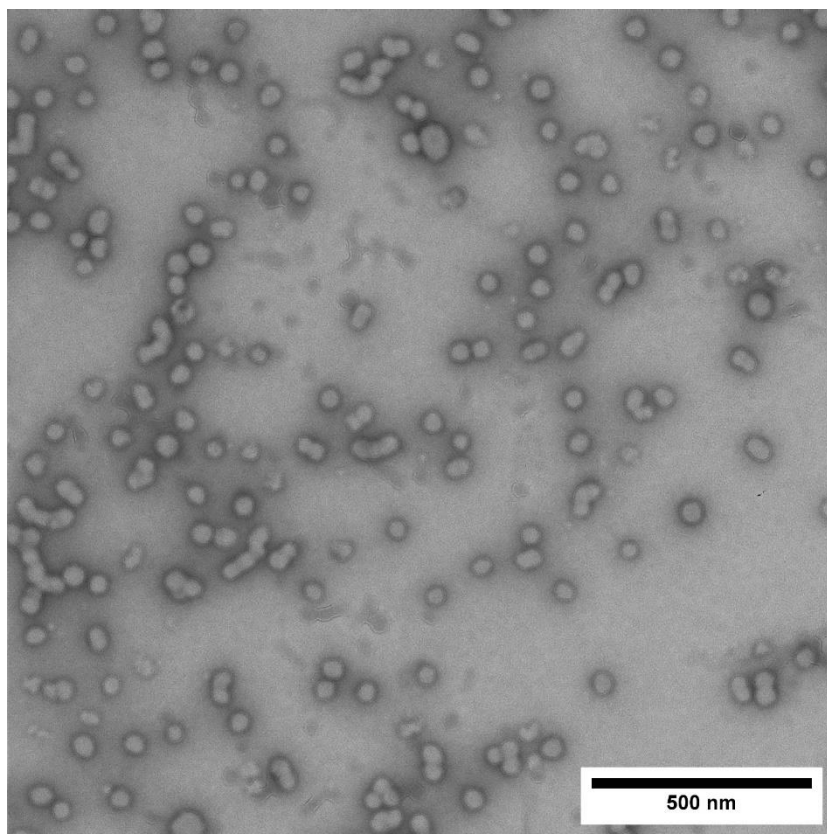


Figure 7.38 – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH - 333 μ L/min)

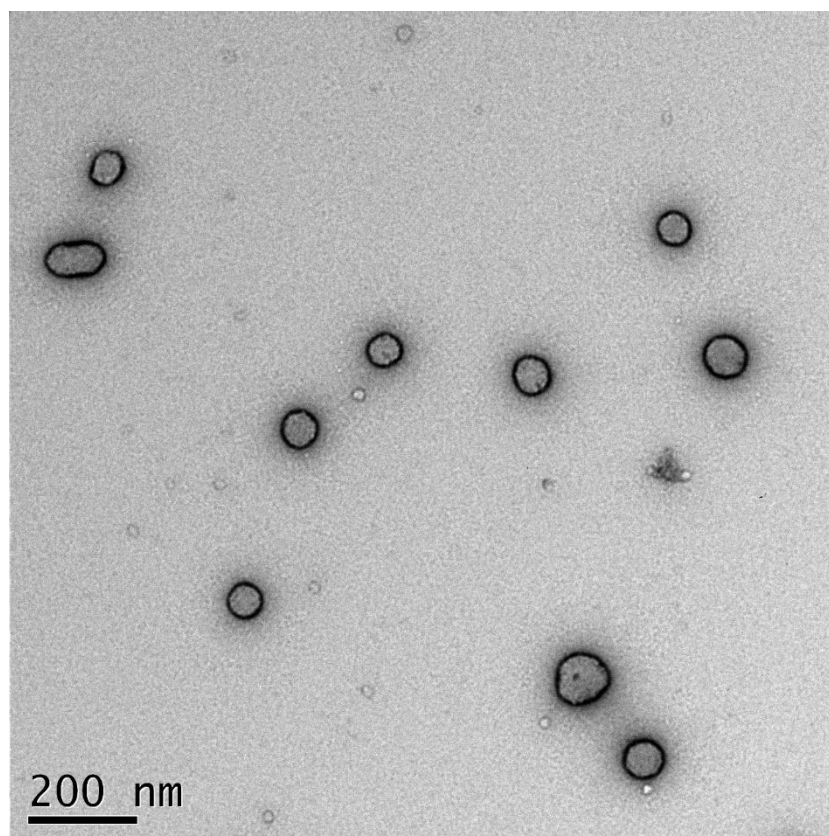


Figure 7.39:: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH - 333 μ L/min)

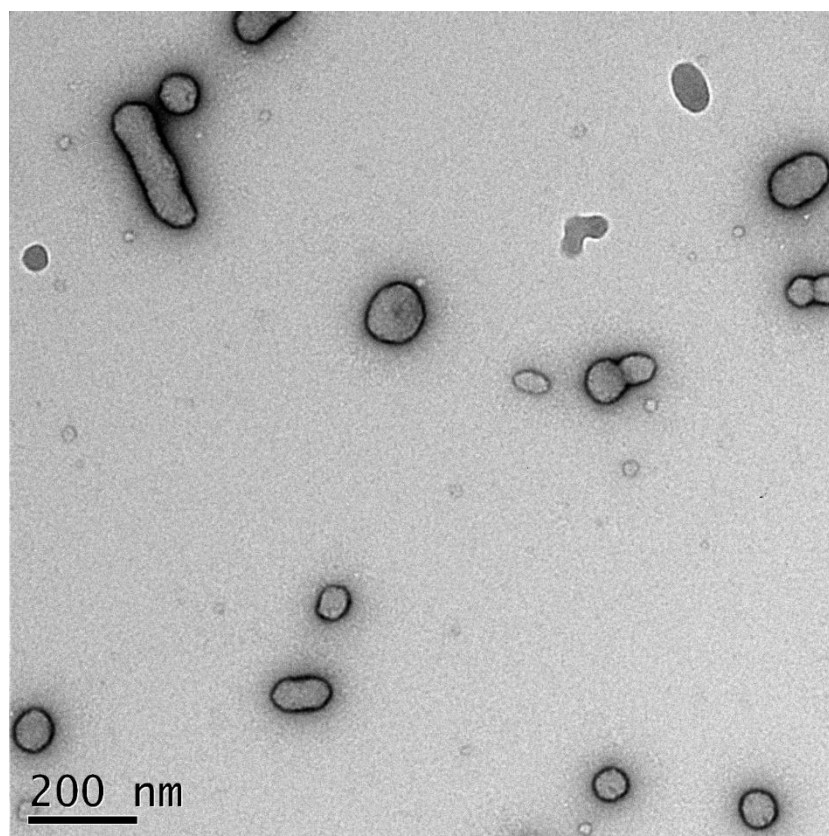


Figure 7.40: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH - 333 μ L/min)

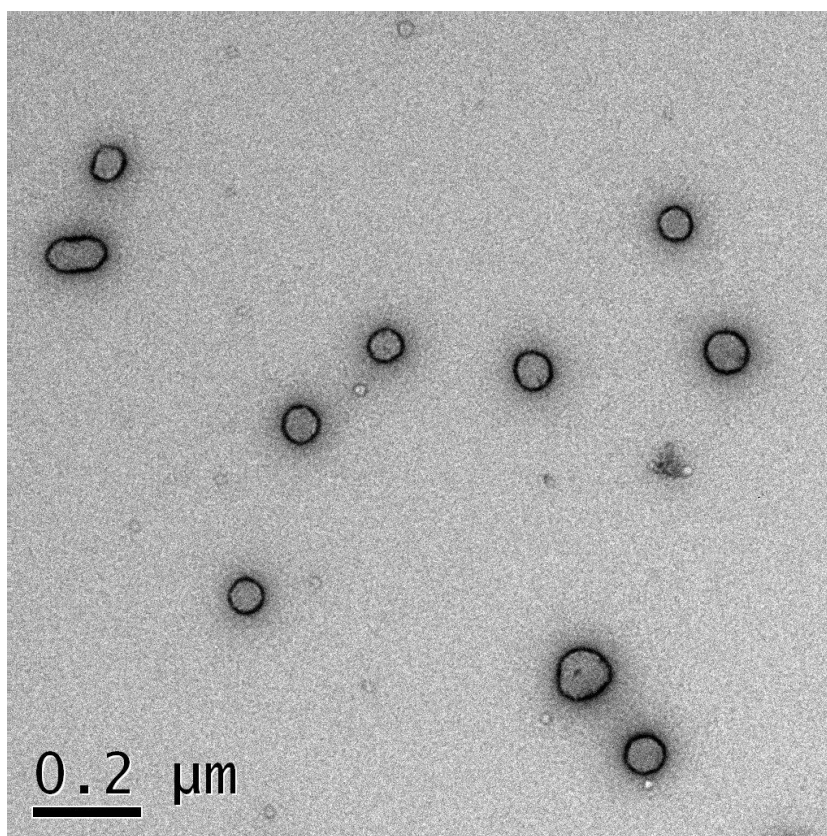


Figure 7.41: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH - 333μL/min)

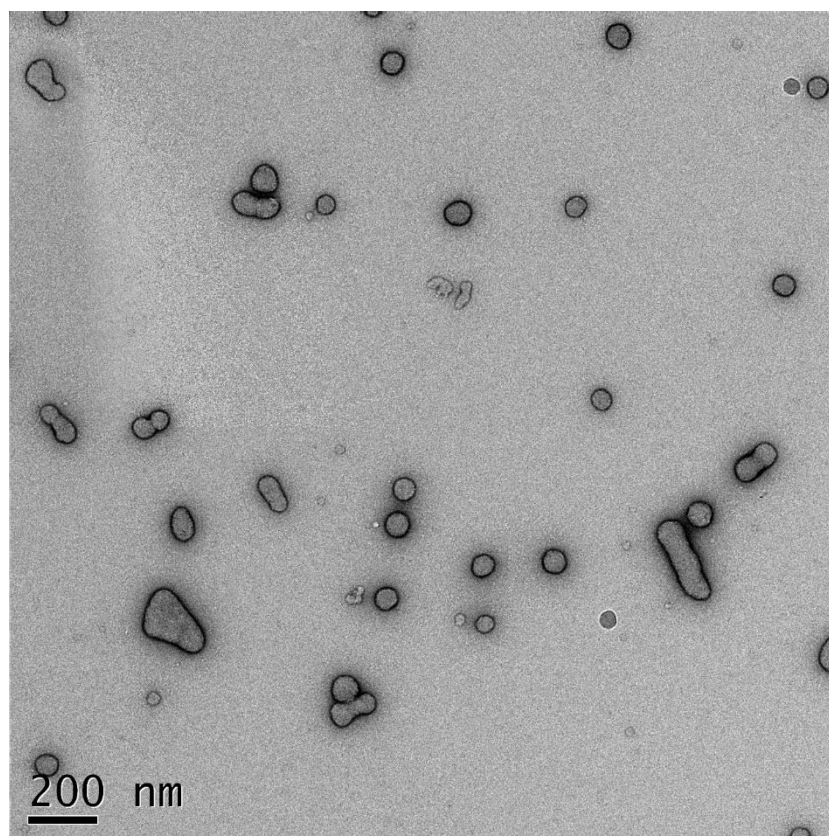


Figure 7.42: – TEM micrograph for PDMA₅₀-b-PDAA₃₀₀ nano-objects made via SE (10% MeOH - 333 μ L/min)

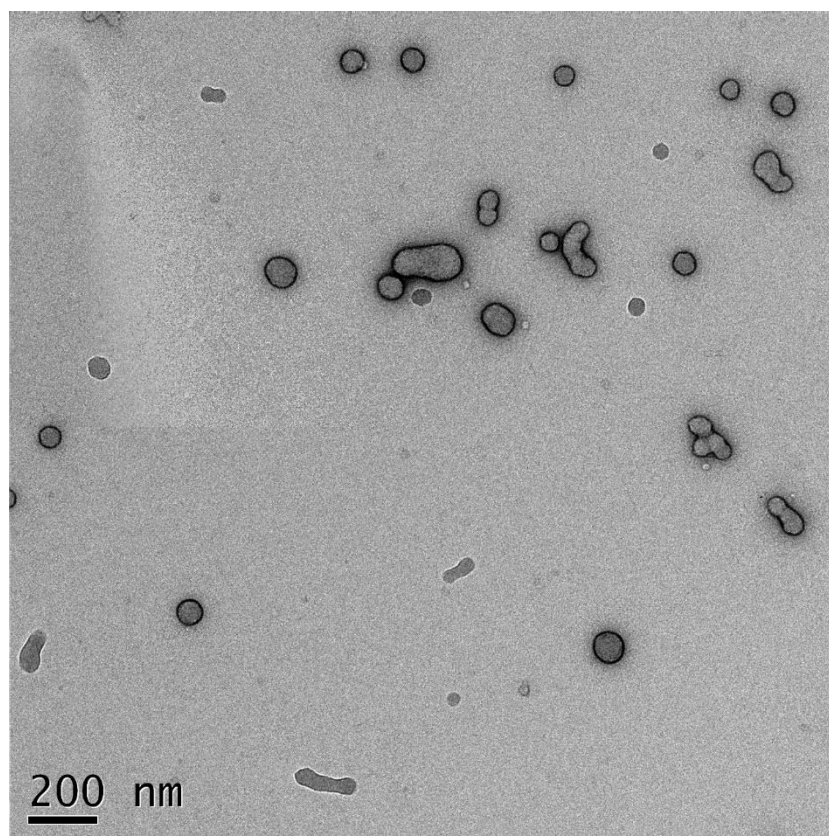


Figure 7.43:: – TEM micrograph for PDMA₅₀-b-PDAA₃₀₀ nano-objects made via SE (10% MeOH - 333 μ L/min)

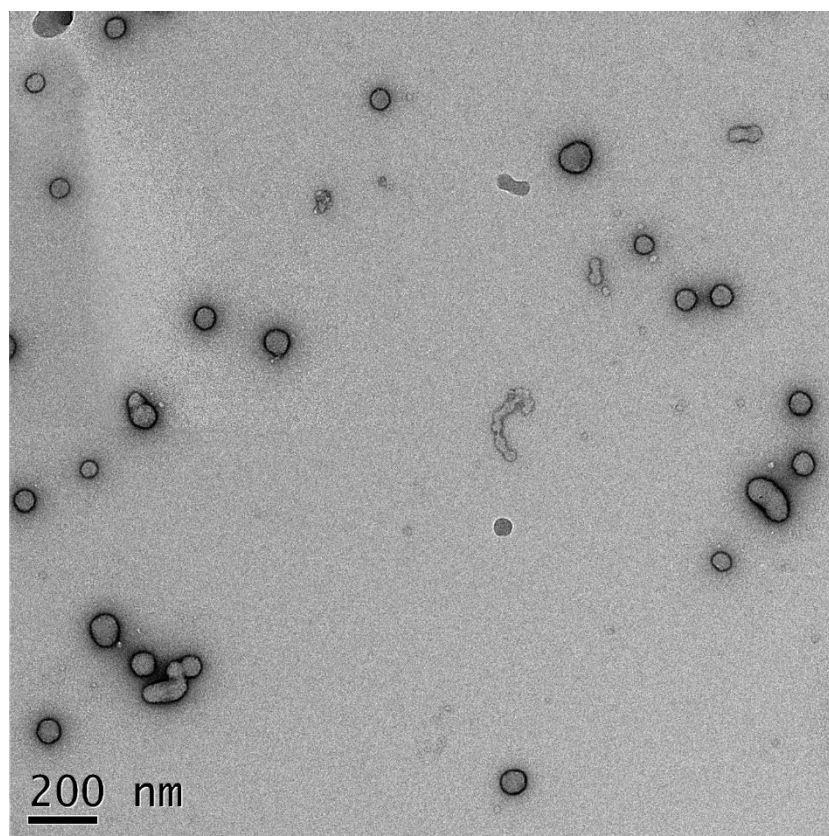


Figure 7.44: – TEM micrograph for PDMA₅₀-b-PDAA₃₀₀ nano-objects made via SE (10% MeOH - 333 μ L/min)

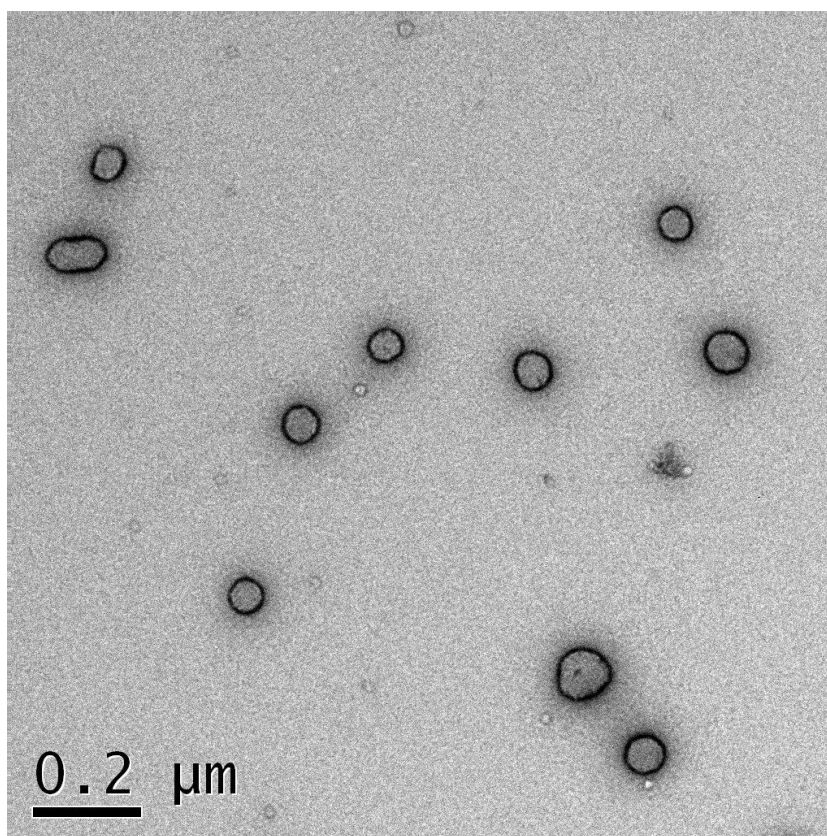


Figure 7.45: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH - 333μL/min)

SE PDMAm₅₀-b-PDAAm₃₀₀ 10% MeOH – Rapid addition

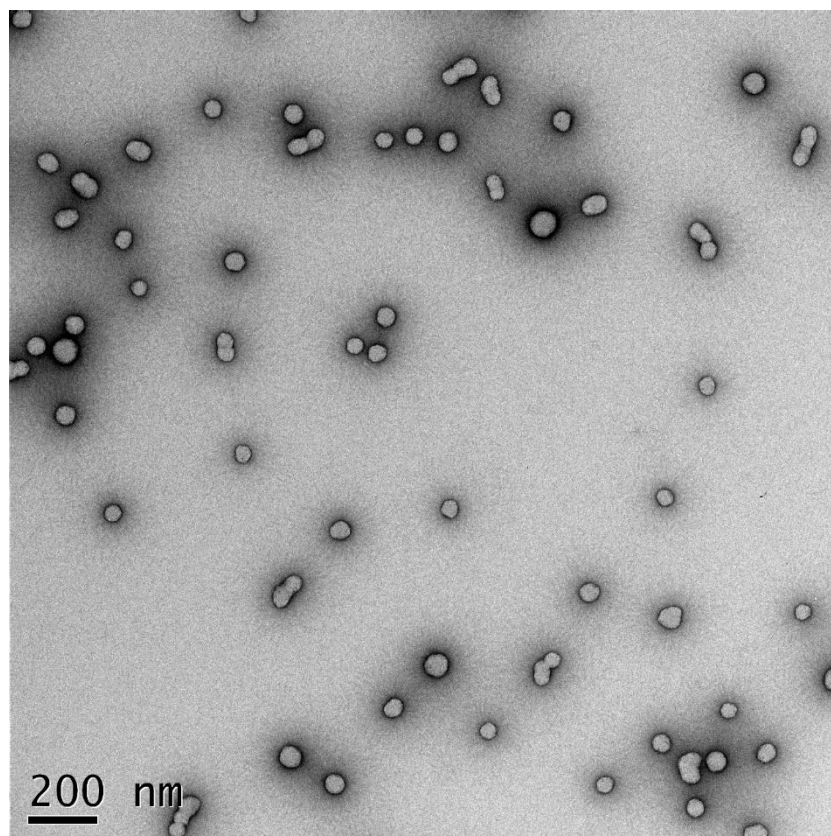


Figure 7.46: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH – Rapid Addition)

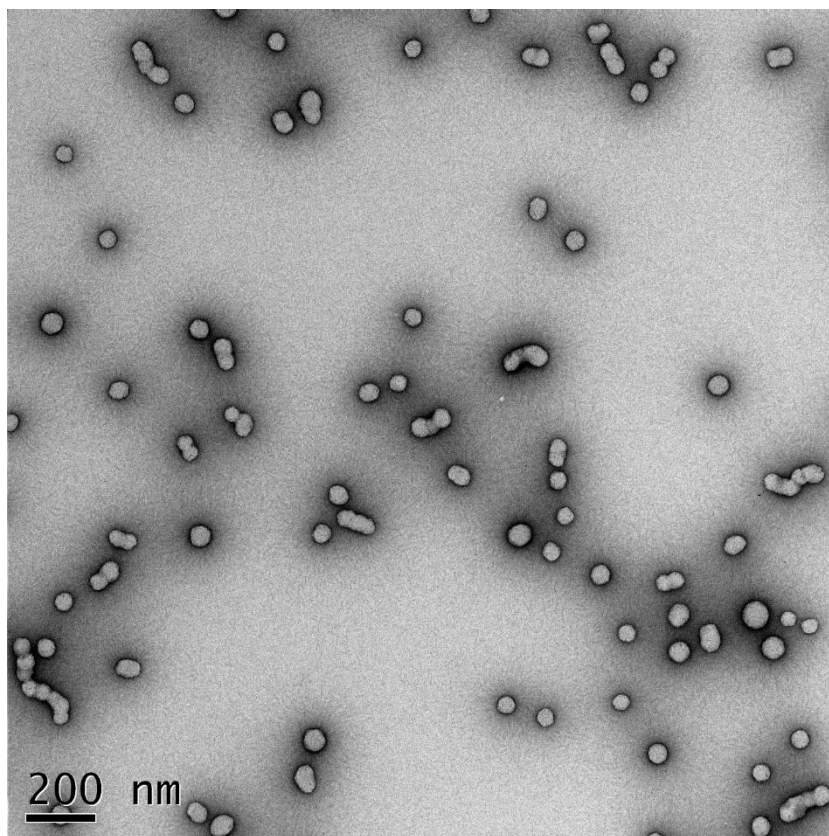


Figure 7.47: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH – Rapid Addition)

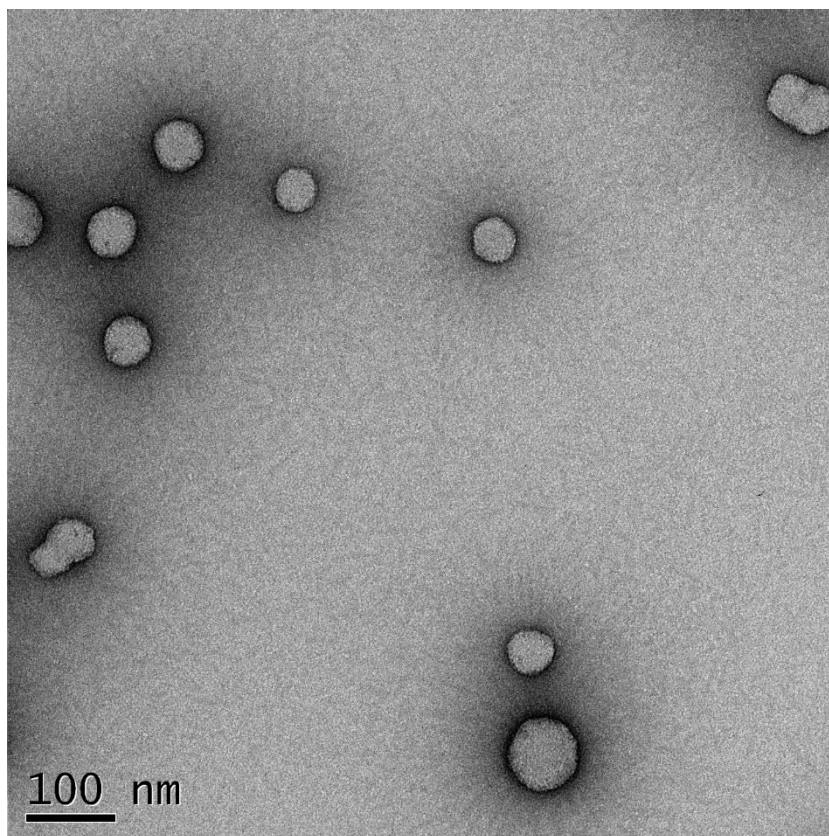


Figure 7.48: – TEM micrograph for PDMA₅₀-b-PDAA₃₀₀ nano-objects made via SE (10% MeOH – Rapid Addition)

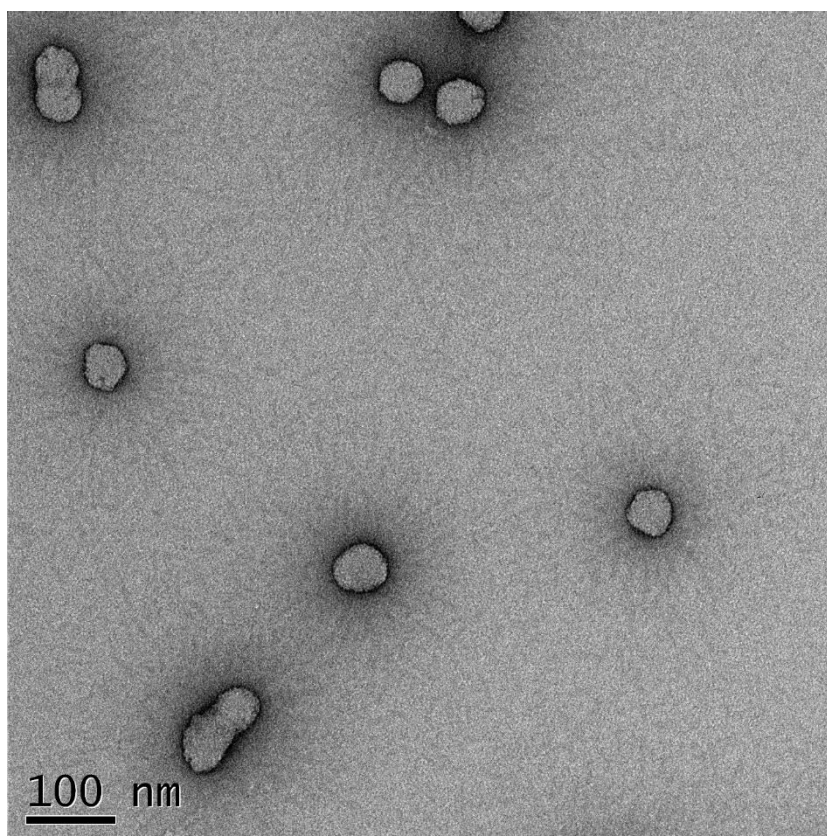


Figure 7.49 – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH – Rapid Addition)

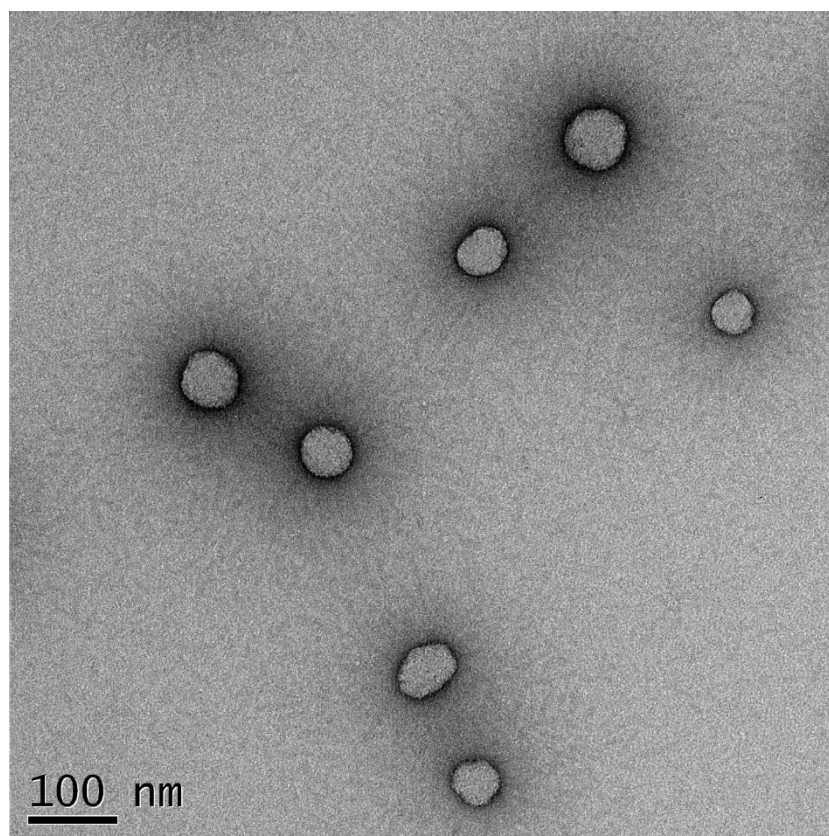


Figure 7.50: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH – Rapid Addition)

SE PDMAM₅₀-b-PDAAM₃₀₀ 10% MeOH – Rapid addition – Post evaporation for 3 days at room temperature.

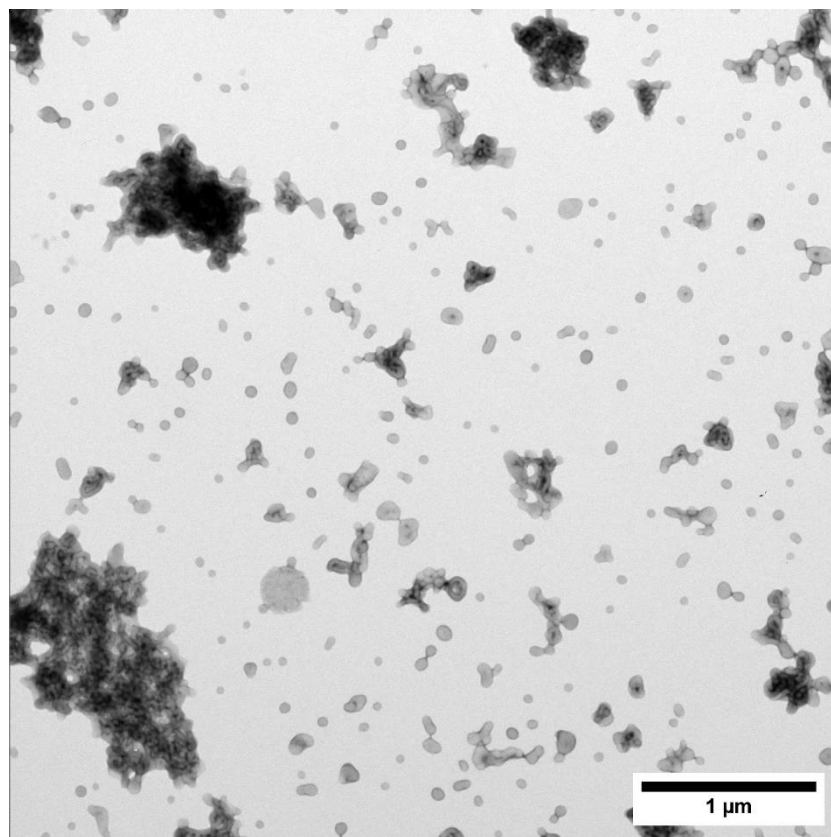


Figure 7.51: – TEM micrograph for PDMAM₅₀-b-PDAAM₃₀₀ nano-objects made via SE (10% MeOH – Rapid Addition – Post evap 72 hours)

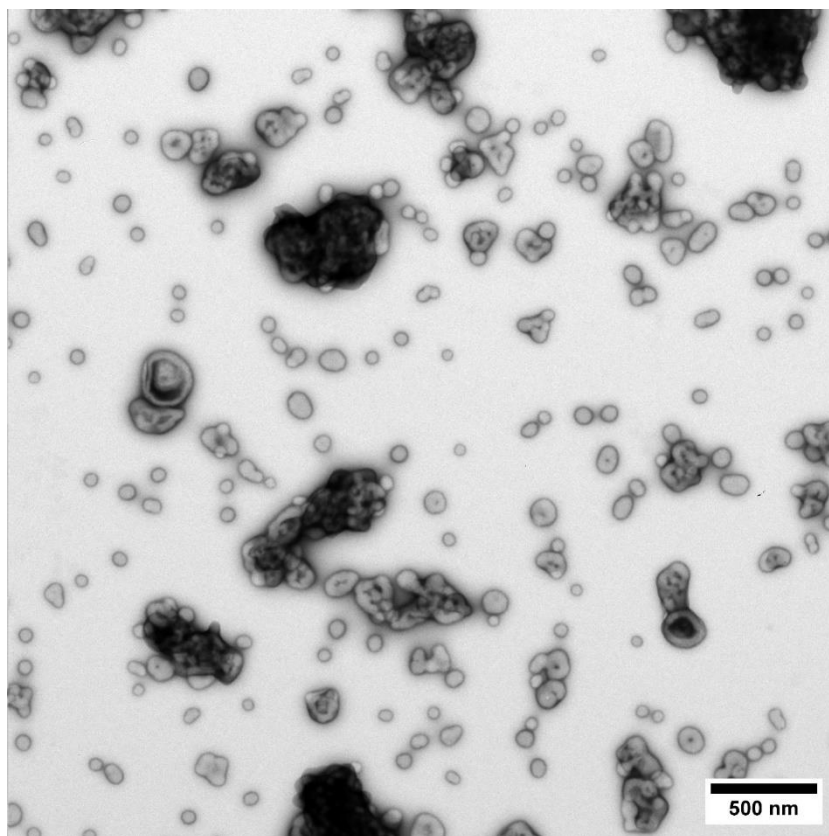


Figure 7.52: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH – Rapid Addition – Post evap 72 hours)

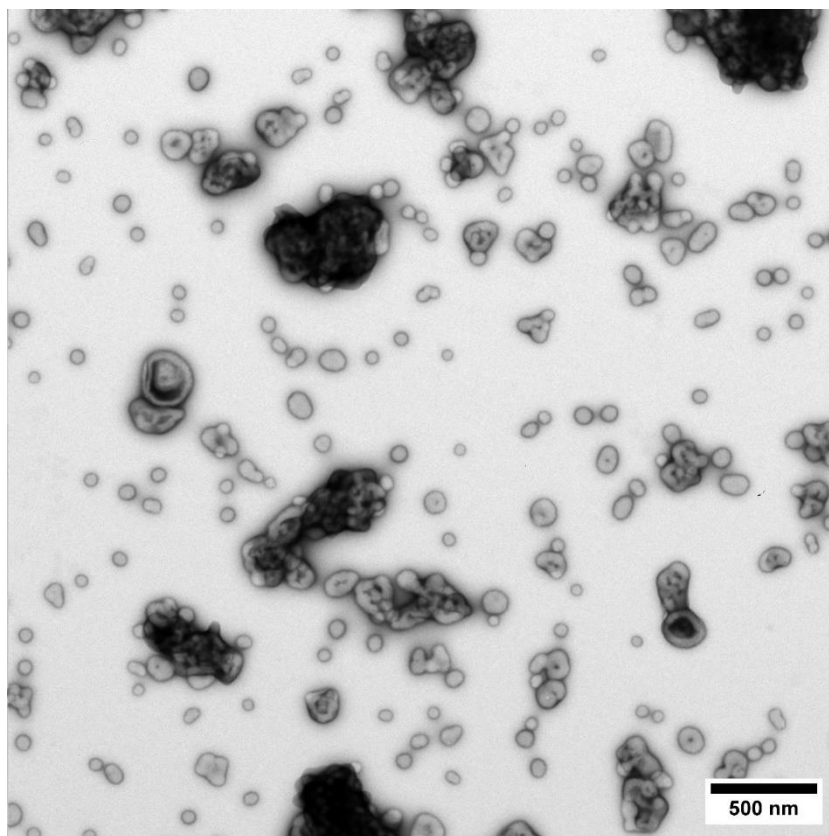


Figure 7.53: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH – Rapid Addition – Post evap 72 hours)

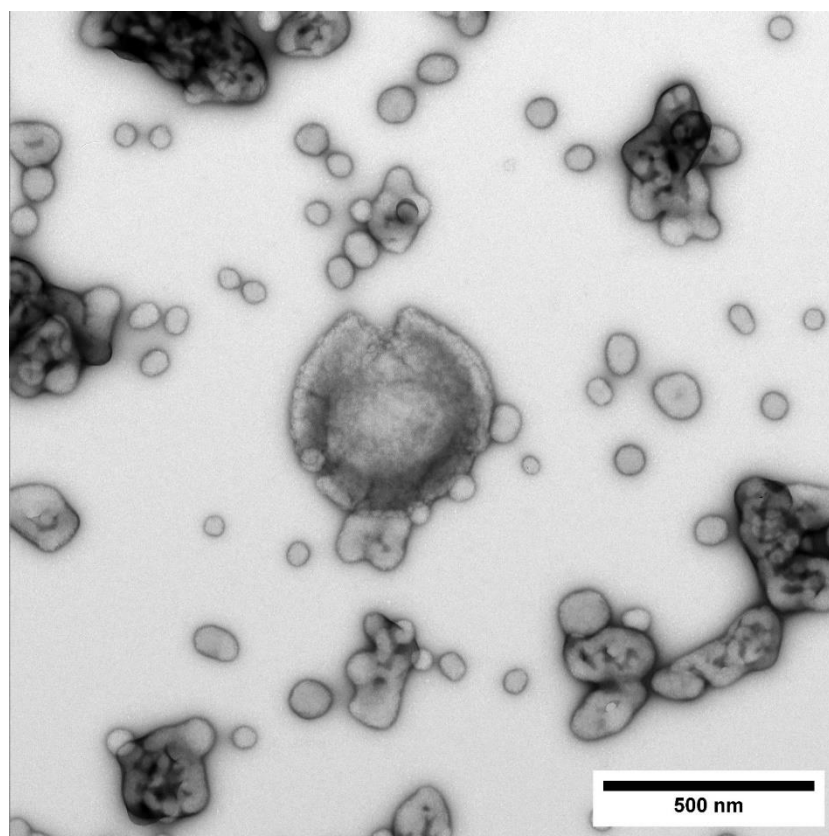


Figure 7.54: – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH – Rapid Addition – Post evap 72 hours)

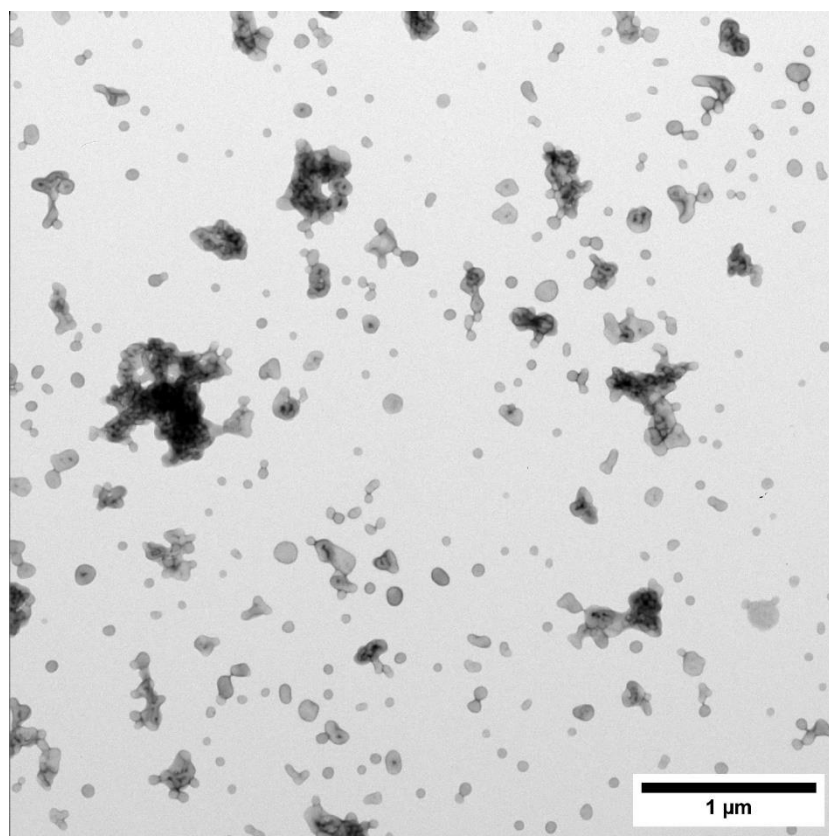


Figure 7.55: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH – Rapid Addition – Post evap 72 hours)

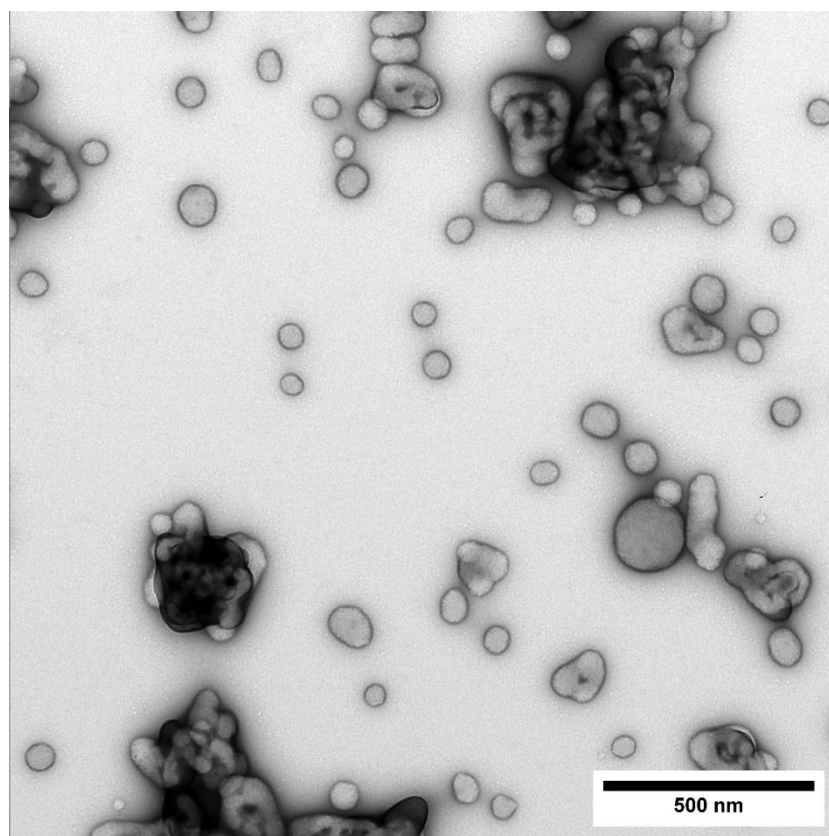


Figure 7.56: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH – Rapid Addition – Post evap 72 hours)

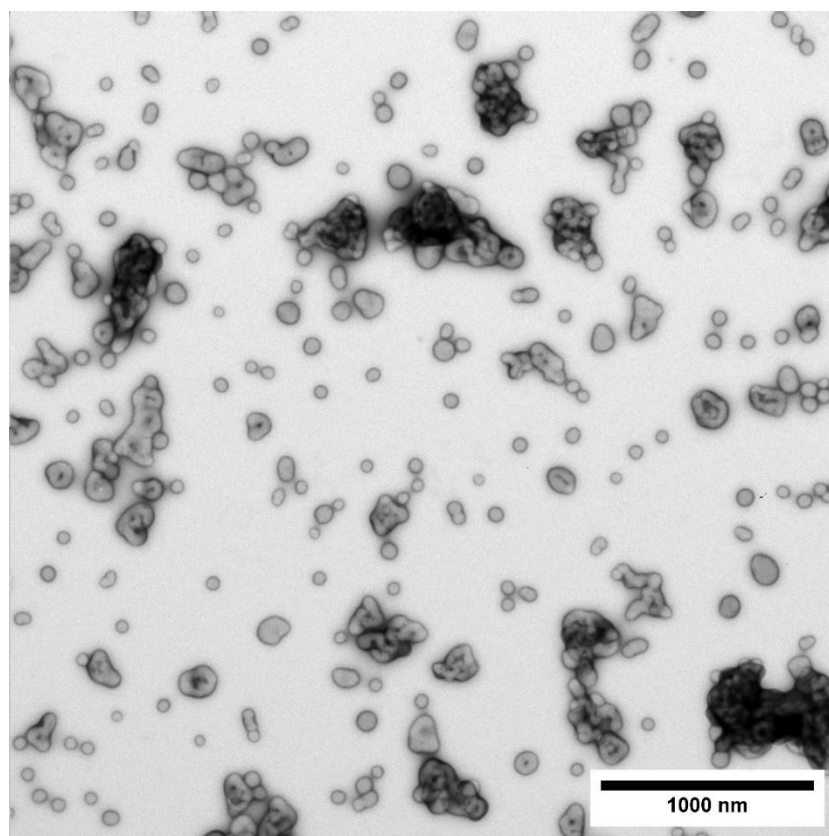


Figure 7.57: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH – Rapid Addition – Post evap 72 hours)

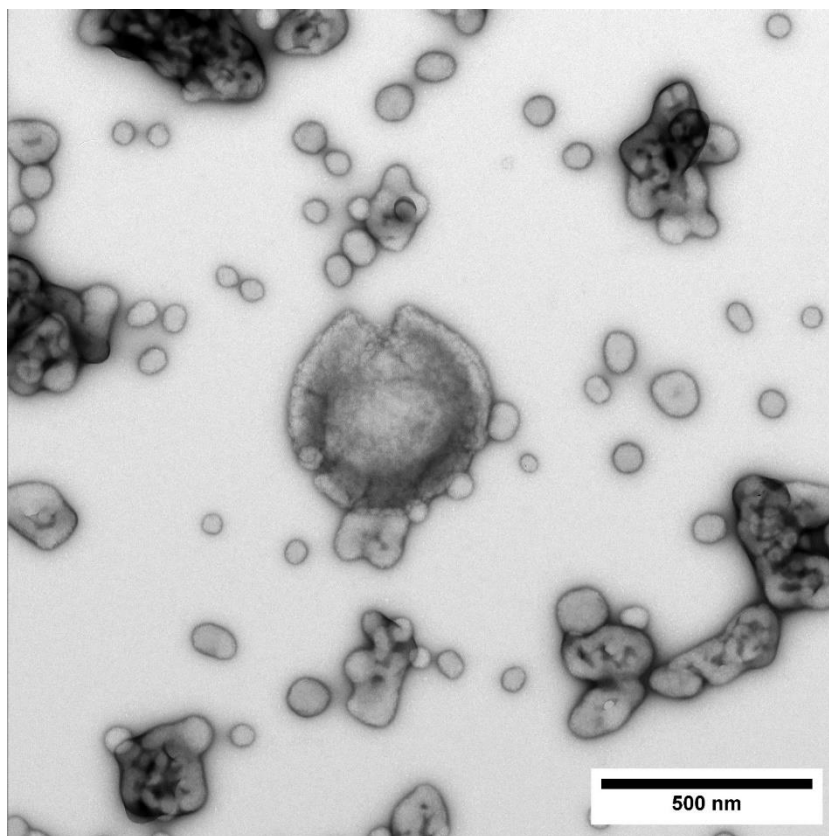


Figure 7.58: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH – Rapid Addition – Post evap 72 hours)

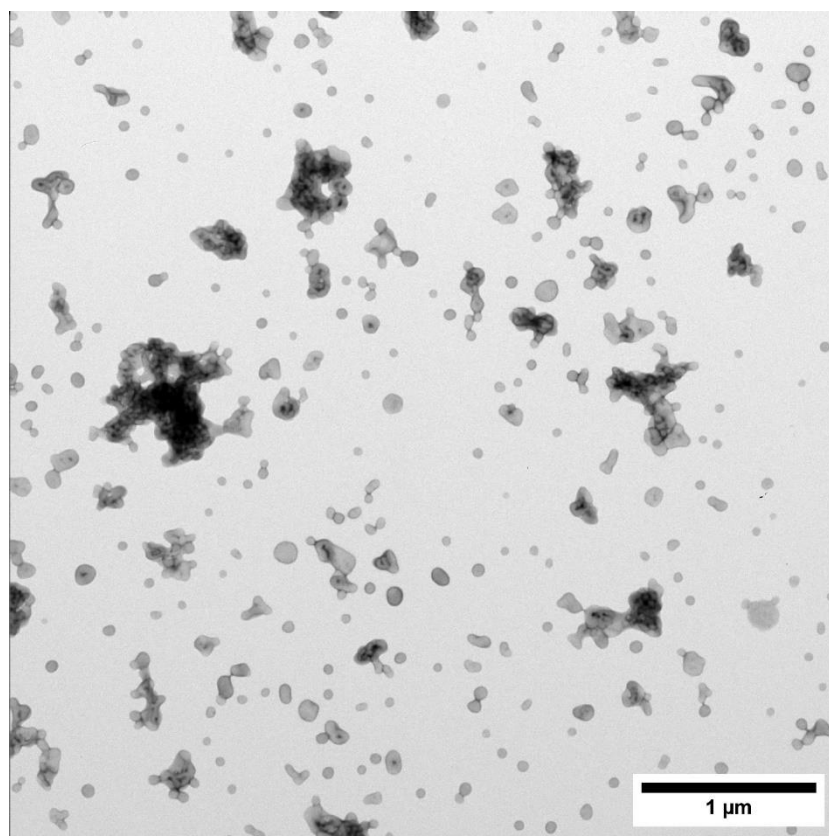


Figure 7.59: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH – Rapid Addition – Post evap 72 hours)

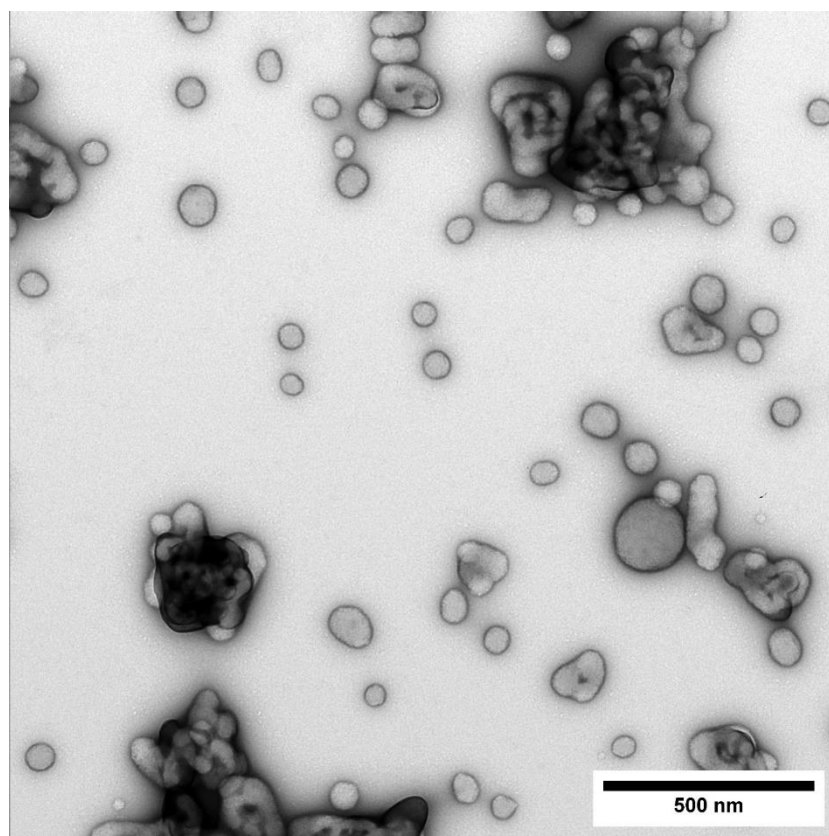


Figure 7.60: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH – Rapid Addition – Post evap 72 hours)

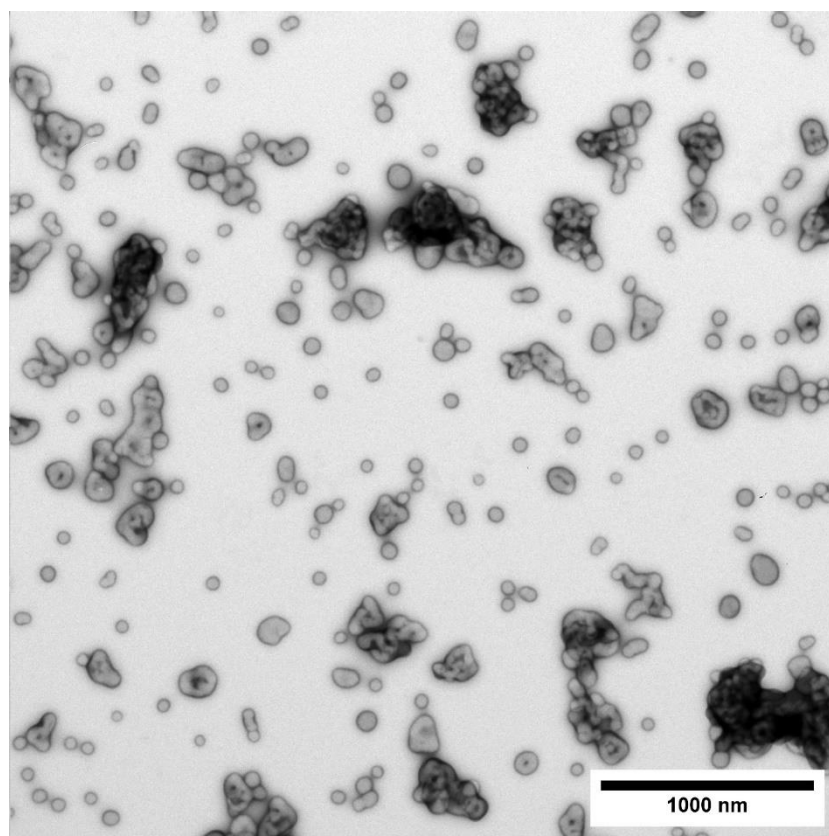


Figure 7.61: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (10% MeOH – Rapid Addition – Post evap 72 hours)

SE PDMAm₅₀-b-PDAAm₃₀₀ 50% MeOH – Rapid addition, t=0 (5 min of mixing)

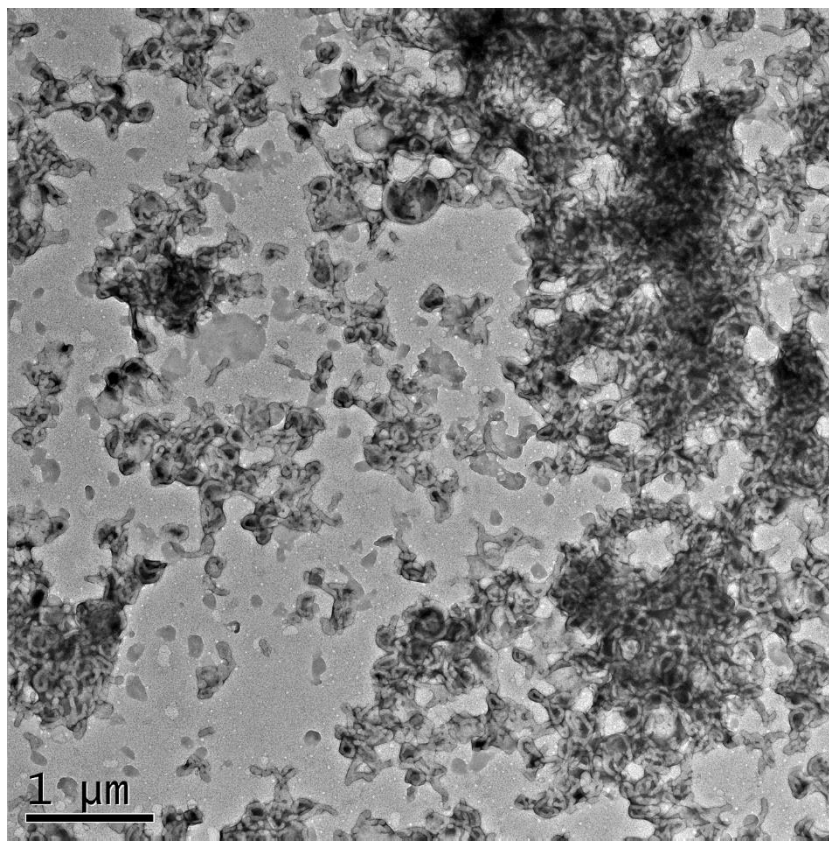


Figure 7.62: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – t=0)

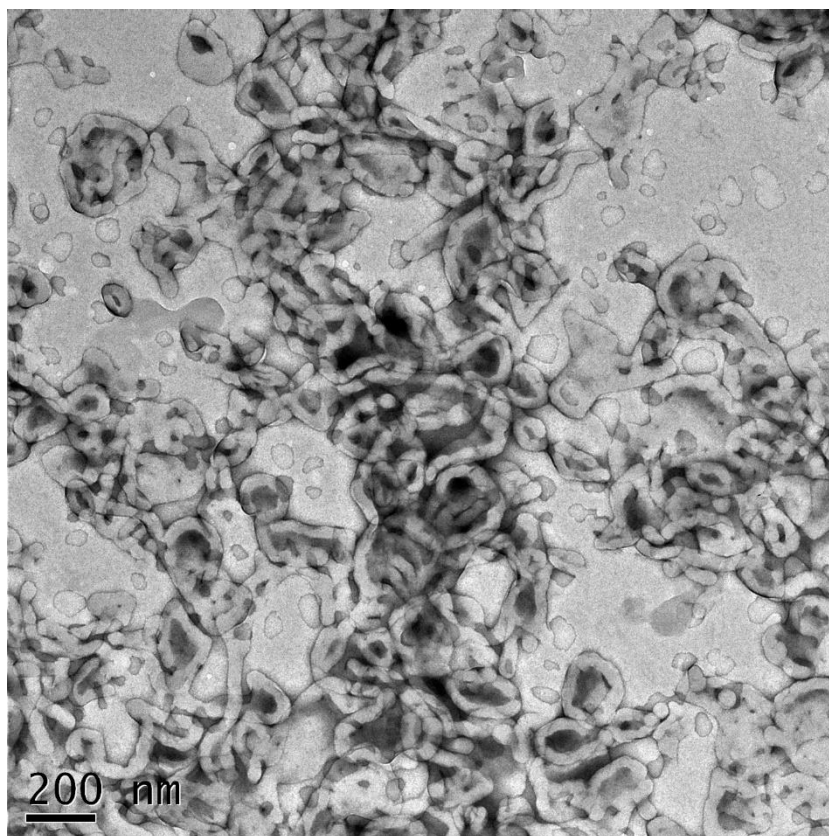


Figure 7.63: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – t=0)

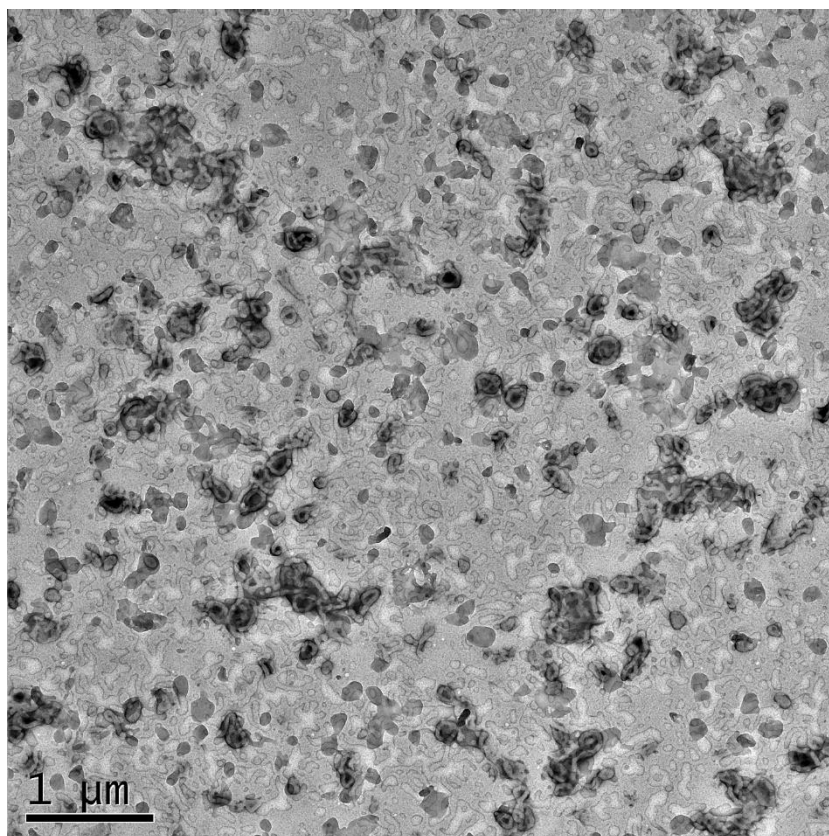


Figure 7.64: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – t=0)

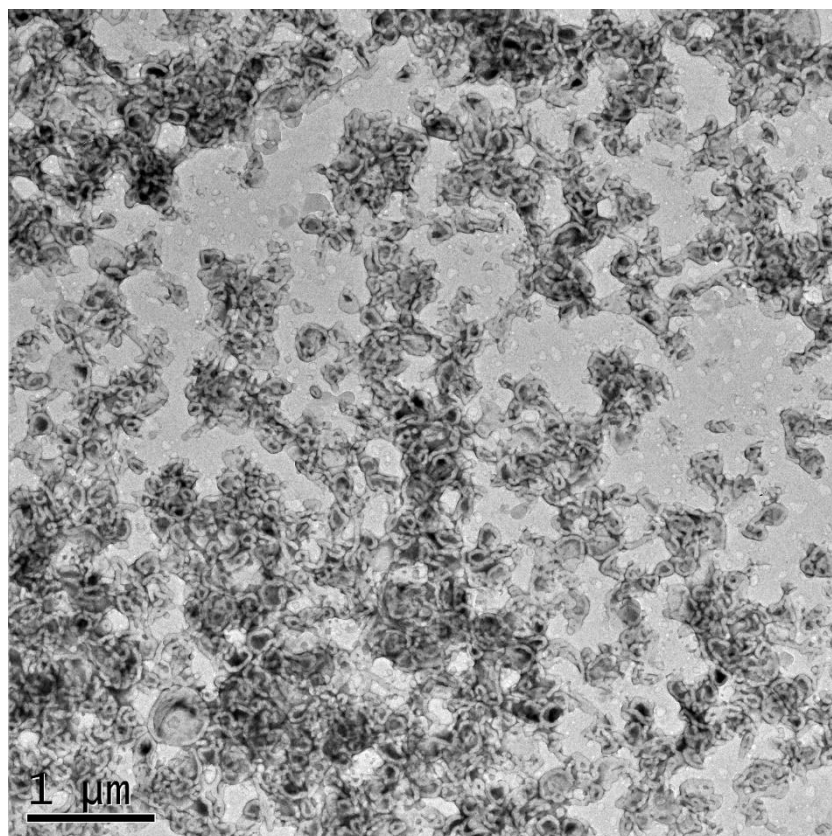


Figure 7.65: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – t=0)

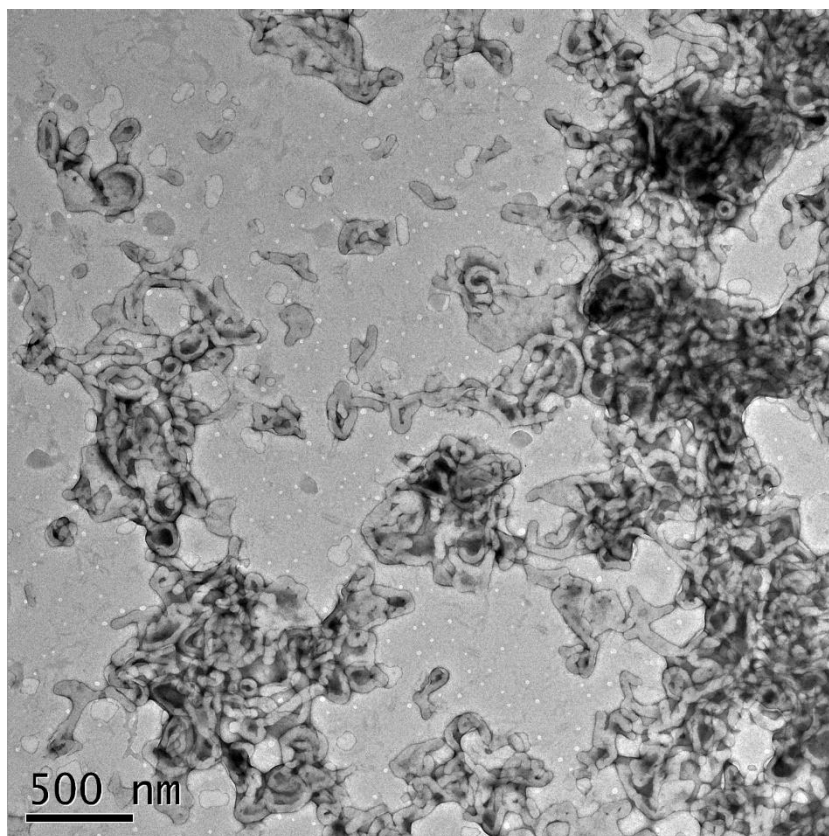


Figure 7.66: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – t=0)

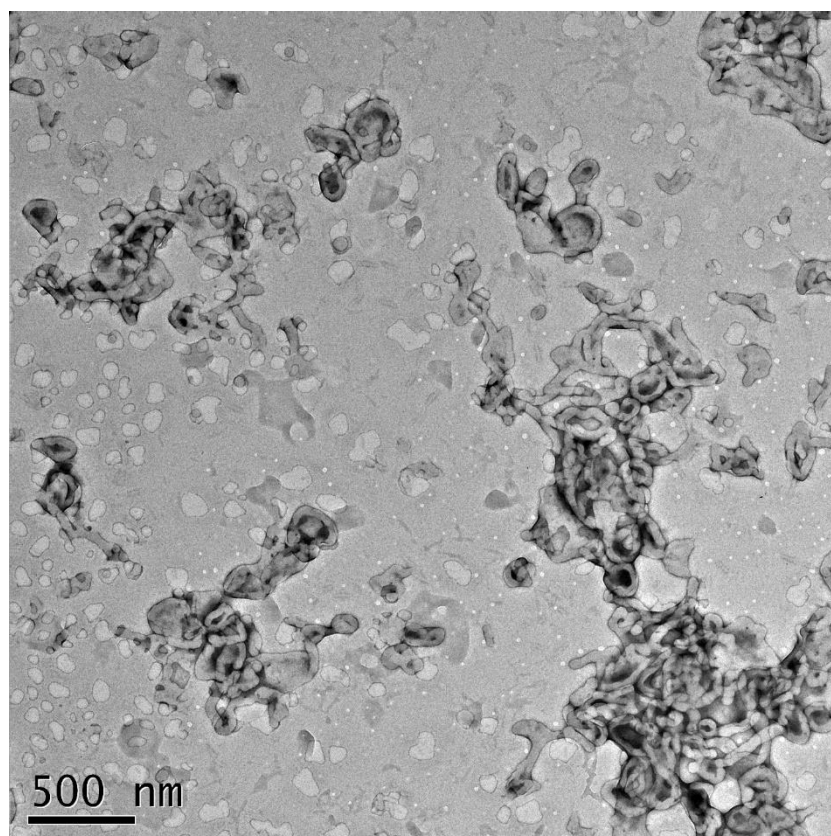


Figure 7.67: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – t=0)

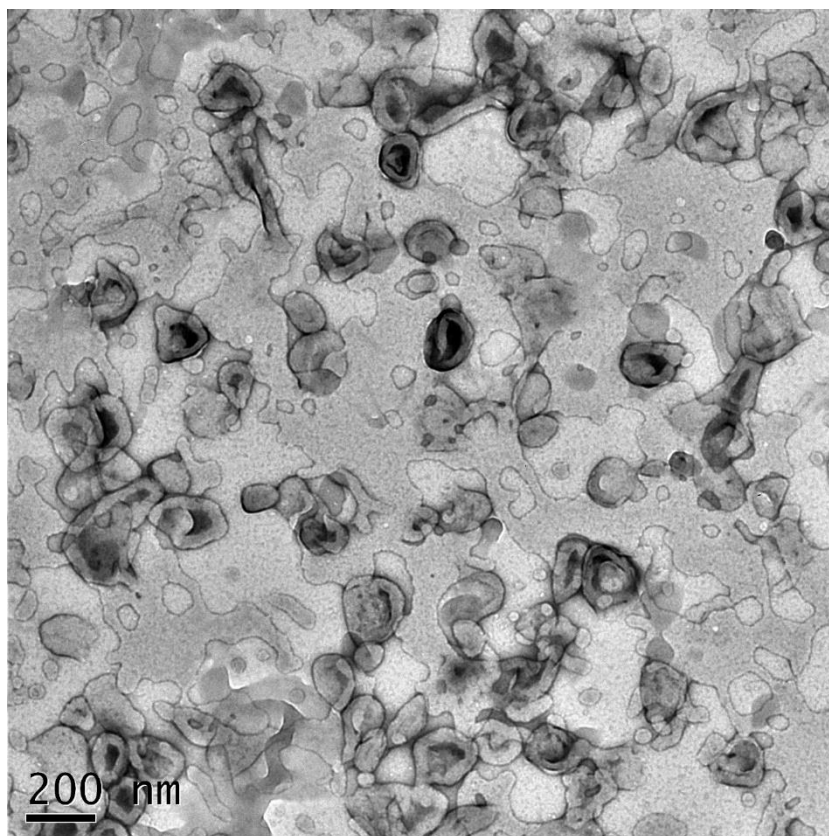


Figure 7.68: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – $t=0$)

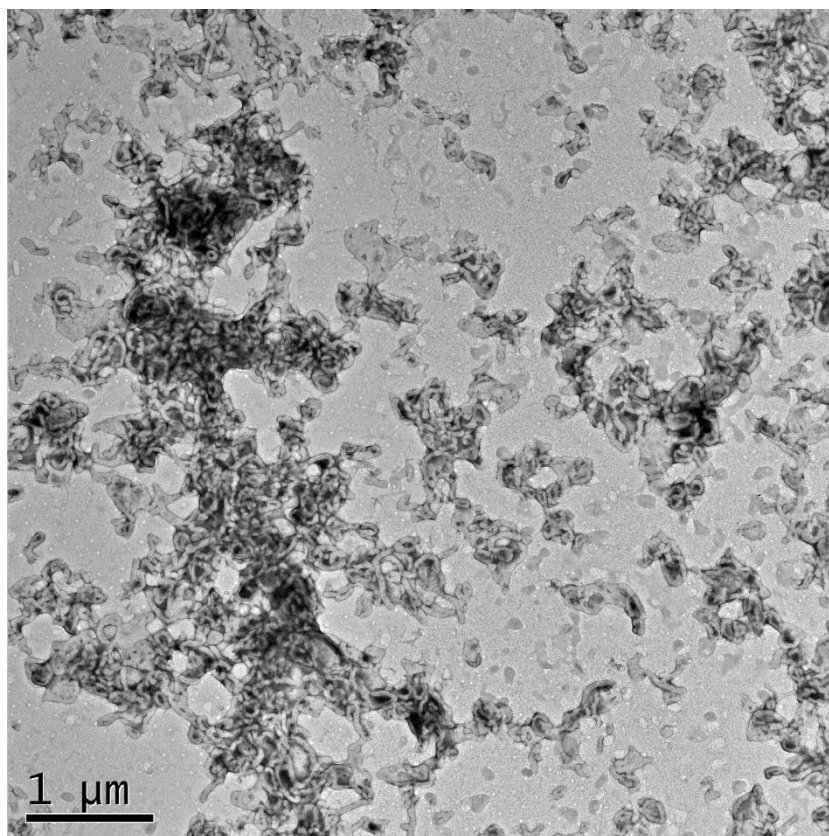


Figure 7.69: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – t=0)

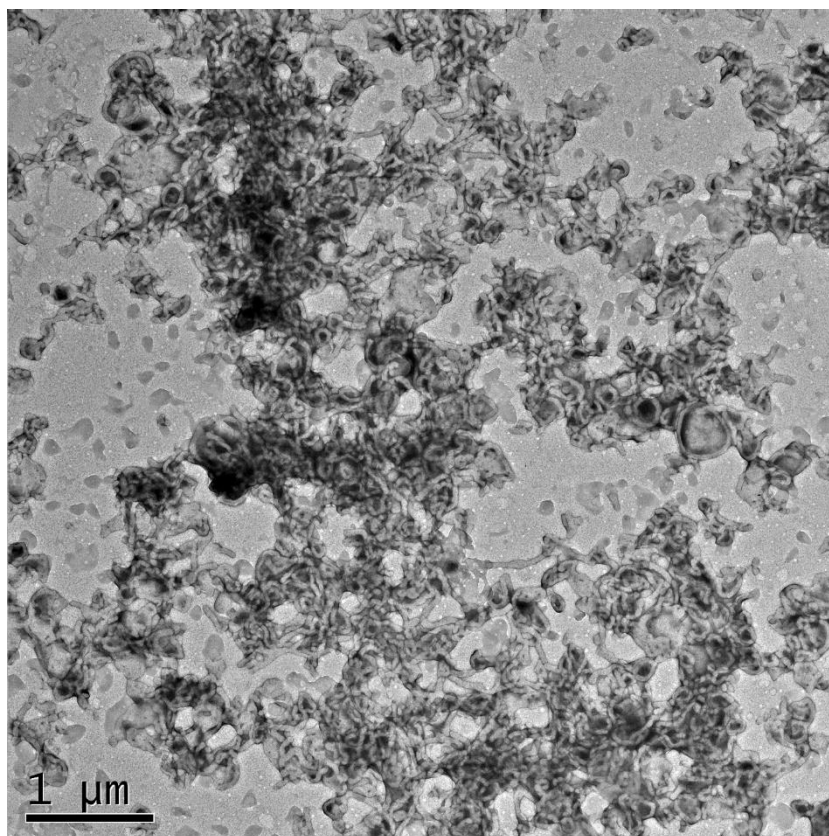


Figure 7.70: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – $t=0$)

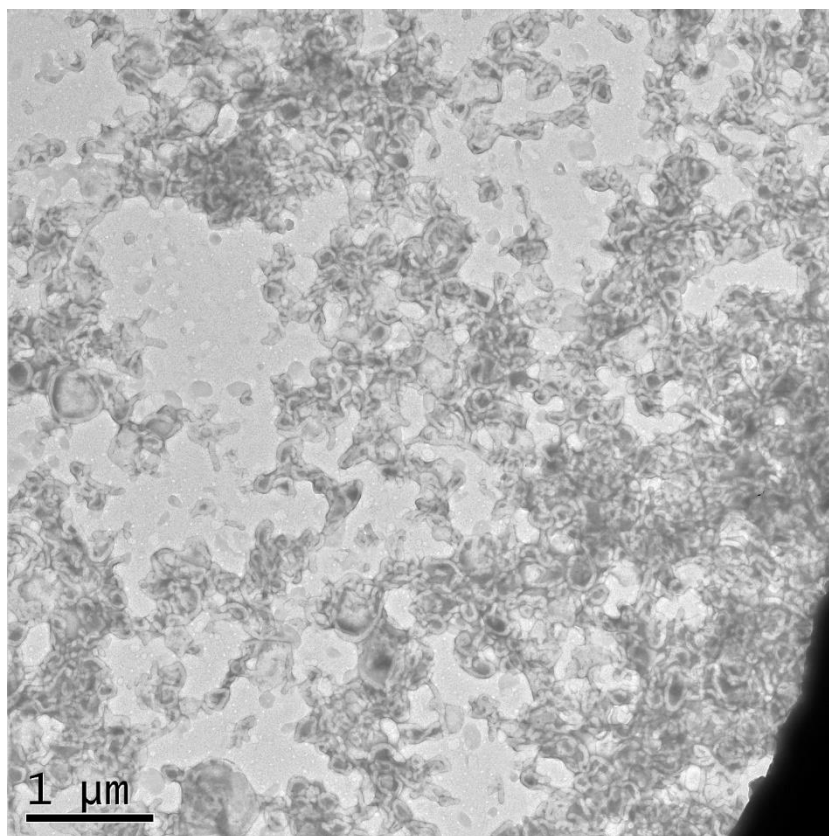


Figure 7.71: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – $t=0$)

SE PDMAm₅₀-b-PDAAm₃₀₀ 50% MeOH – Rapid addition, t=30 min

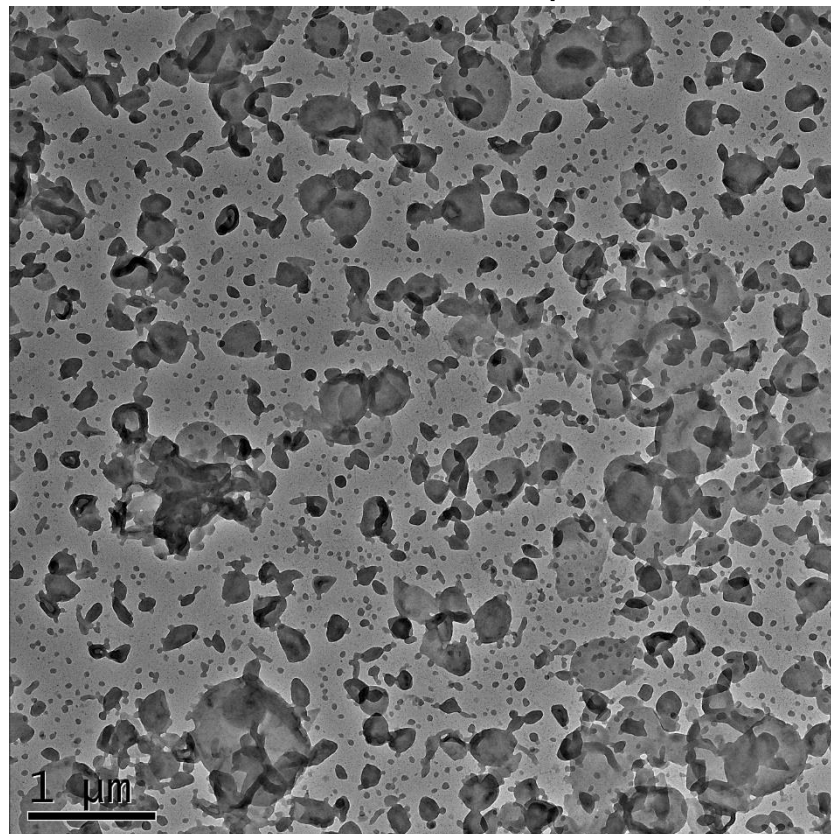


Figure 7.72: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – t = 30 min)

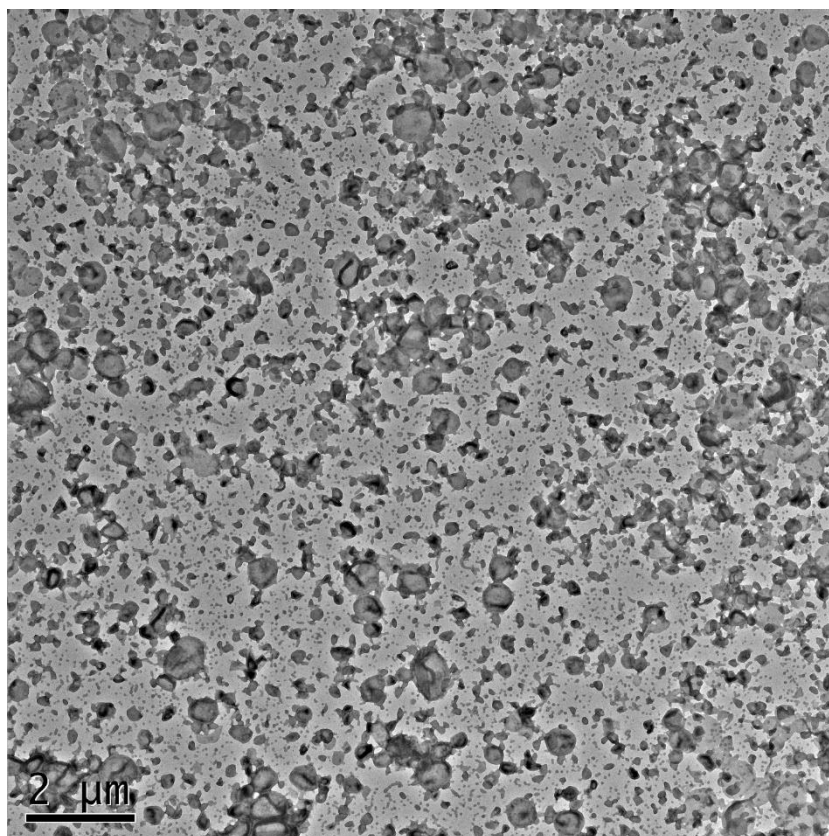


Figure 7.73: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – t = 30 min)

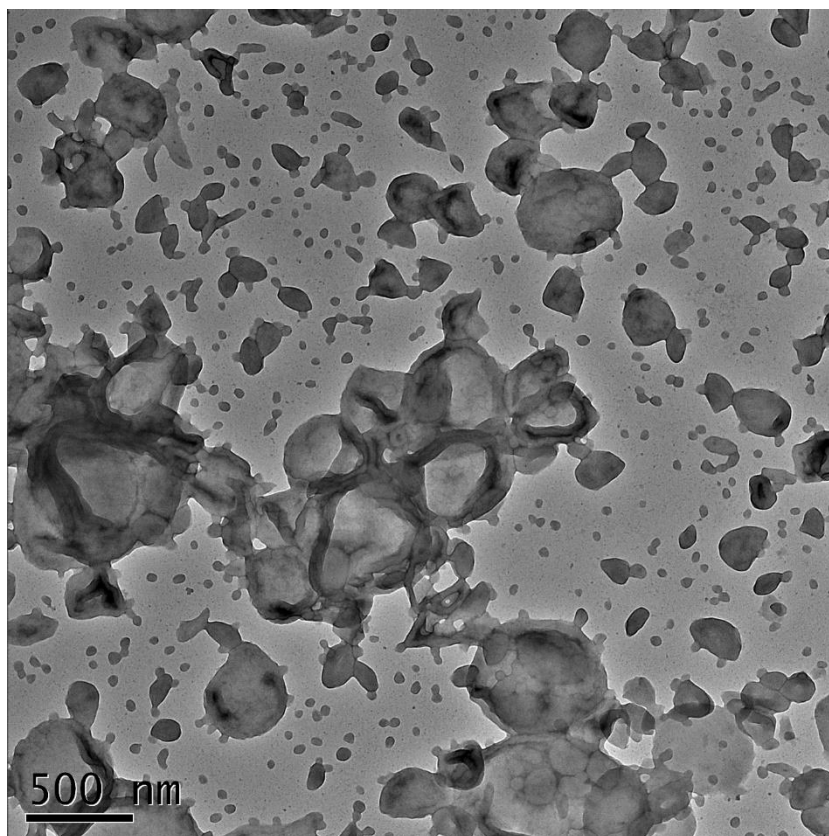


Figure 7.74: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – t = 30 min)

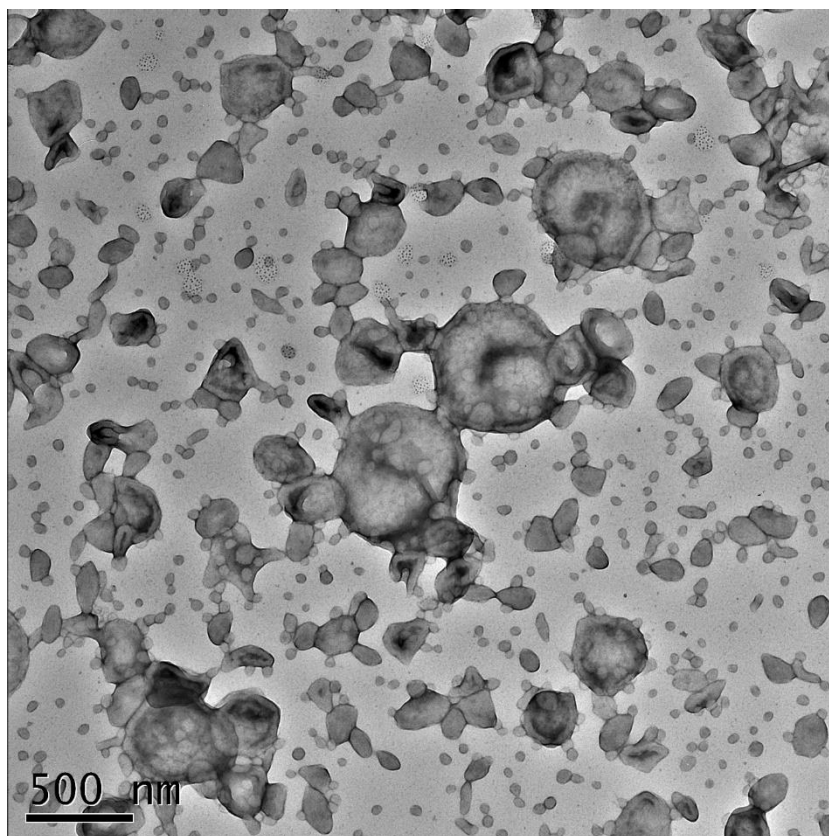


Figure 7.75: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – t = 30 min)

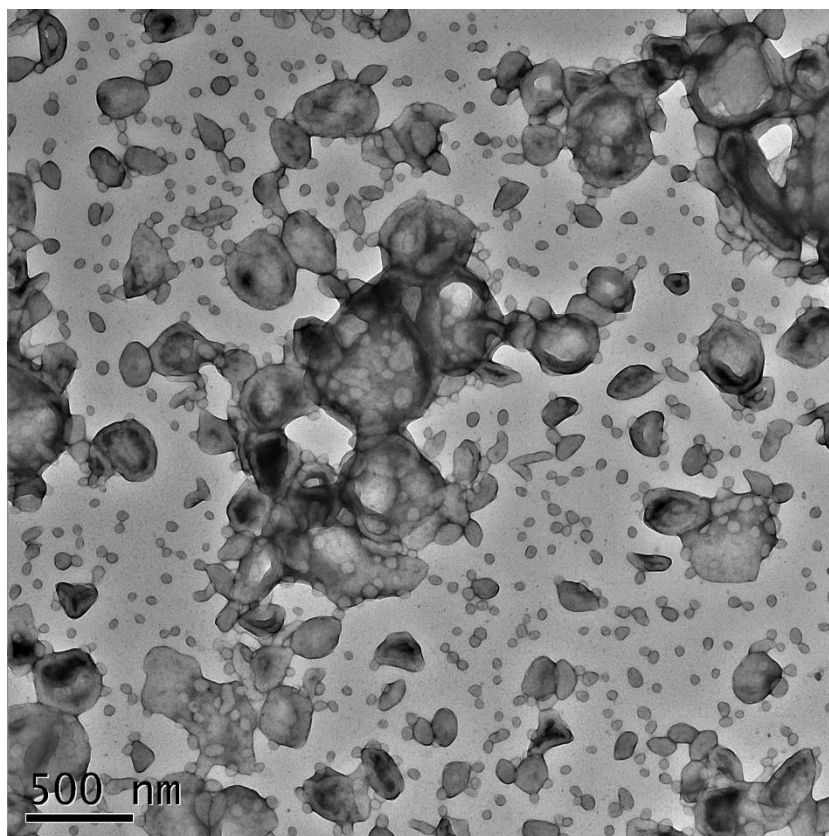


Figure 7.76: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – t = 30 min)

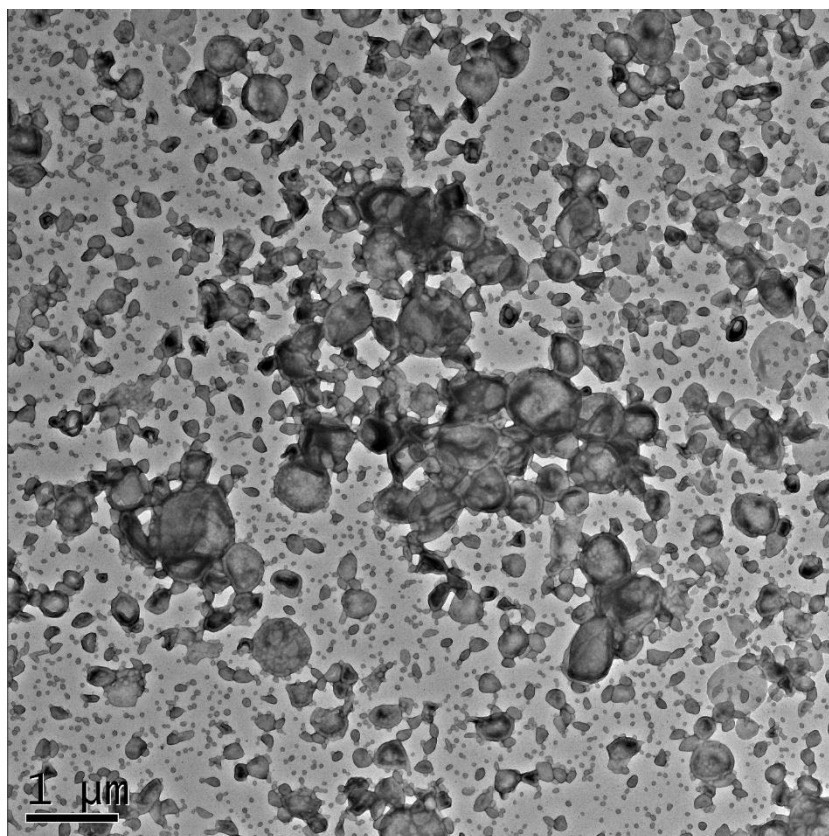


Figure 7.77: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – t = 30 min)

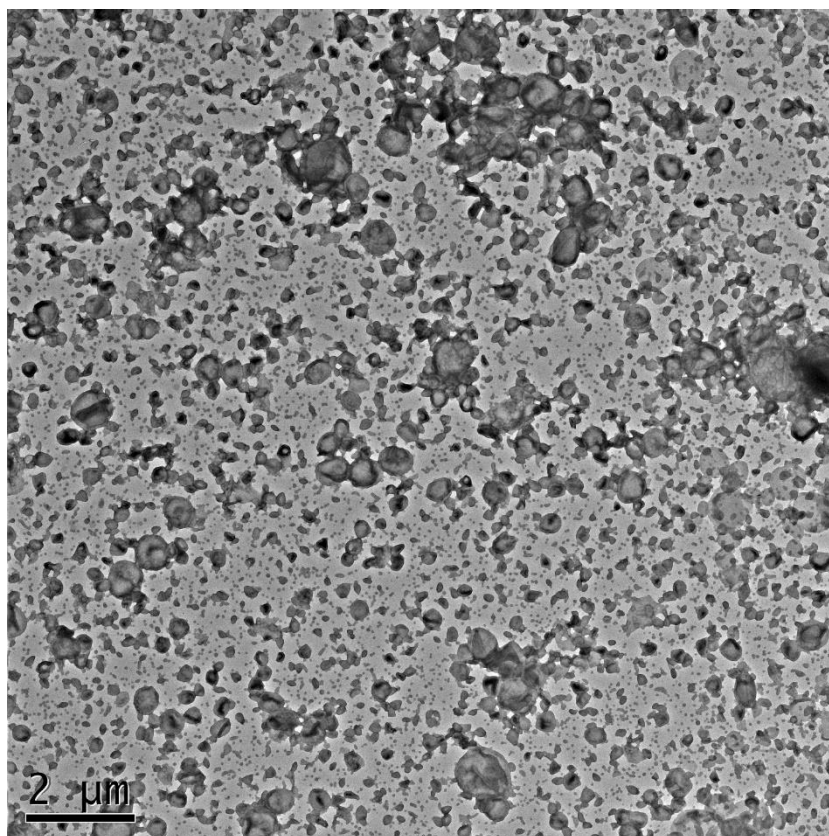


Figure 7.78 : – TEM micrograph for PDMA_{m50}-b-PDAA_{m300} nano-objects made via SE (50% MeOH – Rapid Addition – t = 30 min)

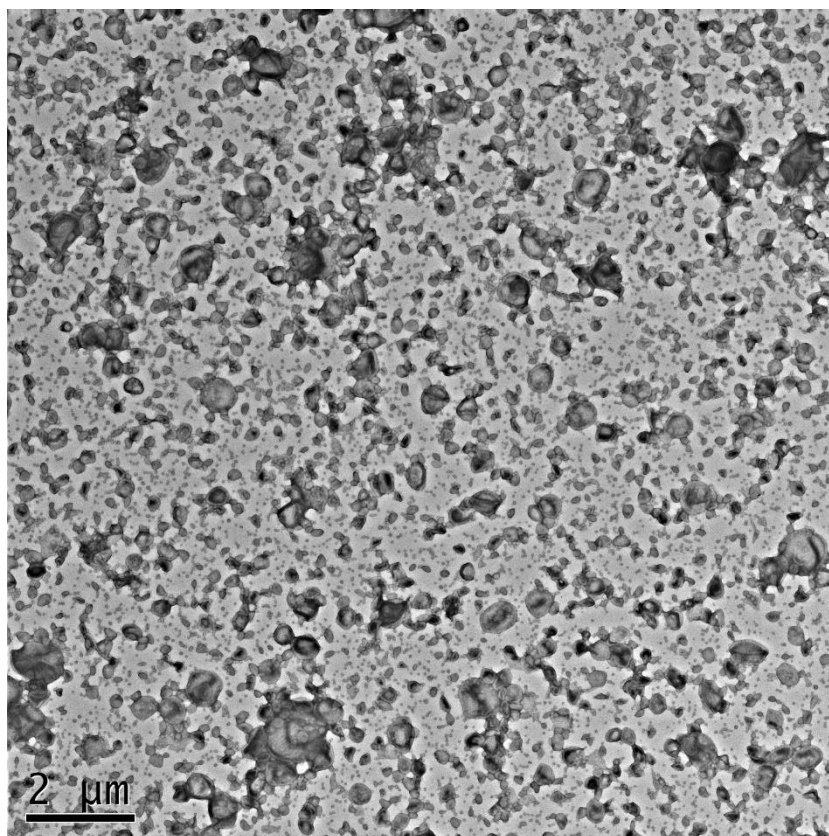


Figure 7.79: – TEM micrograph for PDMAm_{50.b}-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – t = 30 min)

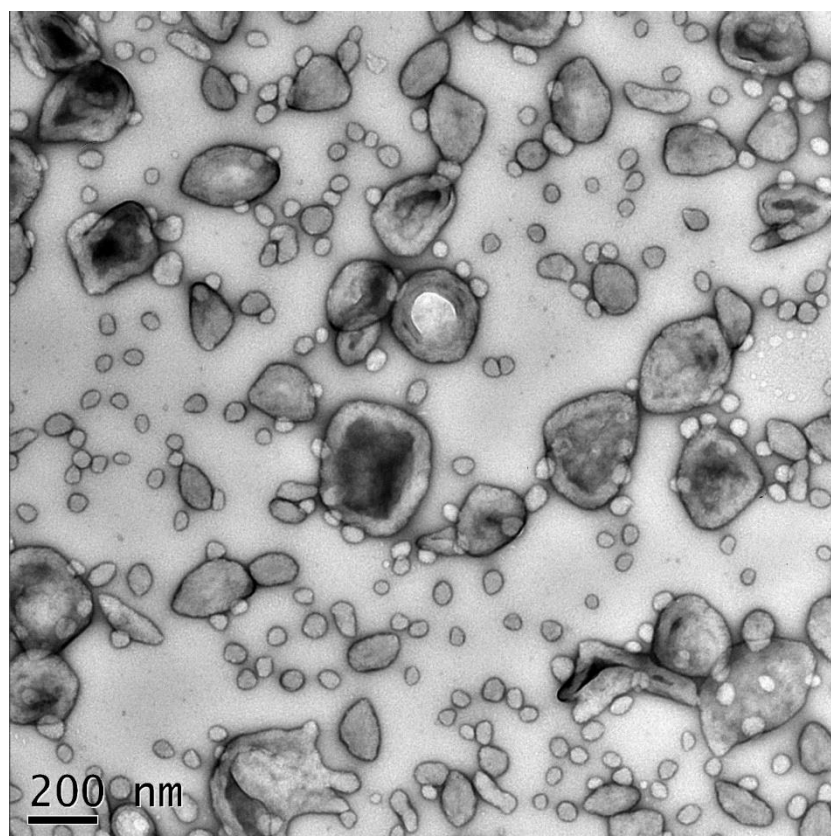


Figure 7.80 – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – t = 30 min)

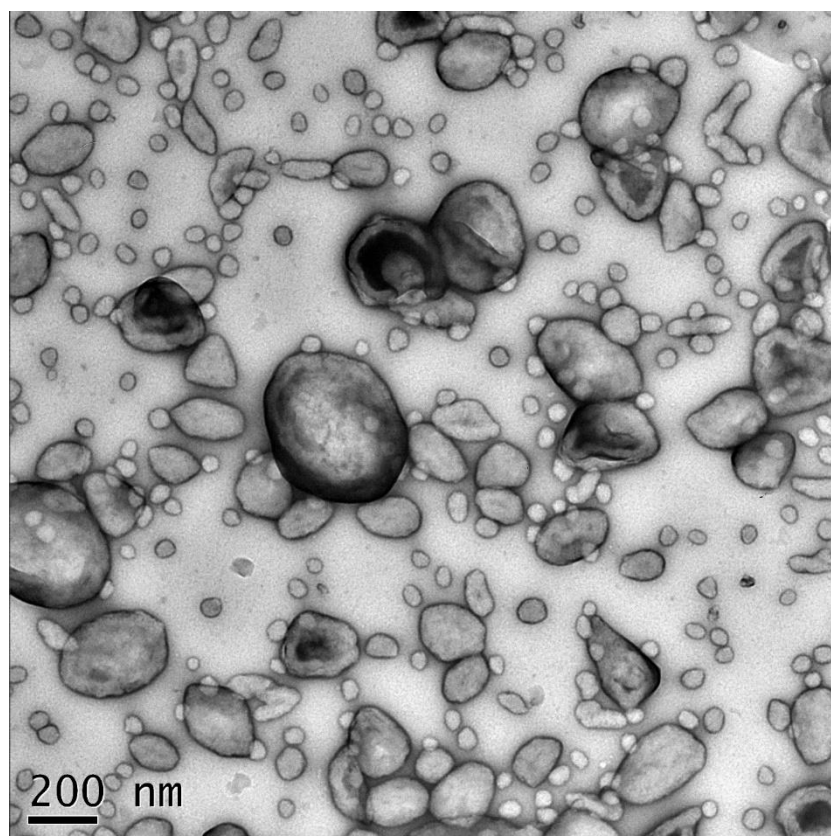


Figure 7.81: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – t = 30 min)

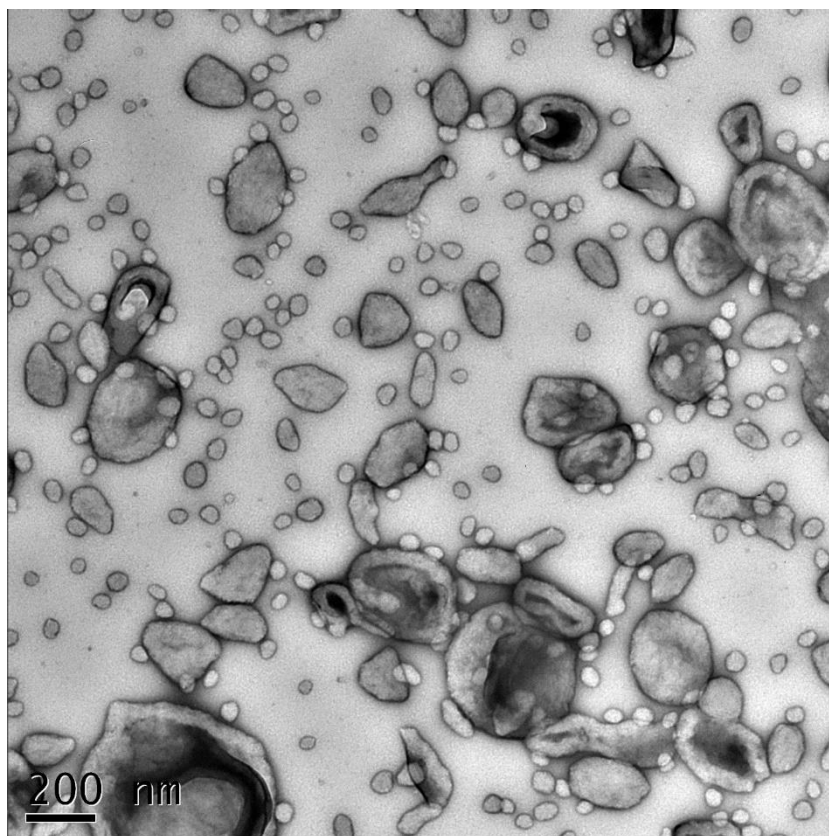


Figure 7.82: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – t = 30 min)

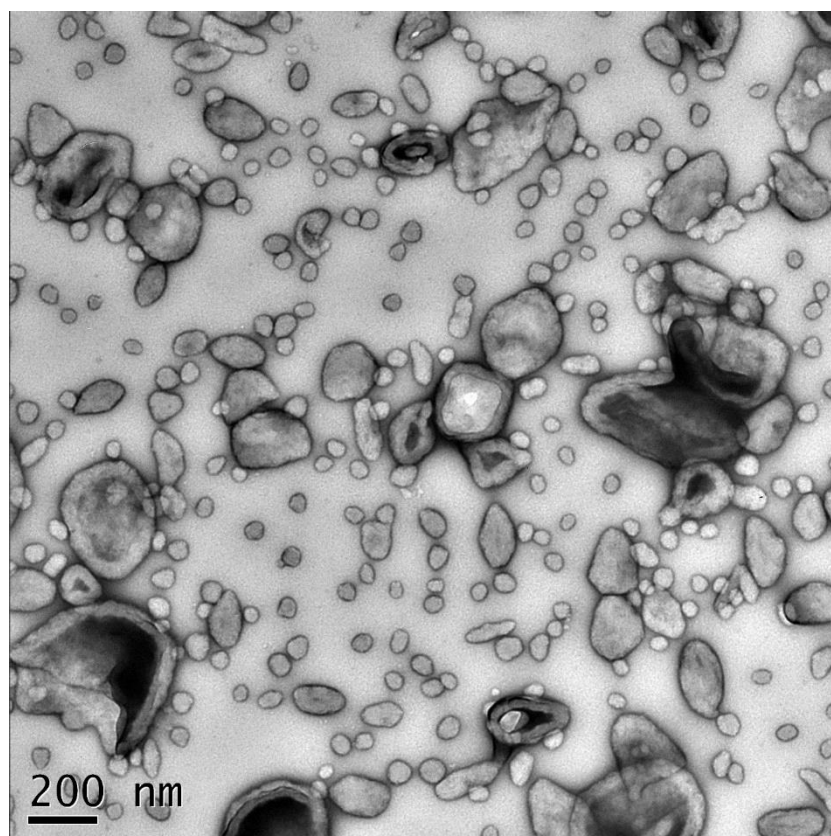


Figure 7.83: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – t = 30 min)

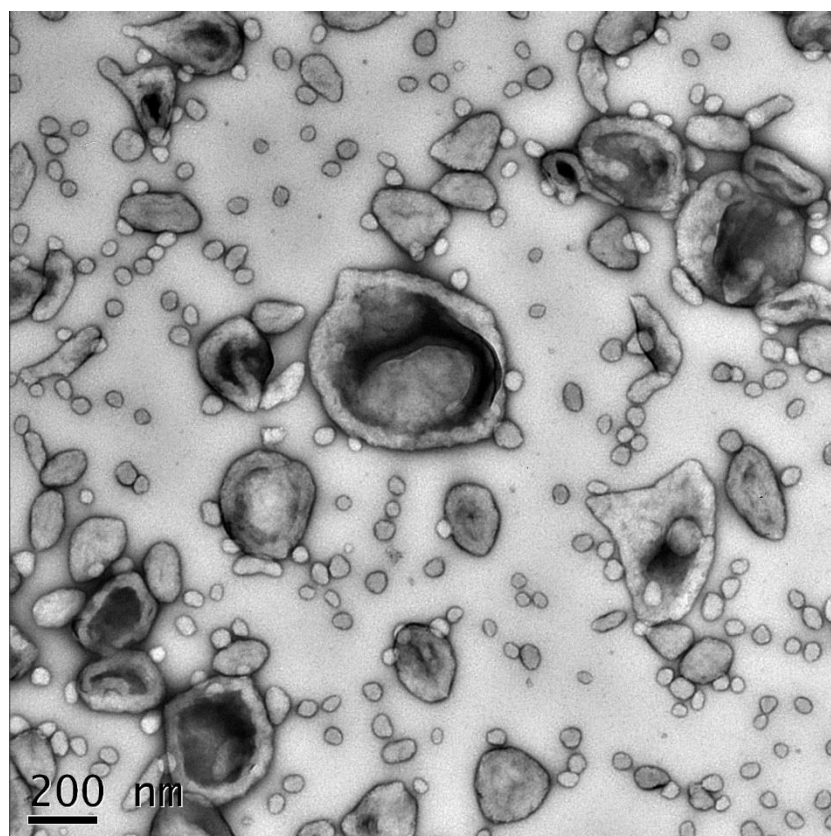


Figure 7.84: – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects made via SE (50% MeOH – Rapid Addition – t = 30 min)

Chapter 5 Supplementary TEM images:

SE PDMA_{m50}-b-PDAAm₃₀₀ pH 7.5 t = 30 min

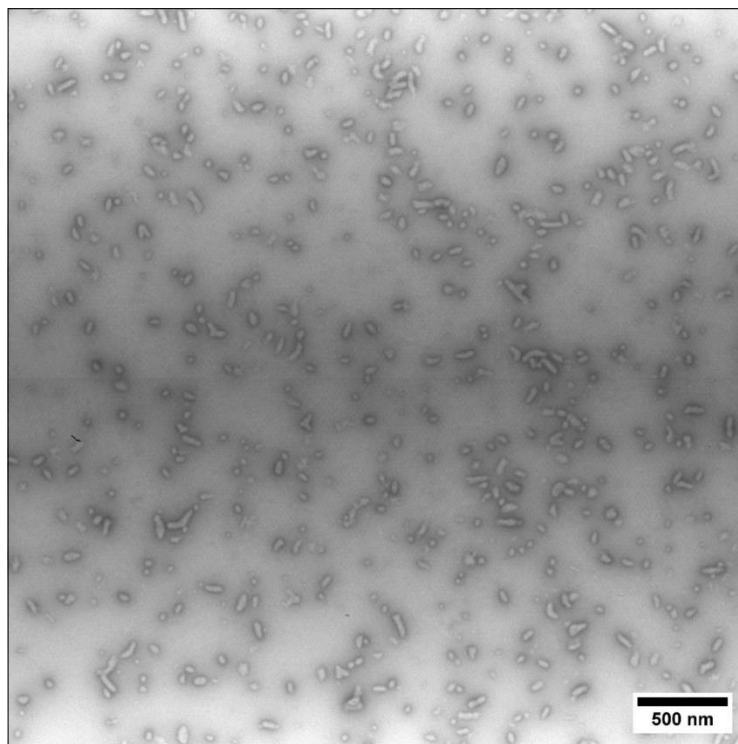


Figure .7.85: – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 (50% MeOH – Rapid Addition – t = 30 min)

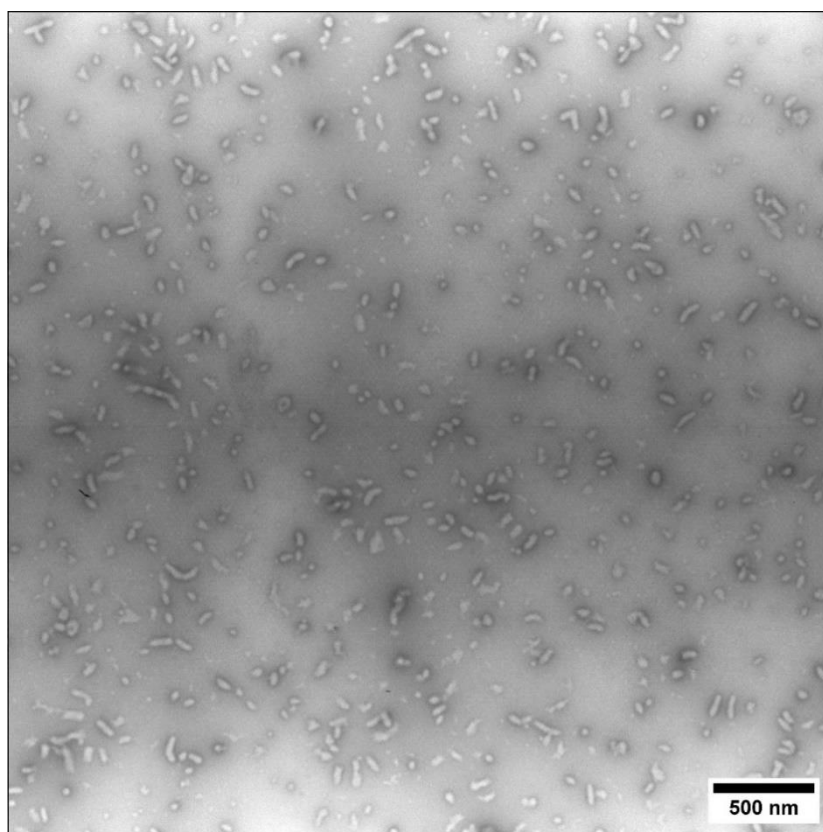


Figure 7.86: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 (50% MeOH – Rapid Addition – t = 30 min)

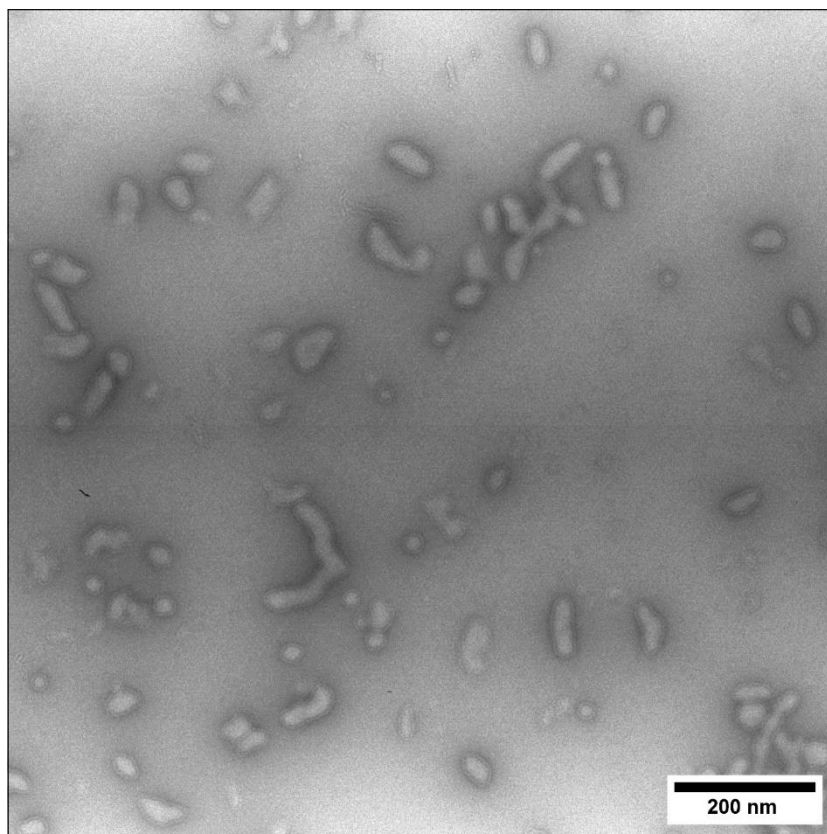


Figure 7.87: – TEM micrograph for PDMA_{m50}-b-PDAA_{m300} nano-objects synthesised via SE at pH 7.5 (50% MeOH – Rapid Addition – t = 30 min)

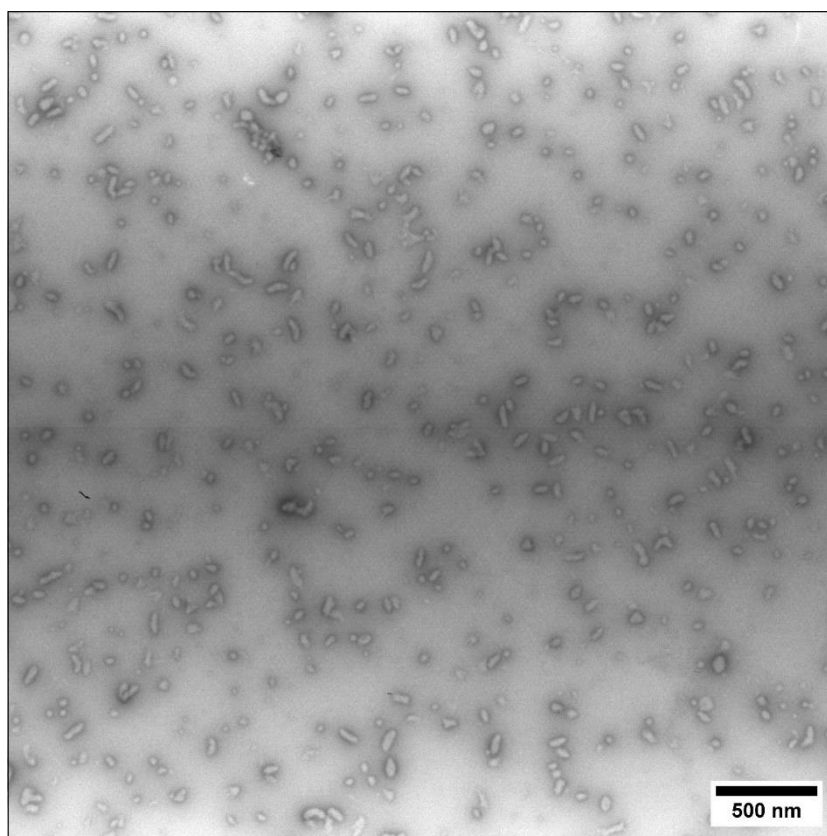


Figure 7.88: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 (50% MeOH – Rapid Addition – t = 30 min)

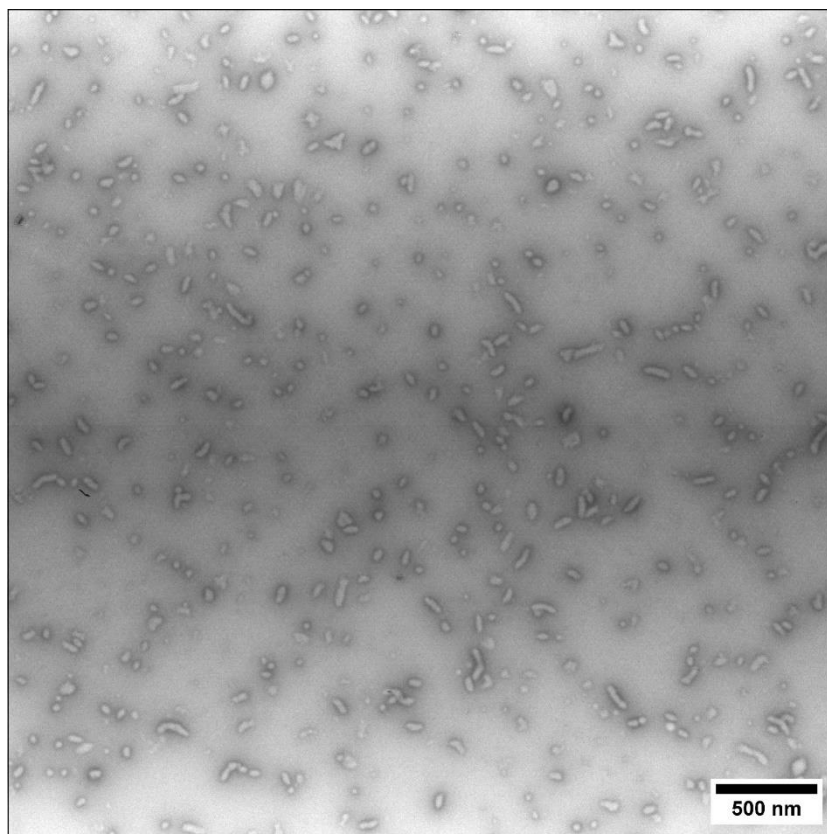


Figure 7.89: – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 (50% MeOH – Rapid Addition – t = 30 min)

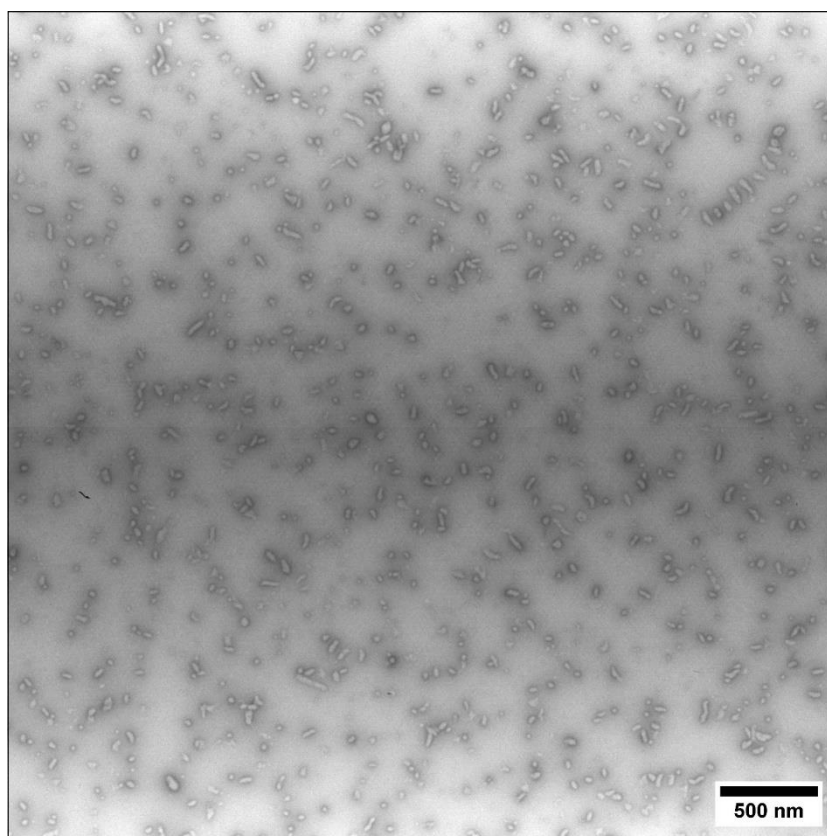


Figure 7.90: – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 (50% MeOH – Rapid Addition – $t = 30$ min)

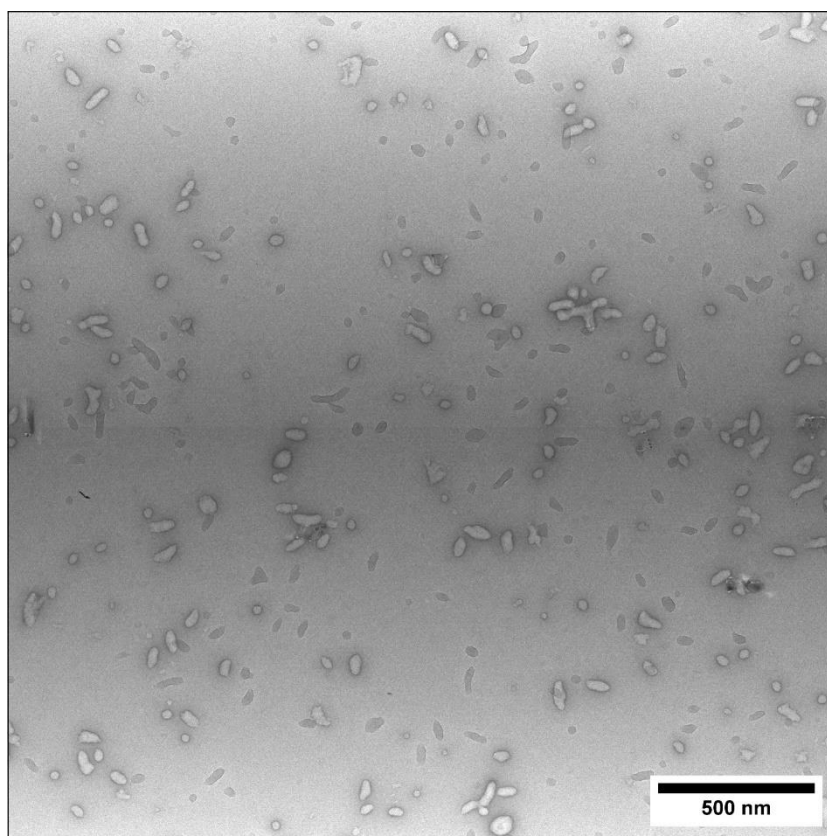


Figure 7.91: – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 (50% MeOH – Rapid Addition – t = 30 min)

SE PDMA₅₀-b-PDAAm₃₀₀ pH 7.5, 140 mM NaCl t = 30 min

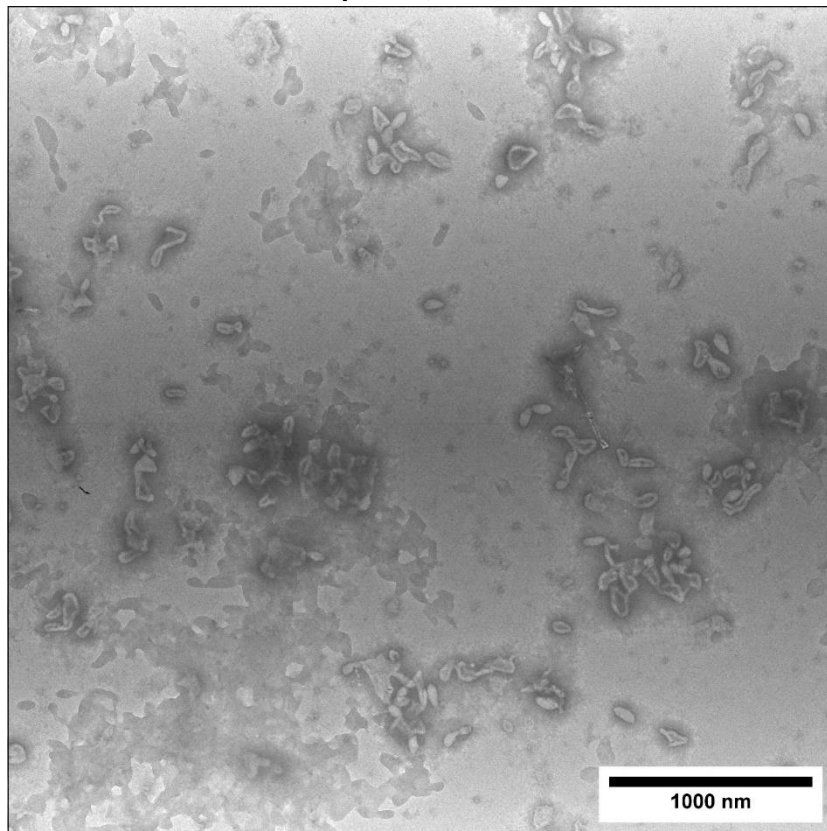


Figure 7.92: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 30 min)

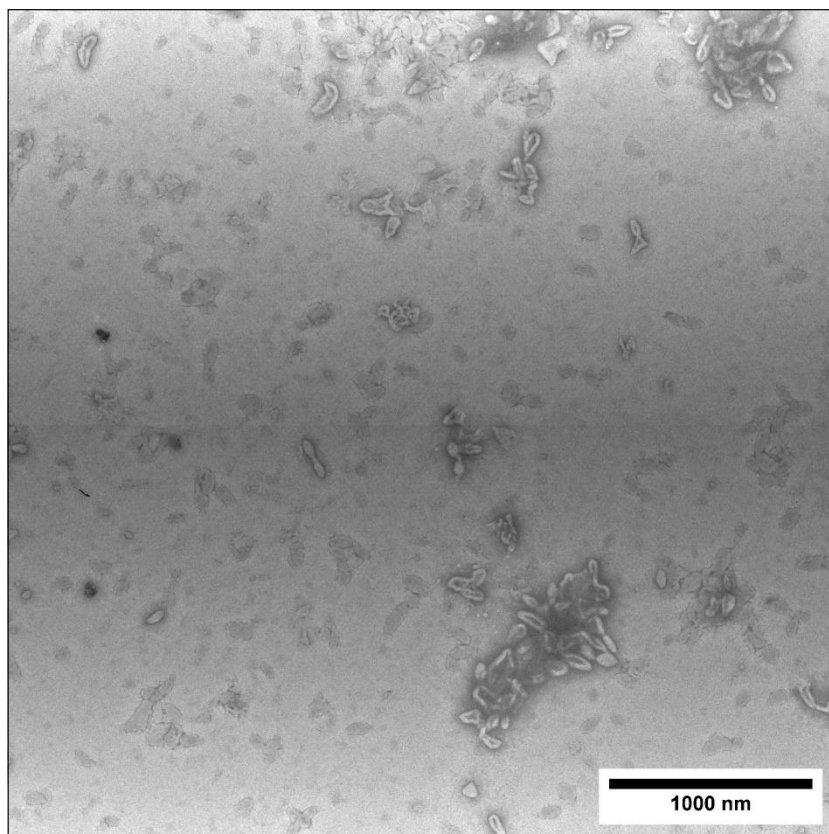


Figure 7.93: – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 30 min)

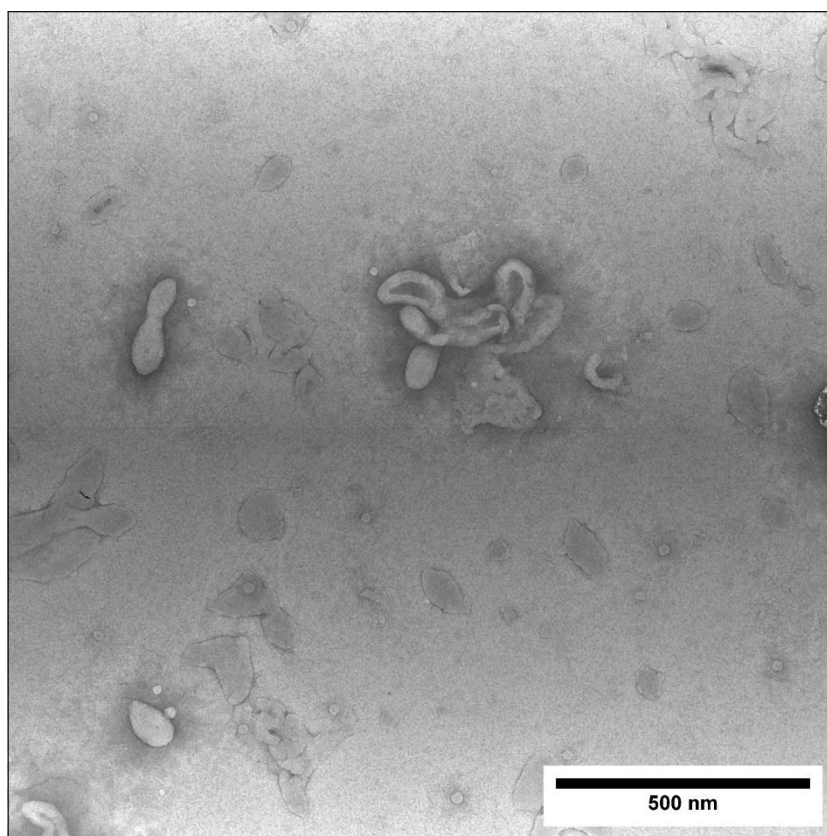


Figure 7.94: – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 30 min)

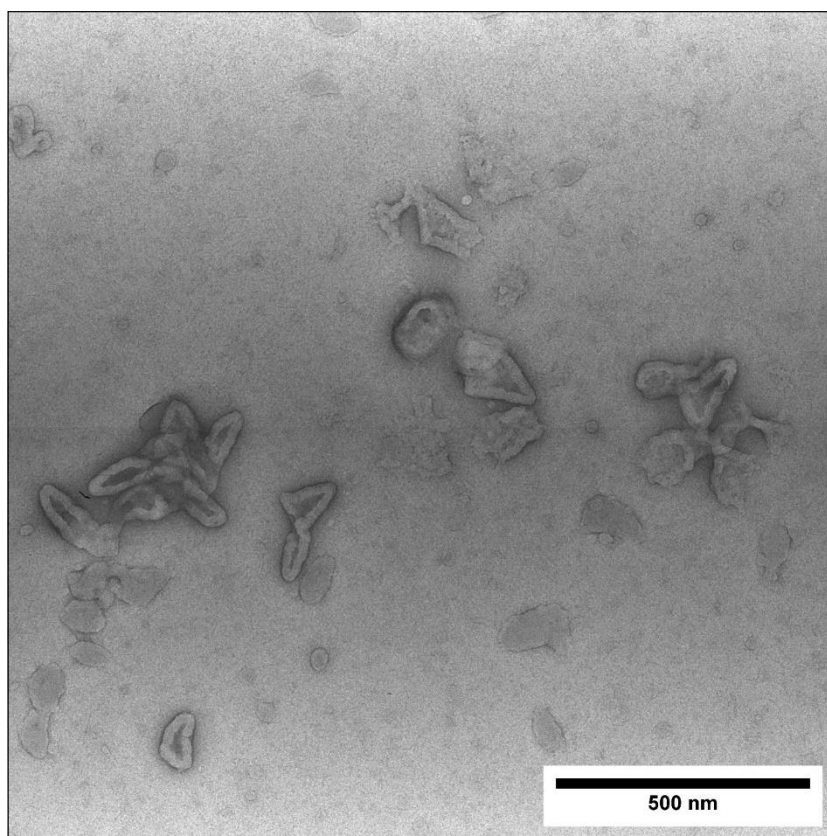


Figure 7.95: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 30 min)

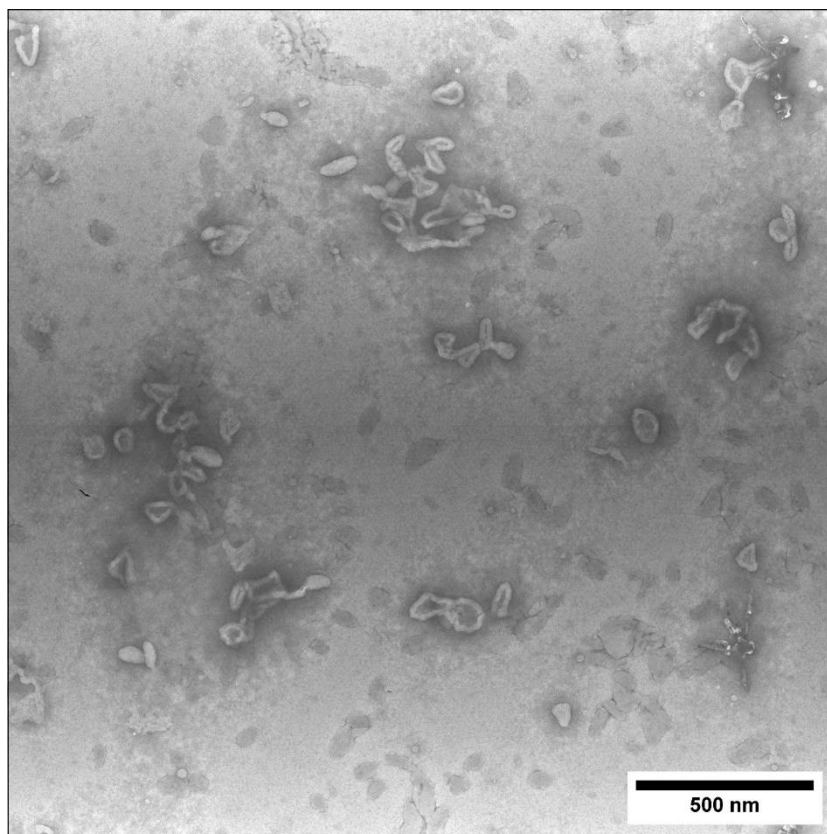


Figure 7.96: – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 30 min)

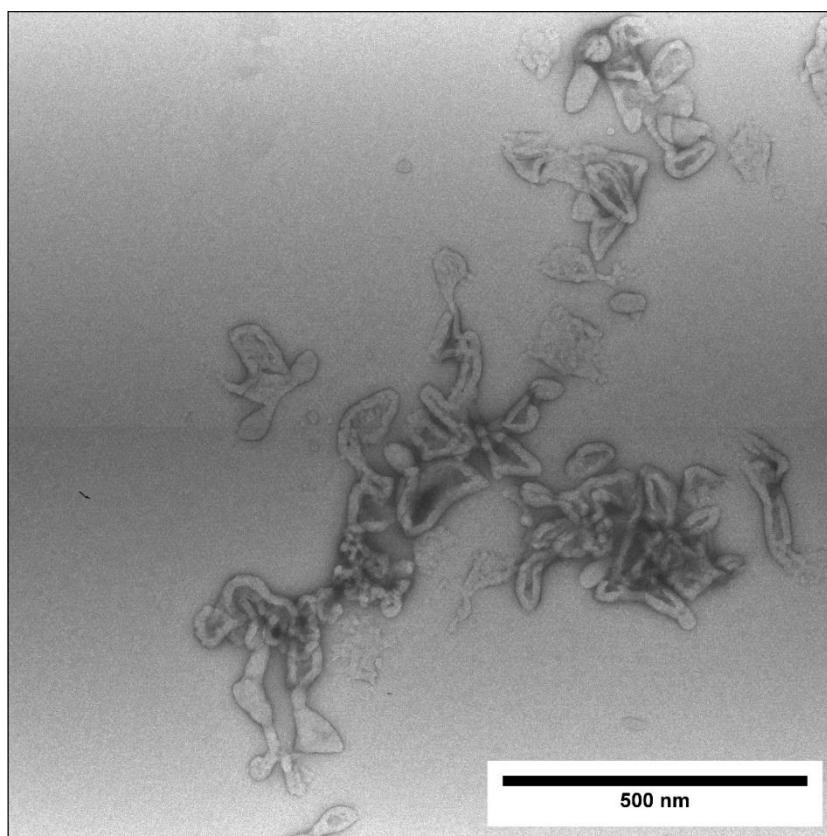


Figure 7.97: – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 30 min)

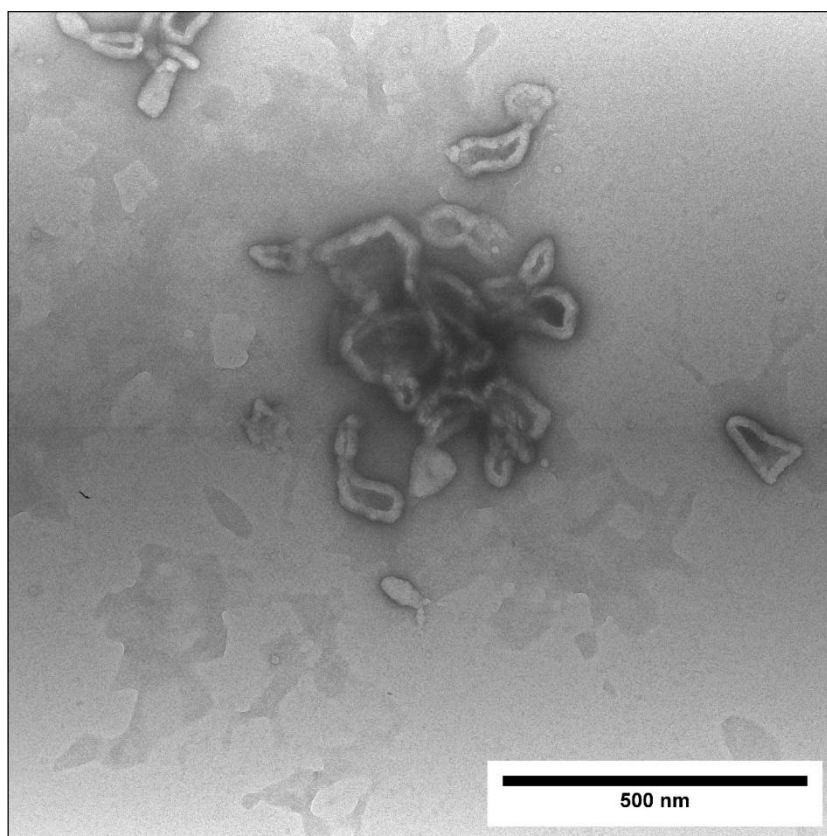


Figure 7.98: – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 30 min)

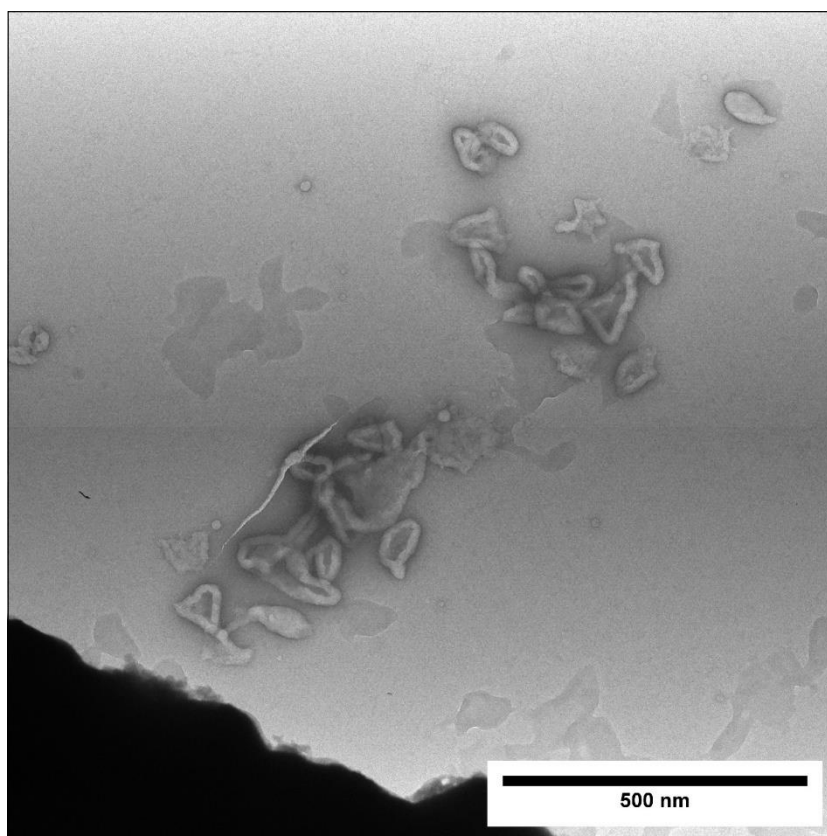


Figure 7.99: – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 30 min)

3-hour time resolved SE varying ionic strength and pH on block co-polymer.

PDMAm₅₀PDAAm₃₀₀ Doubly charged pH 2.5

t = 5 min

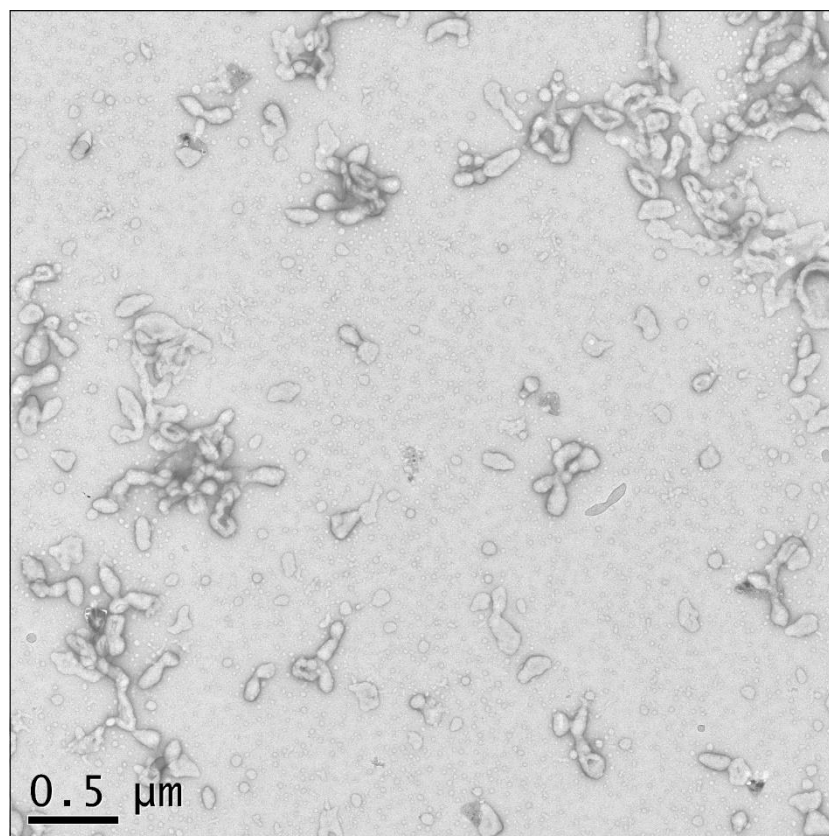


Figure 7.100: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 5 min)

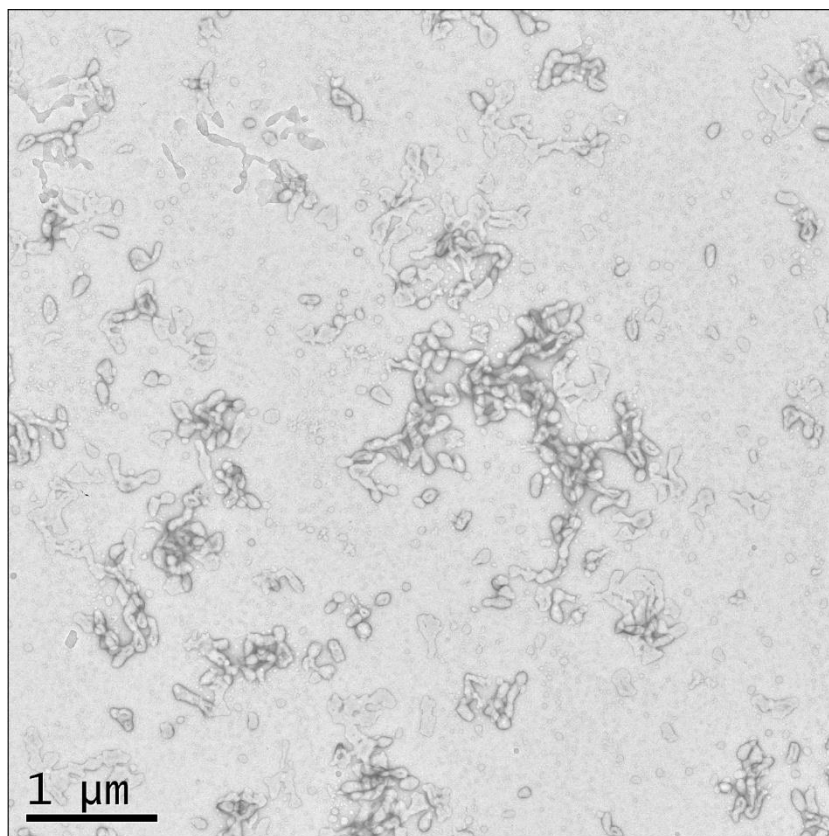


Figure 7.101: – TEM micrograph for PDMA₅₀-b-PDAA₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 5 min)

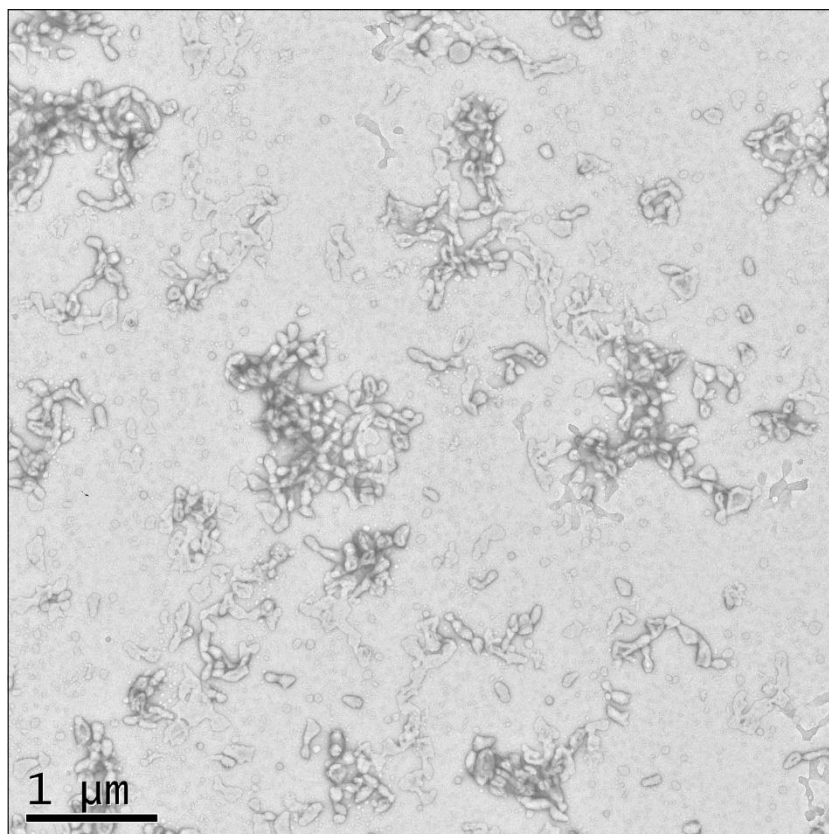


Figure 7.102: – TEM micrograph for PDMA_{m50}-b-PDAA_{m300} nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 5 min)

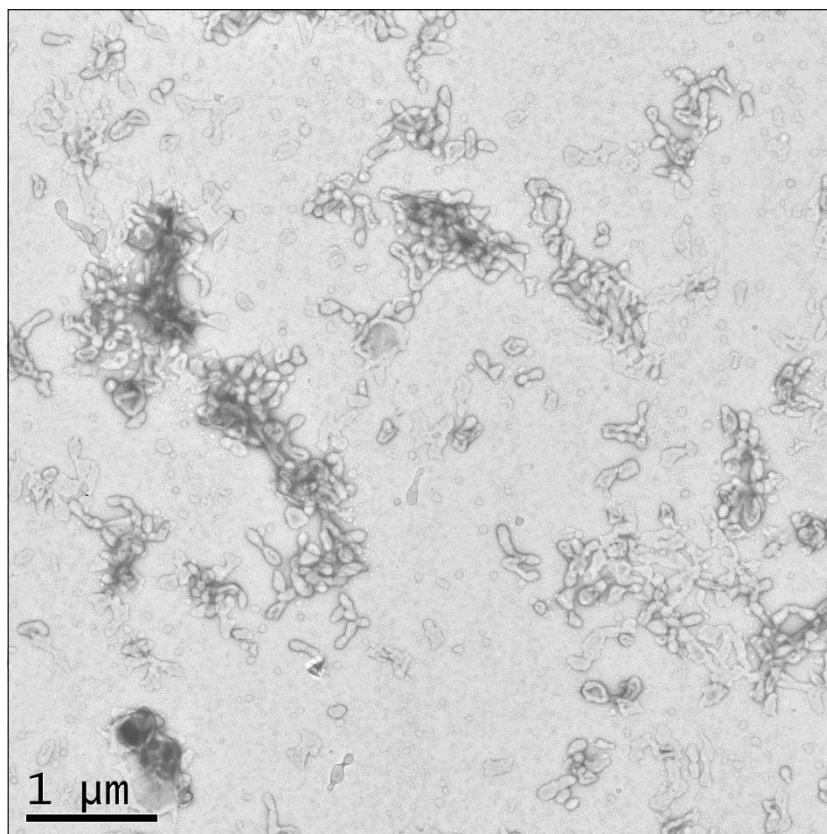


Figure 7.103: – TEM micrograph for PDMA_{m50}-b-PDAA_{m300} nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 5 min)

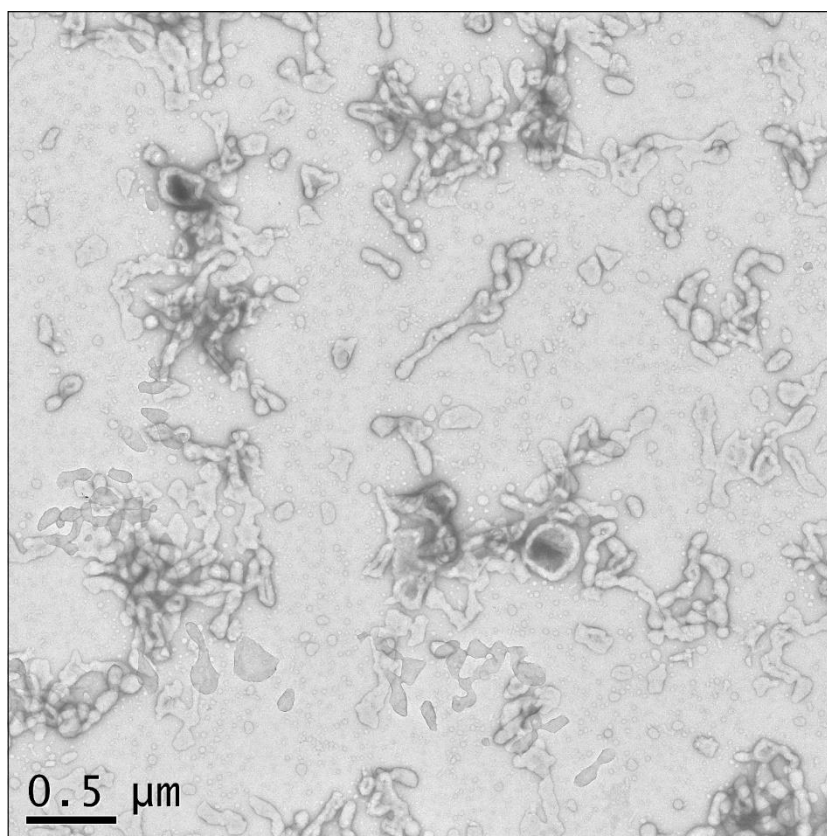


Figure 7.104: – TEM micrograph for PDMA₅₀-b-PDAA₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 5 min)

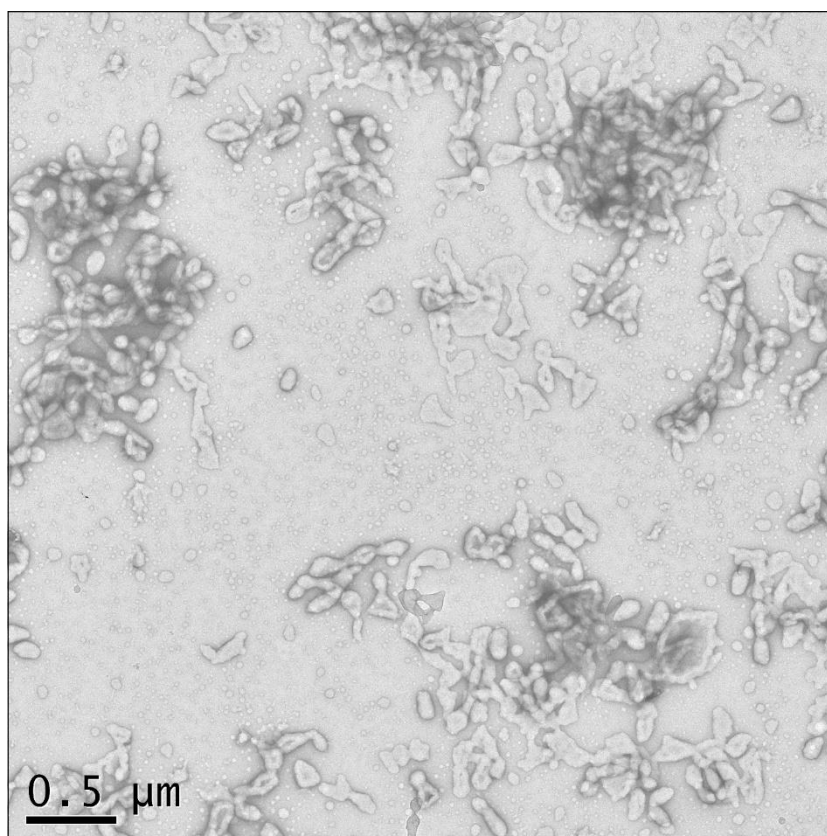


Figure 7.105: – TEM micrograph for PDMA₅₀-b-PDAA₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 5 min)

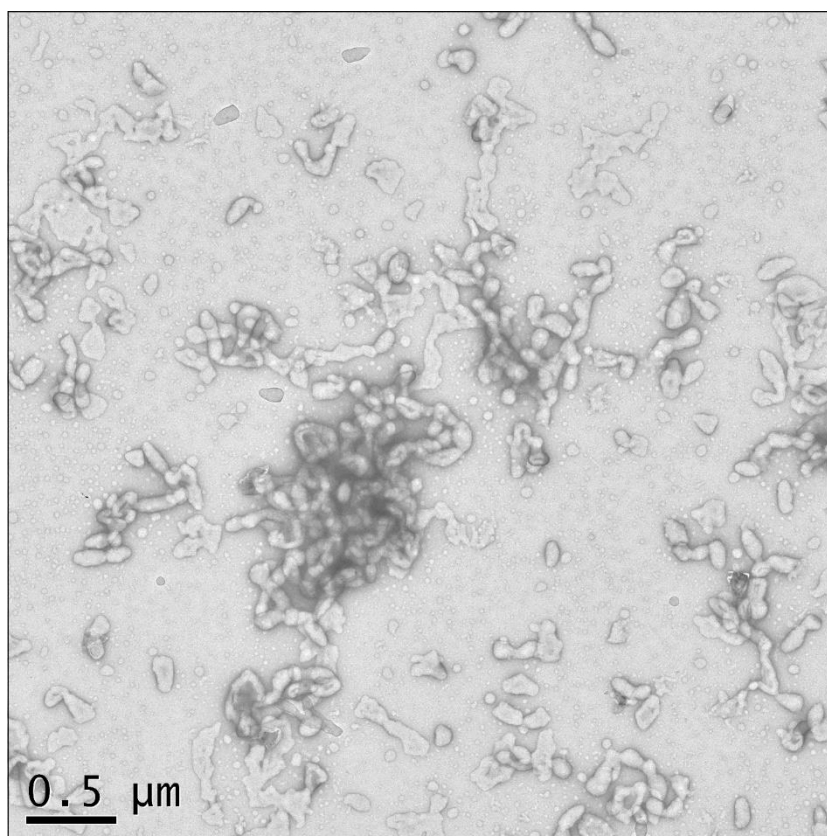


Figure 7.106: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 5 min)

t = 15 min

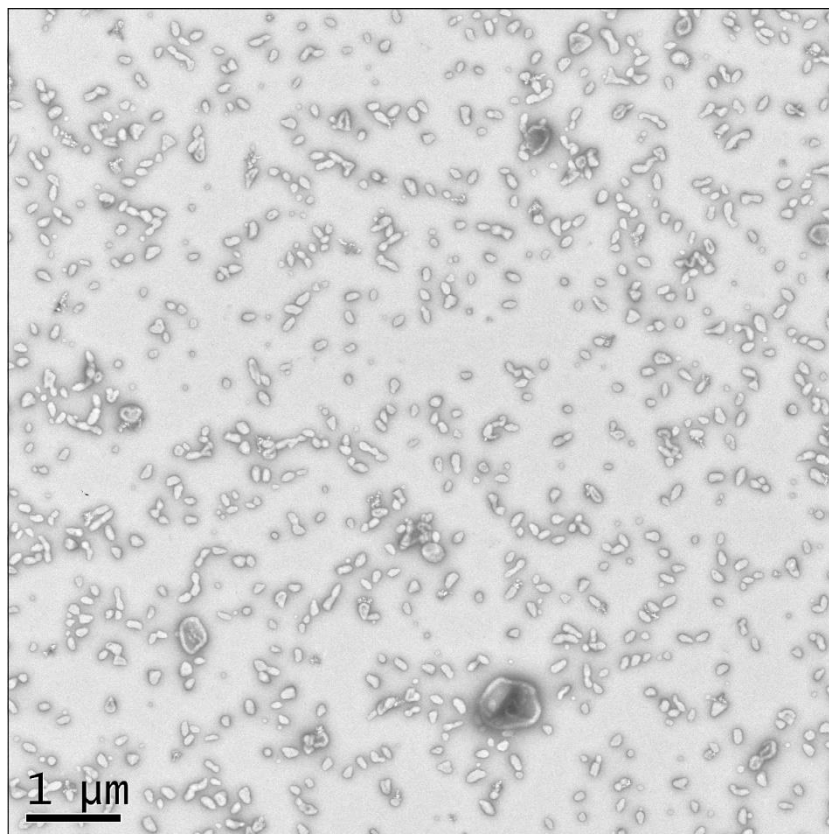


Figure 7.107: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 15 min)

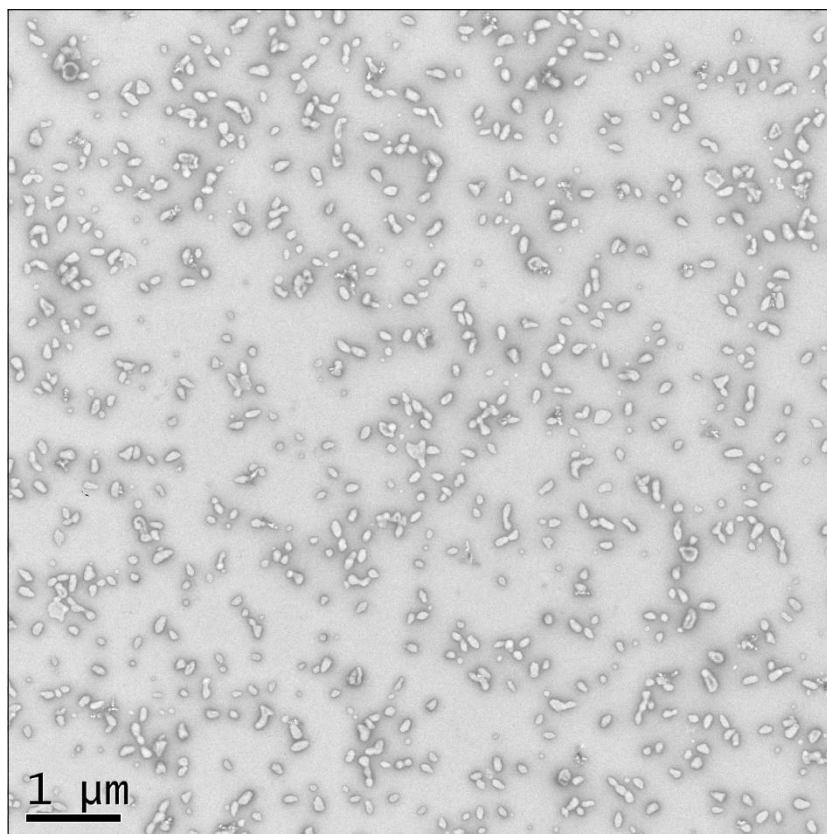


Figure 7.108: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 15 min)

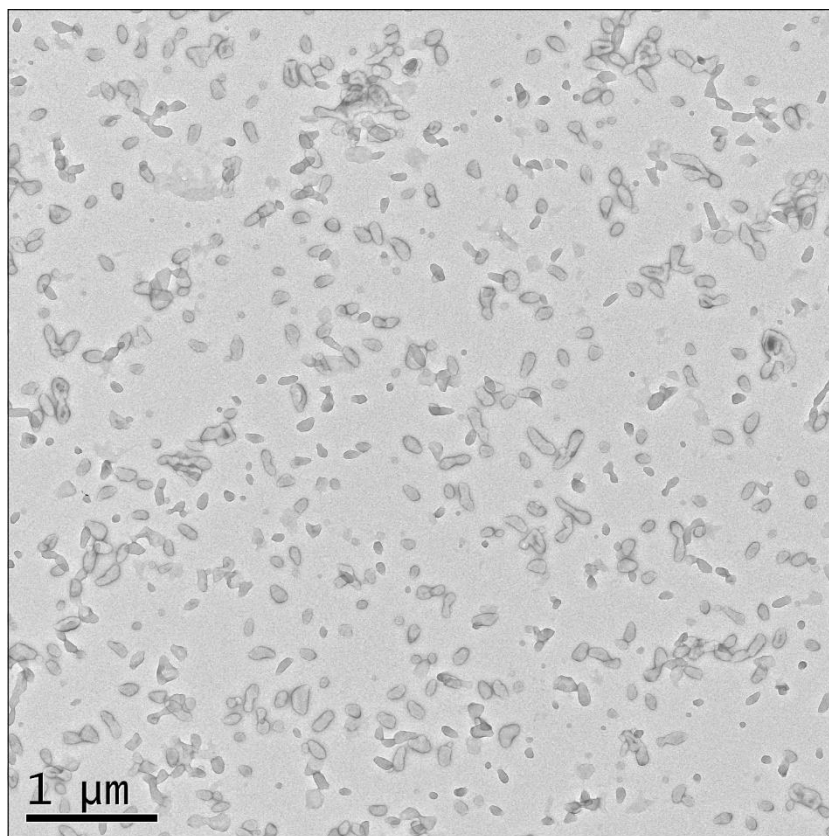


Figure 7.109: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 15 min)

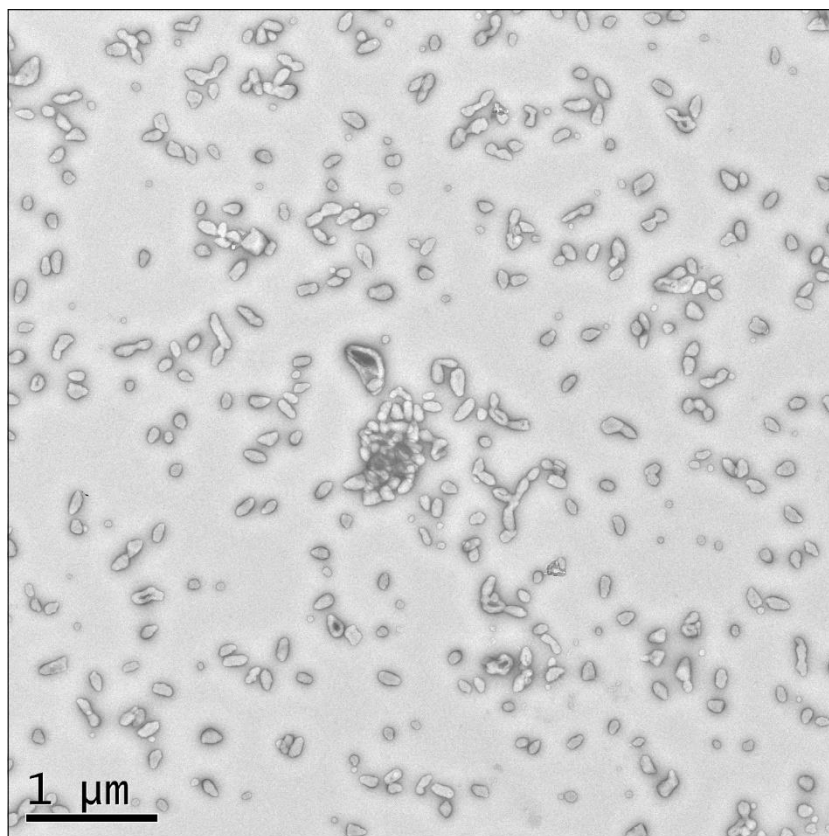


Figure 7.110: – TEM micrograph for PDMA_{m50}-b-PDAA_{m300} nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 15 min)

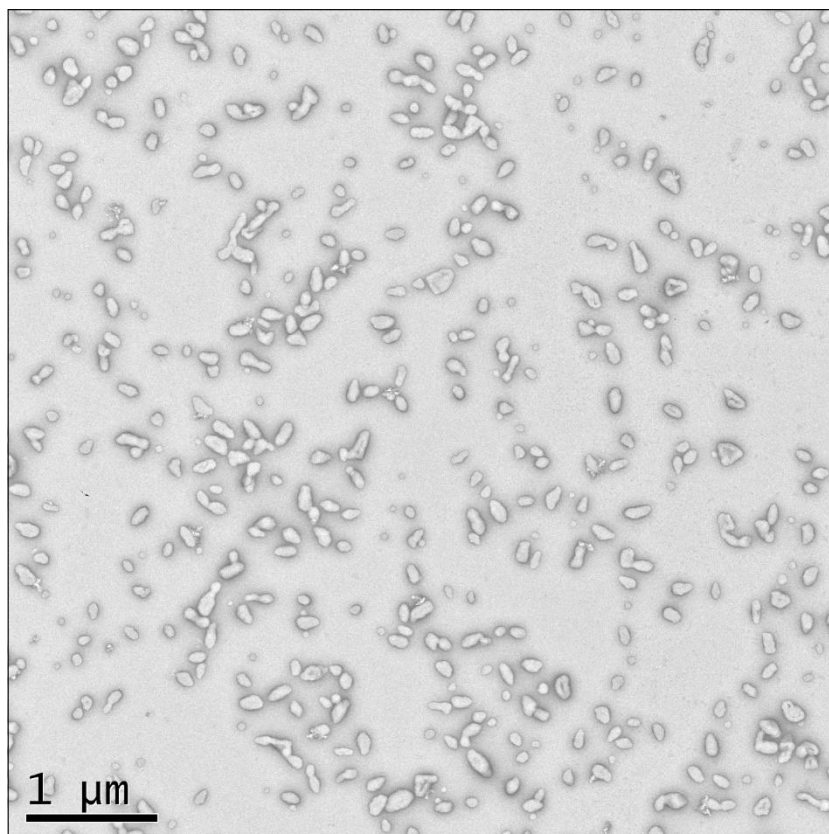


Figure 7.111: TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 15 min)

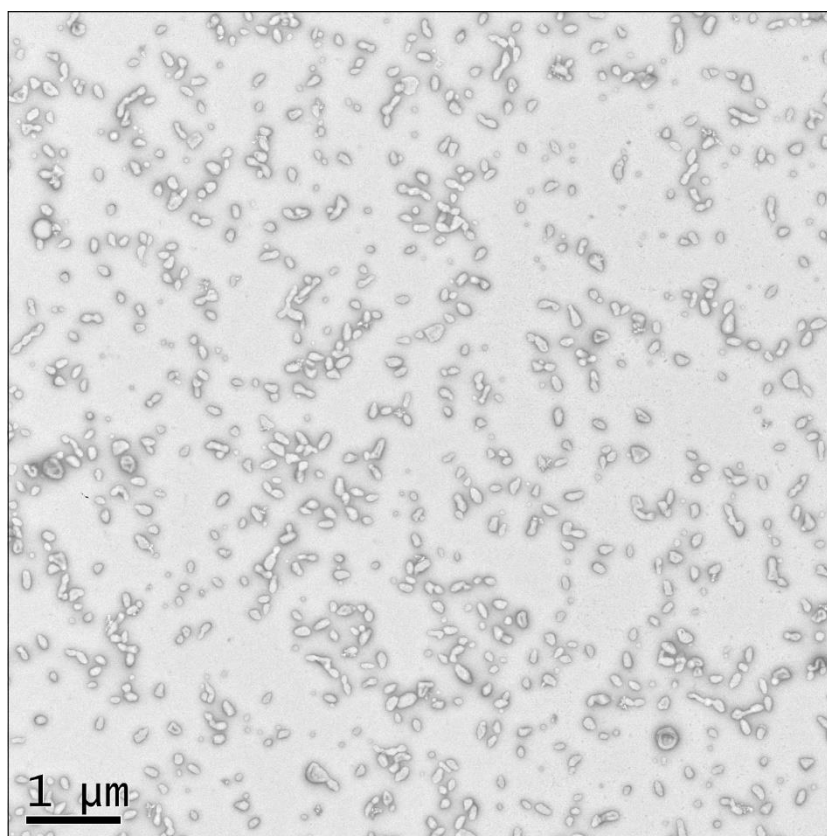


Figure 7.112: – TEM micrograph for PDMA_{m50}-b-PDAA_{m300} nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 15 min)

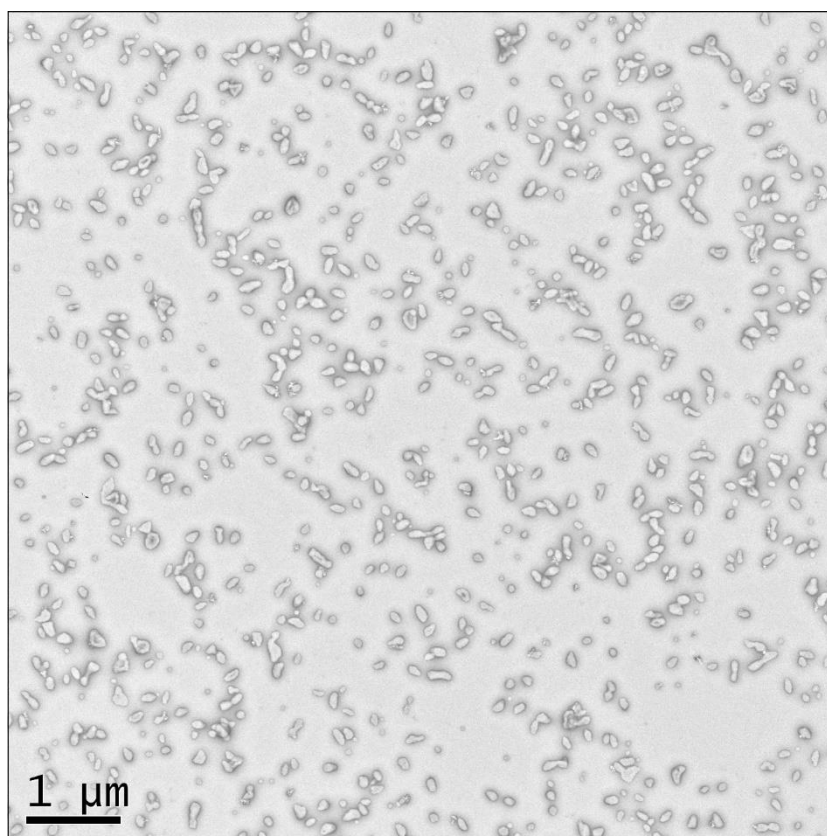


Figure 7.113: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 15 min)

t = 30 min

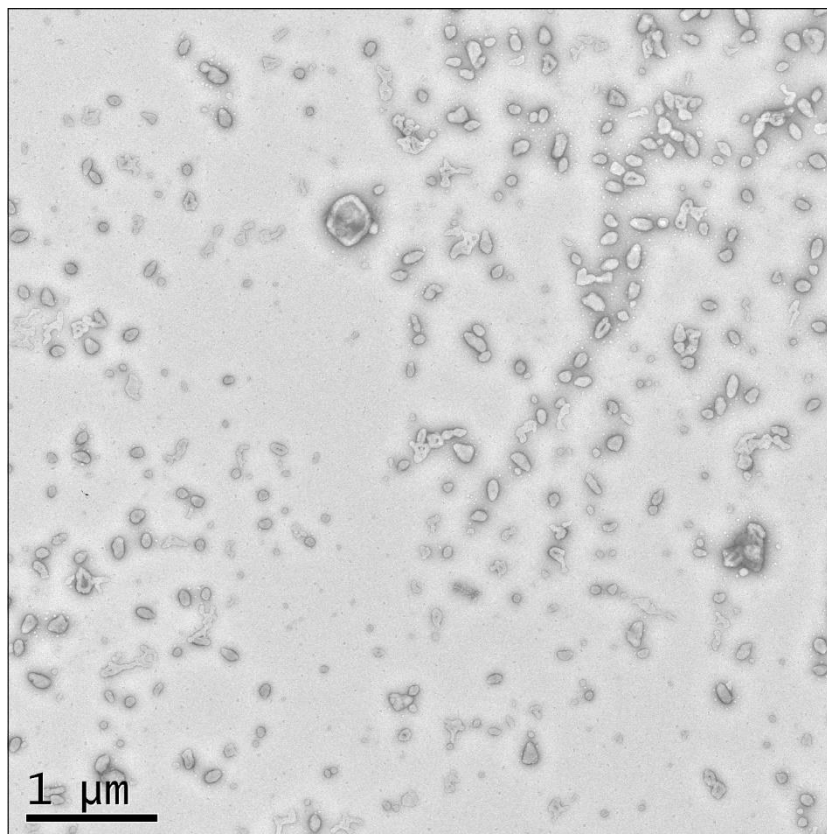


Figure 7.114: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 30 min)

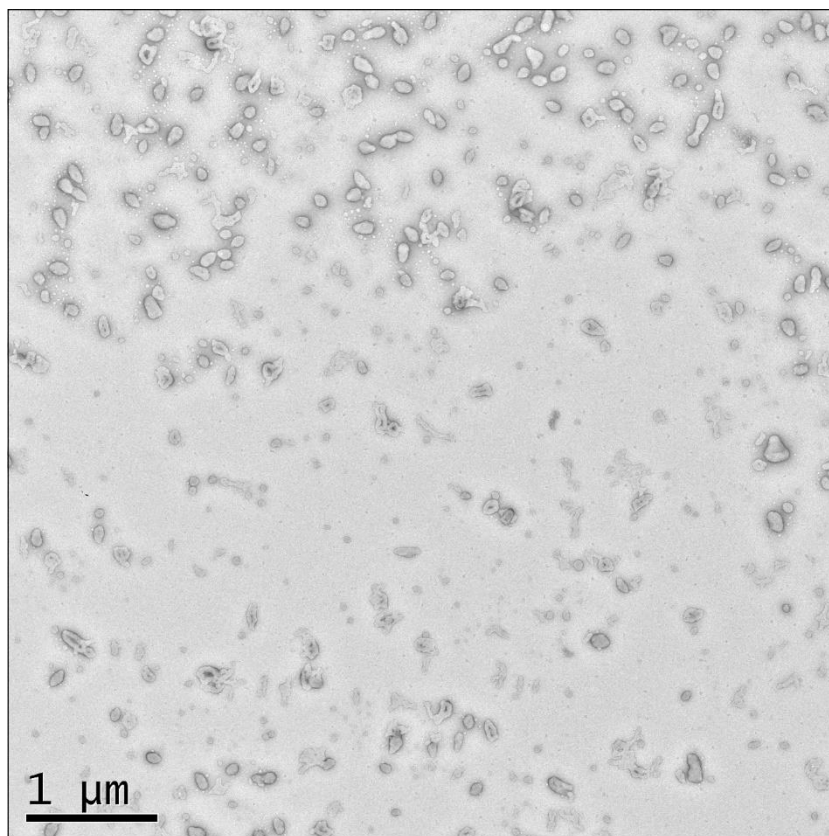


Figure 7.115: – TEM micrograph for PDMA_{m50}-b-PDAA_{m300} nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 30 min)

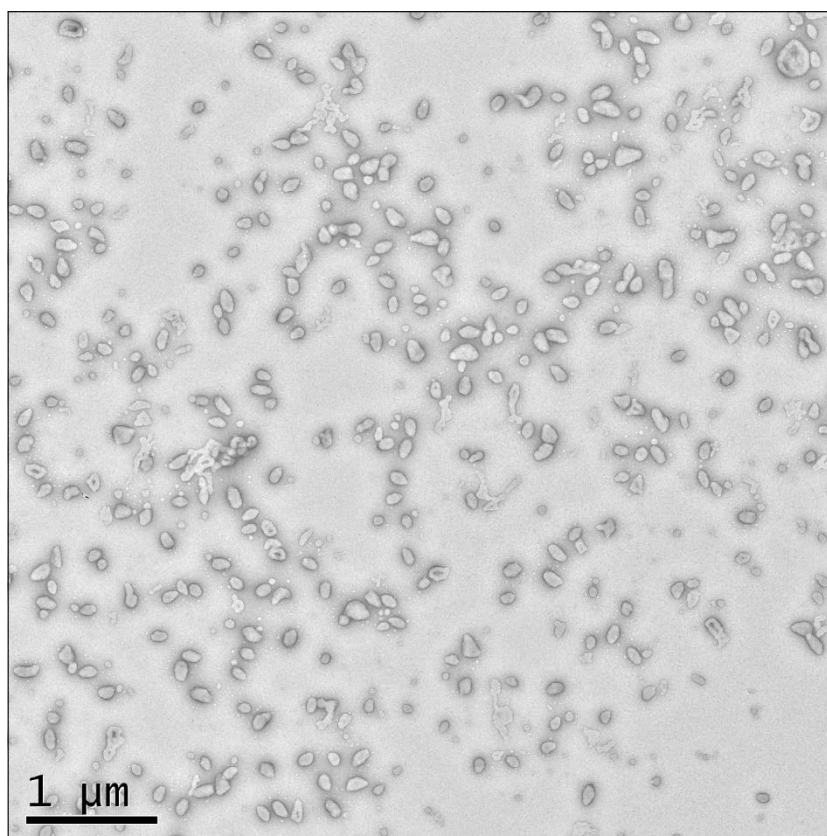


Figure 7.116: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 30 min)

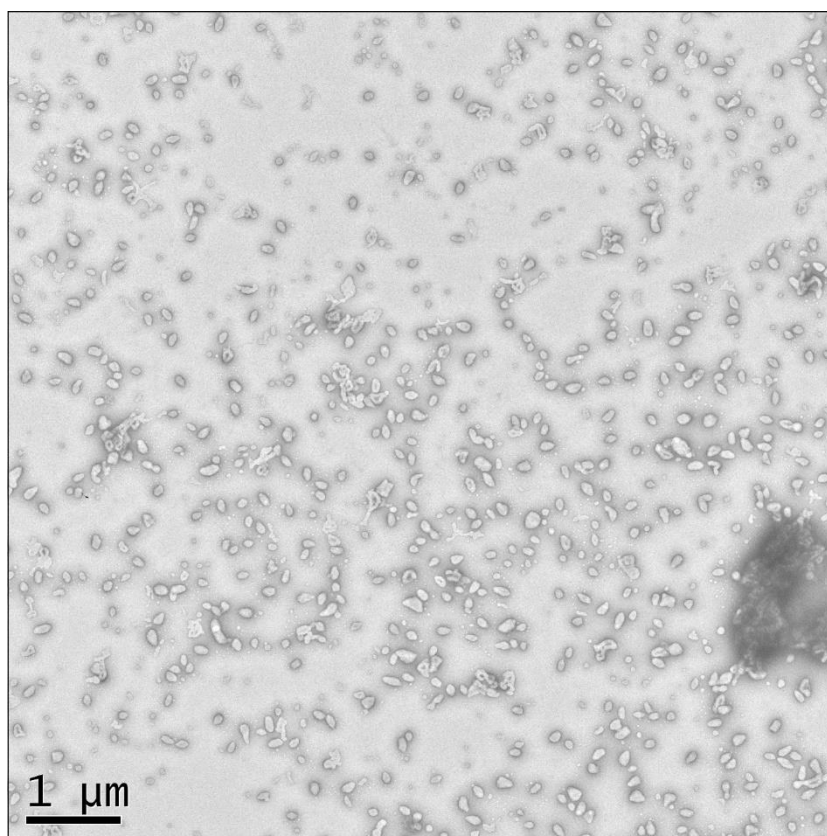


Figure 7.117: – TEM micrograph for PDMA_{m50}-b-PDAA_{m300} nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 30 min)

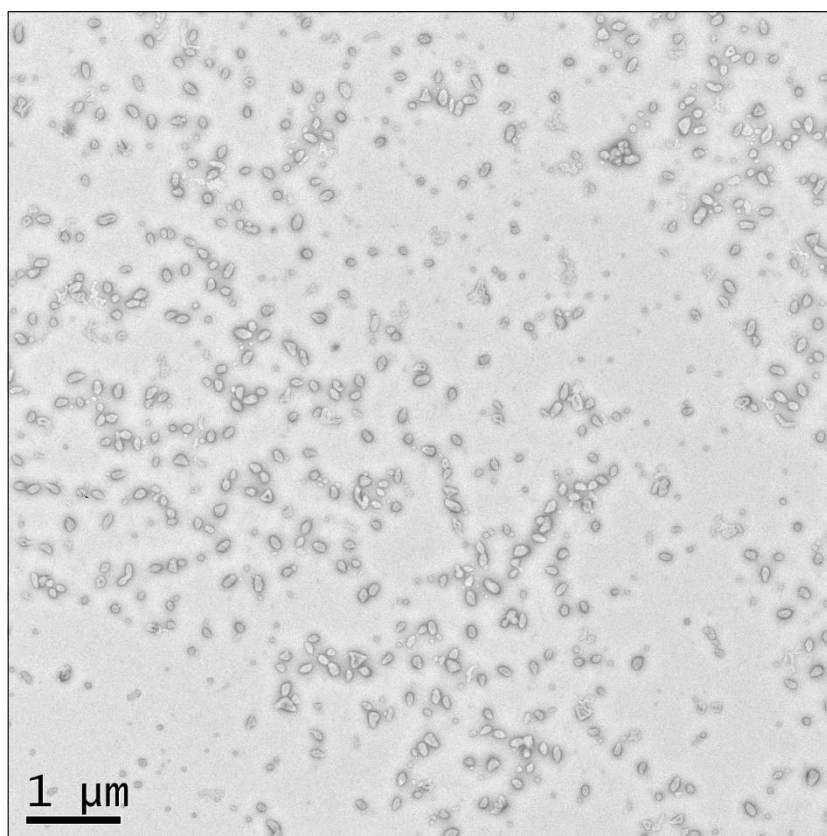


Figure 7.118: – TEM micrograph for PDMA_{m50}-b-PDAA_{m300} nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 30 min)

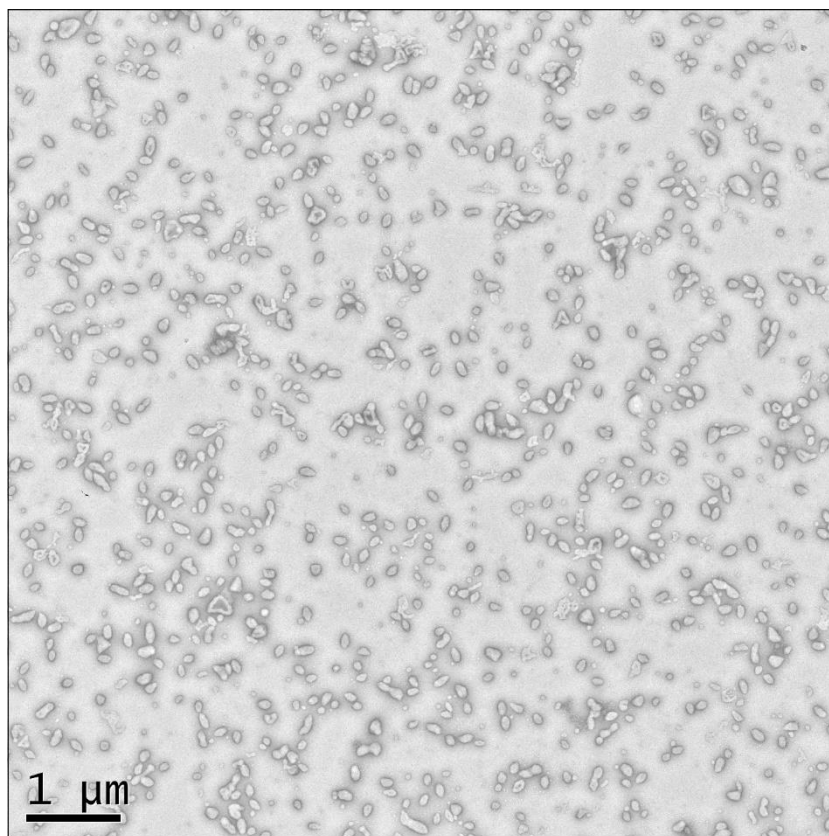


Figure 7.119: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 30 min)

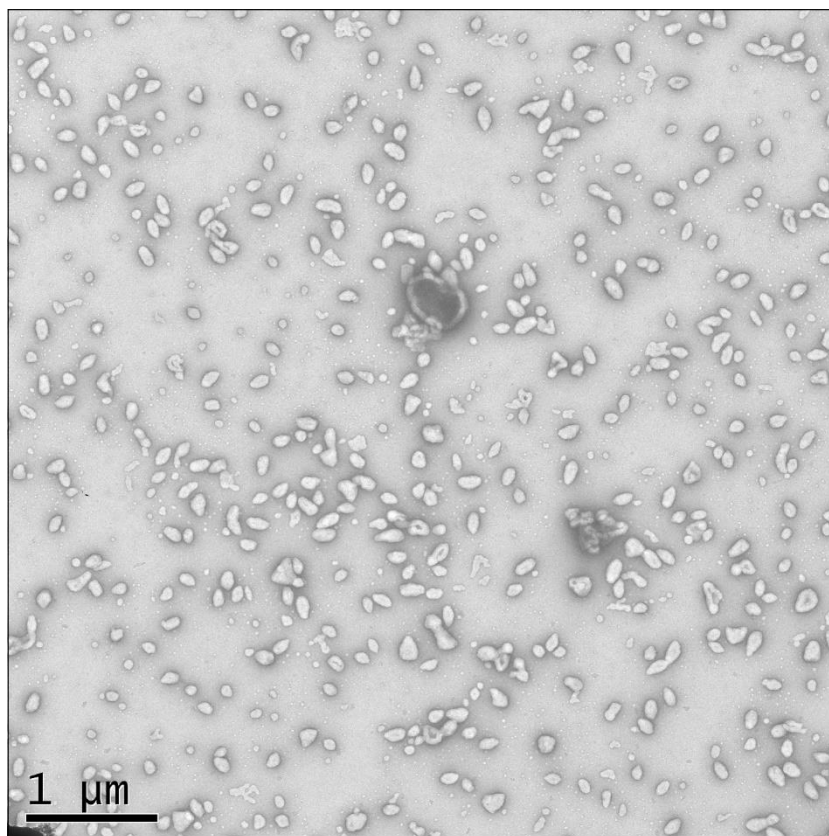


Figure 7.120: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 30 min)

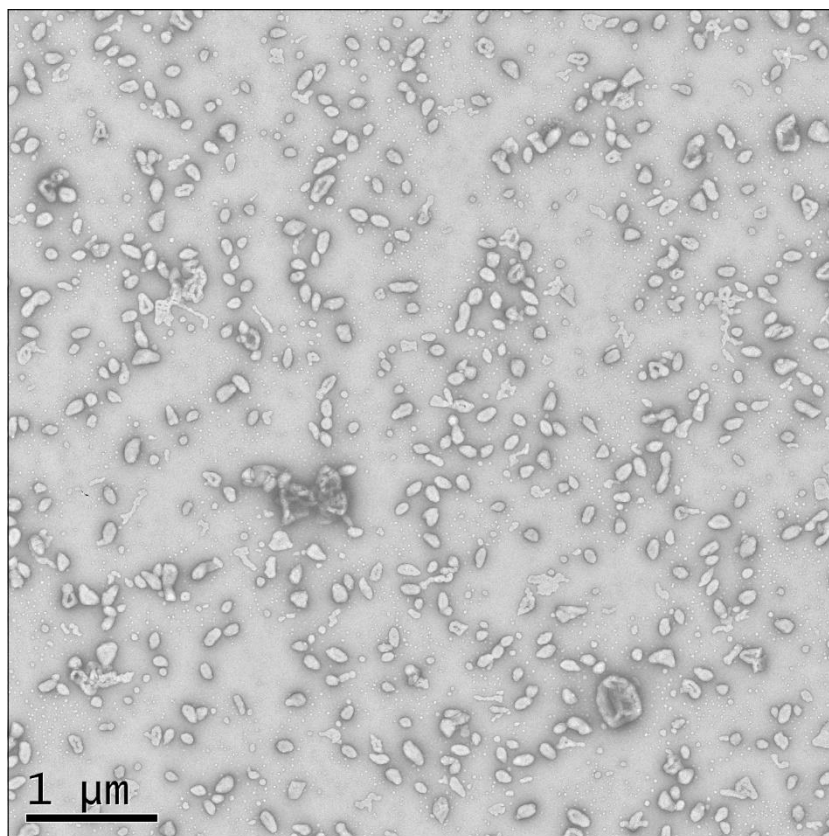


Figure 7.121: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 30 min)

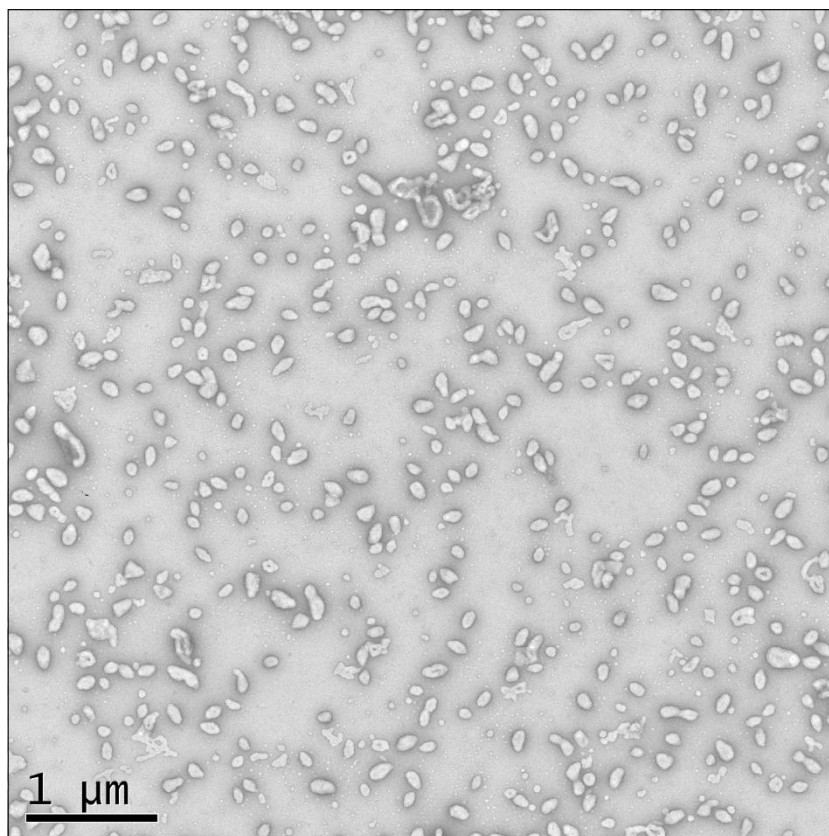


Figure 7.122: – TEM micrograph for PDMA₅₀-b-PDAA₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 30 min)

t = 60 min

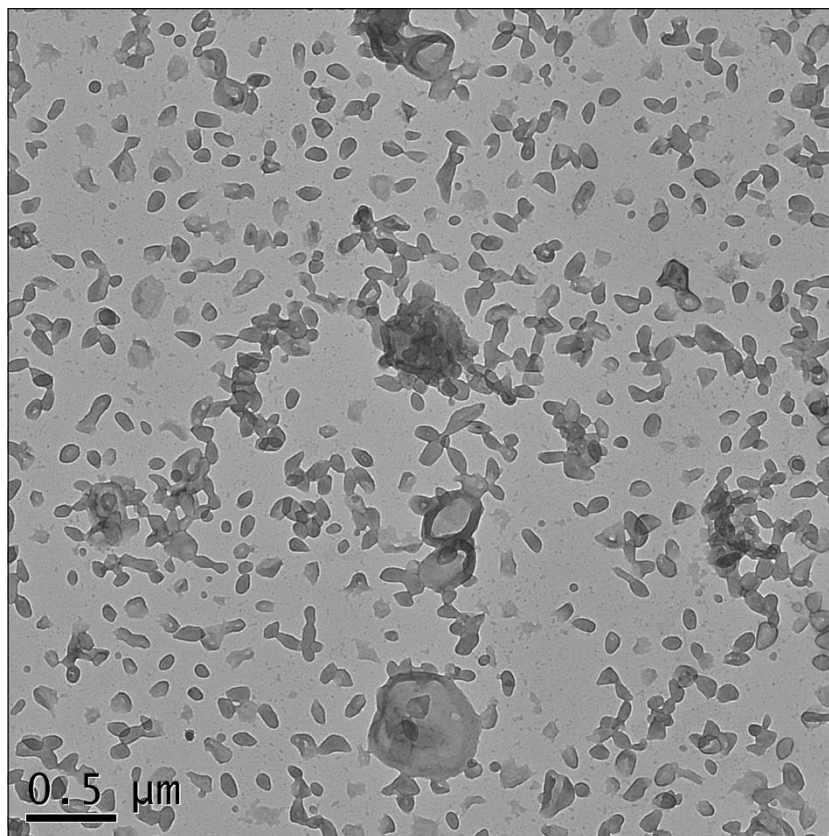


Figure 7.123: – TEM micrograph for PDMA₅₀-b-PDAA₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 60 min)

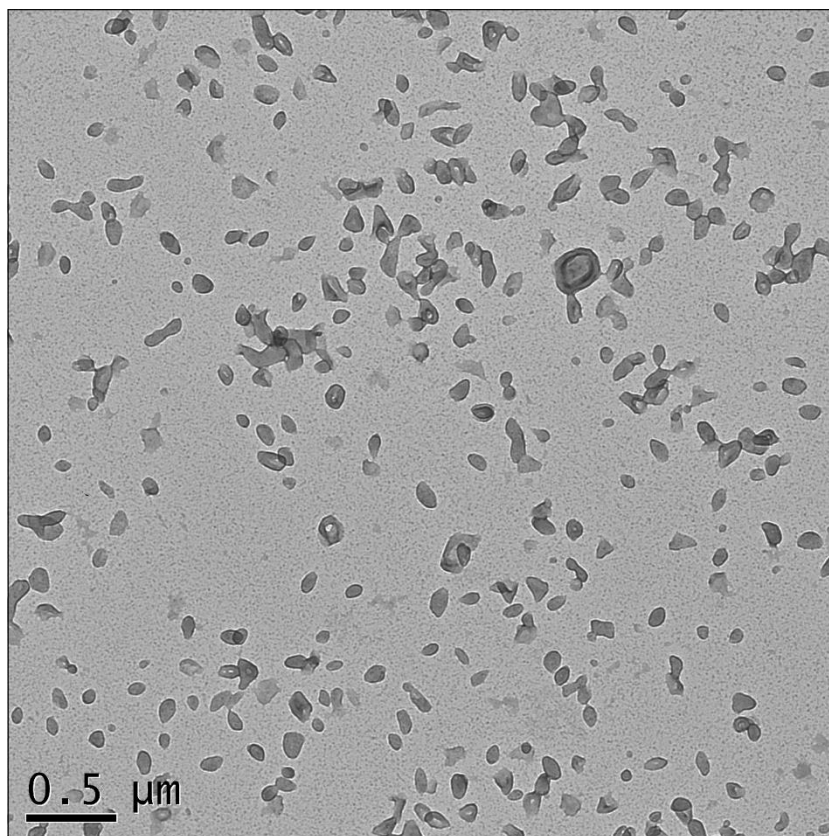


Figure 7.124: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 60 min)

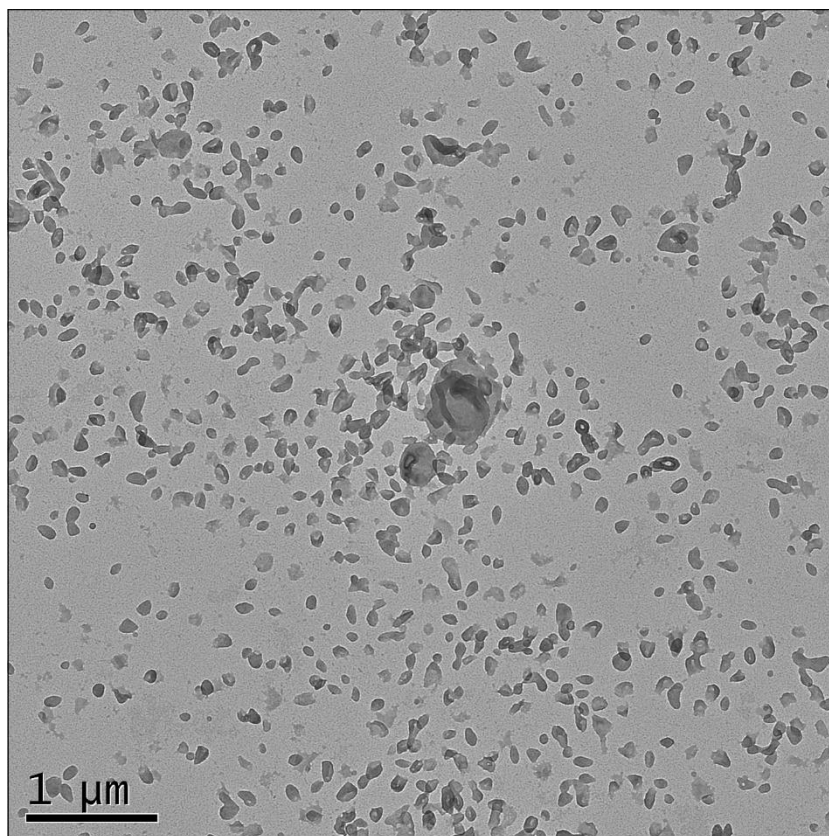


Figure 7.125: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 60 min)

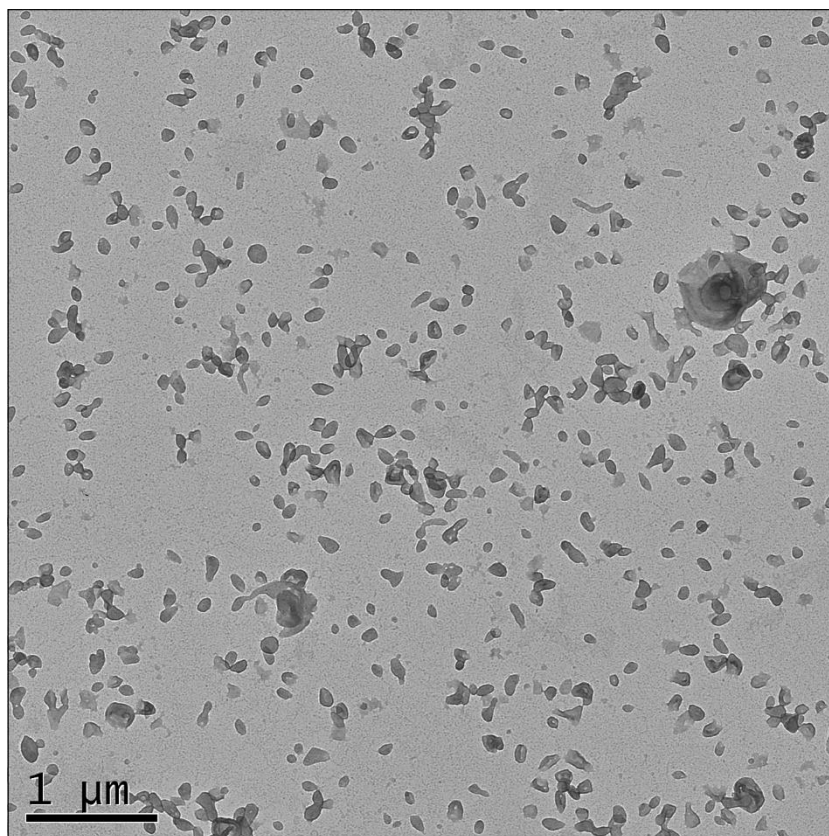


Figure 7.126 – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 60 min)

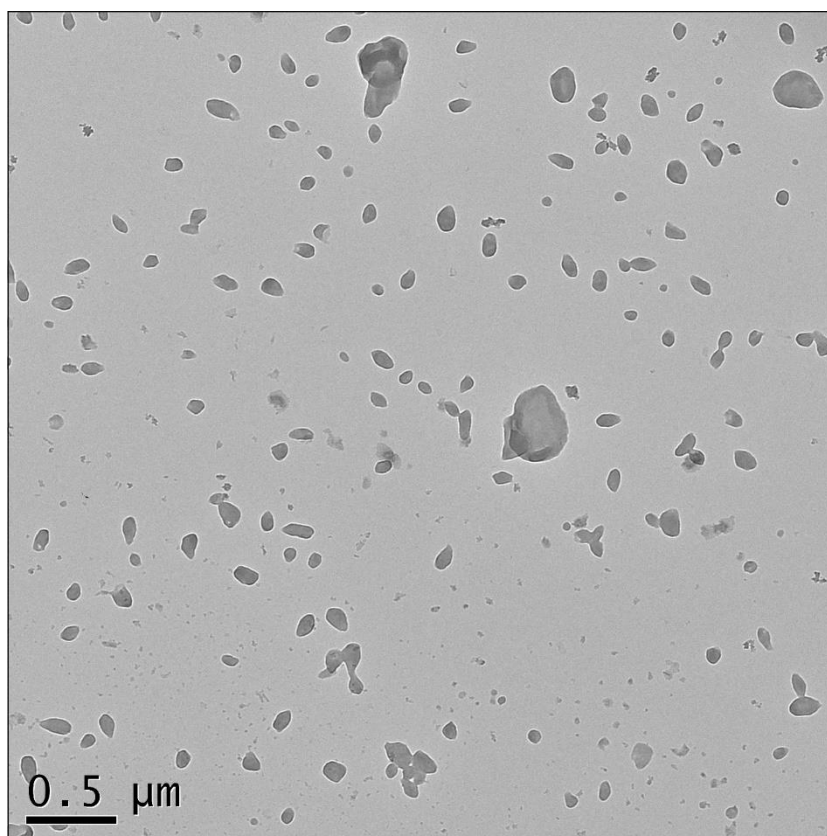


Figure 7.127: – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 60 min)

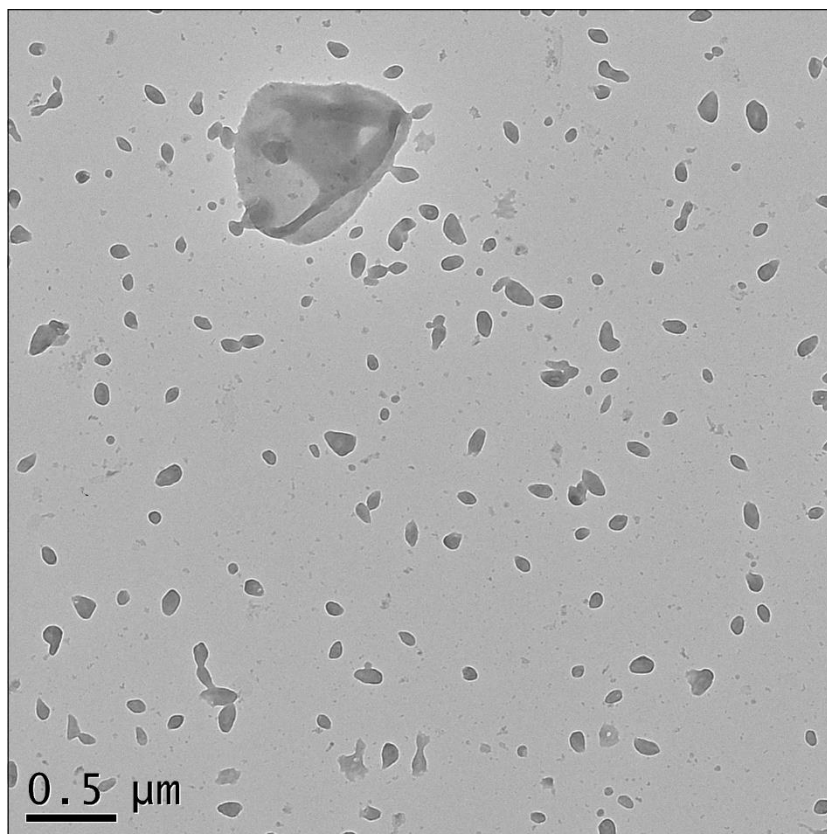


Figure 7.128: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 60 min)

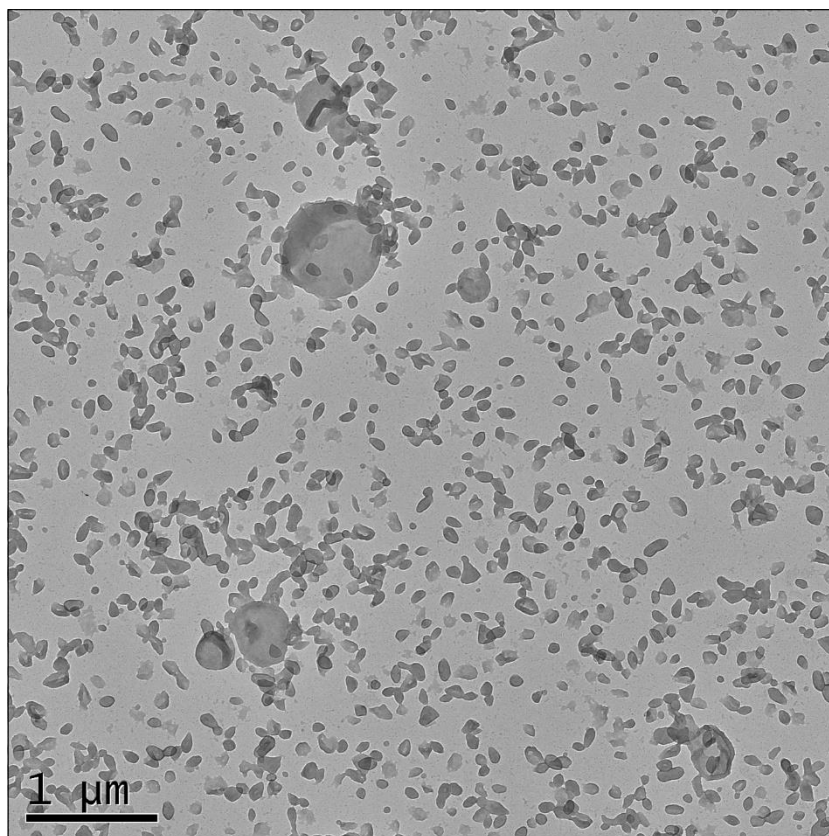


Figure 7.129: – TEM micrograph for PDMA_{m50}-b-PDAA_{m300} nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 60 min)

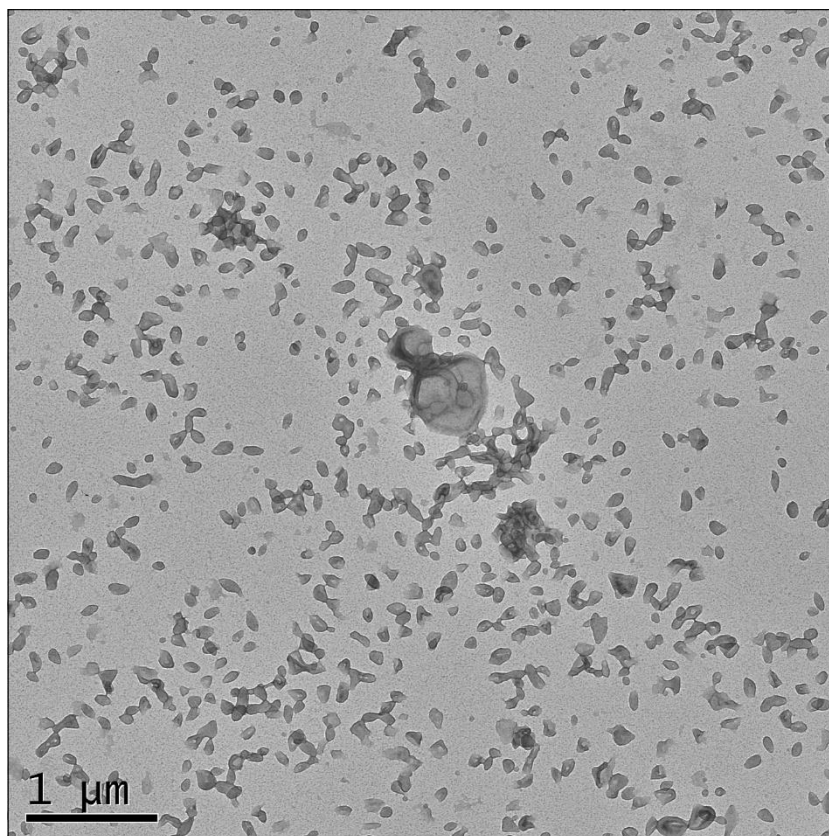


Figure 7.130: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 60 min)

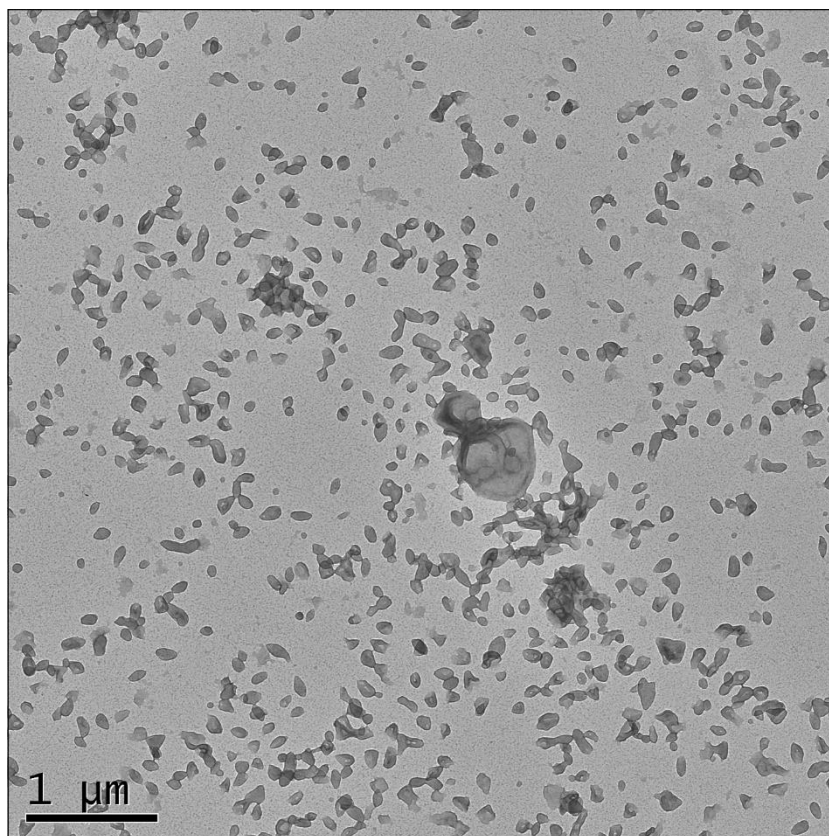


Figure 7.131: – TEM micrograph for PDMA₅₀-b-PDAA₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 60 min)

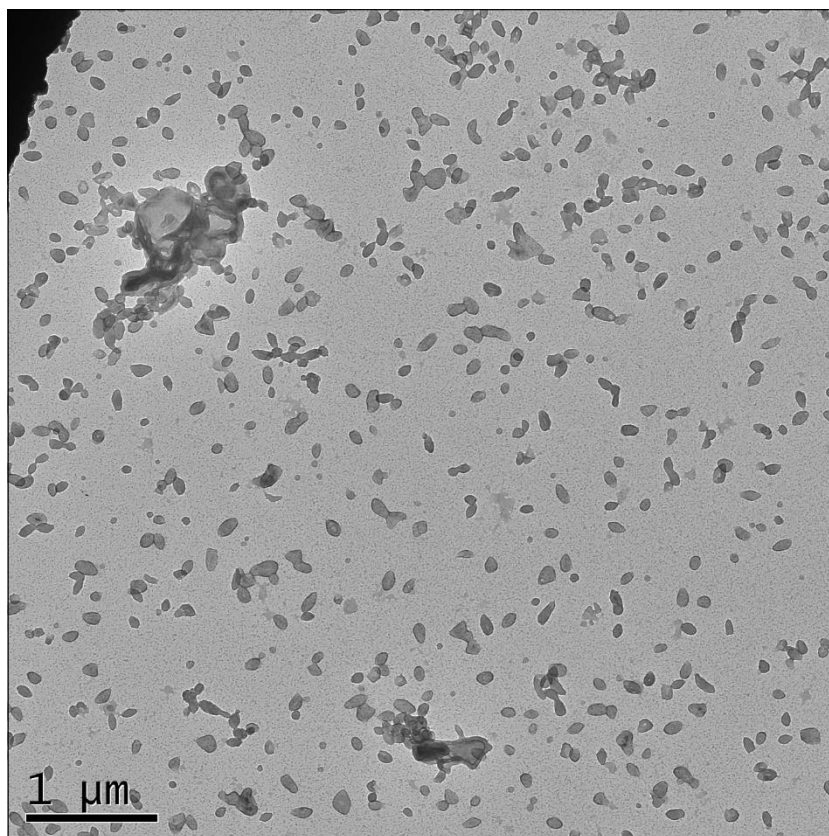


Figure 7.132: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 60 min)

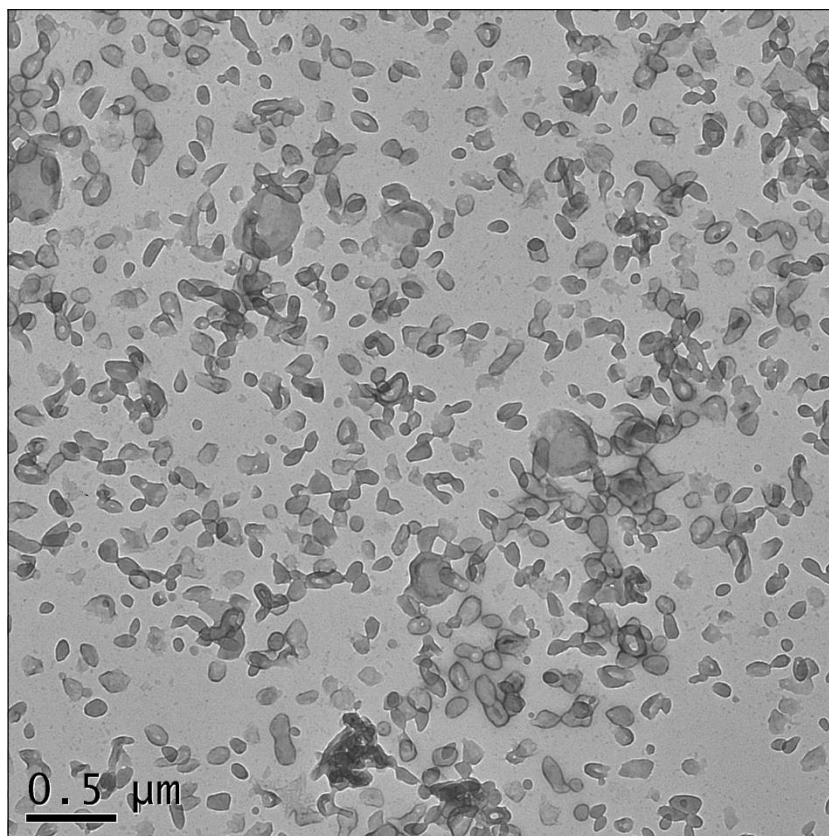


Figure 7.133: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 60 min)

t = 120 min

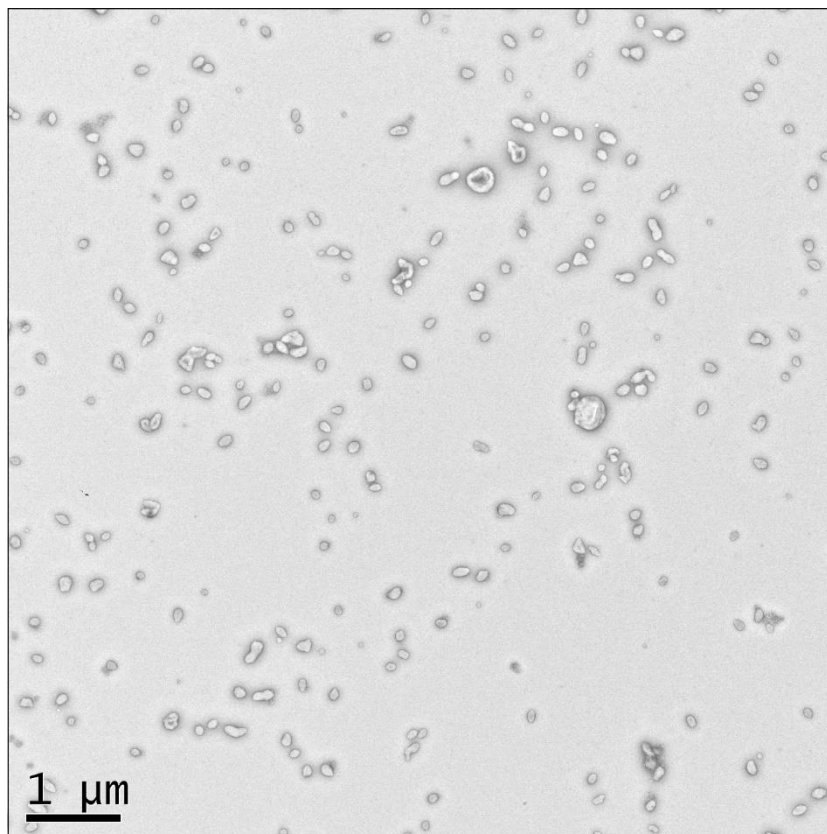


Figure 7.134: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 120 min)

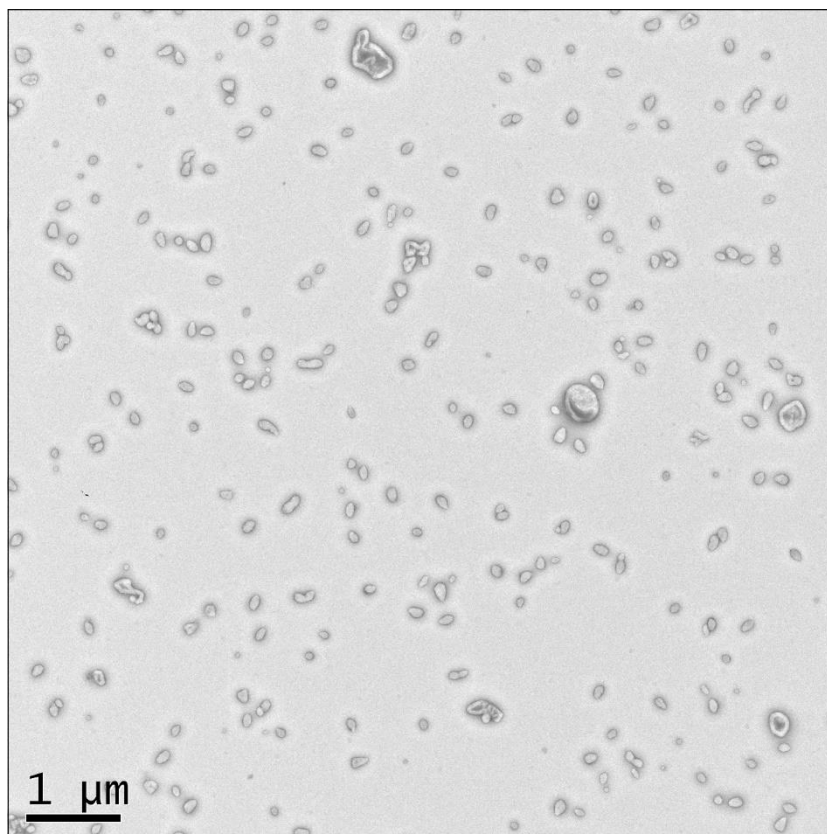


Figure 7.135: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 120 min)

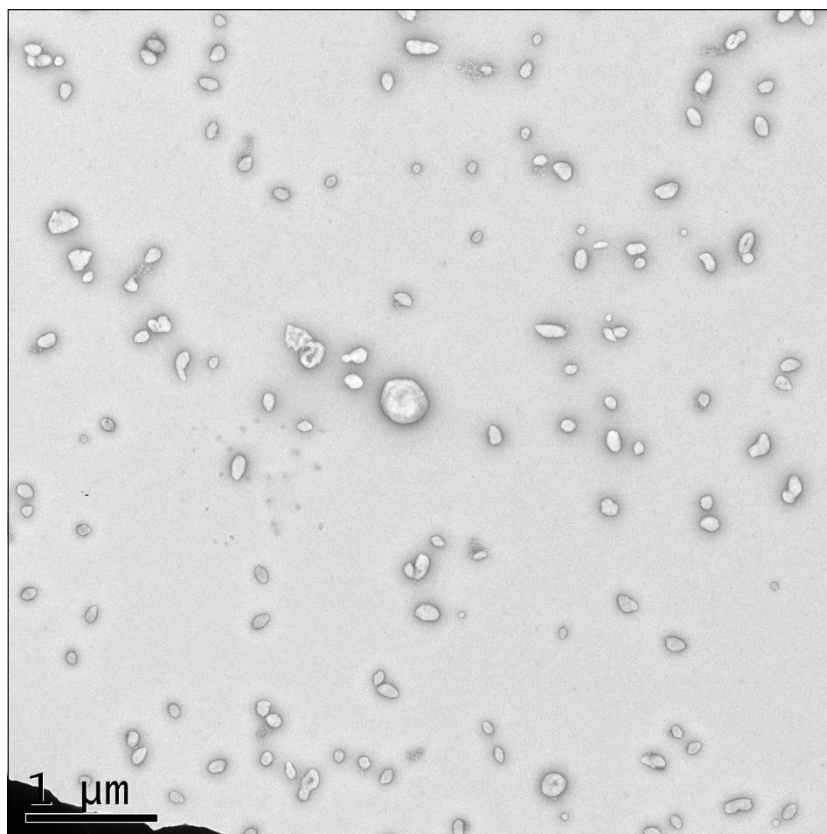


Figure 7.136: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 120 min)

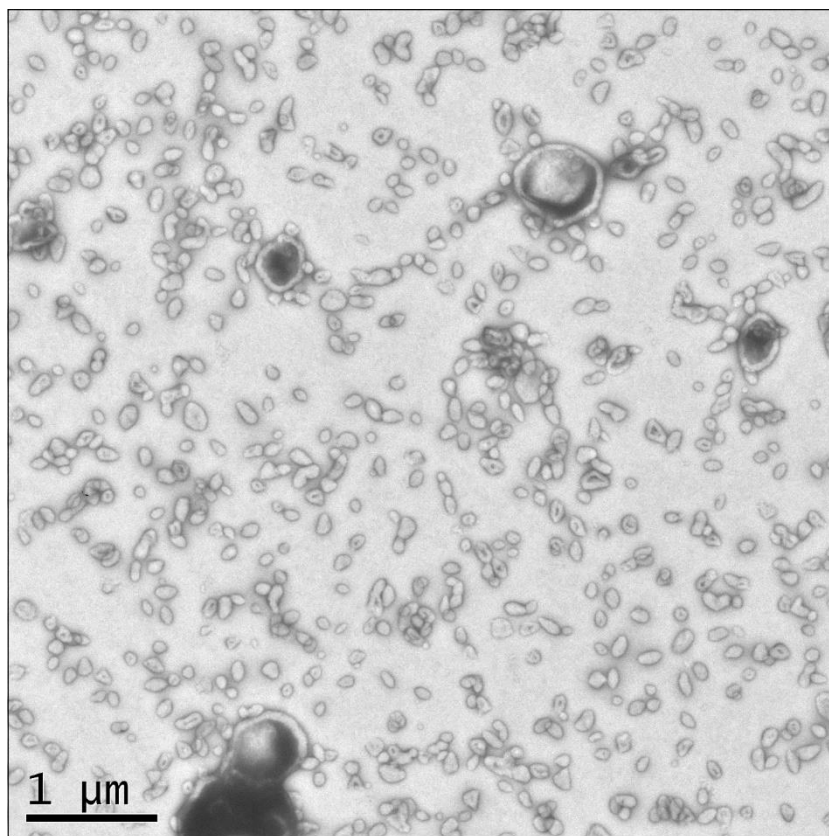


Figure 7.137: – TEM micrograph for PDMA_{m50}-b-PDAA_{m300} nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 120 min)

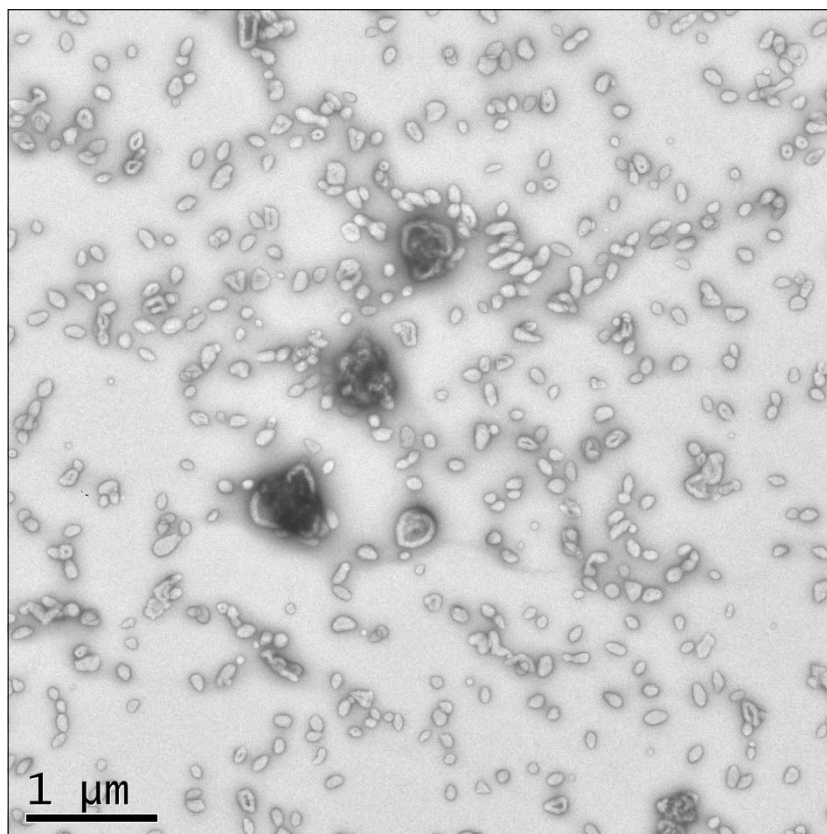


Figure 7.138: – TEM micrograph for PDMA_{m50}-b-PDAA_{m300} nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 120 min)

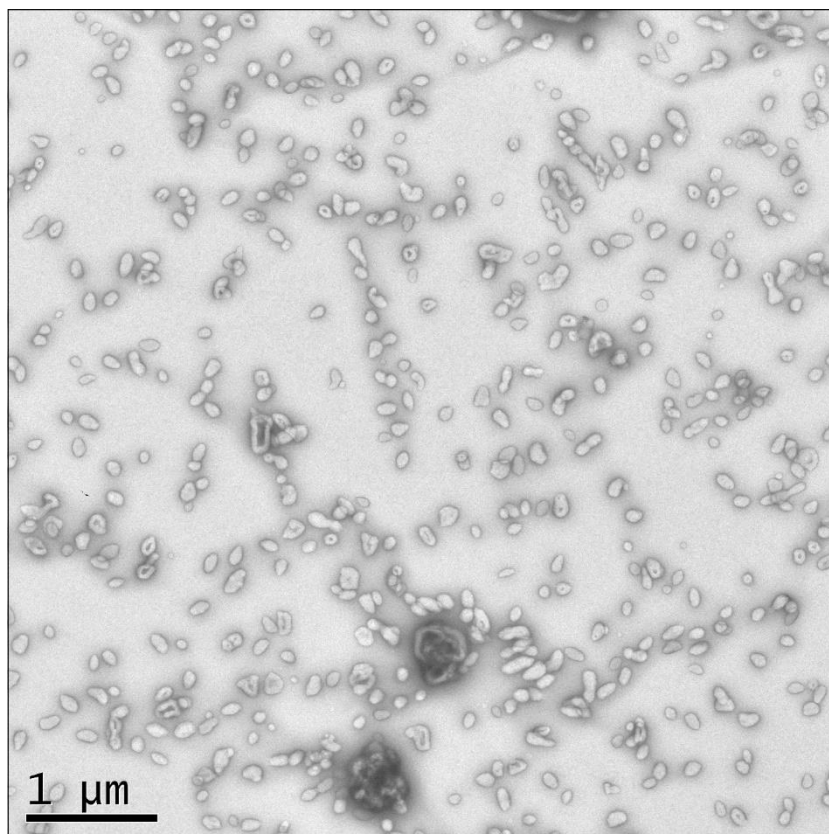


Figure 7.139: – TEM micrograph for PDMA_{m50}-b-PDAA_{m300} nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 120 min)

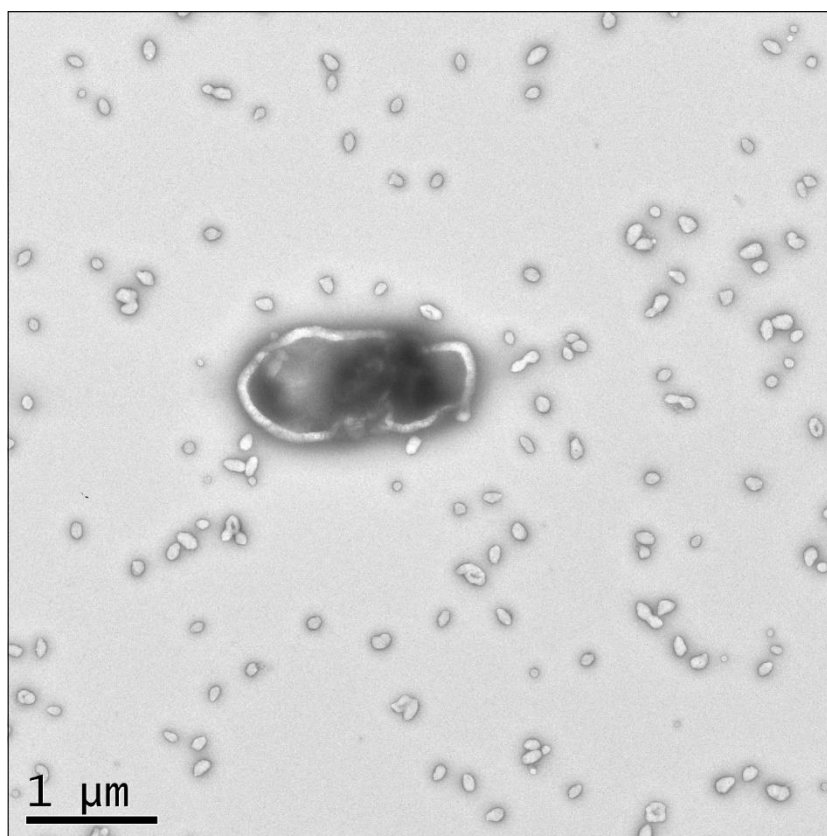


Figure 7.140: – TEM micrograph for PDMA₅₀-b-PDAA₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 120 min)

t = 180 min

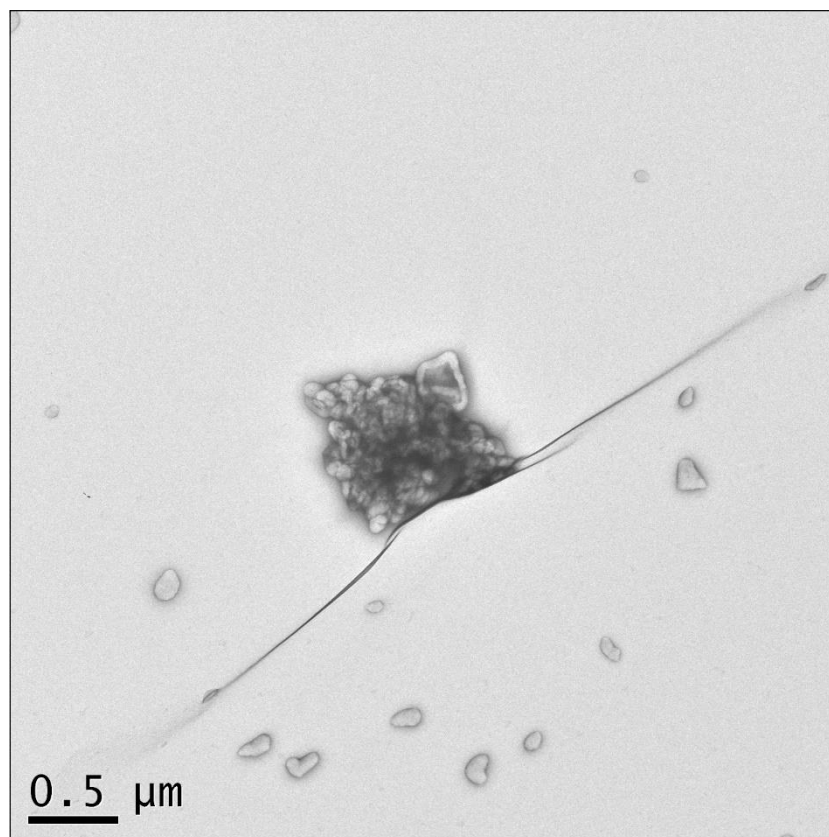


Figure 7.141: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 180 min)

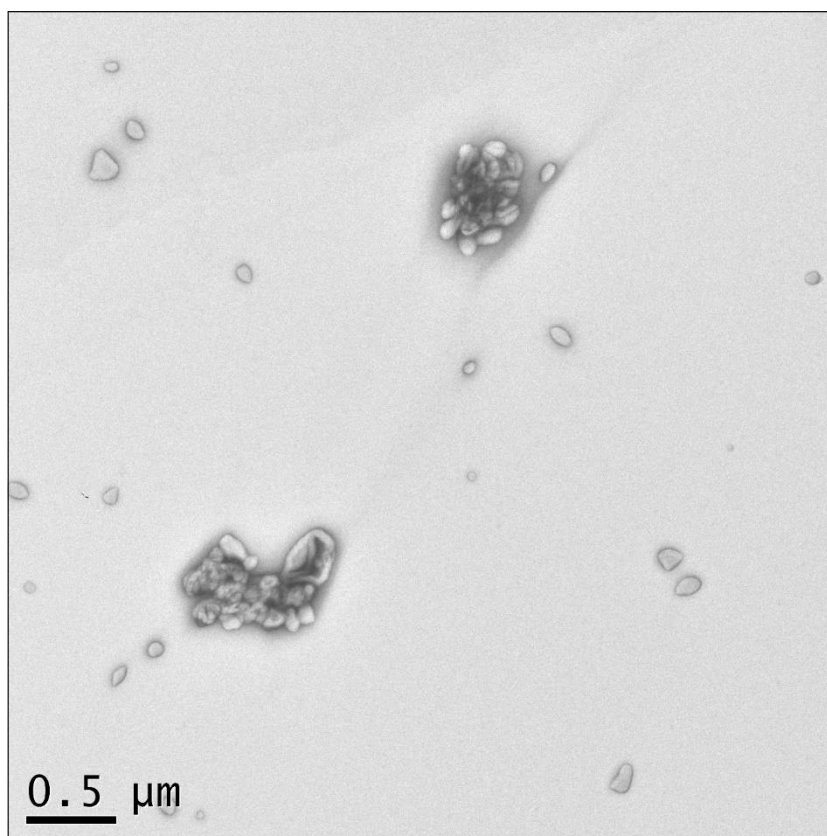


Figure 7.142: – TEM micrograph for PDMA₅₀-b-PDAA₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 180 min)

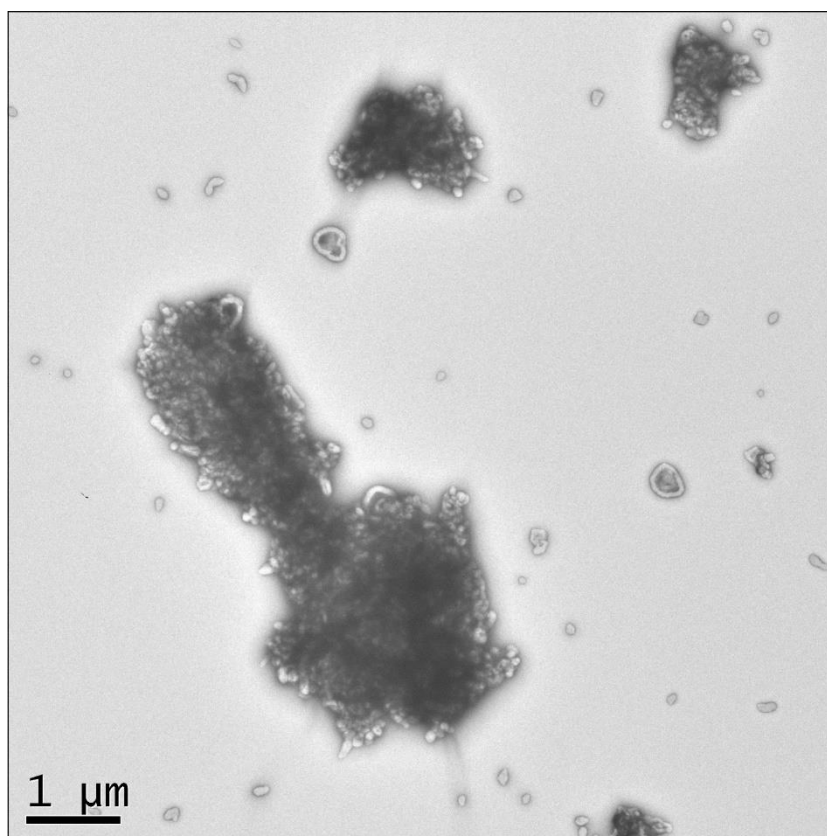


Figure 7.143: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 180 min)

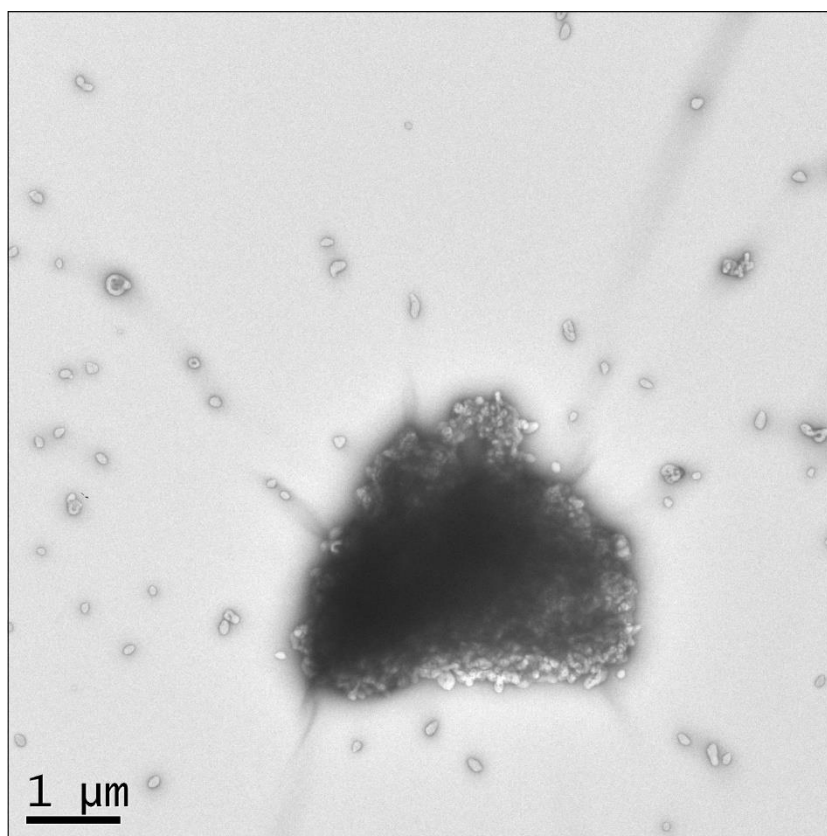


Figure 7.144: – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 180 min)

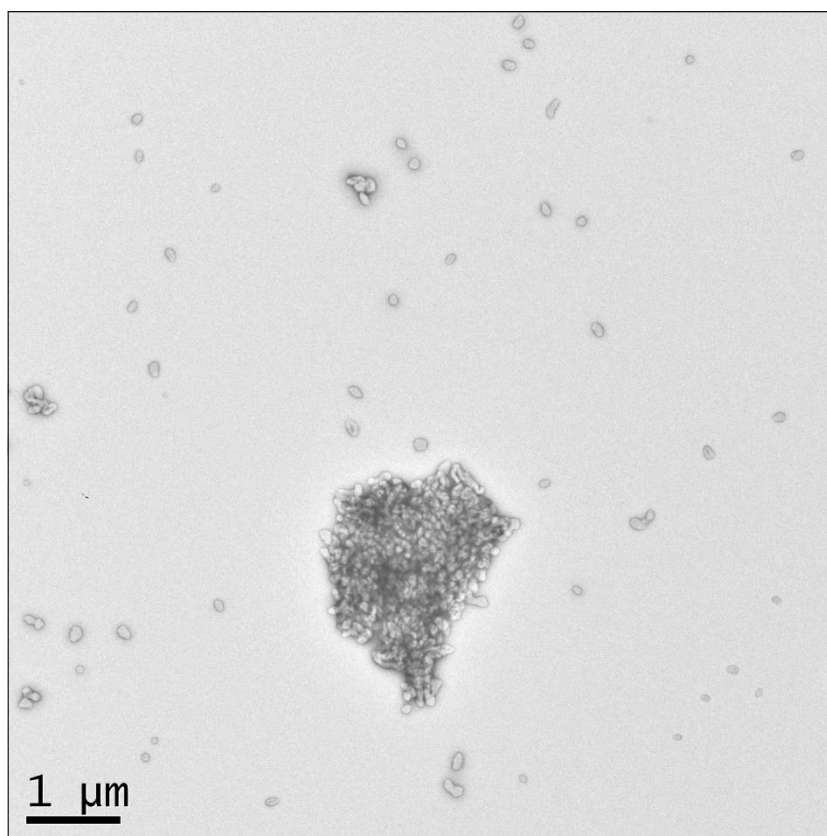


Figure 7.145: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 180 min)

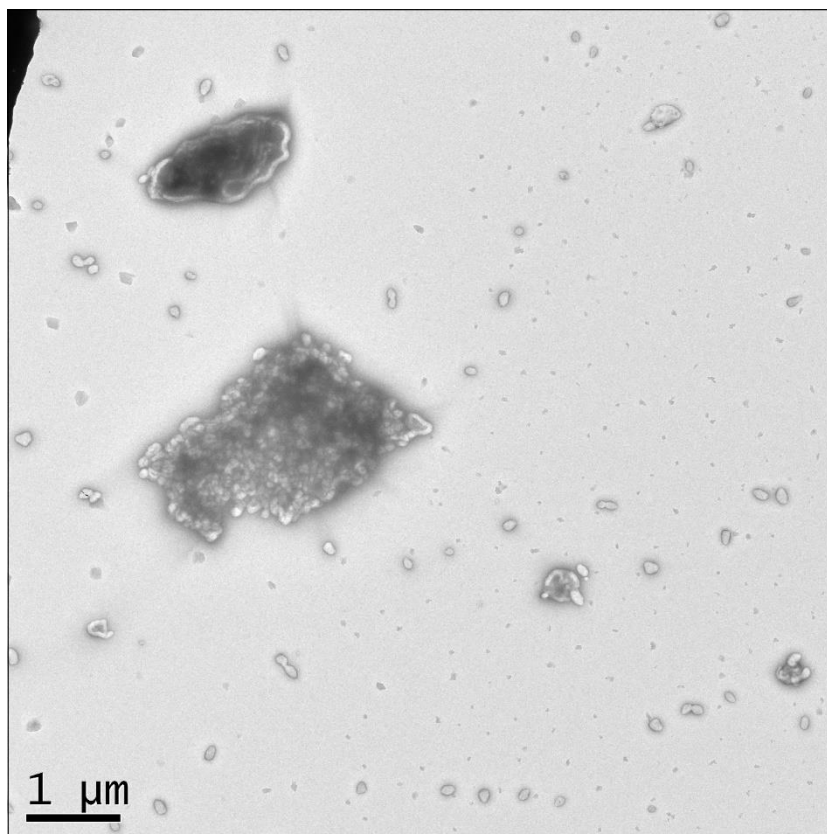


Figure 7.146: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 180 min)

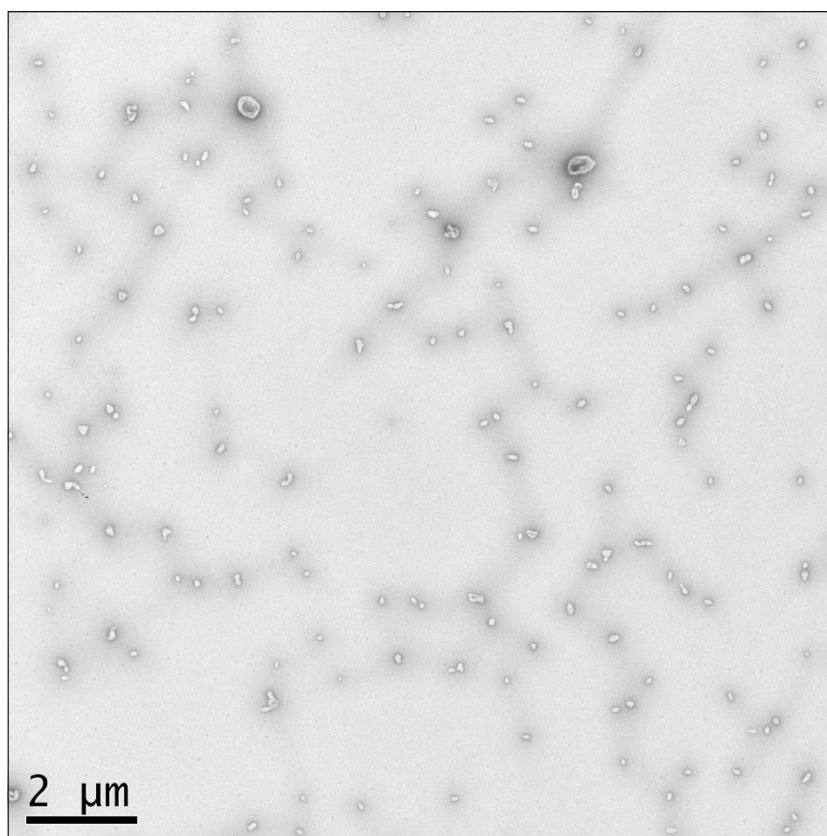


Figure 7.147: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 2.5 (50% MeOH – Rapid Addition – t = 180 min)

PDMAm₅₀PDAAm₃₀₀ Doubly charged pH 7.5 500 mM NaCl

t = 0 min

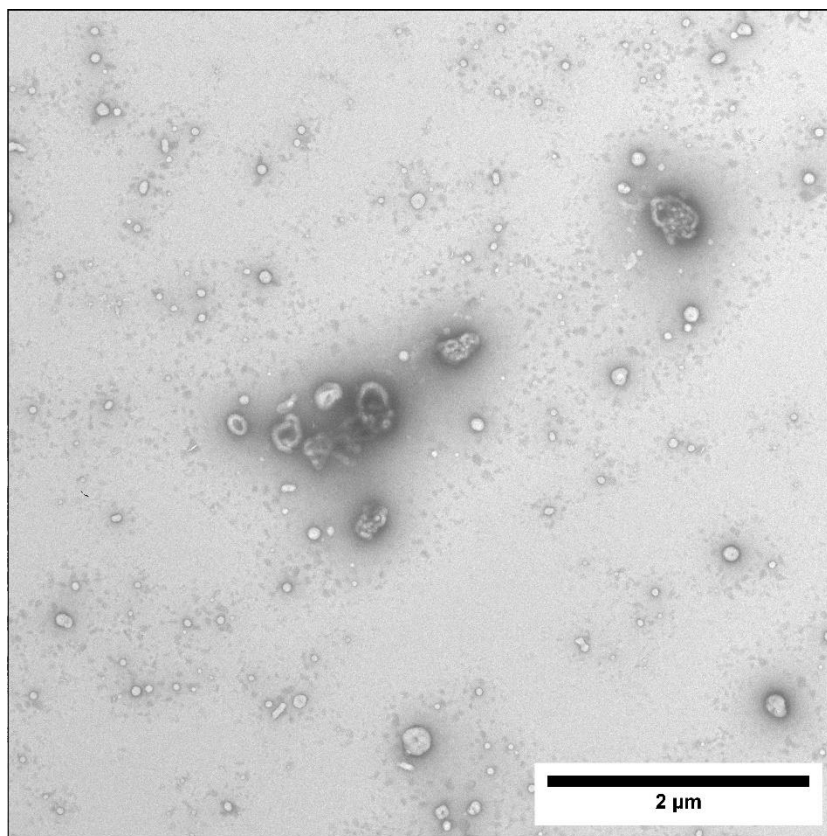


Figure 7.148: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

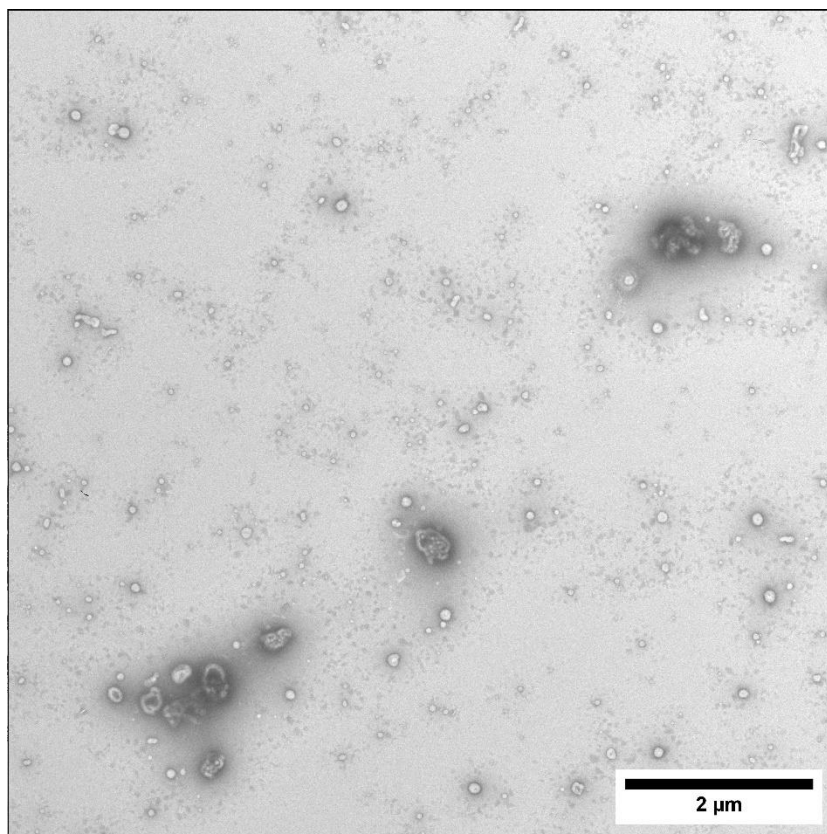


Figure 7.149: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

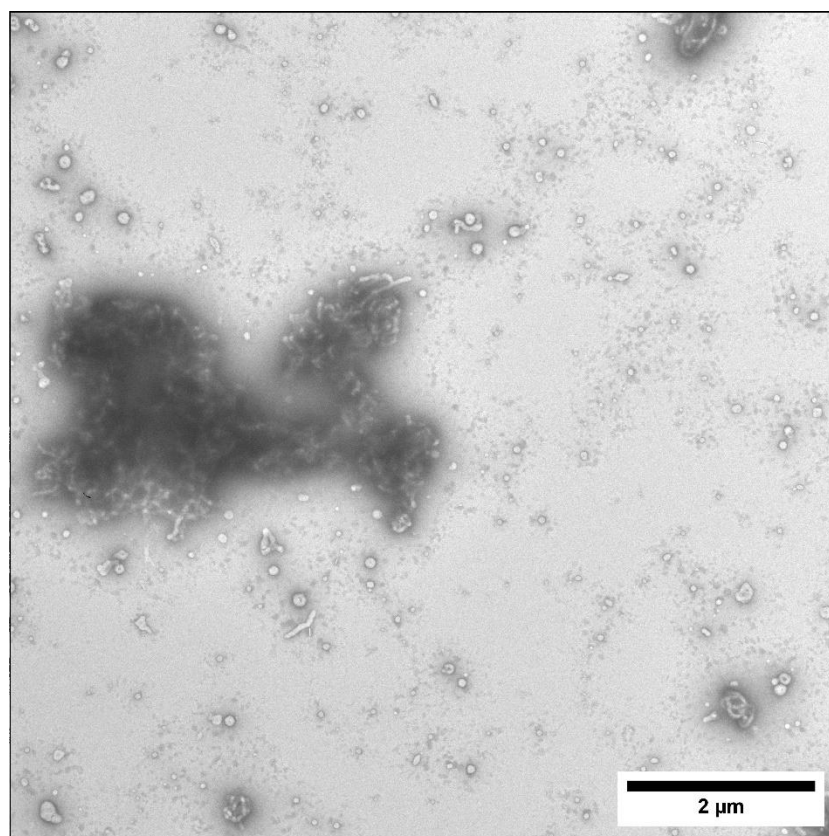


Figure 7.150: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

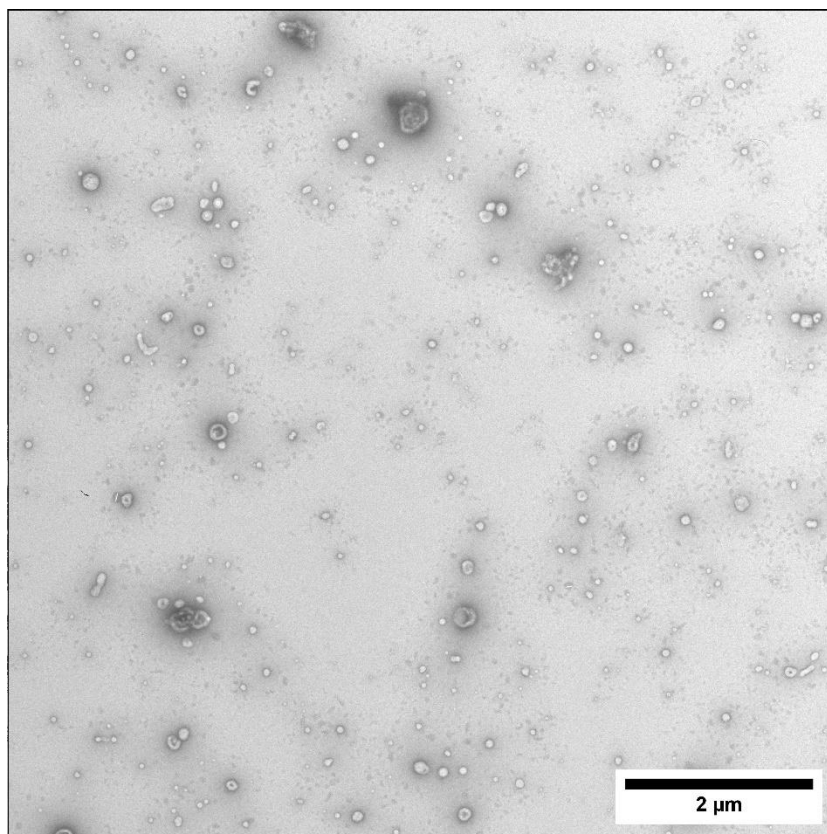


Figure 7.151: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

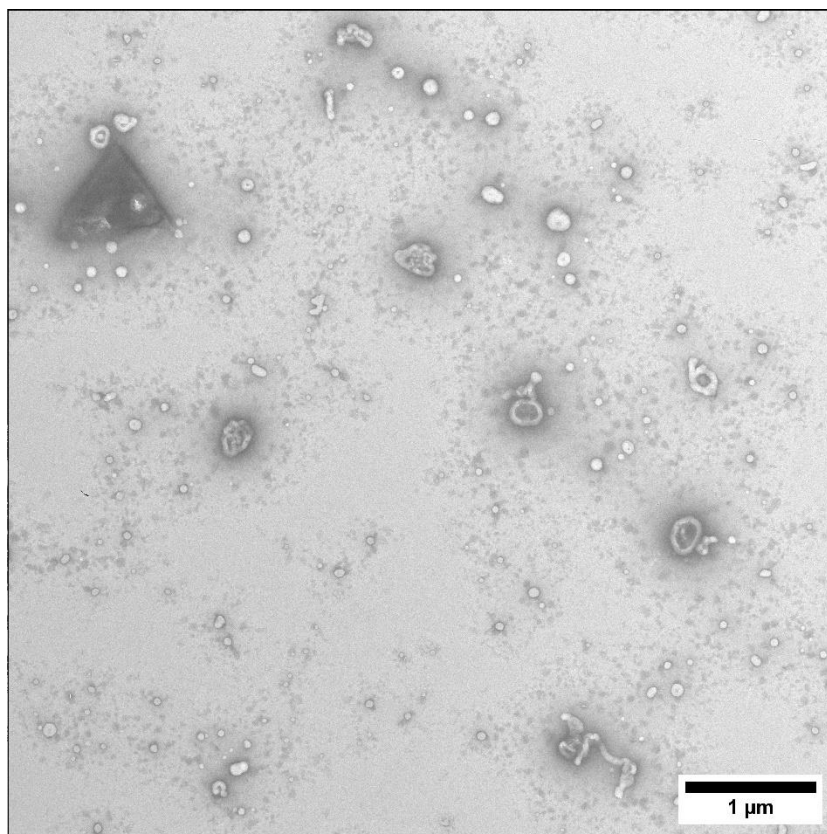


Figure 7.152: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

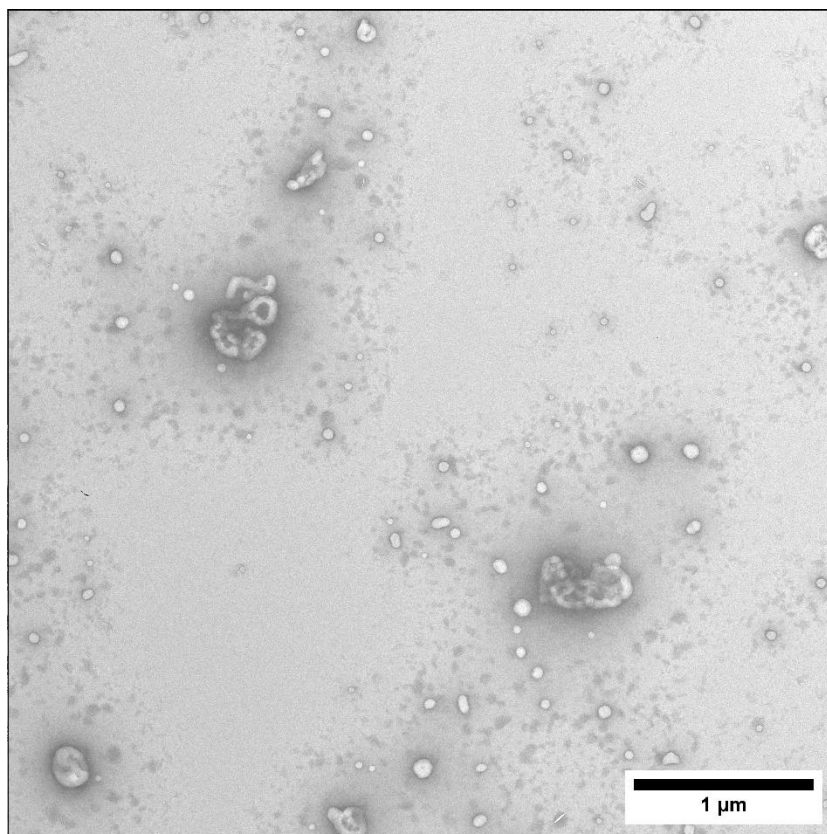


Figure 7.153: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

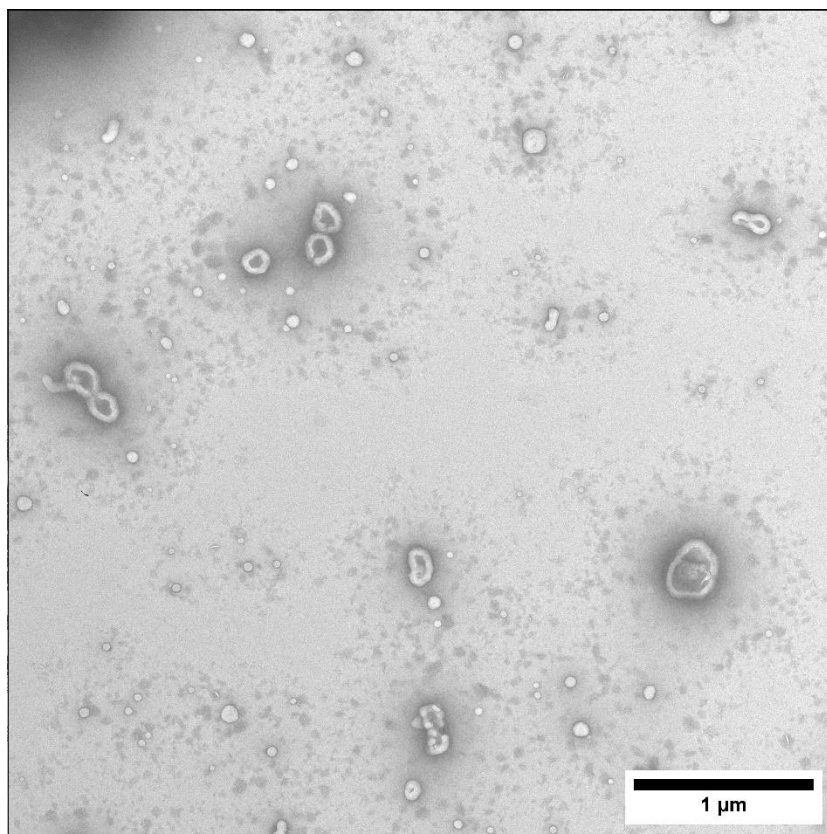


Figure 7.154 – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

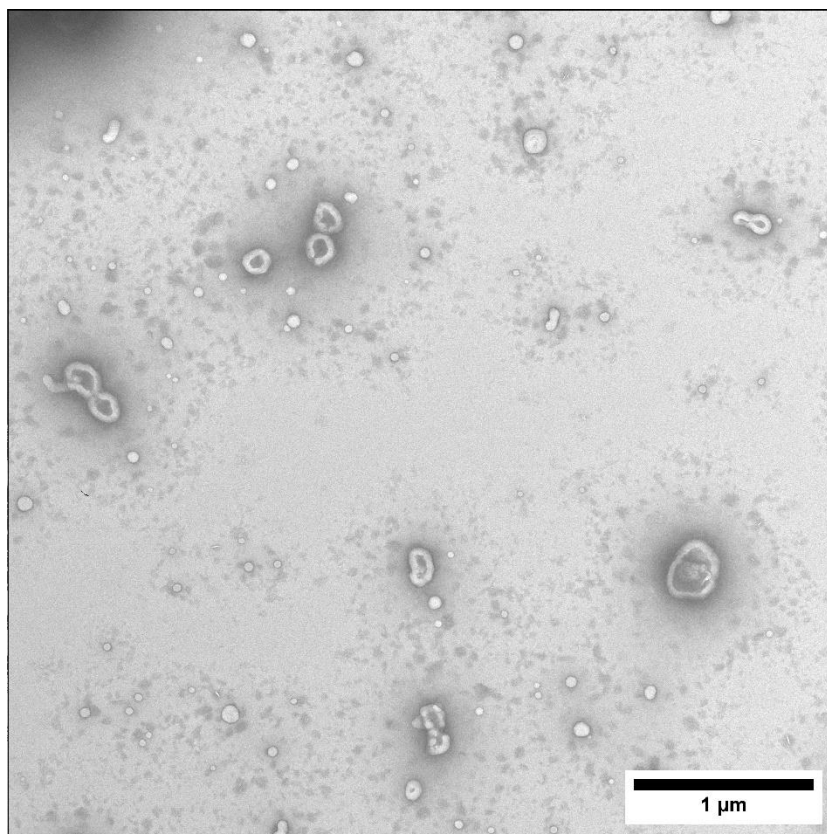


Figure 7.155: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

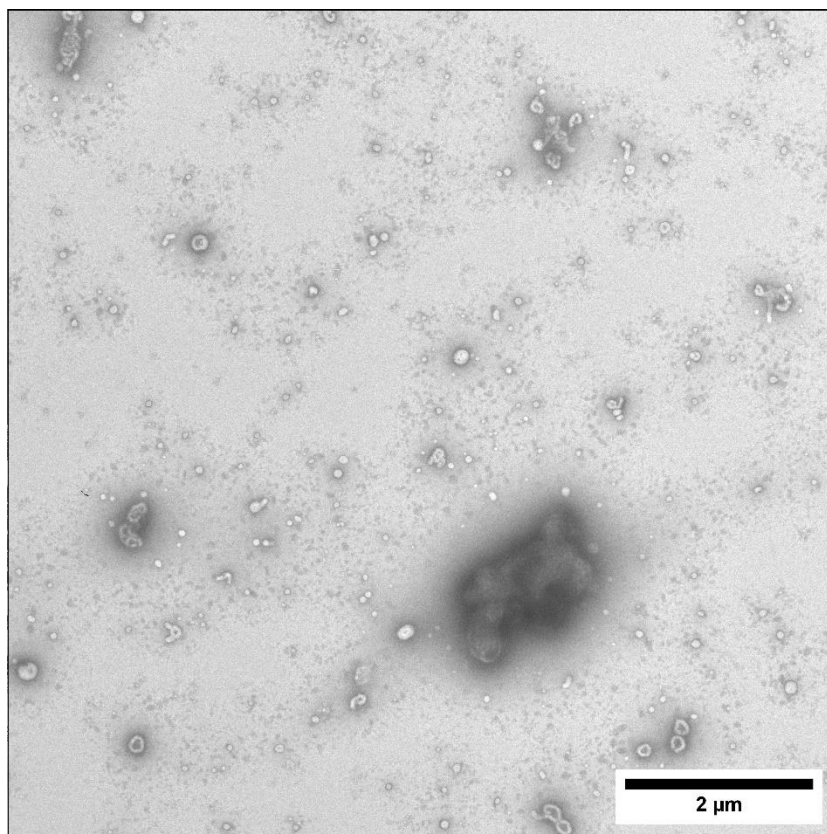


Figure 7.156: – TEM micrograph for PDMA₅₀-b-PDAA₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 0 min

t = 20 min

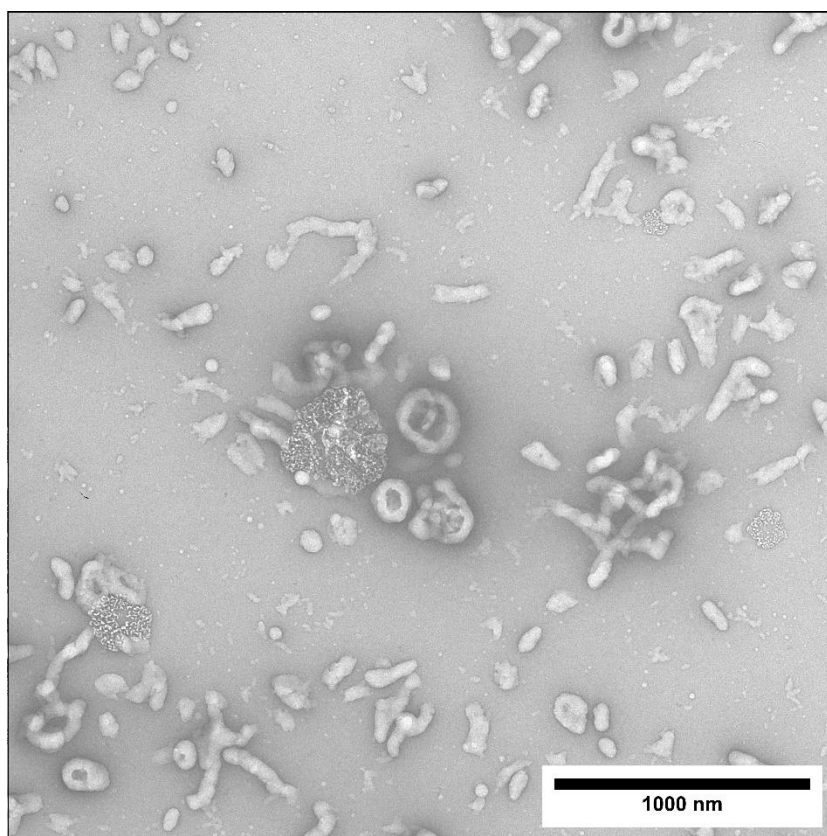


Figure 7.157: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 20 min)

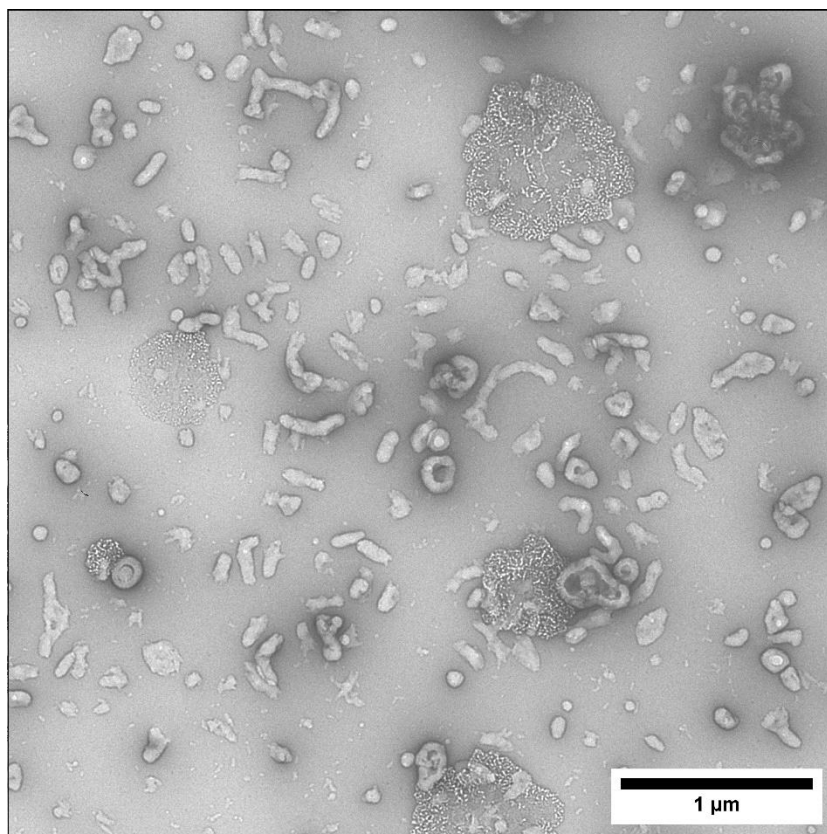


Figure 7.158: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 20 min)

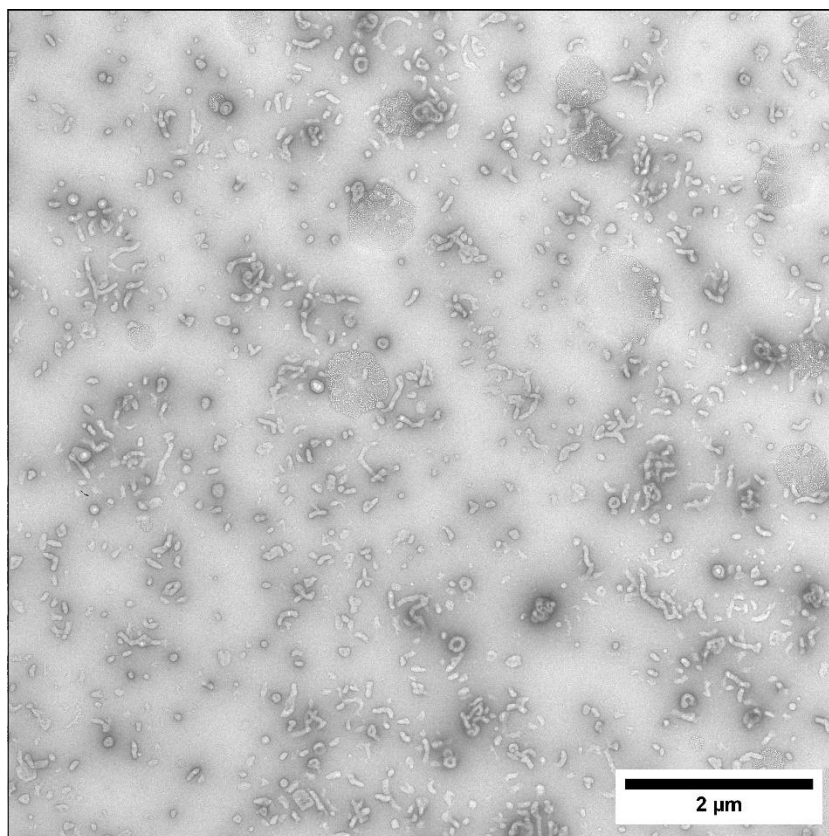


Figure 7.159: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 20 min)

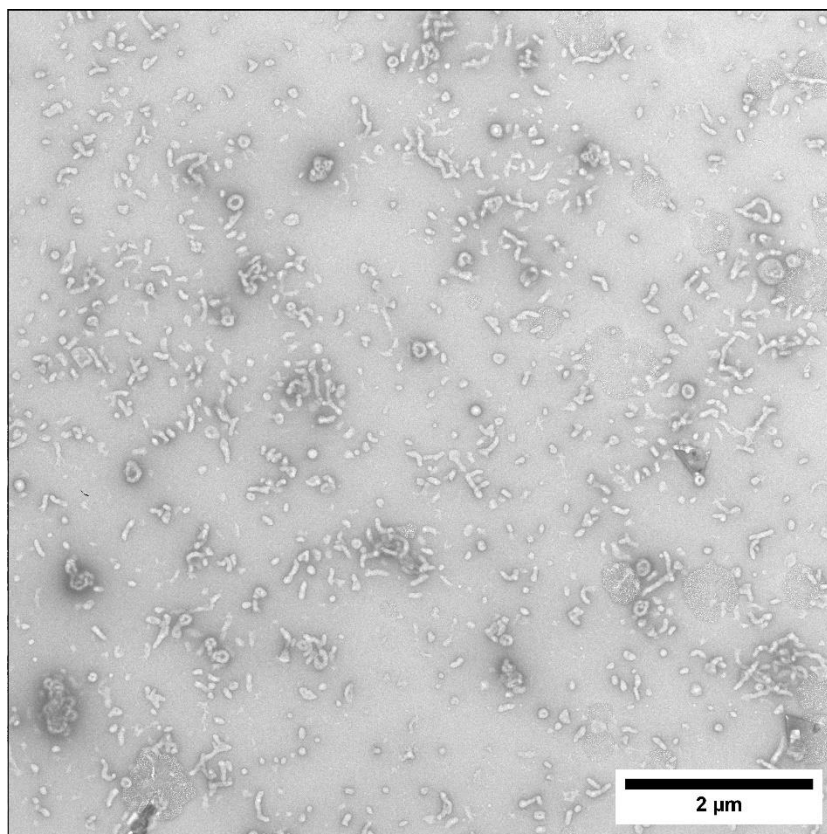


Figure 7.160: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 20 min)

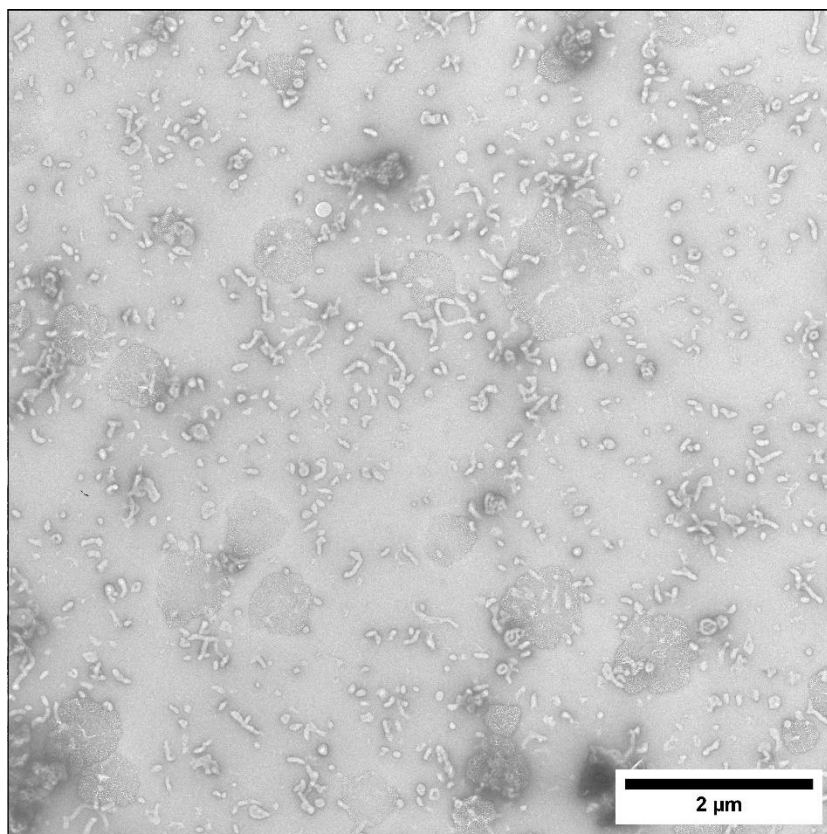


Figure 7.161: – TEM micrograph for PDMA₅₀-b-PDAA₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 20 min)

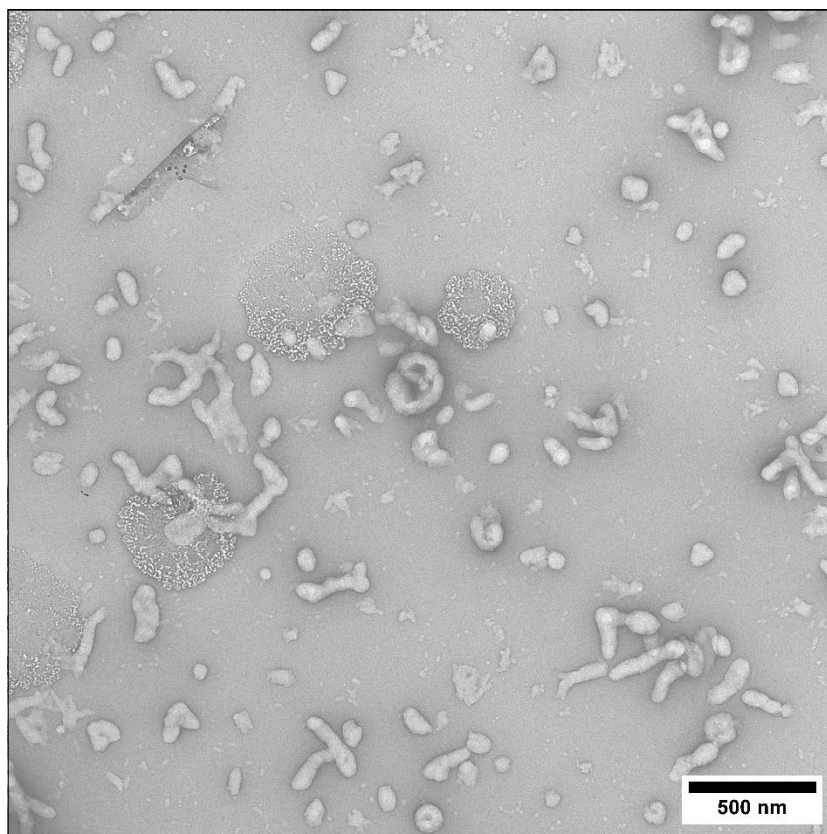


Figure 7.162: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 20 min)

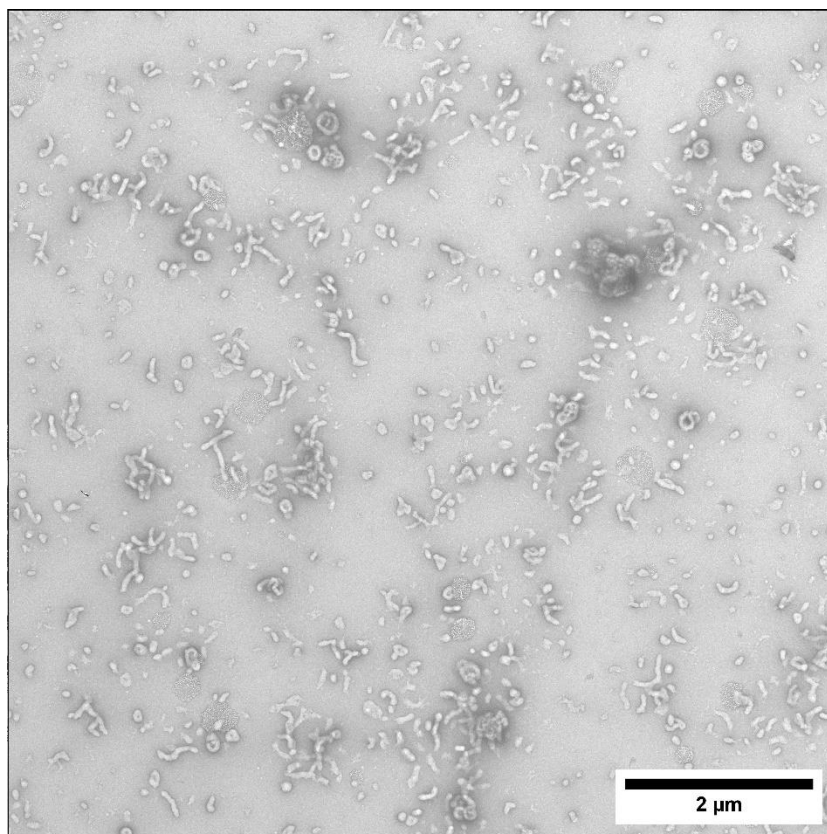


Figure 7.163 – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 20 min)

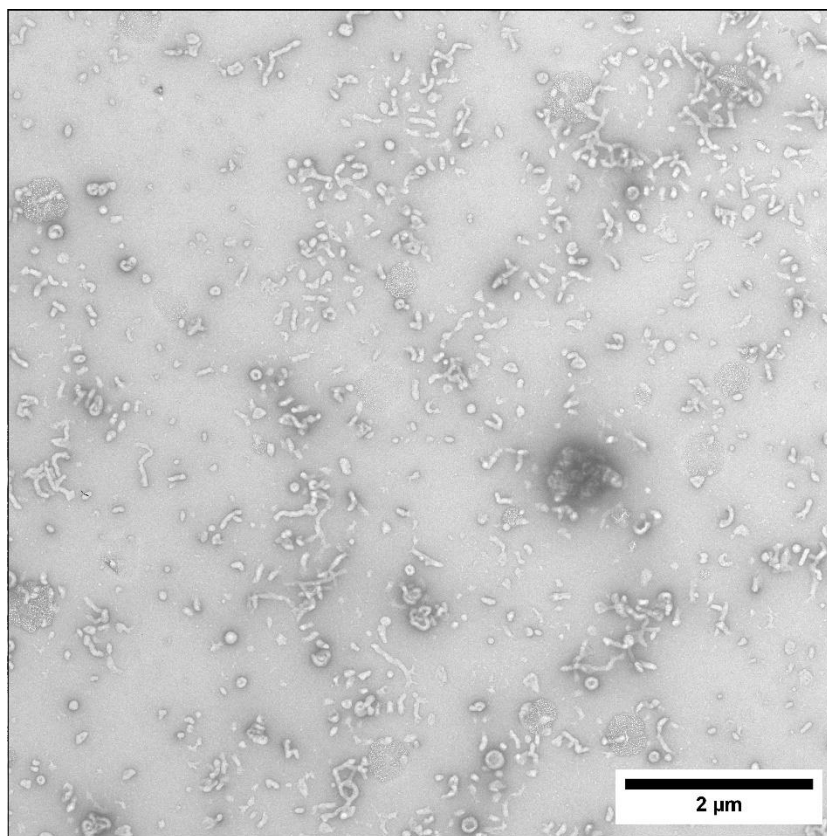


Figure 7.164: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 20 min)

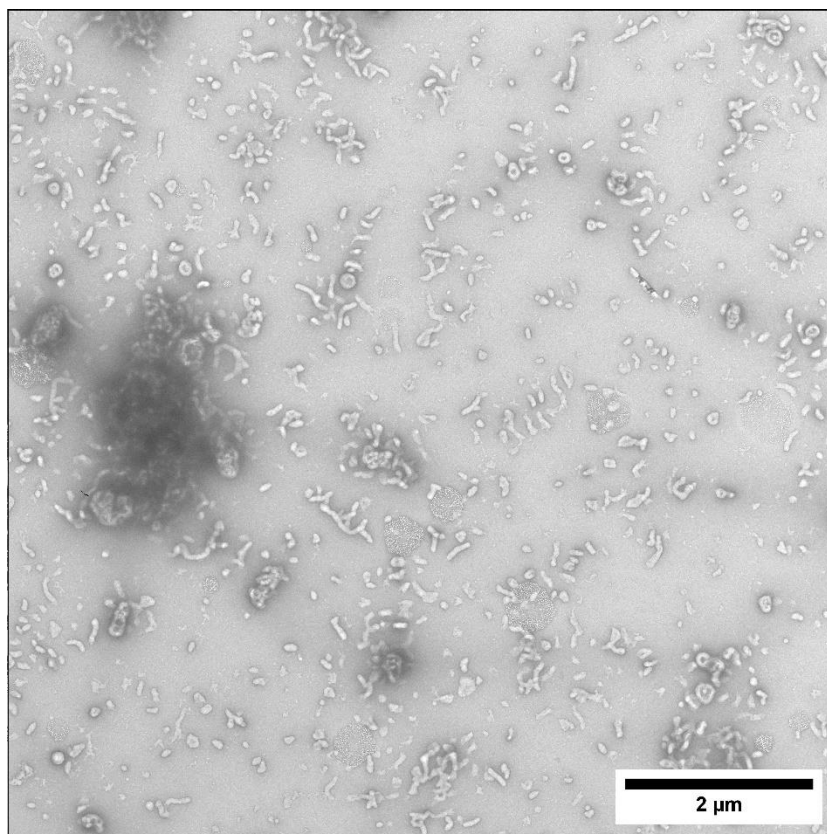


Figure 7.165: – TEM micrograph for PDMA₅₀-b-PDAA₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 20 min)

t = 45 min

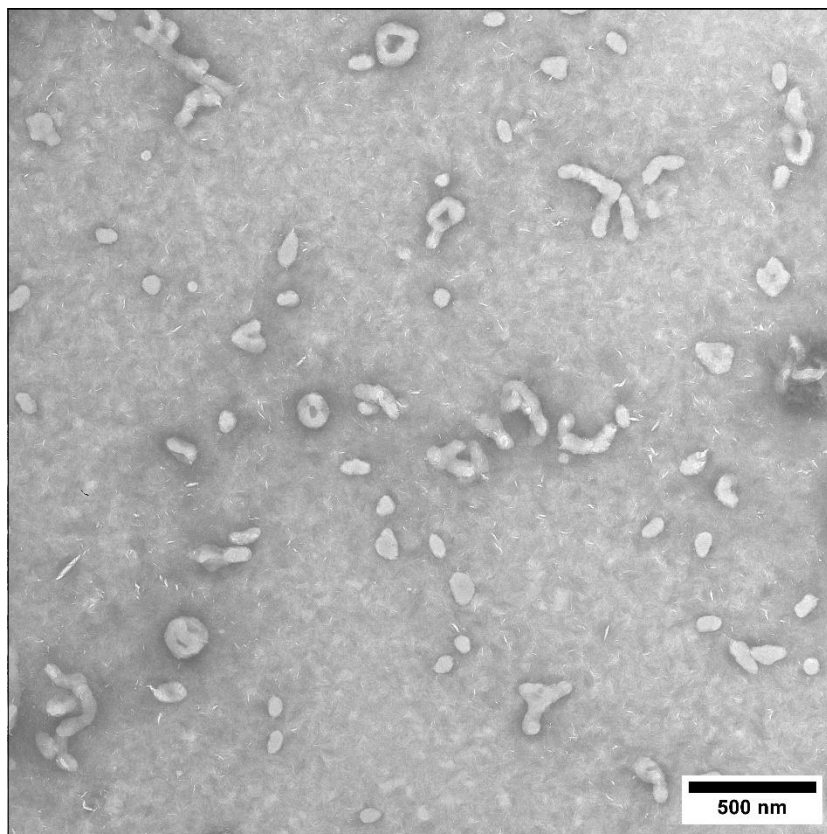


Figure 7.166: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 45 min)

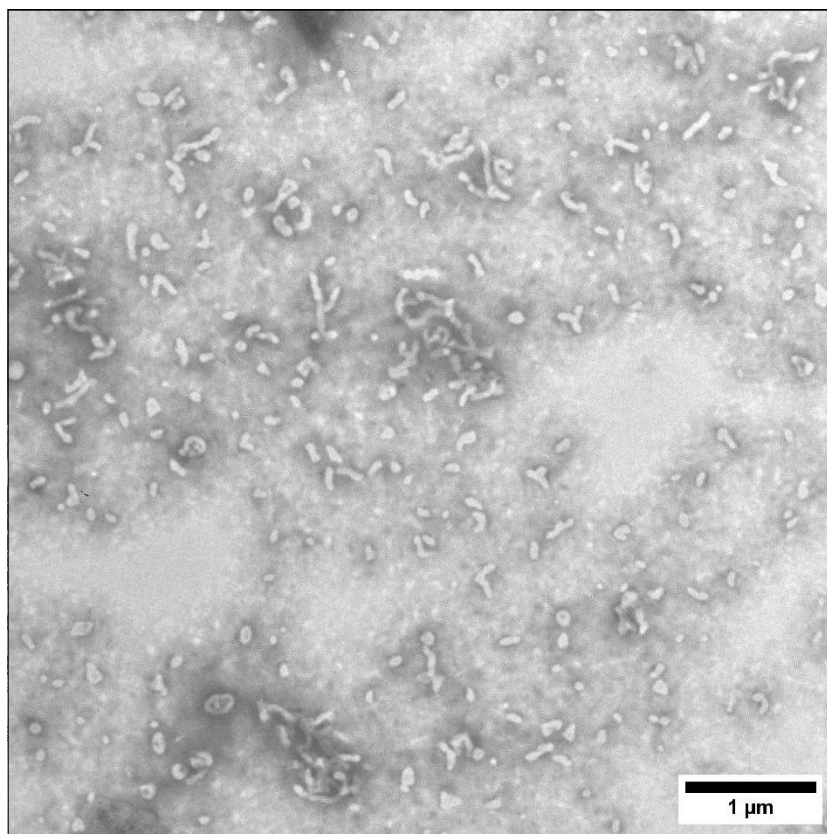


Figure 7.167: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 45 min)

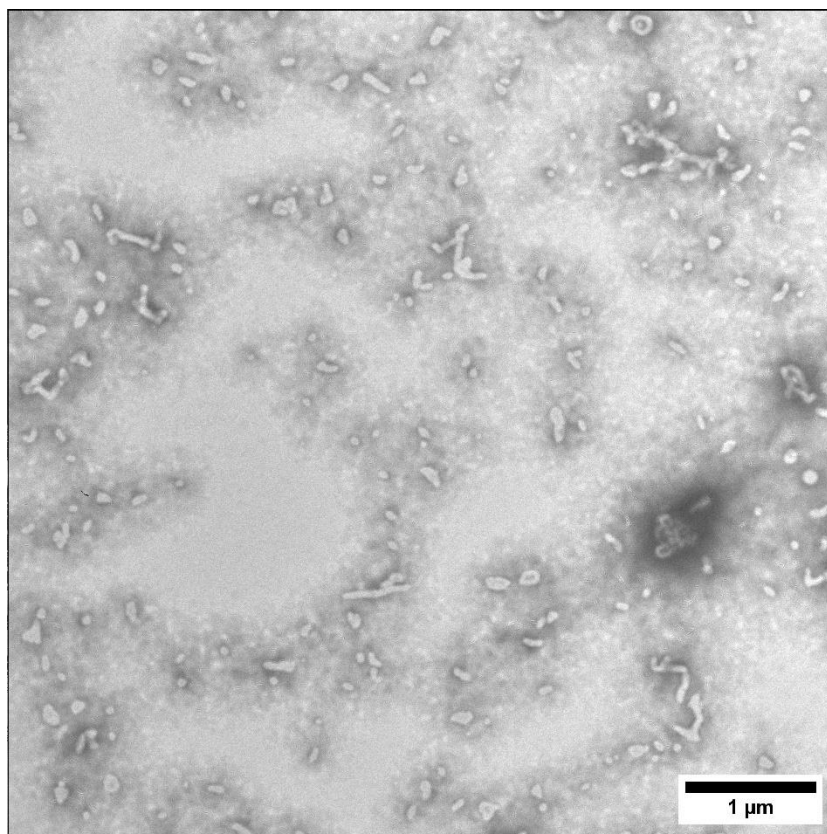


Figure 7.168: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 45 min)

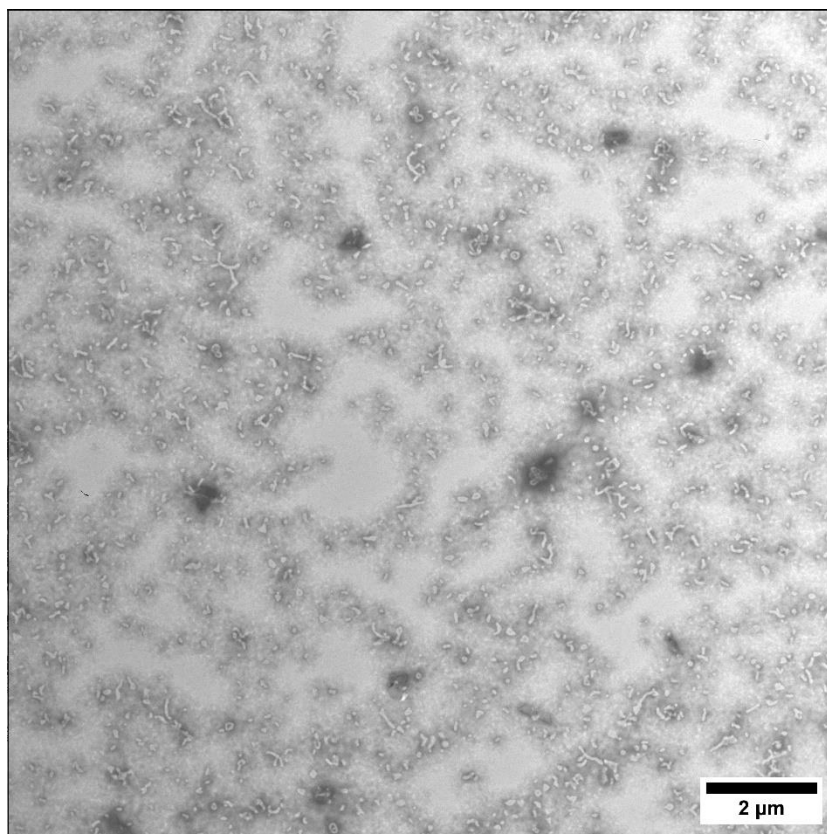


Figure 7.169:
– TEM micrograph for PDMAM₅₀-b-PDAAM₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 45 min)

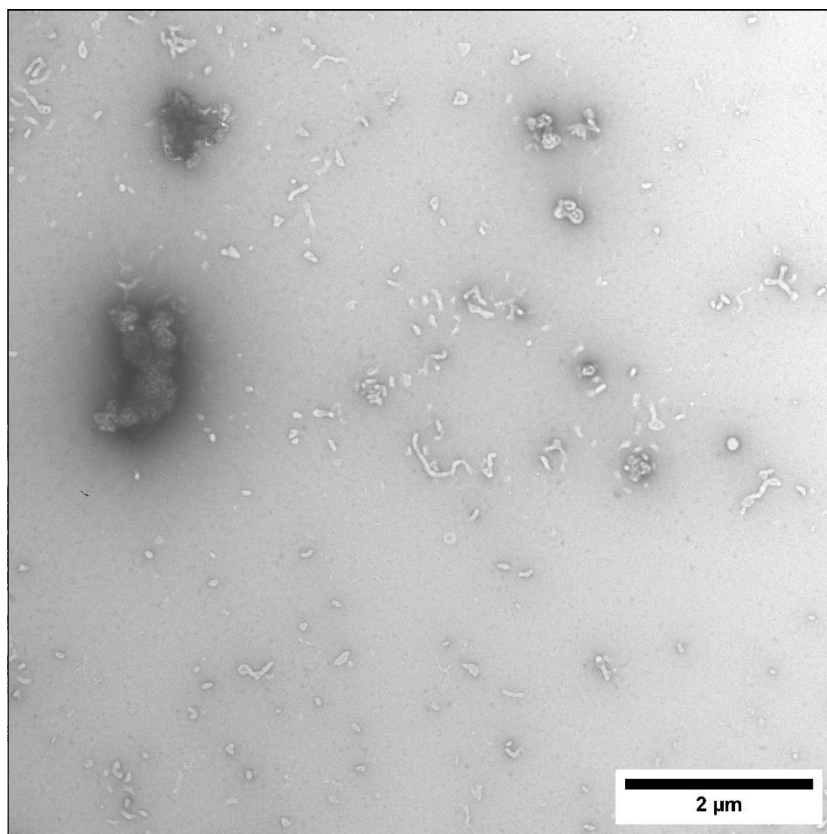


Figure 7.170: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 45 min)

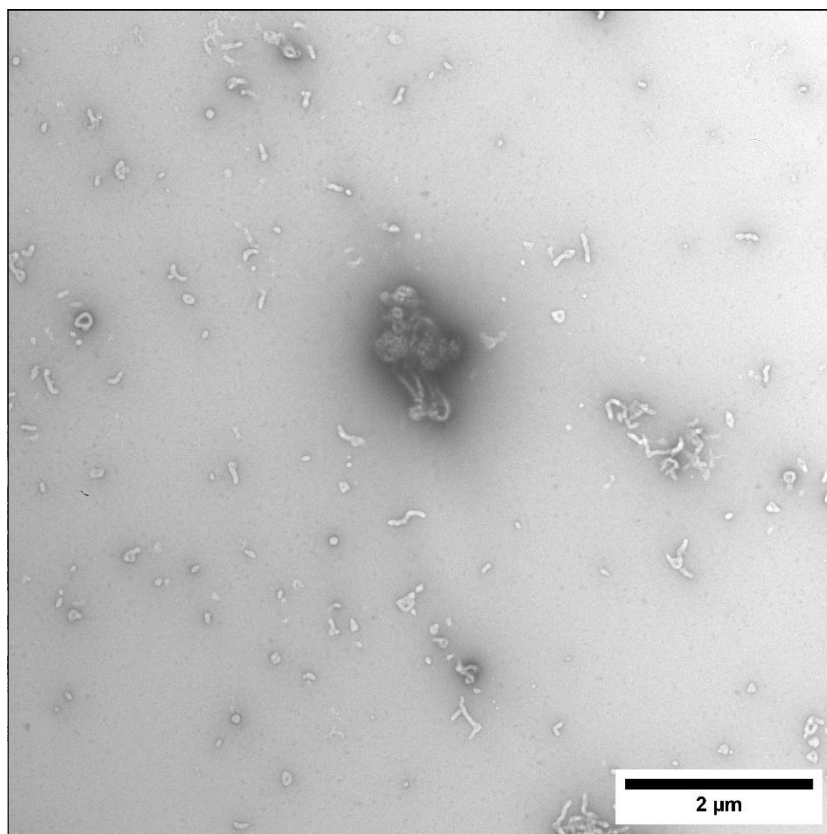


Figure 7.171: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 45 min)

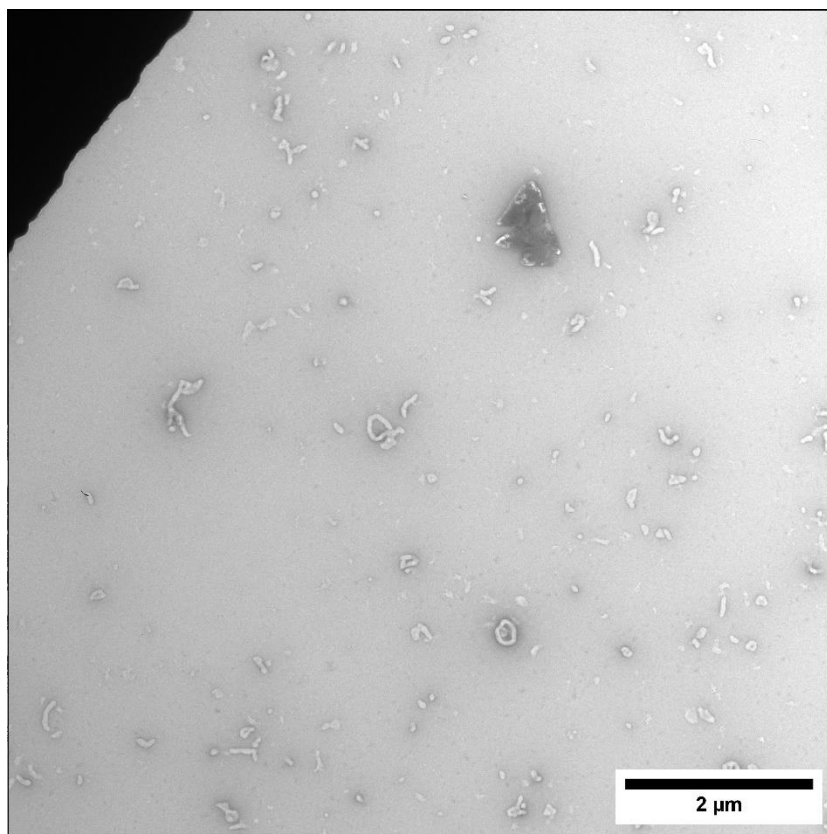


Figure 7.172: – TEM micrograph for PDMA₅₀-b-PDAA₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 45 min)

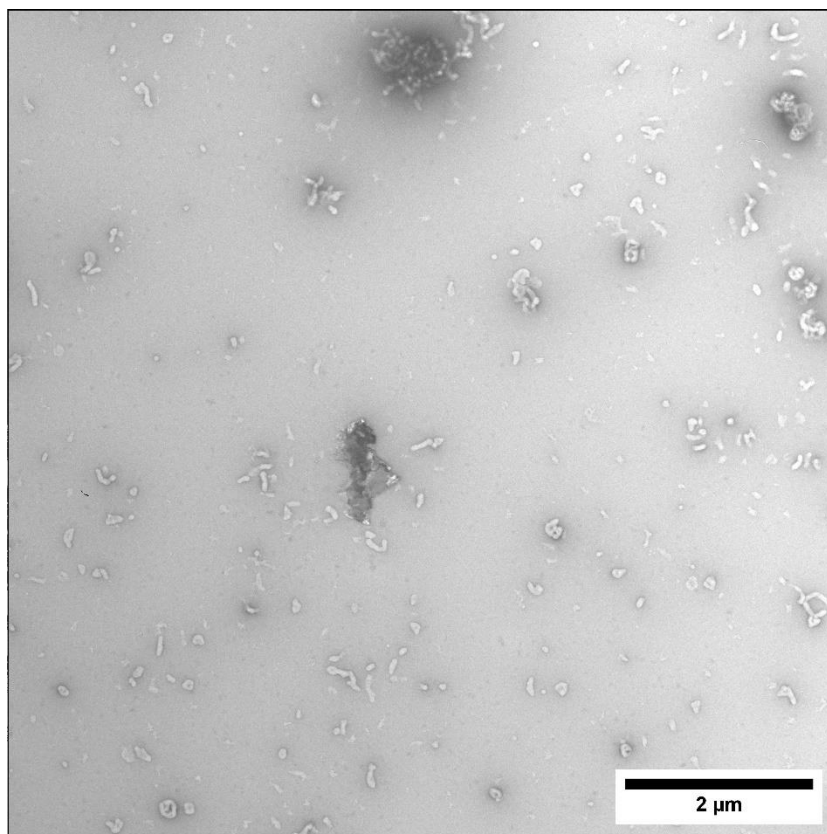


Figure 7.173: – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 45 min)

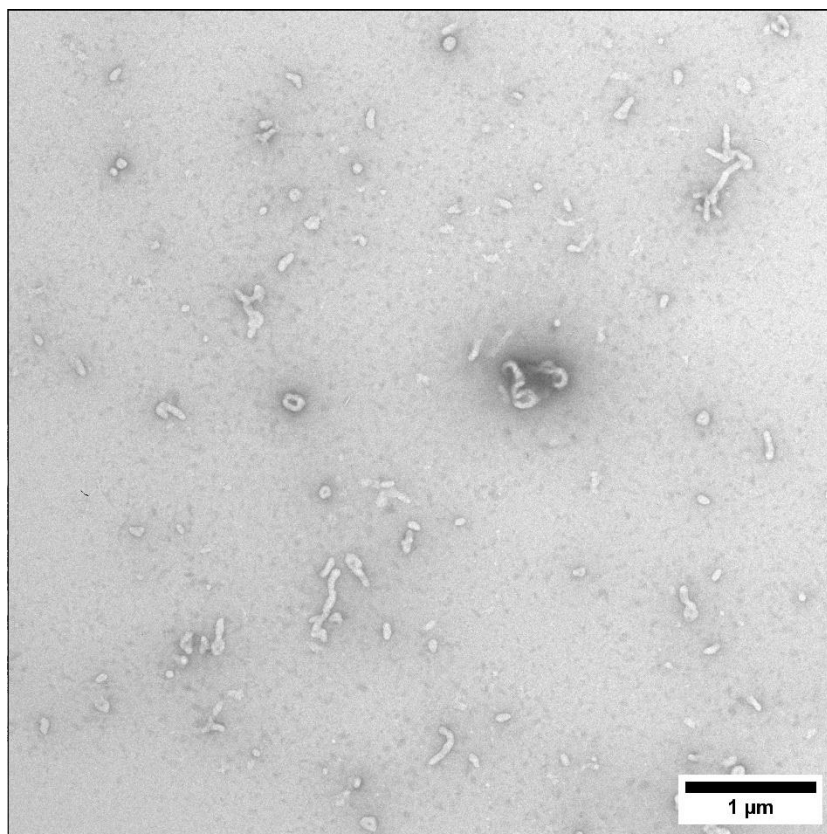


Figure 7.174: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 45 min)

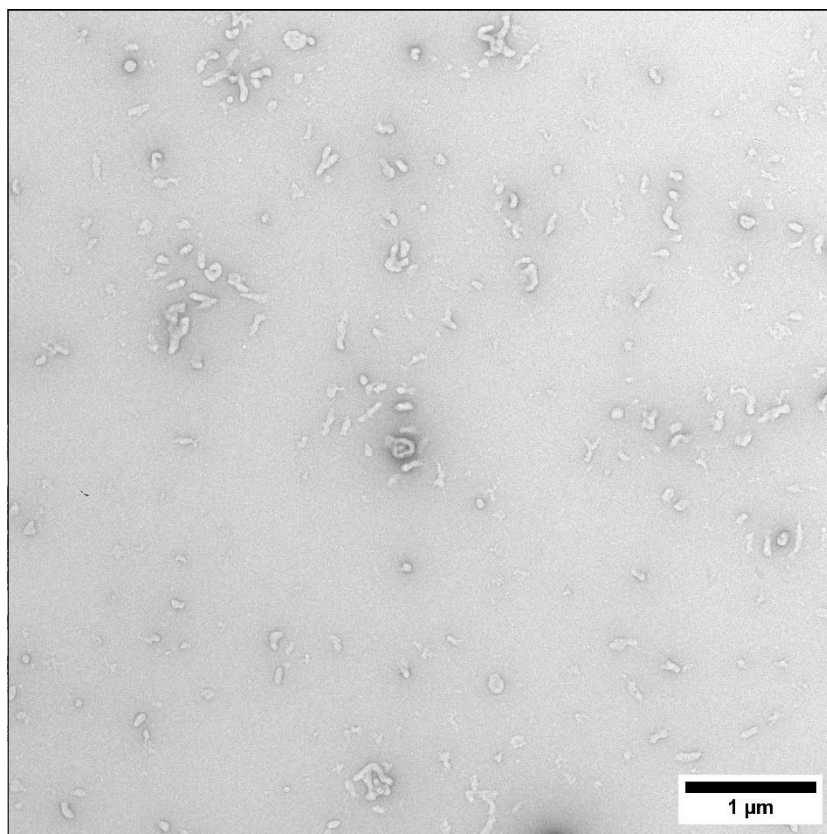


Figure 7.175: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 45 min)

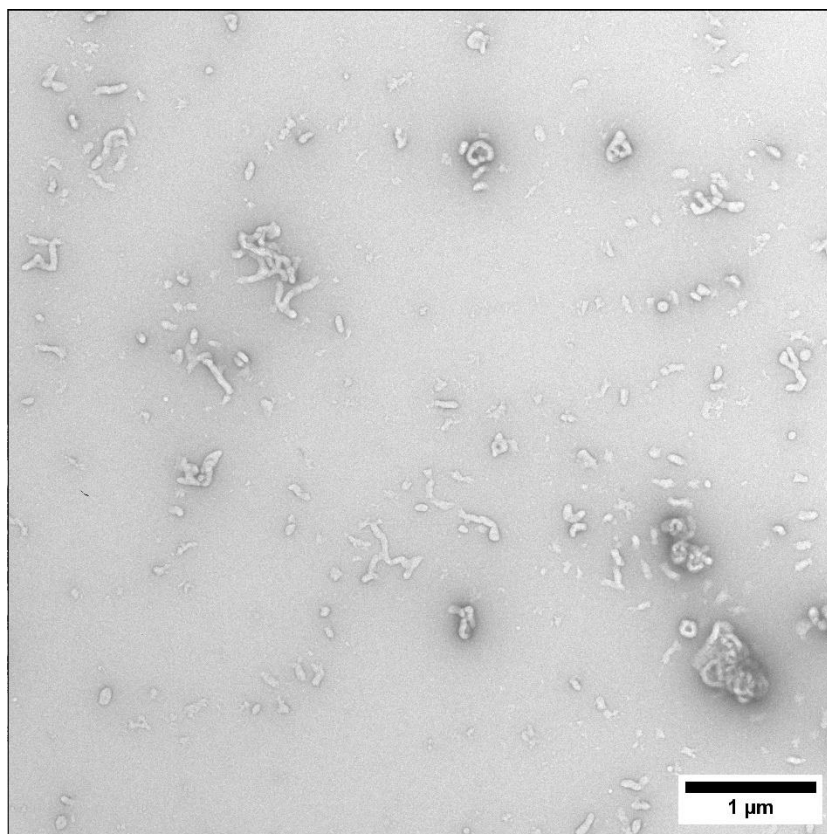


Figure 7.176: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 45 min)

t= 60 min

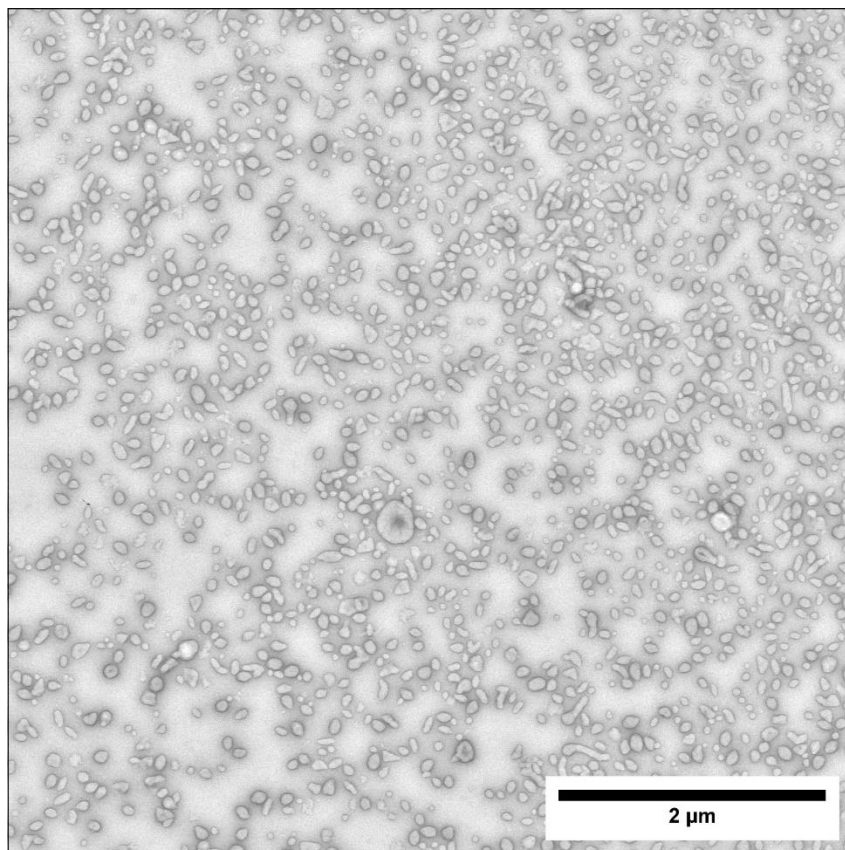


Figure 7.177: – TEM micrograph for PDMA₅₀-b-PDAA₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 60 min)

t = 90 min

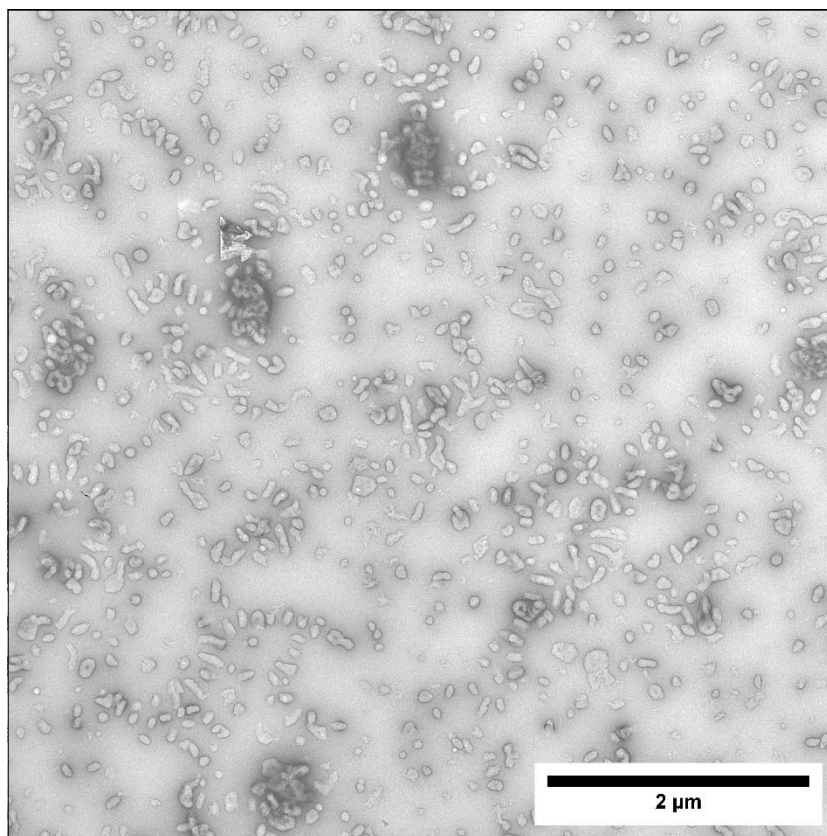


Figure 7.178: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 60 min)

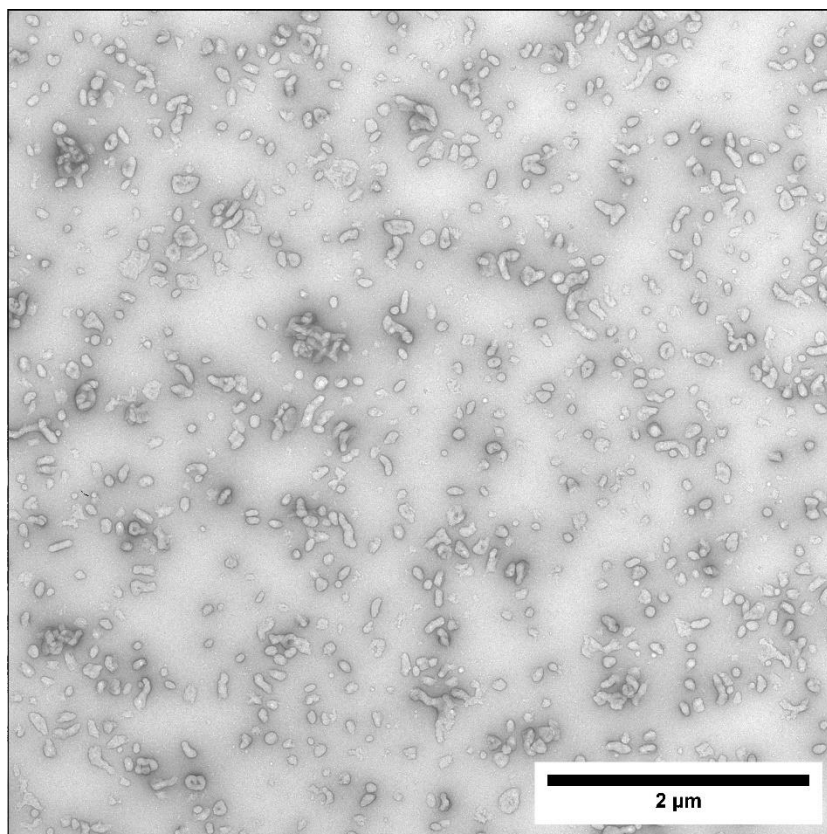


Figure 7.179: – TEM micrograph for PDMA₅₀-b-PDAA₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 60 min)

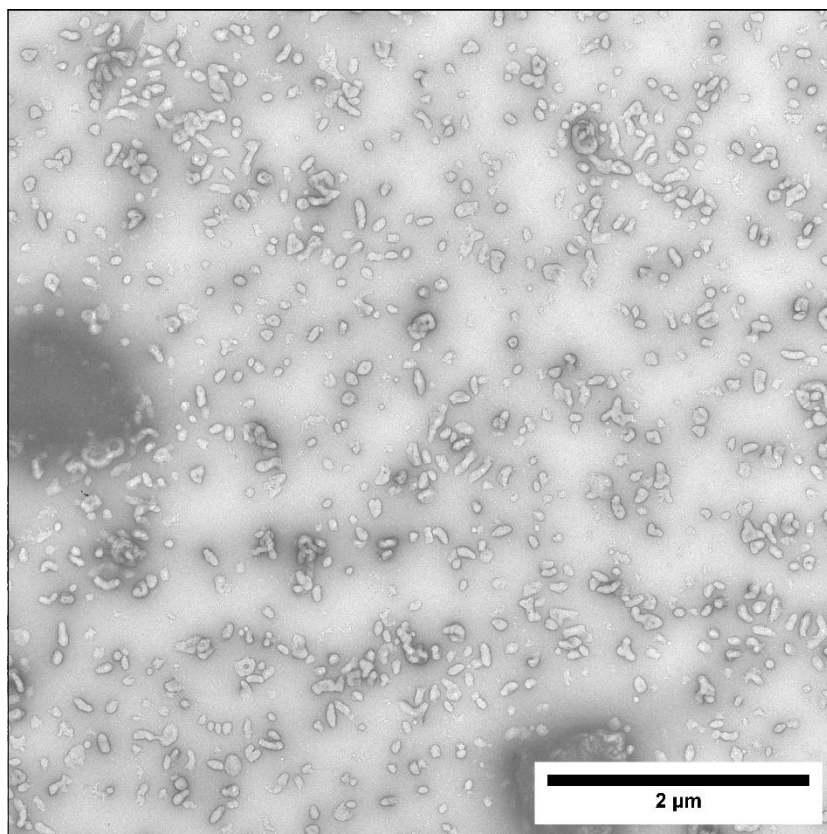


Figure 7.180: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 60 min)

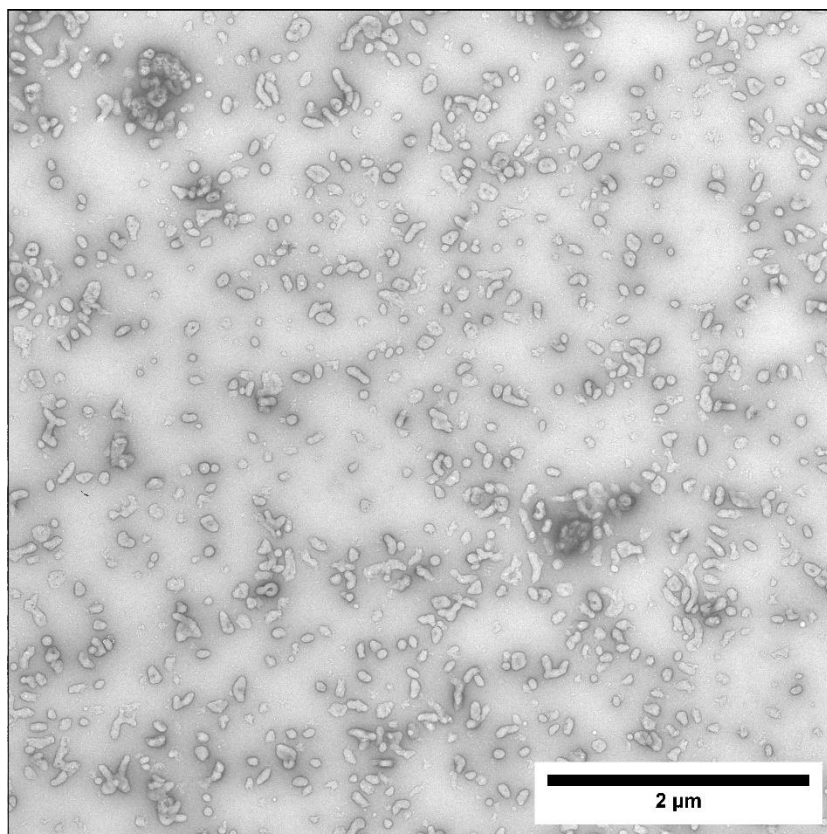


Figure 7.181: – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 60 min)

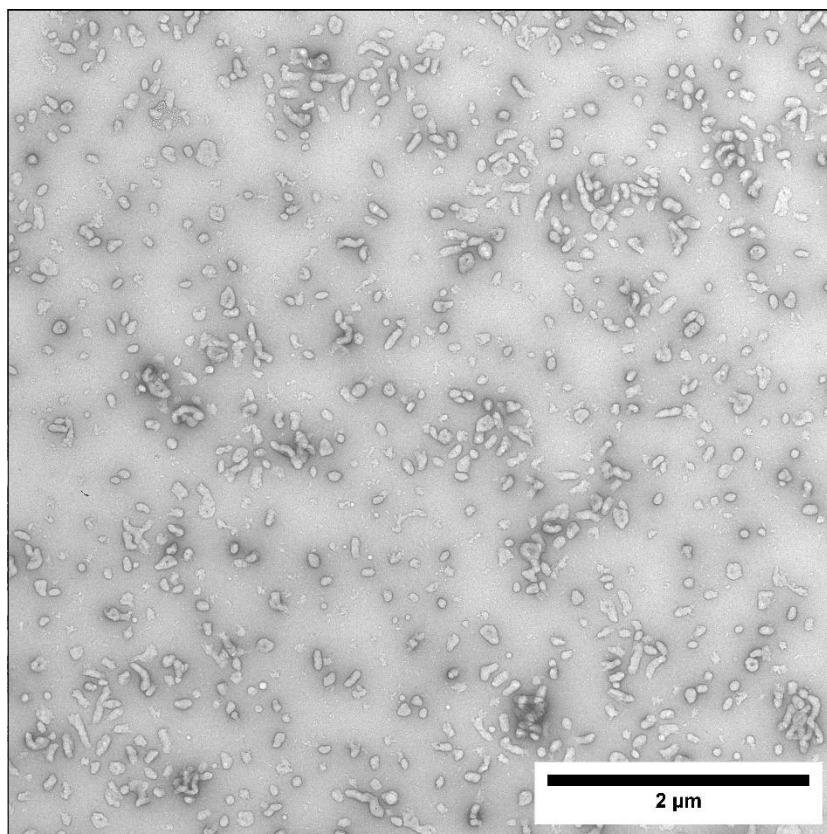


Figure 7.182: – TEM micrograph for PDMA₅₀-b-PDAA₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 60 min)

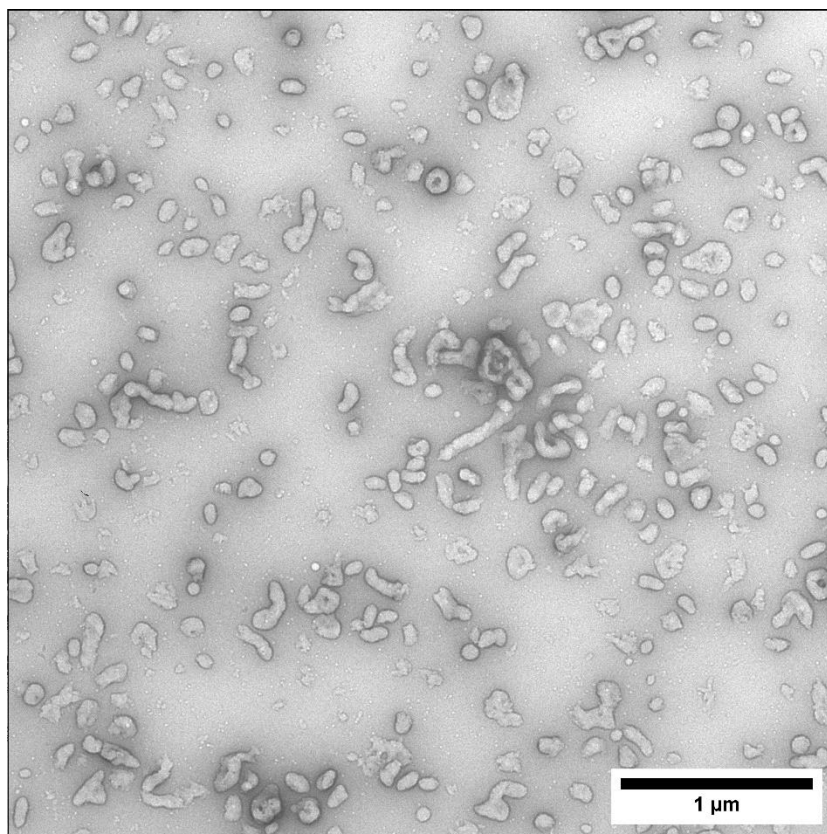


Figure 7.183: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 60 min)

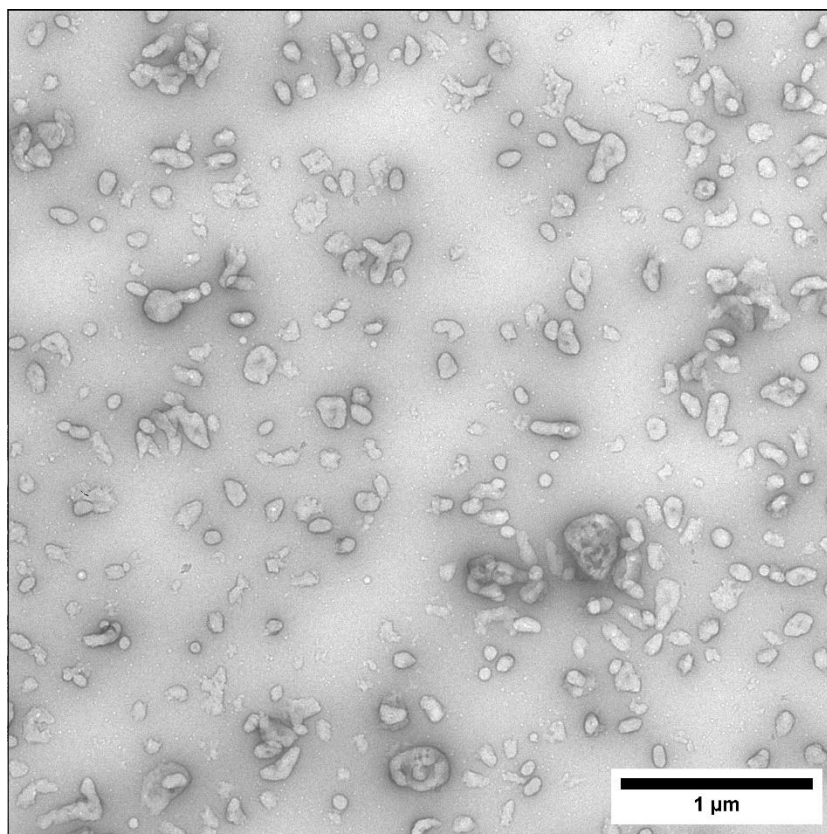


Figure 7.184: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 60 min)

t = 120 min

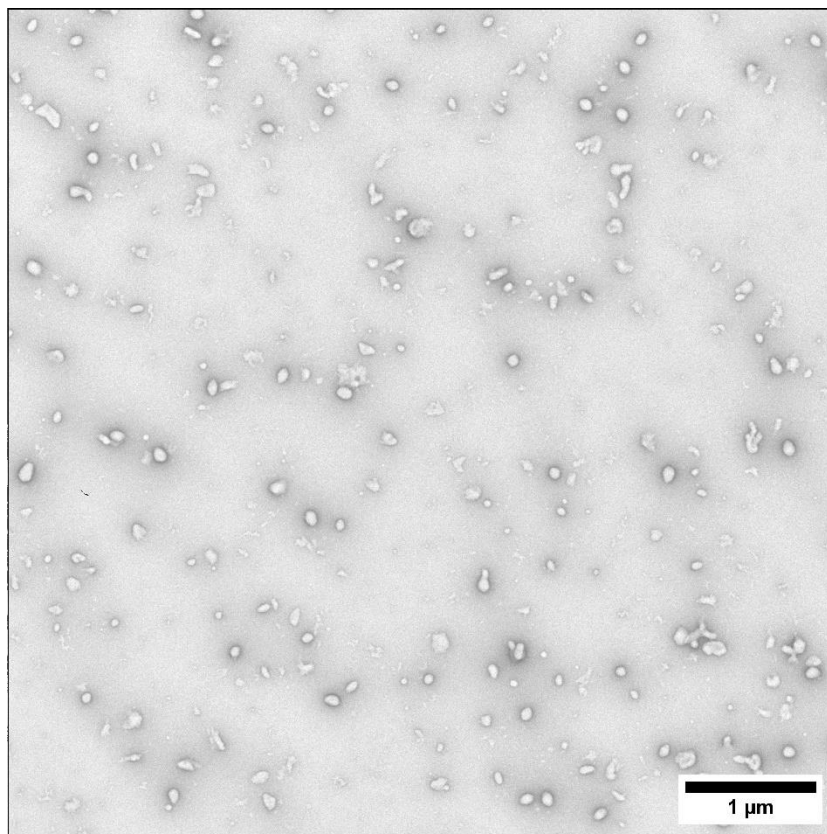


Figure 7.185: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 120 min)

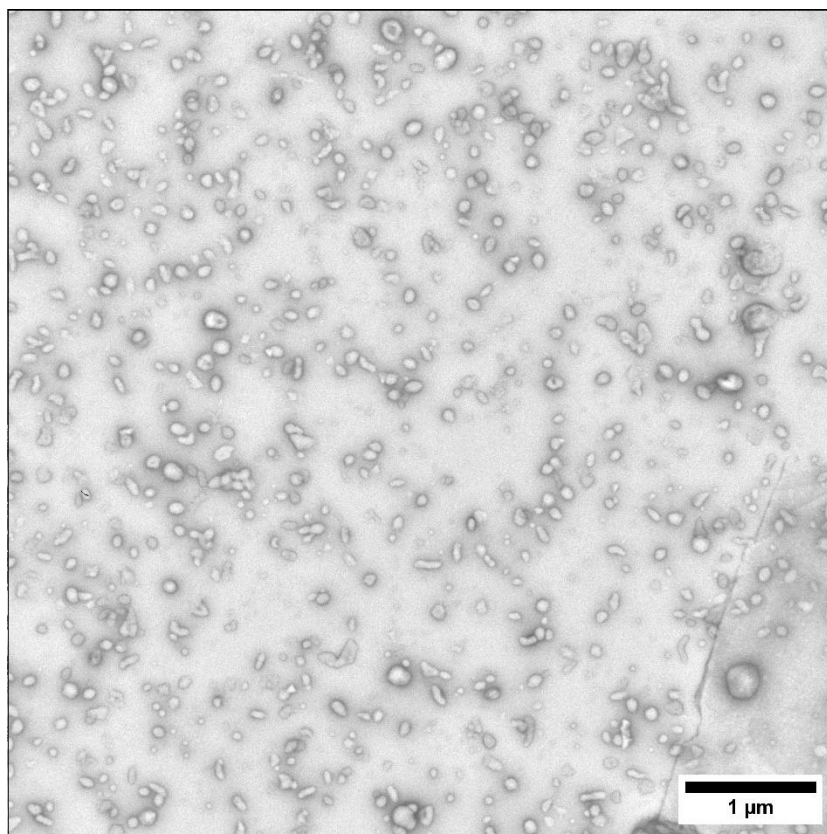


Figure 7.186 – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 120 min)

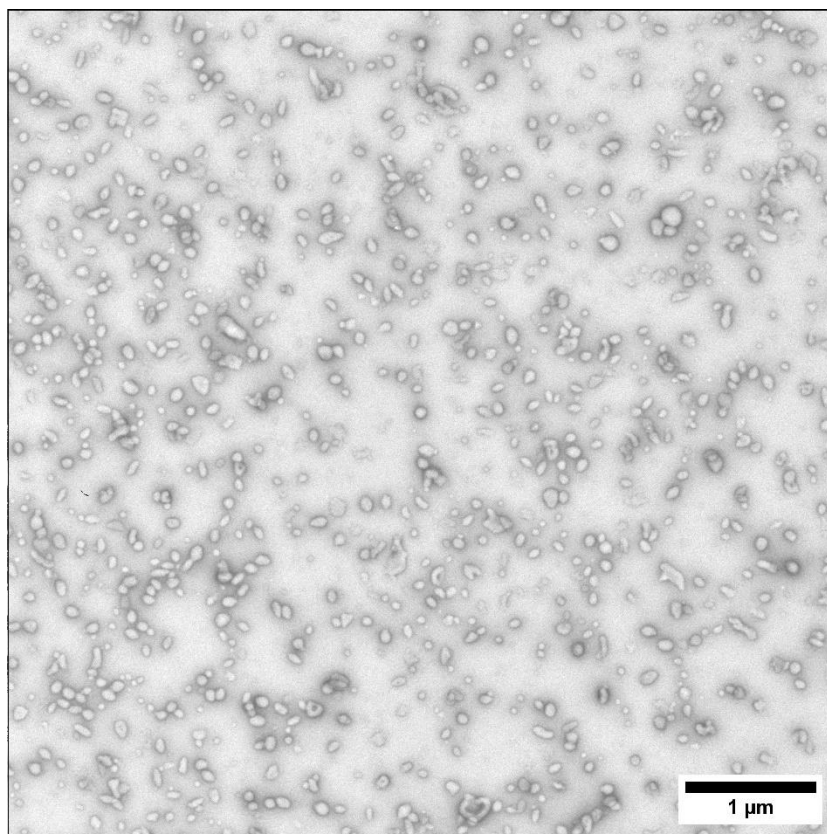


Figure 7.187: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 120 min)

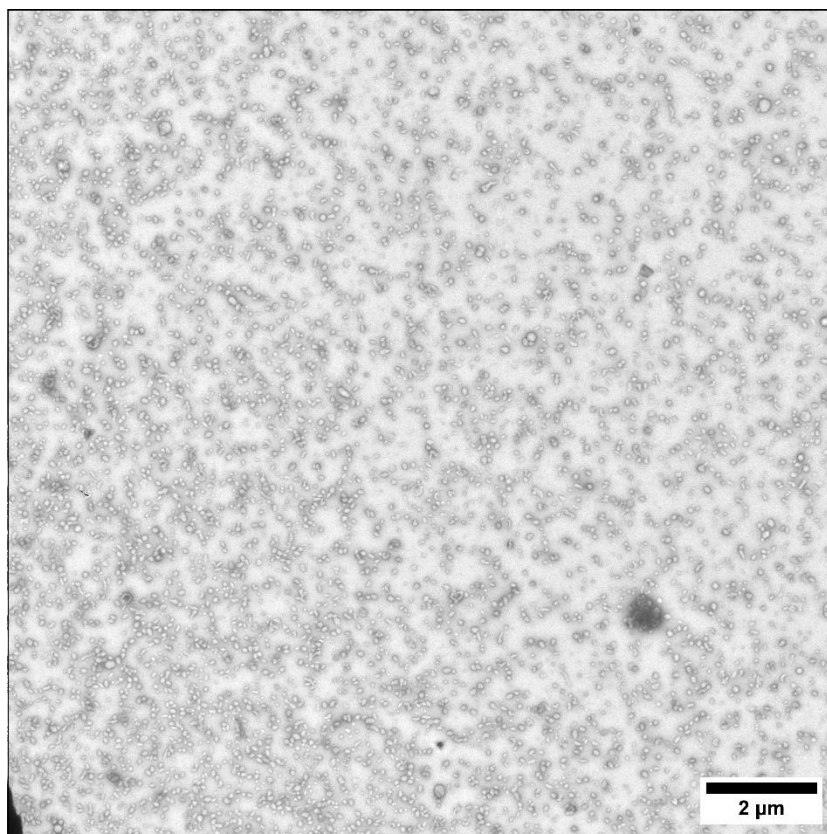


Figure 7.187: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 120 min)

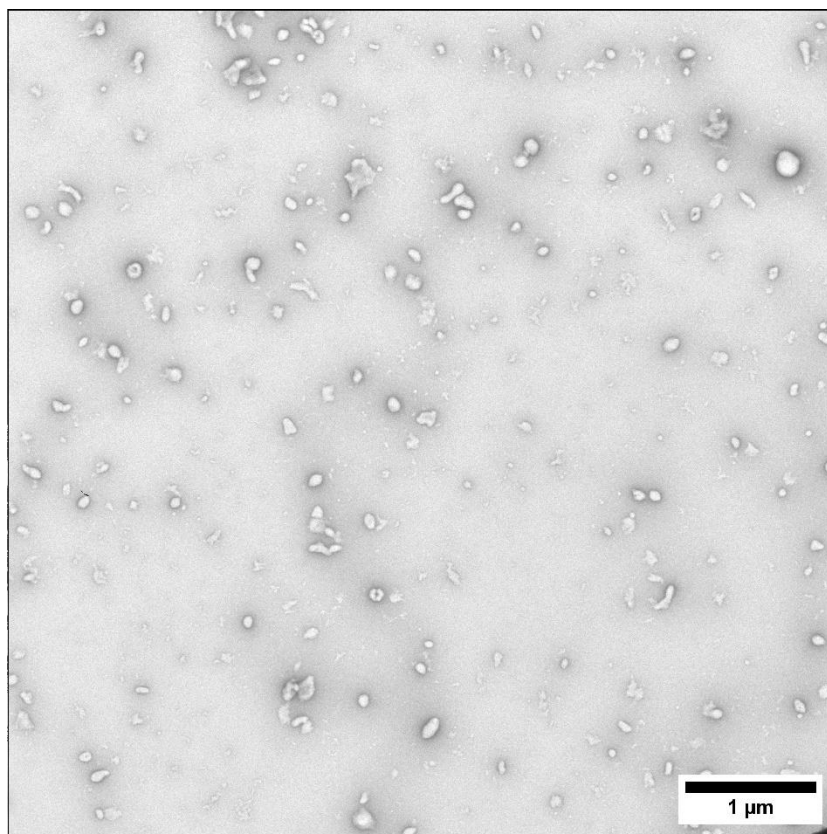


Figure 7.188: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 120 min)

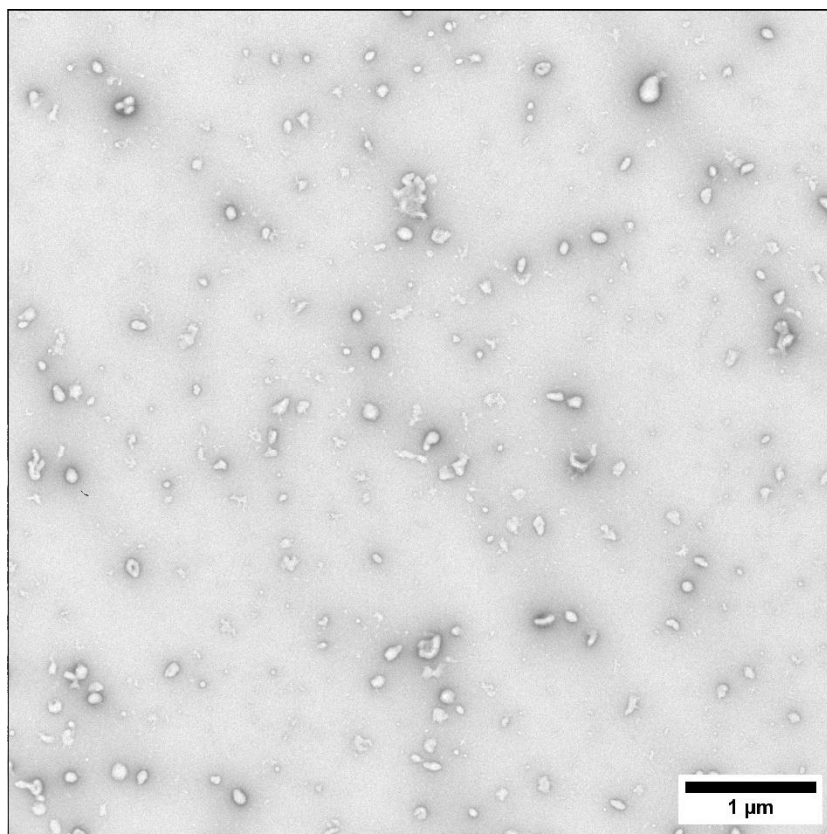


Figure 7.189: – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 120 min)

t = 180 min

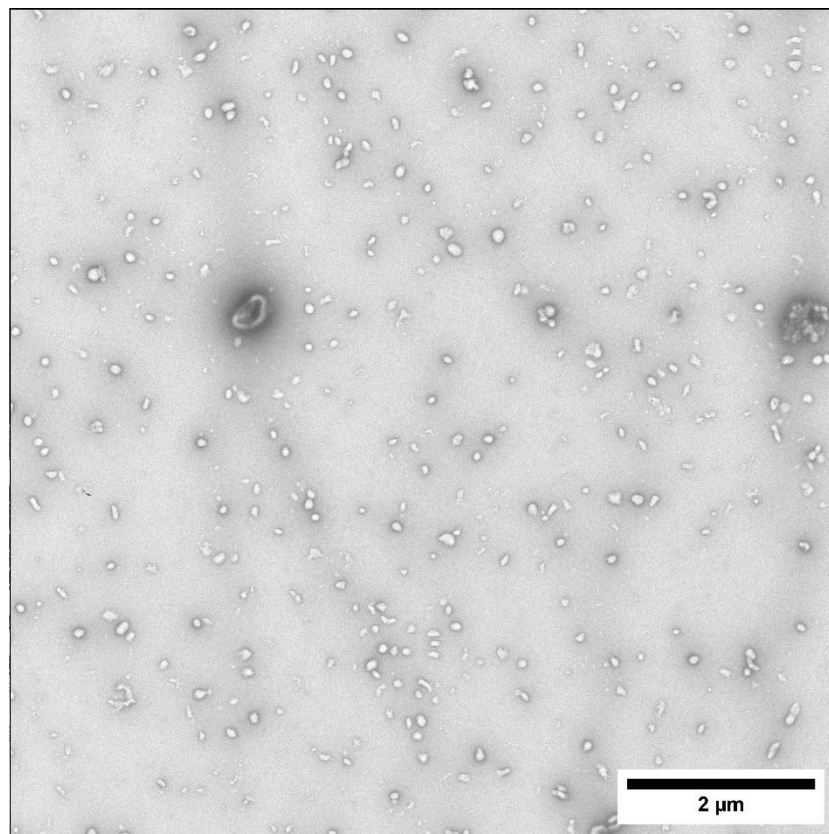


Figure 7.190: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 180 min)

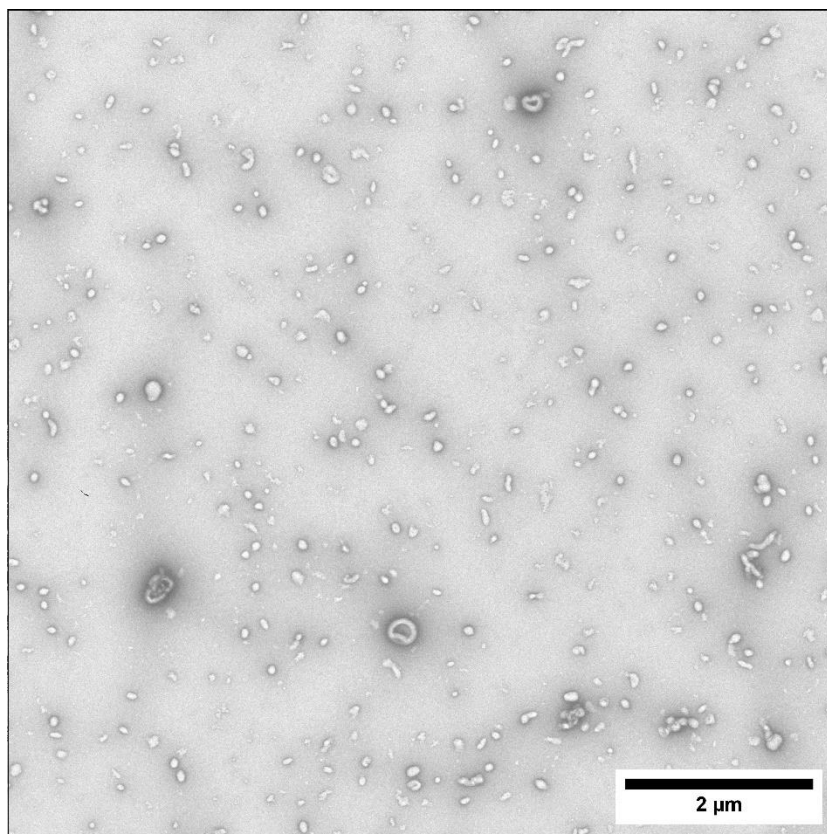


Figure 7.191 – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 180 min)

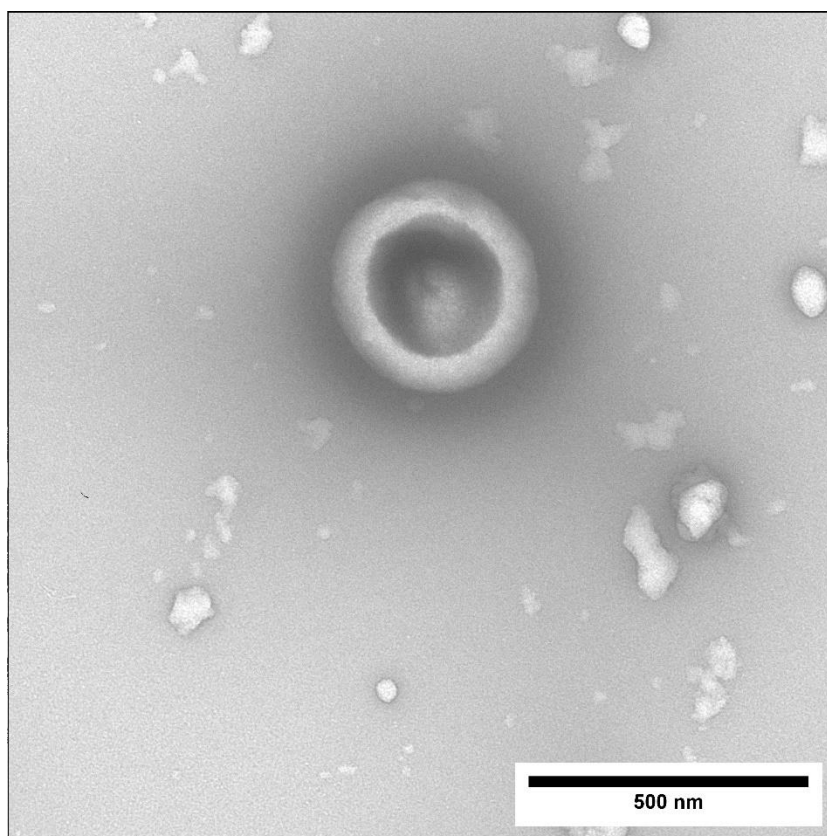


Figure 7.192: – TEM micrograph for PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 180 min)

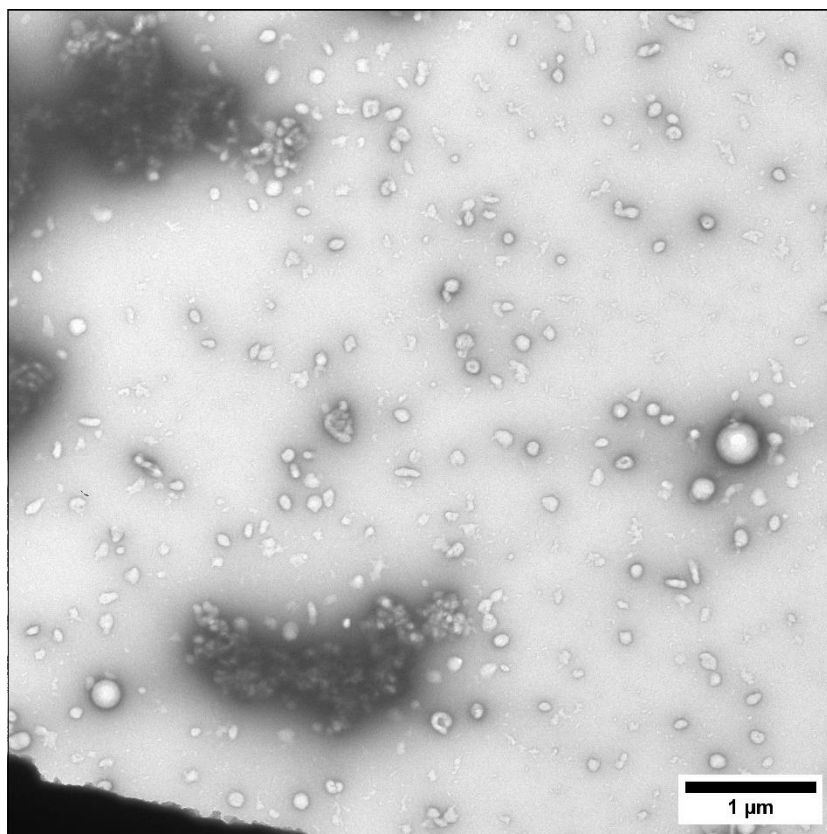


Figure 7.193: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 180 min)

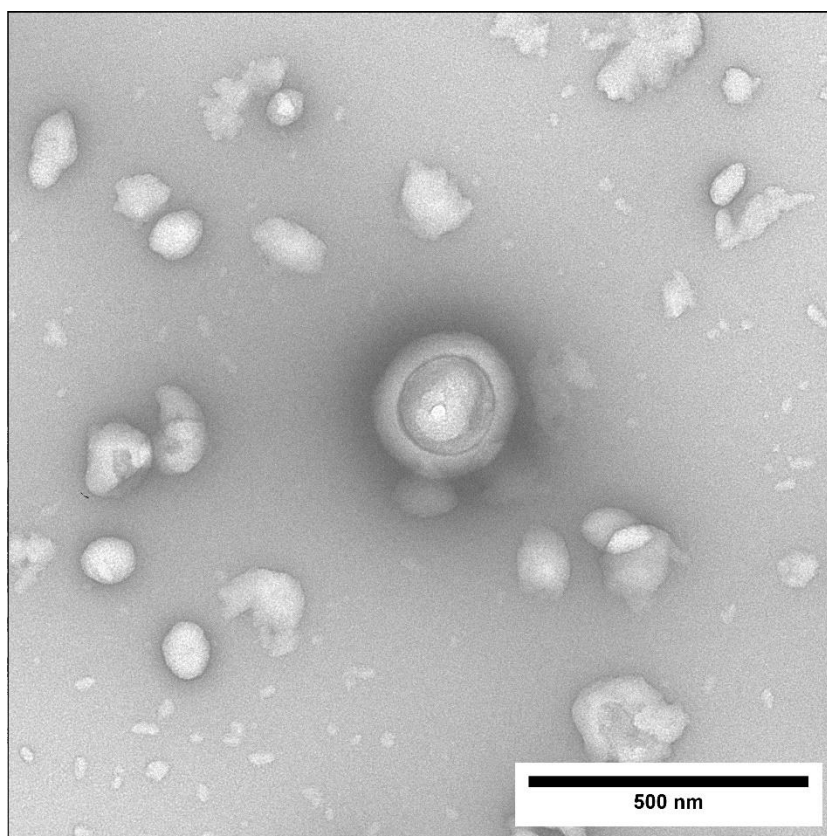


Figure 7.194: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 180 min)

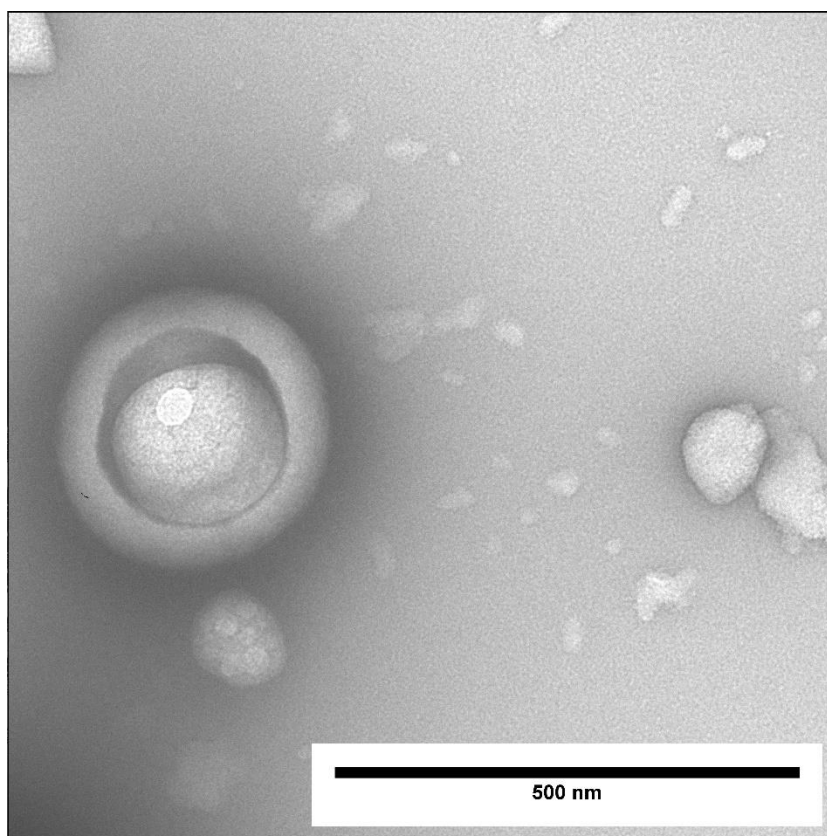


Figure 7.195: – TEM micrograph for PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 180 min)

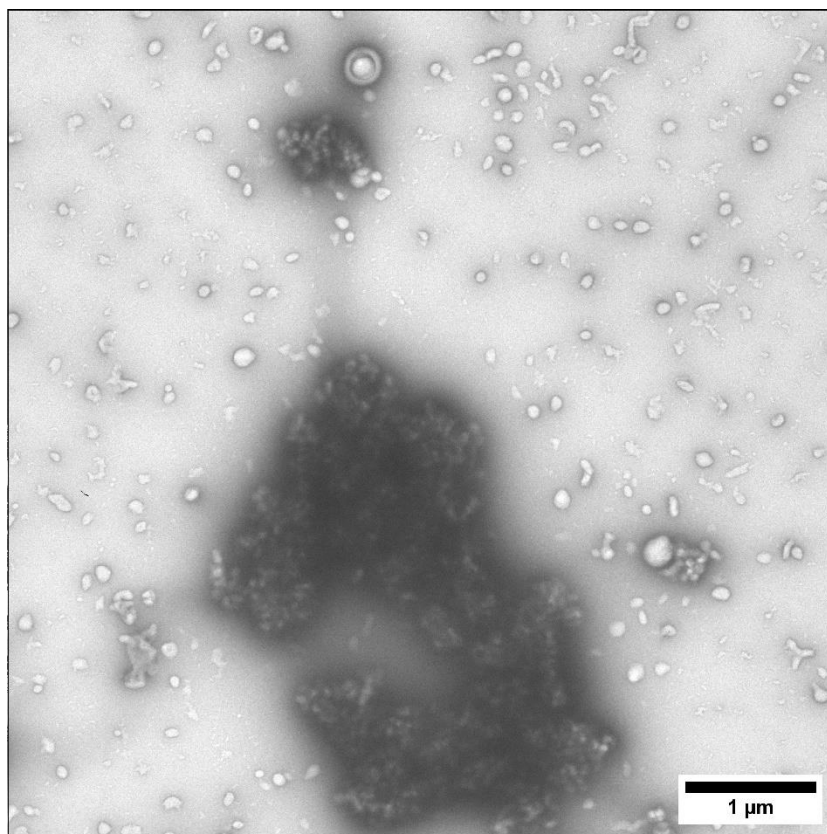


Figure 7.196: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 180 min)

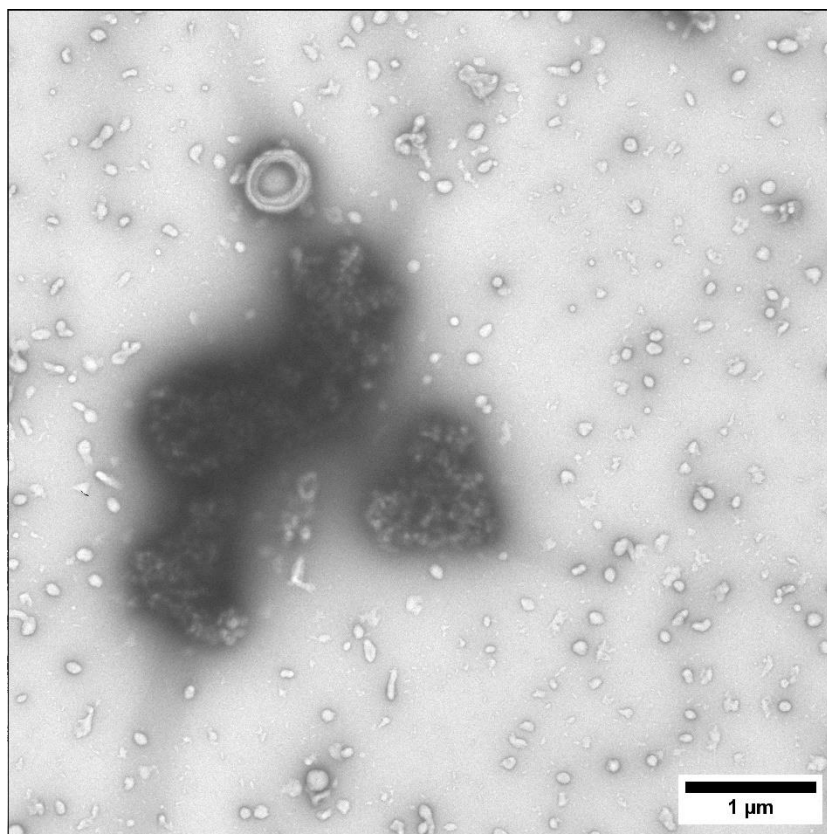


Figure 7.197: – TEM micrograph for PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 180 min)

**SE PDMA_{m50}-b-PDAAm₃₀₀ Singly charged polymer – pH
7.5 500 mM NaCl**

t = 5 min

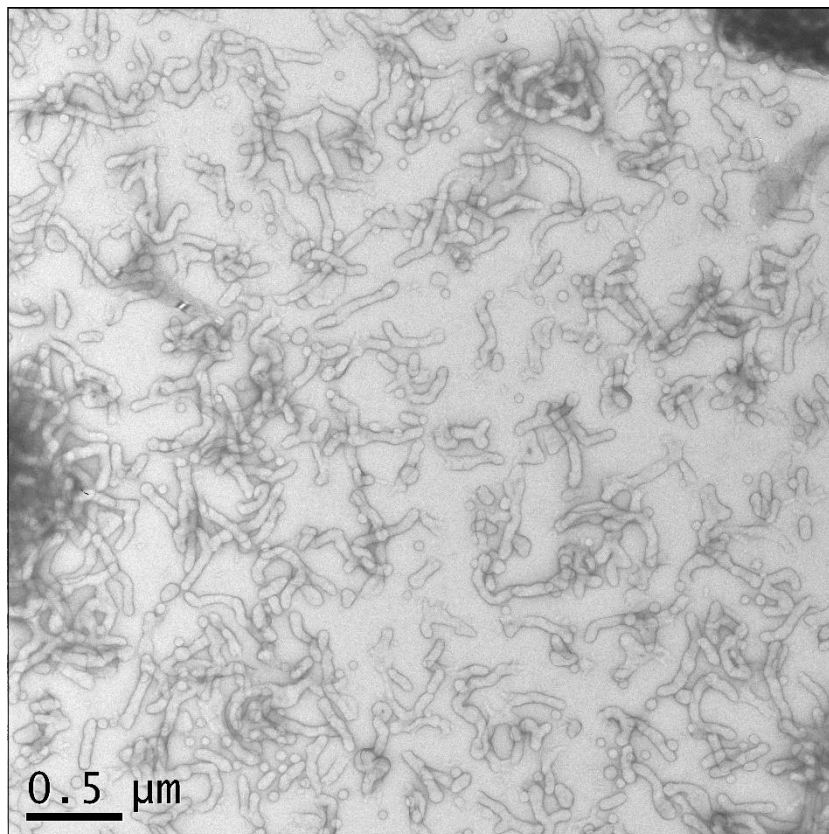


Figure 7.198: – TEM micrograph for SC PDMA_{m50}-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

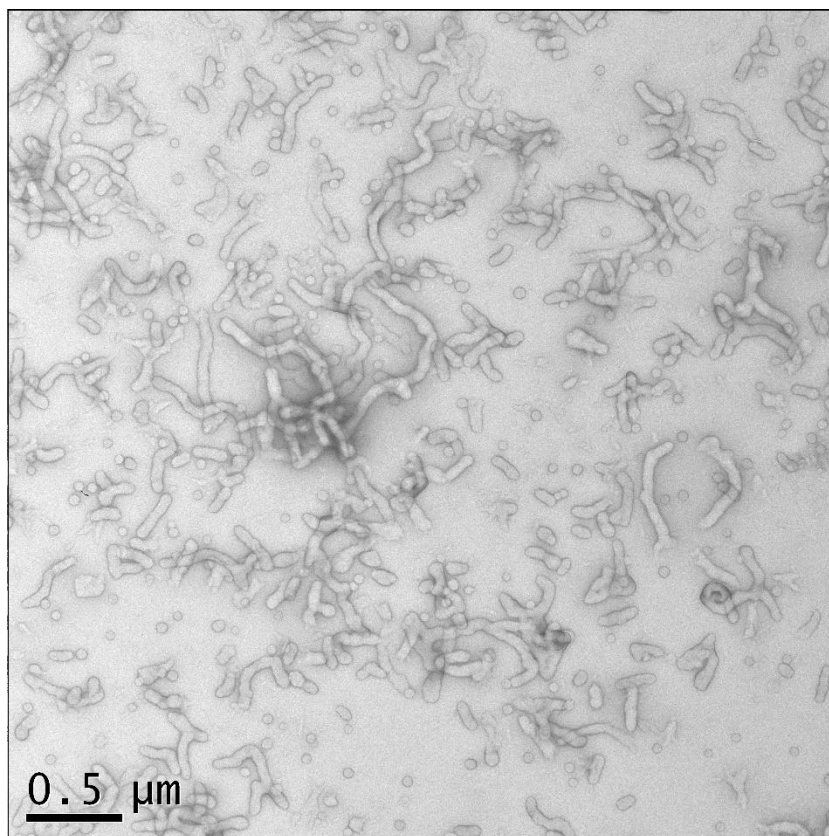


Figure 7.199: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

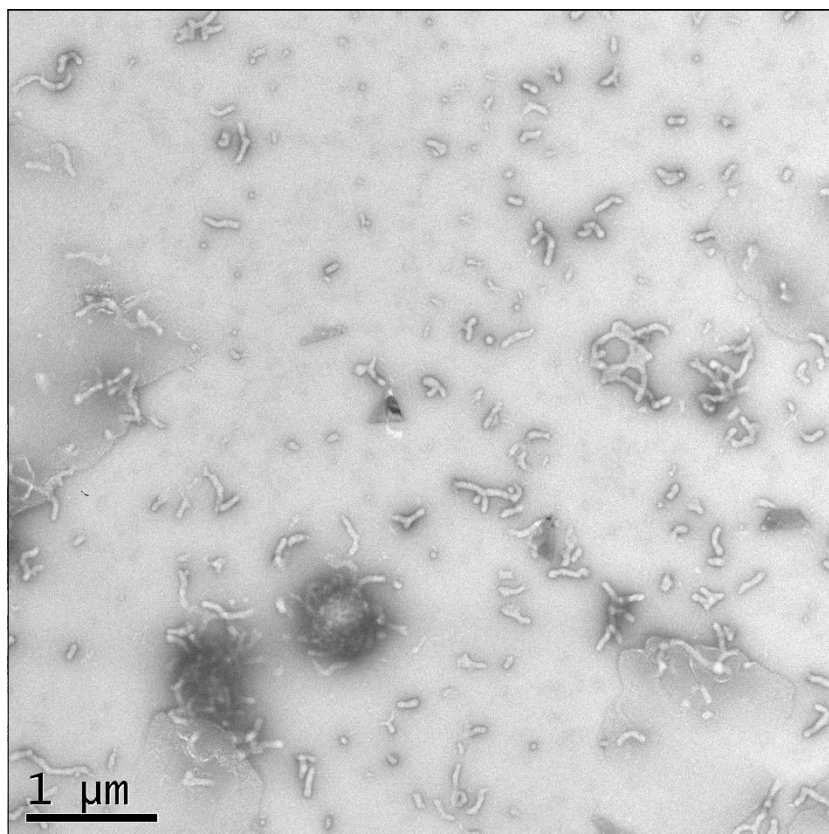


Figure 7.200: – TEM micrograph for SC PDMAm₅₀.b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

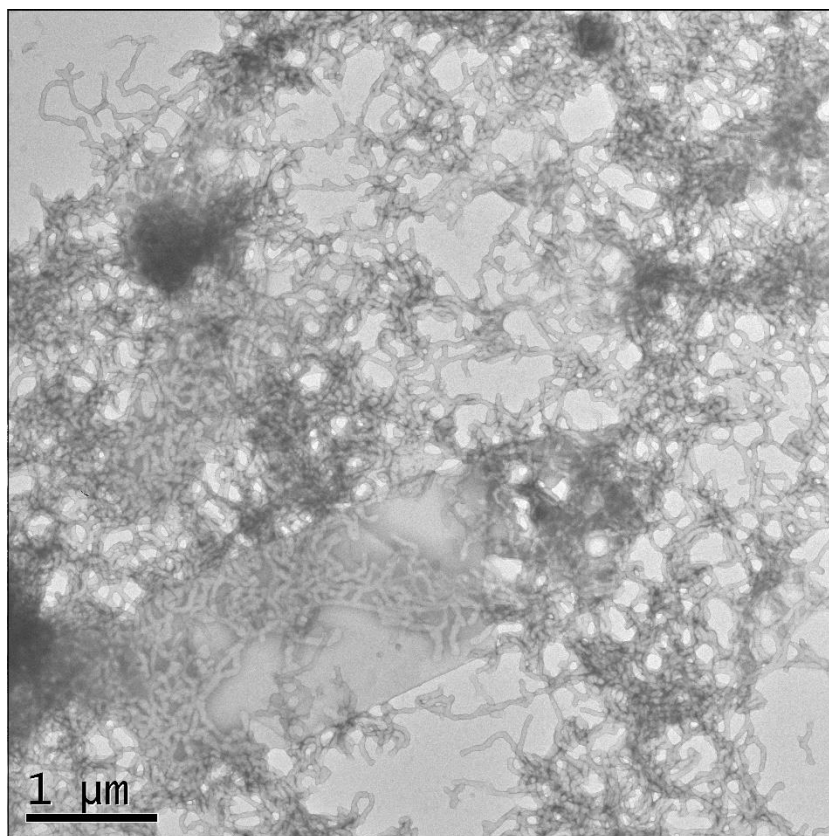


Figure 7.201: – TEM micrograph for SC PDMAm_{50.b}-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

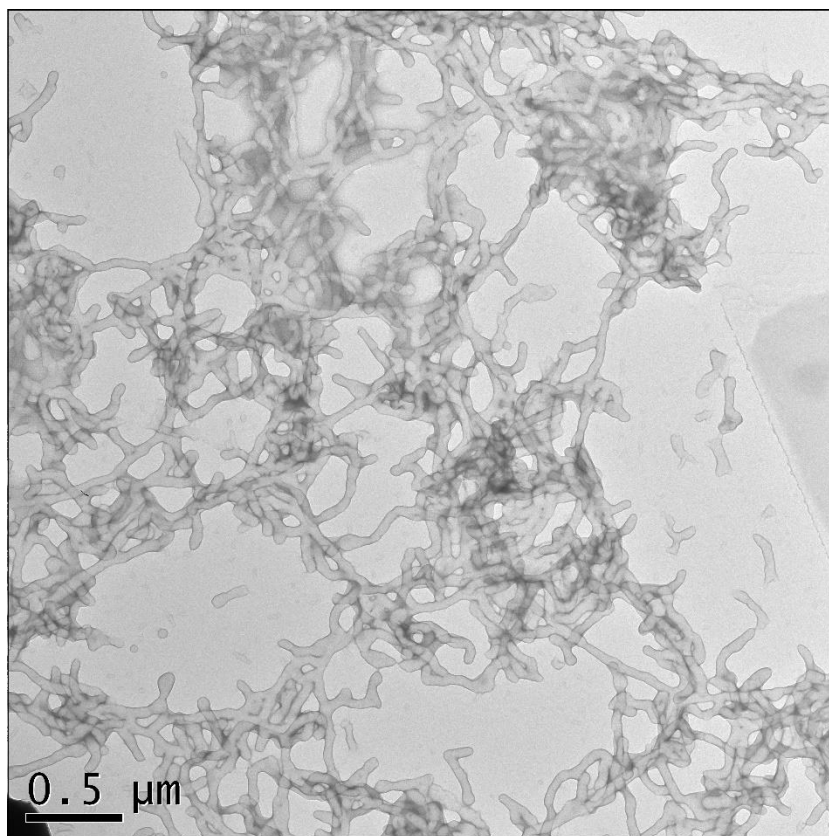


Figure 7.202: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

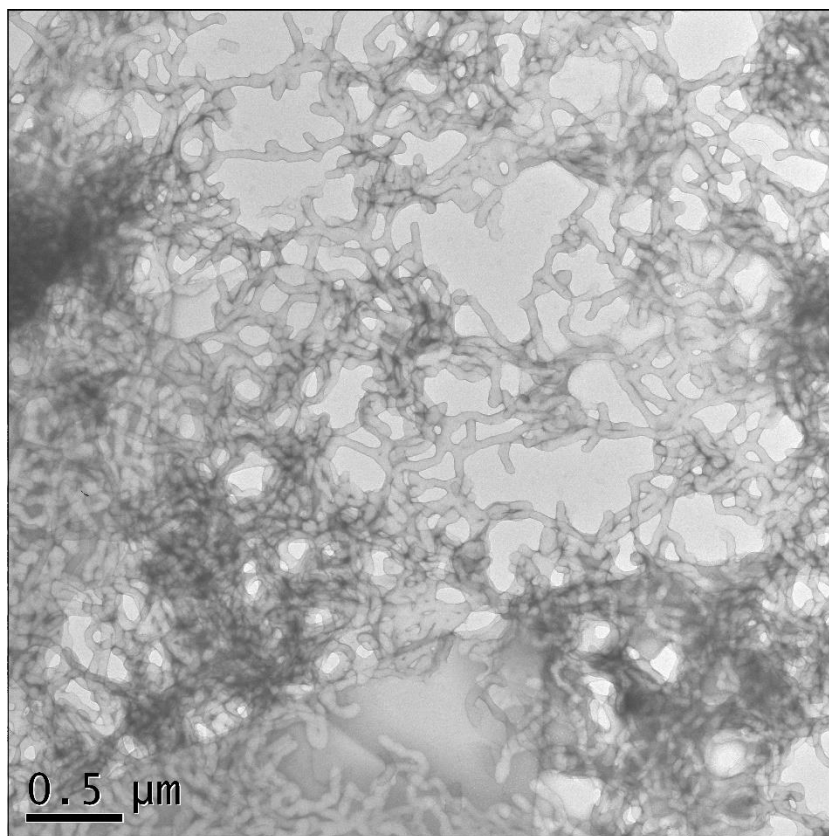


Figure 7.203: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

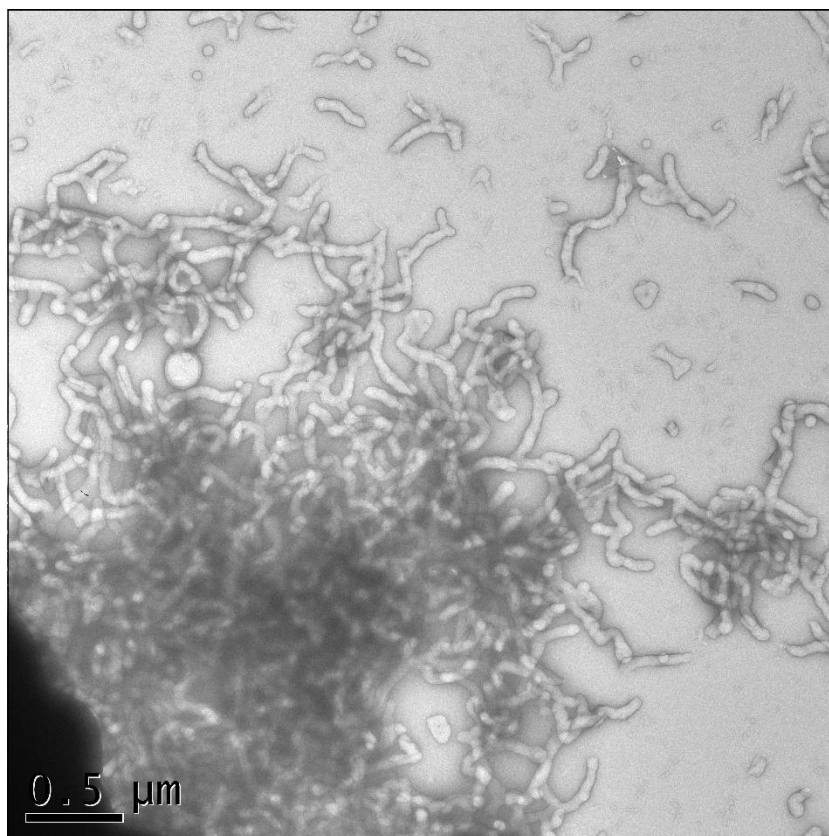


Figure 7.204: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

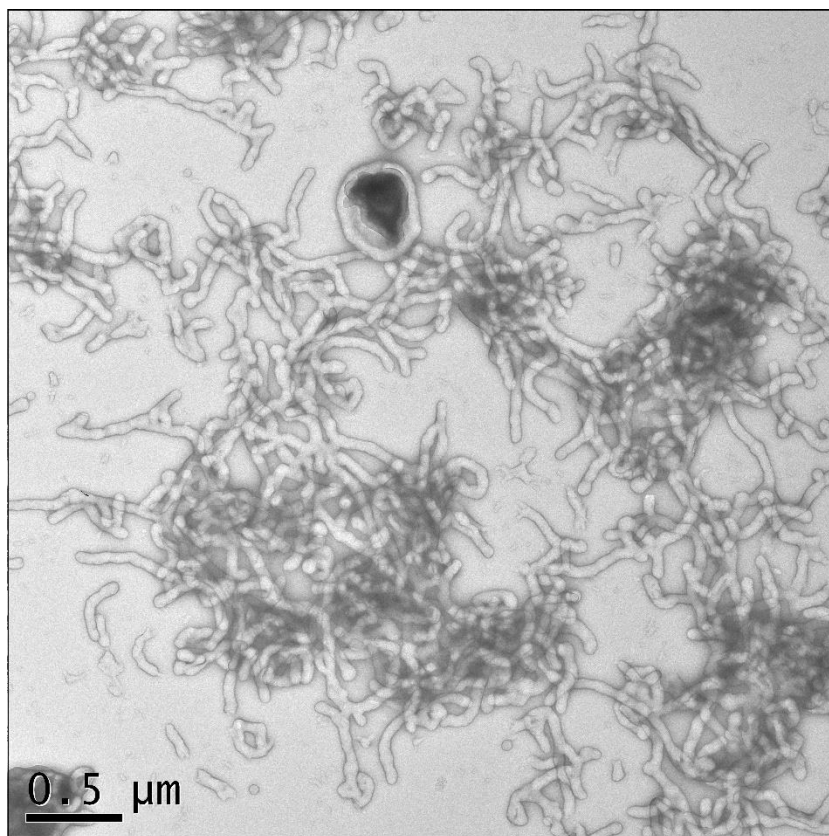


Figure 7.205: – TEM micrograph for SC PDMAm₅₀.b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

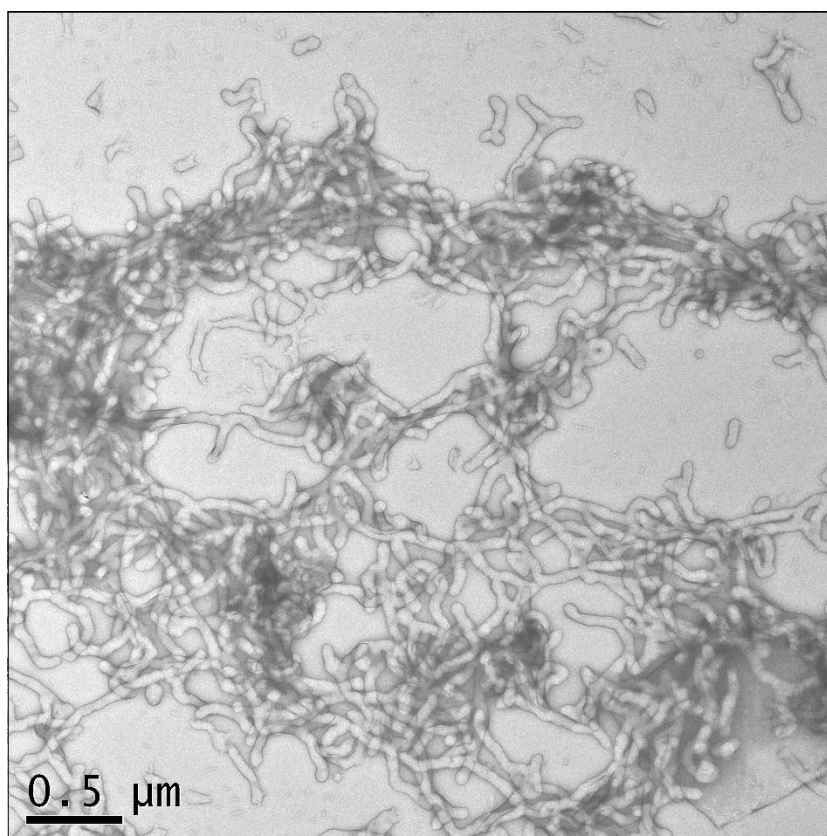


Figure 7.206: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

t = 20 min

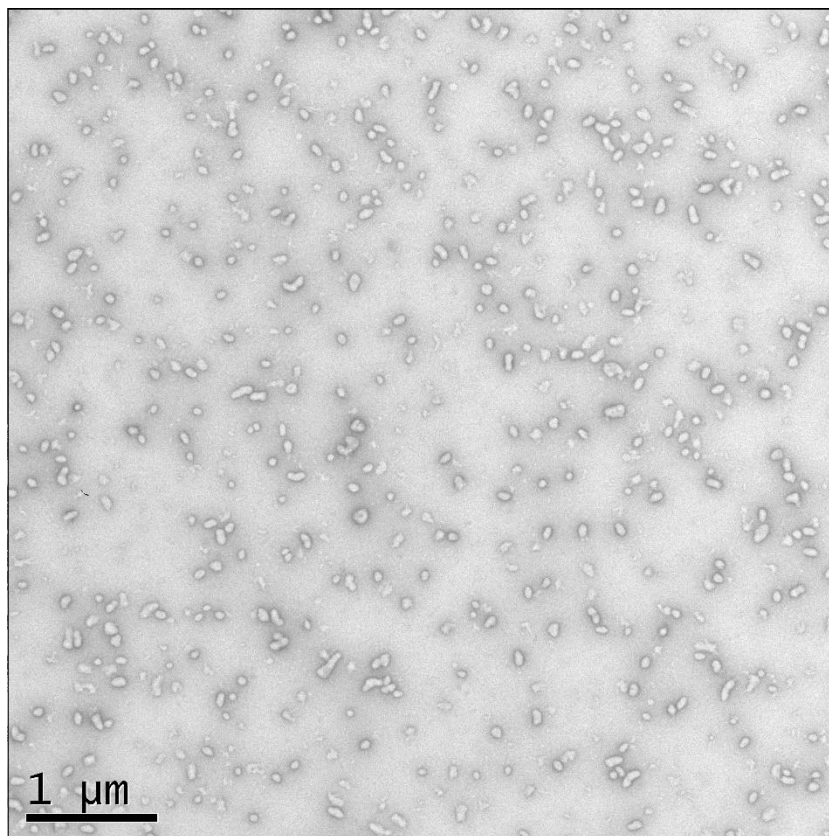


Figure 7.207: – TEM micrograph for SC PDMAm₅₀.b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 20 min)

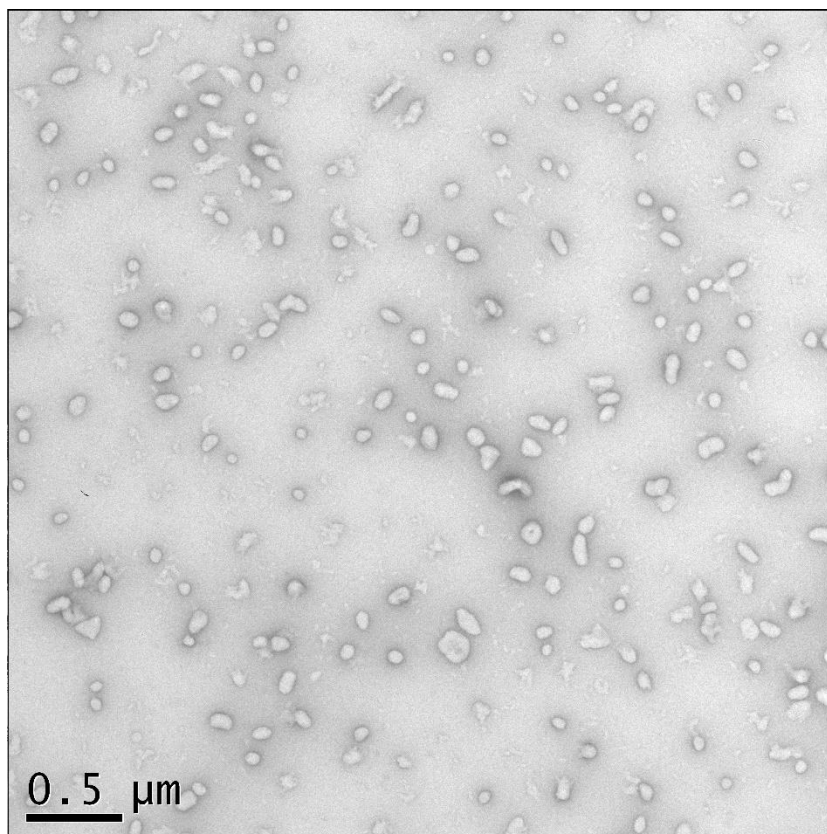


Figure 7.208: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 20 min)

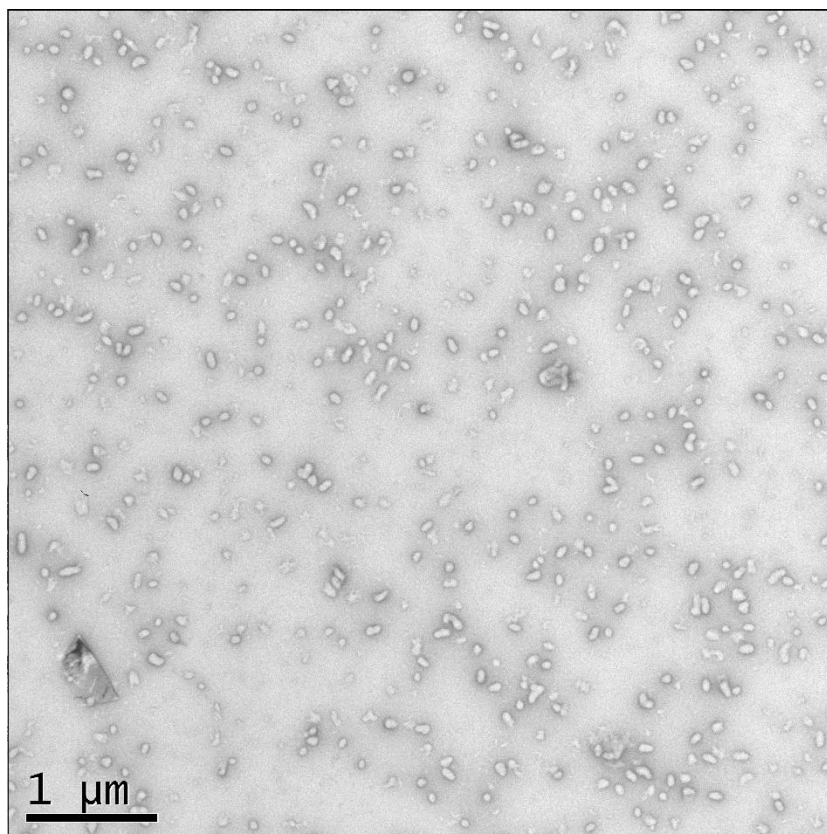


Figure 7.209: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 20 min)

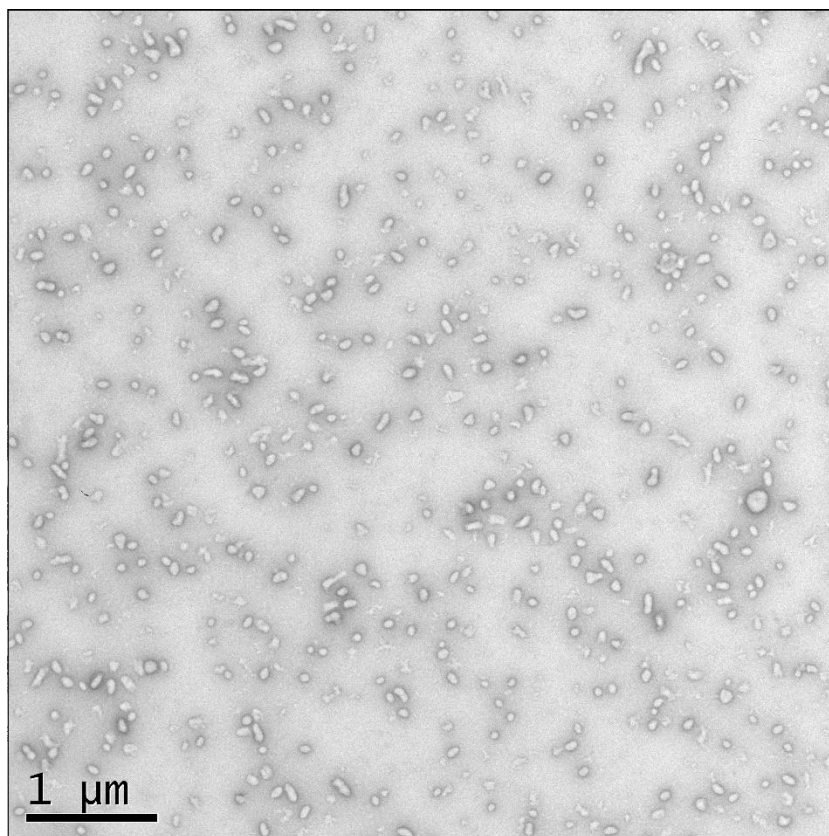


Figure 7.210: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 20 min)

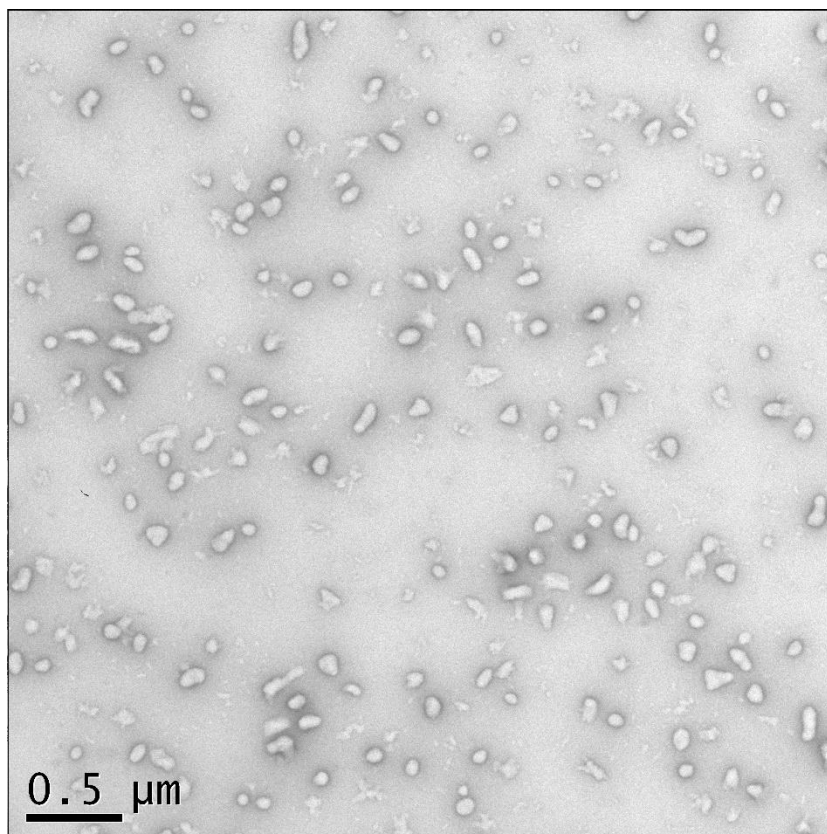


Figure 7.211: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 20 min)

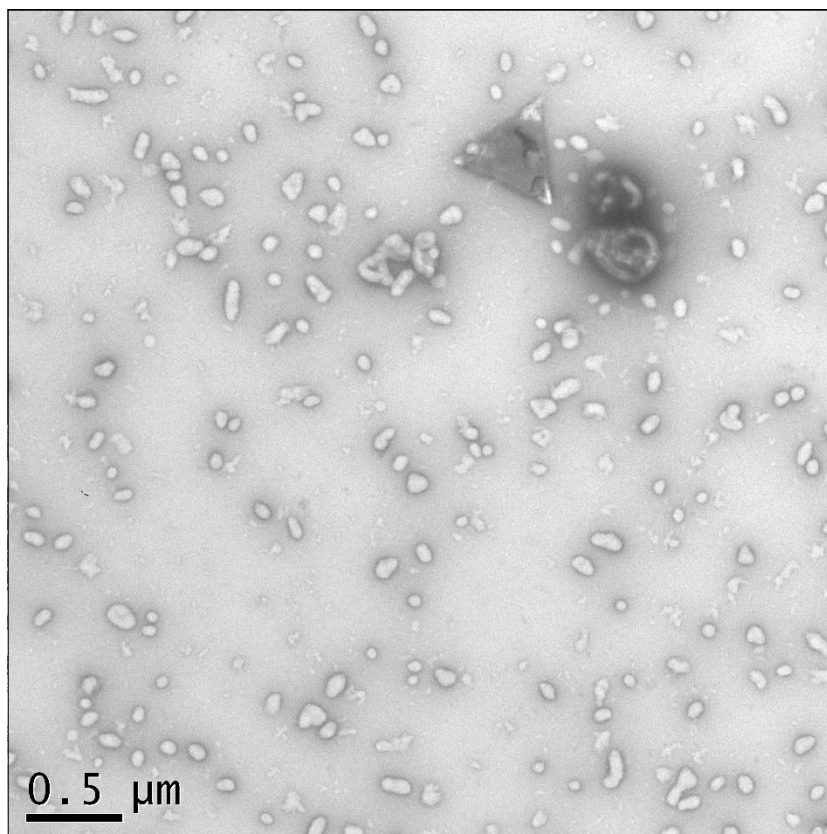


Figure 7.212: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 20 min)

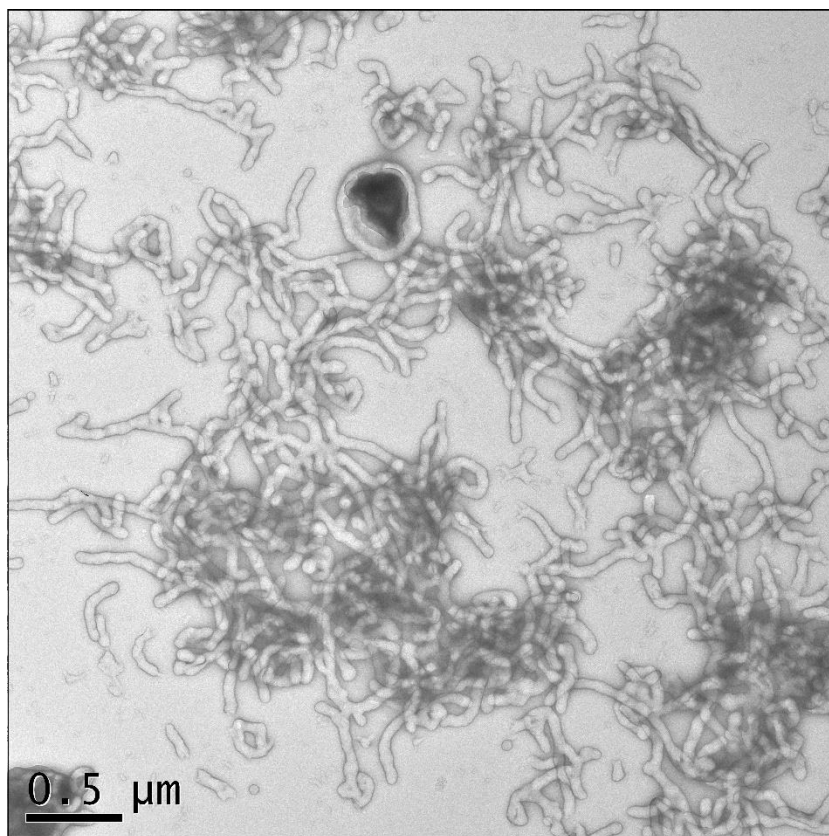


Figure 7.213 – TEM micrograph for SC PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 20 min)

t = 60 min

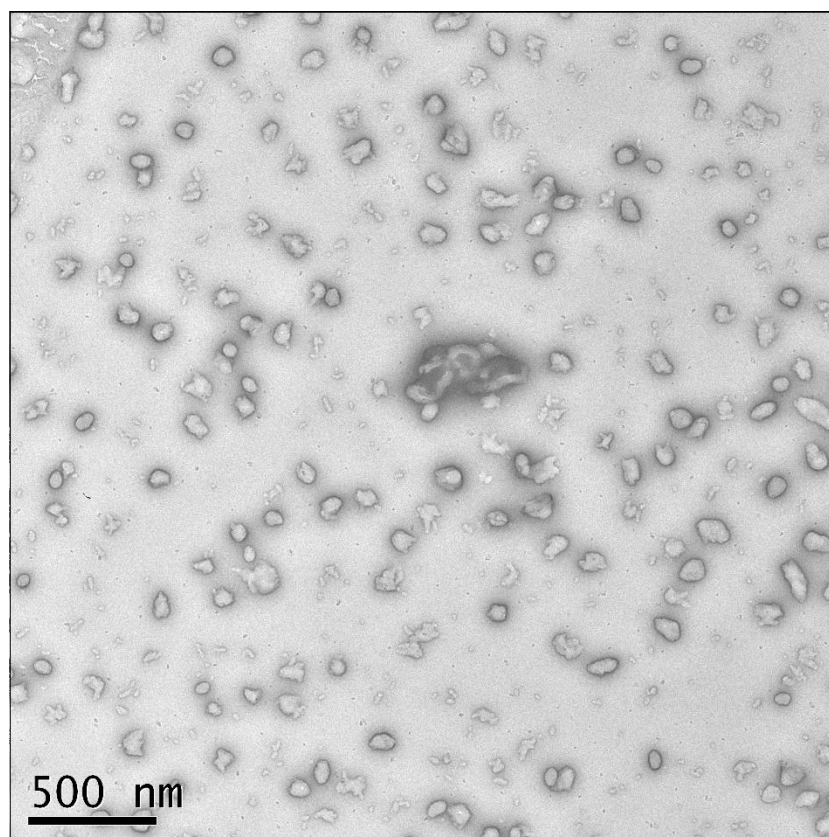


Figure 7.214: – TEM micrograph for SC PDMAm_{50.b}-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 60 min)

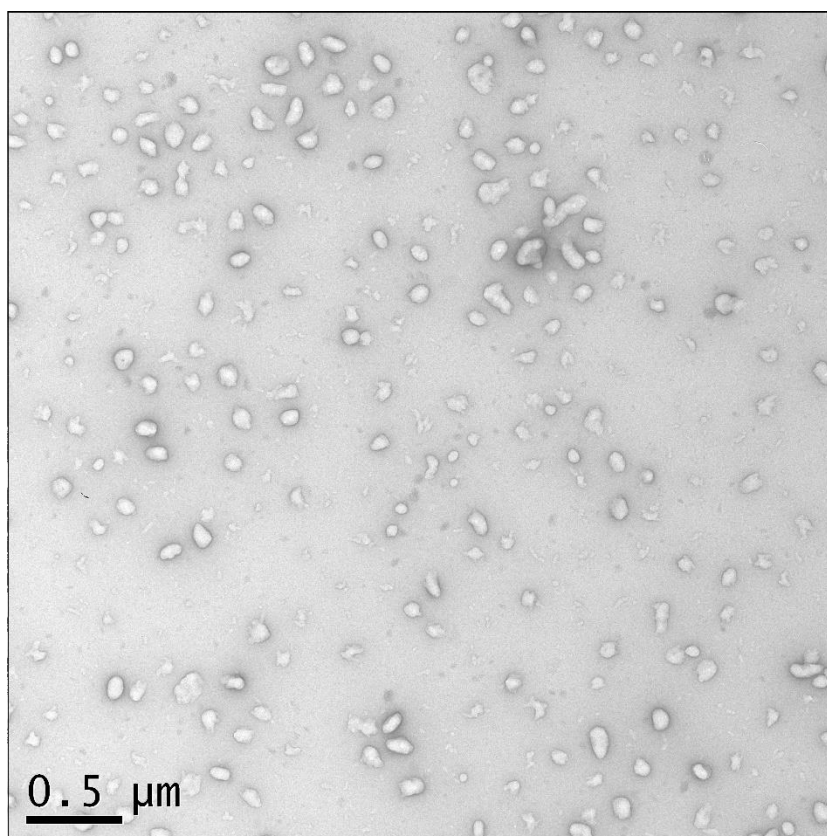


Figure 7.215: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 60 min)

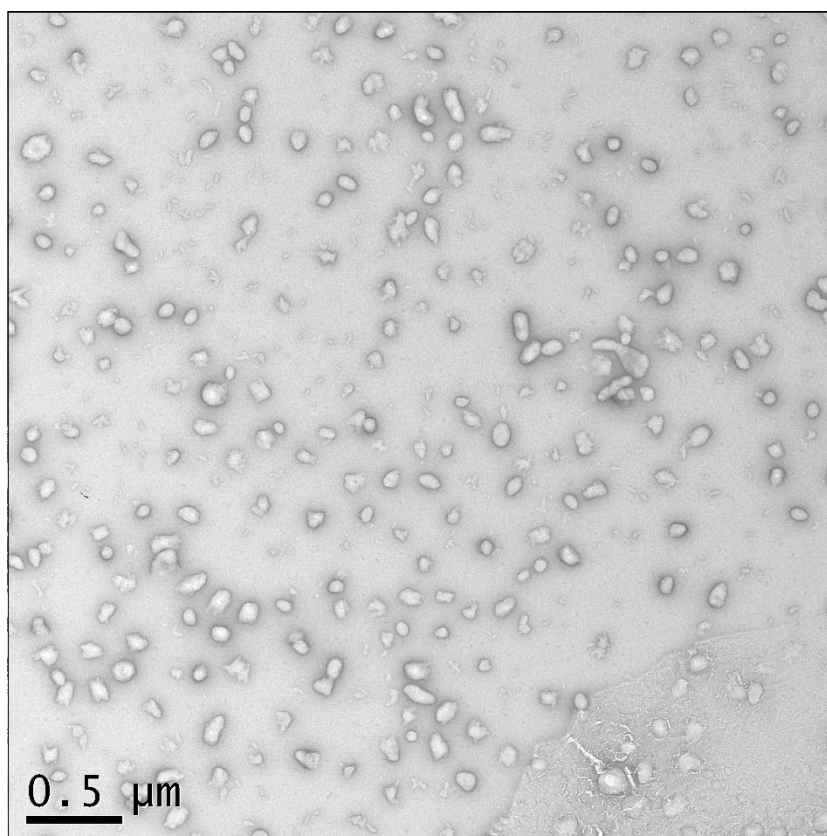


Figure 7.216: – TEM micrograph for SC PDMAm₅₀.b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 60 min)

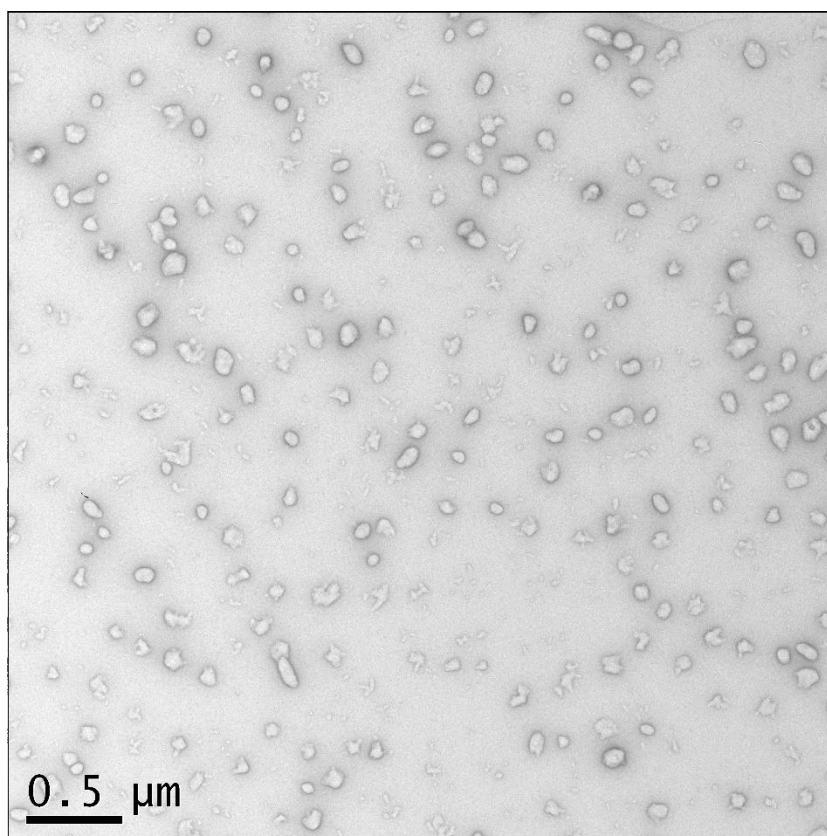


Figure 7.217: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 60 min)

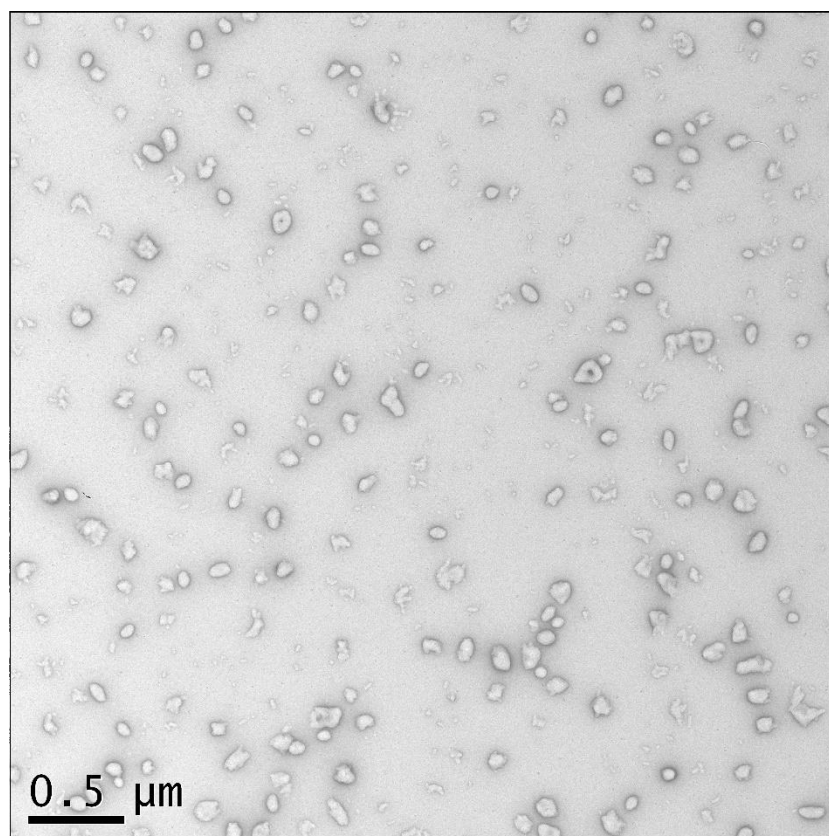


Figure 7.218: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 60 min)

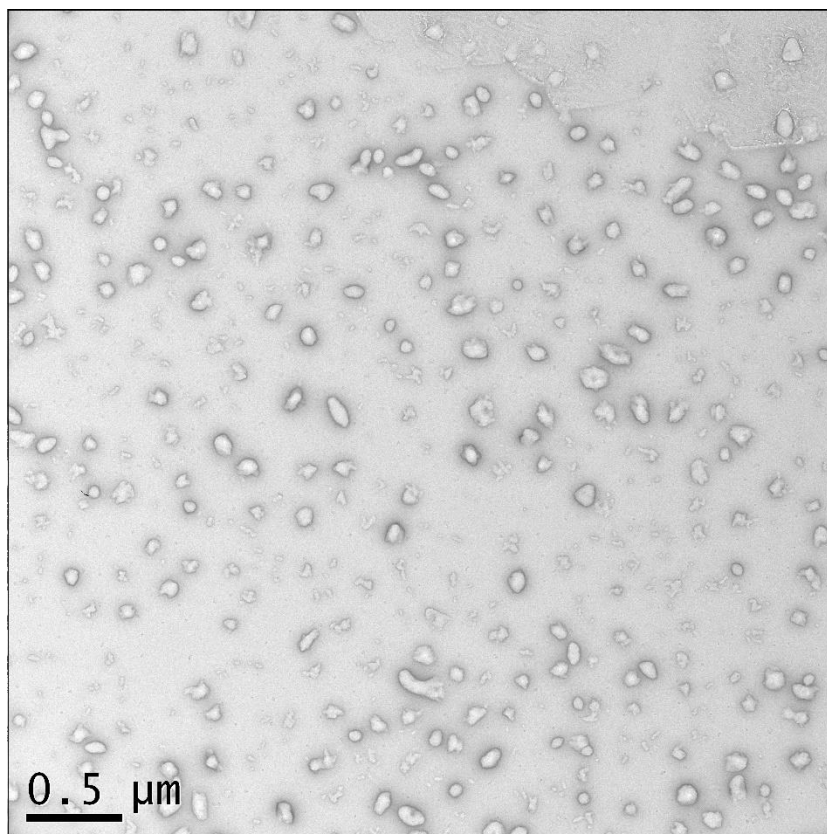


Figure 7.219: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 60 min)

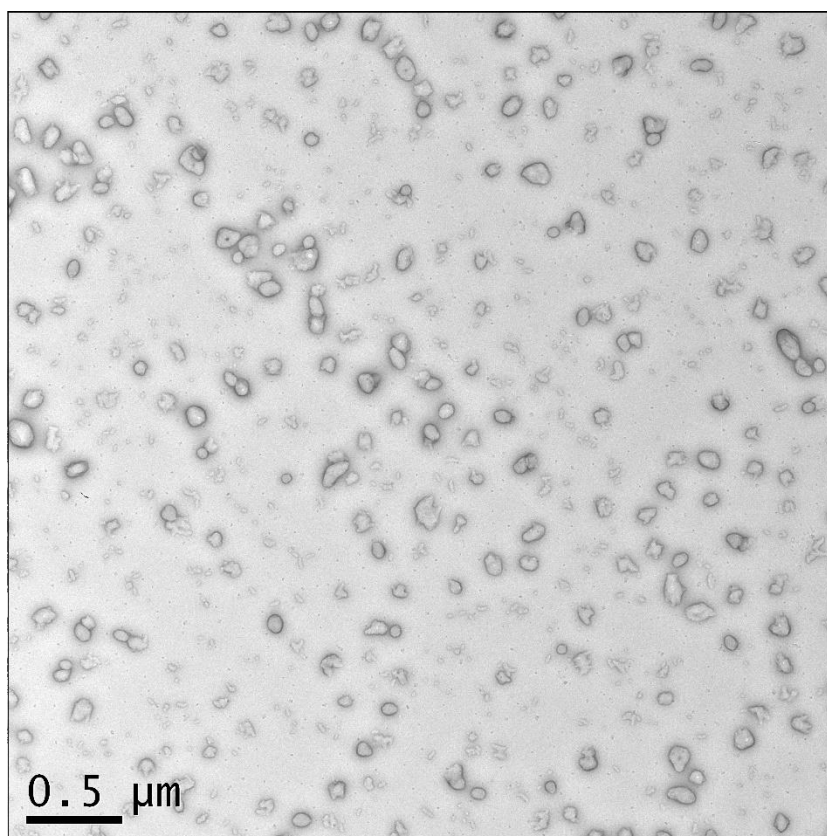


Figure 7.220: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 60 min)

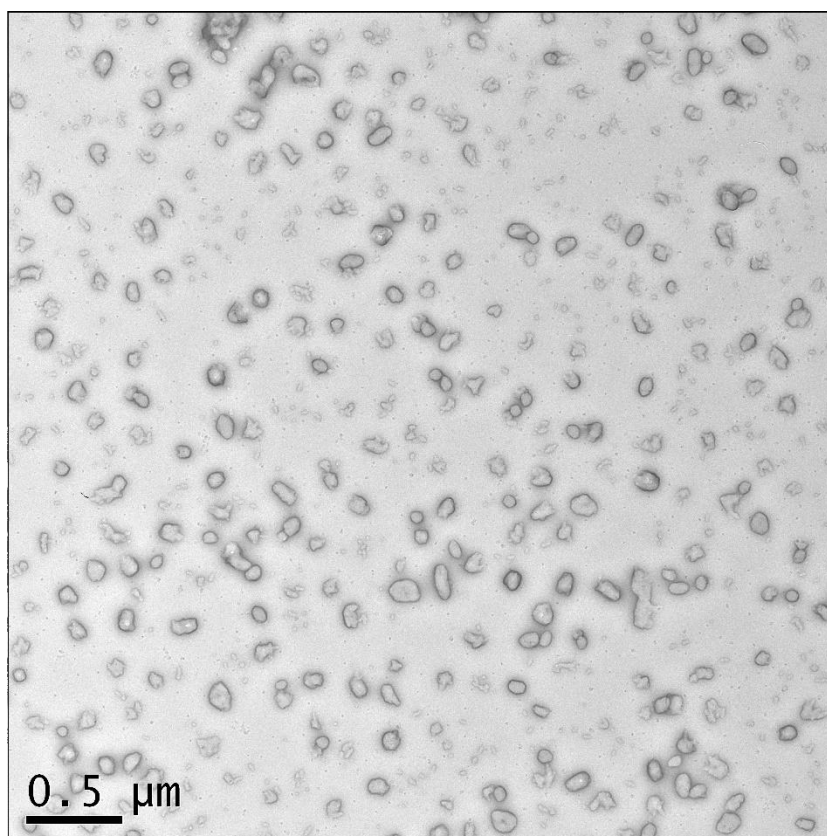


Figure 7.221: – TEM micrograph for SC PDMAM₅₀-b-PDAAM₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 60 min)

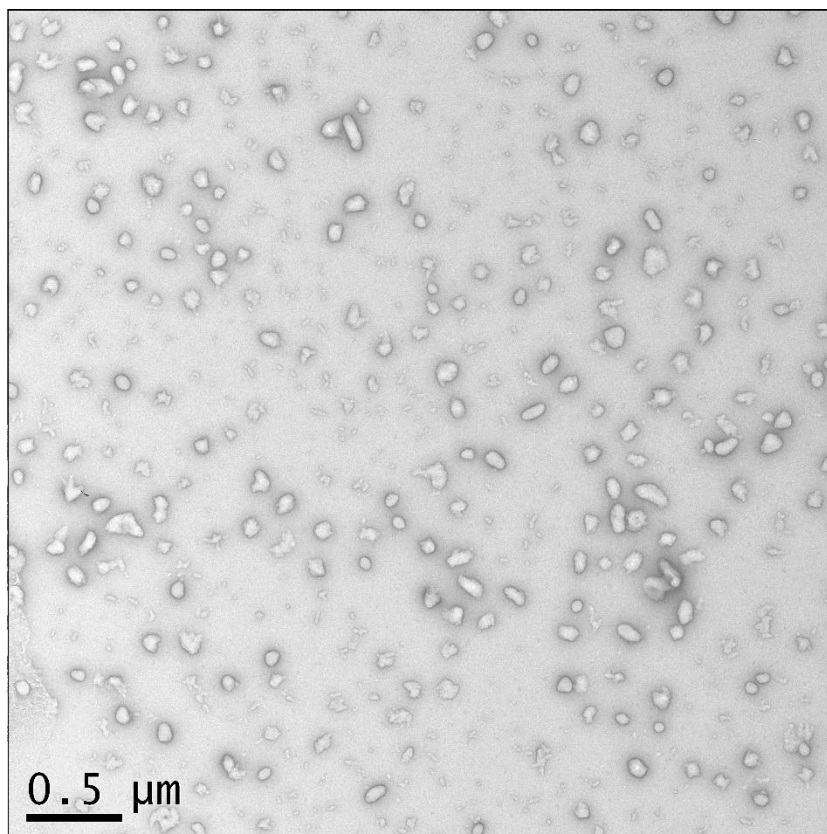


Figure 7.222: – TEM micrograph for SC PDMAm₅₀.b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 60 min)

t = 120 min

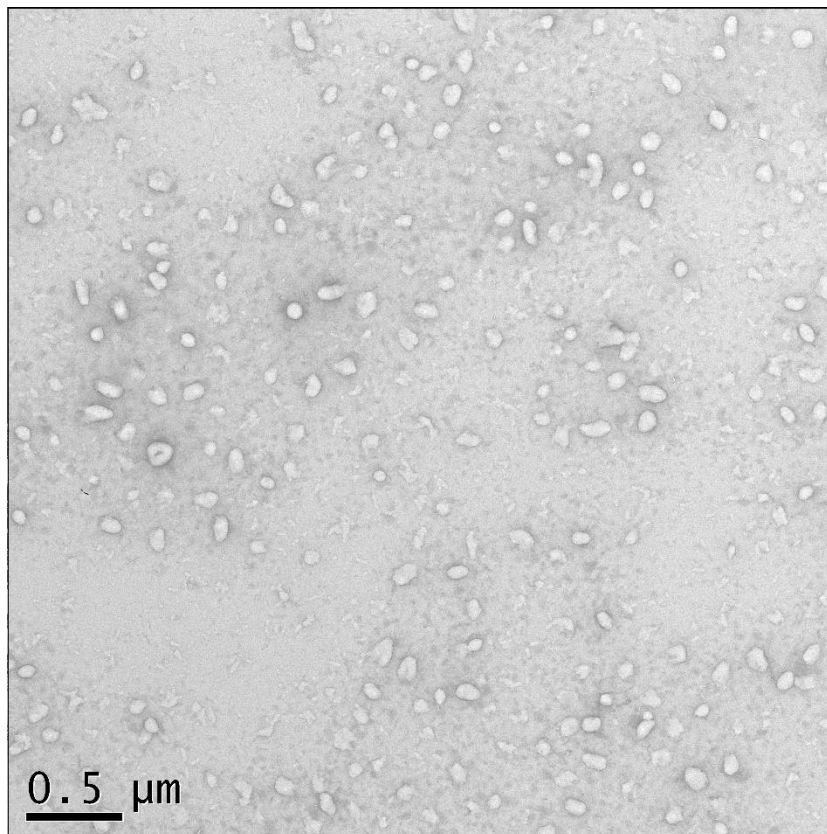


Figure 7.223: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 120 min)

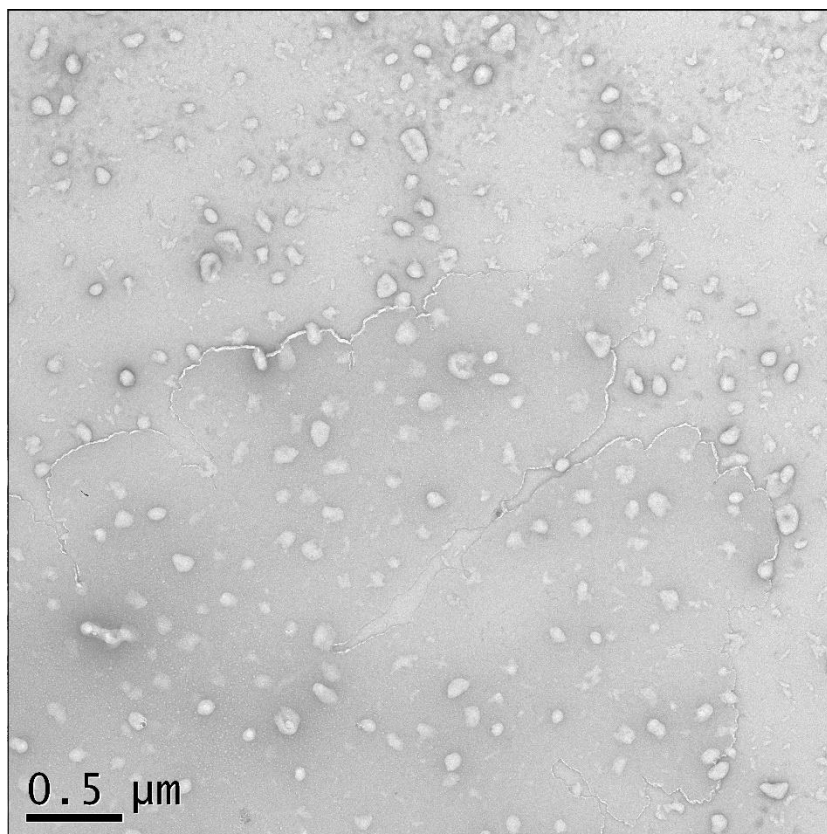


Figure 7.224: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 120 min)

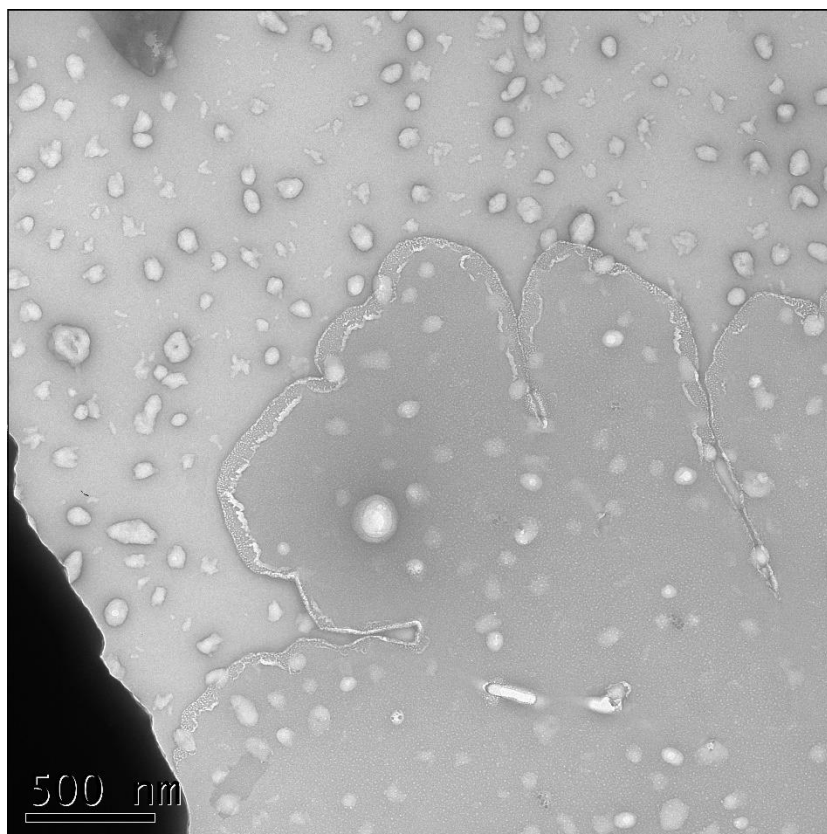


Figure 7.225: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 120 min)

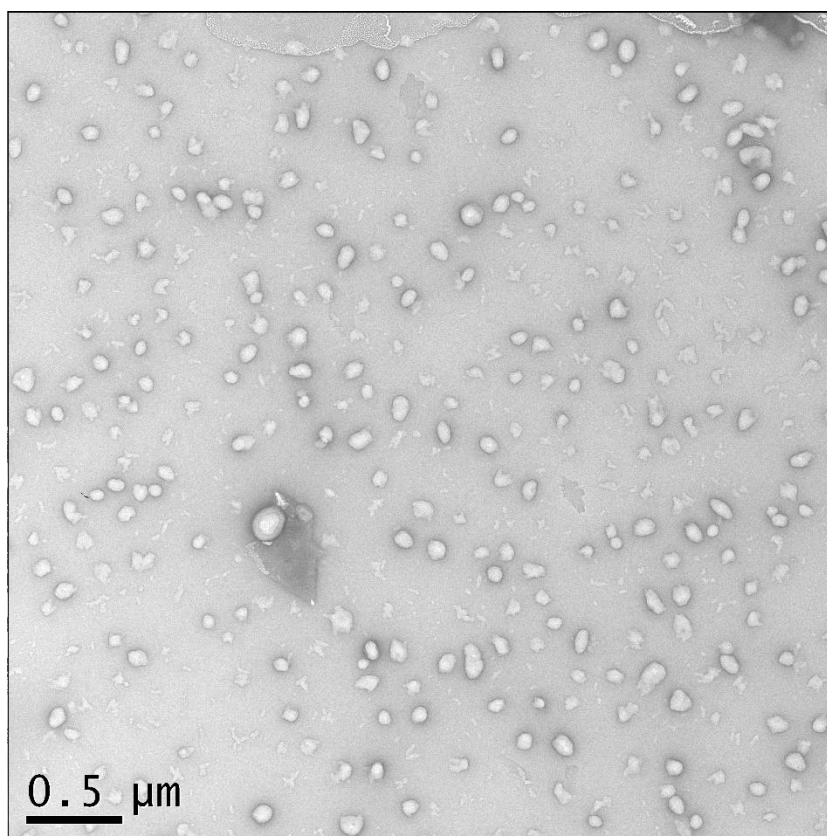


Figure 7.226: – TEM micrograph for SC PDMA₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 120 min)

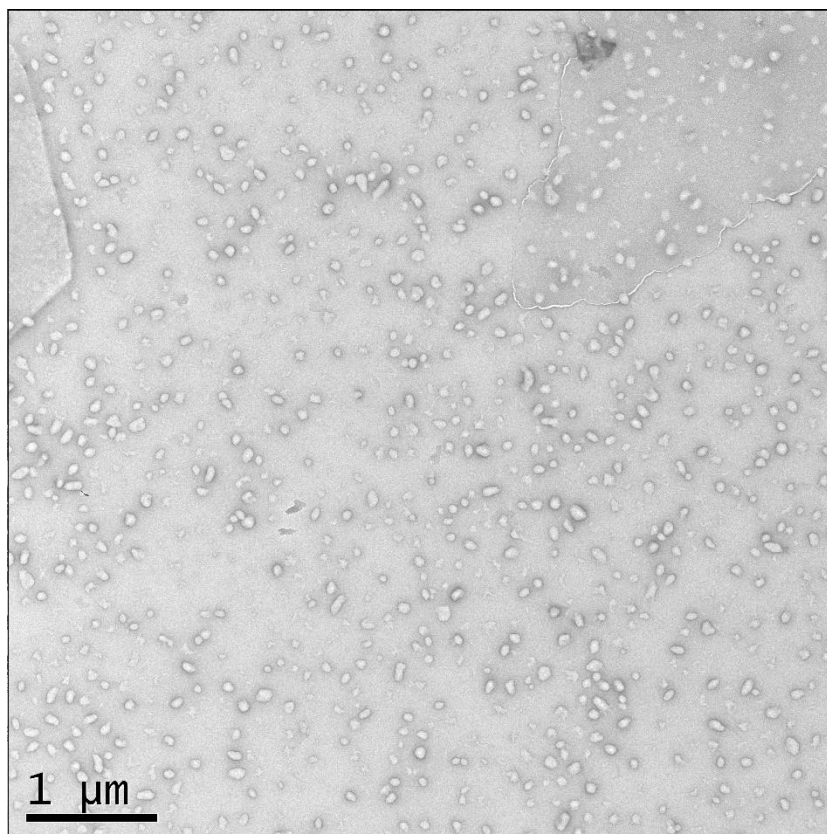


Figure 7.227: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 120 min)

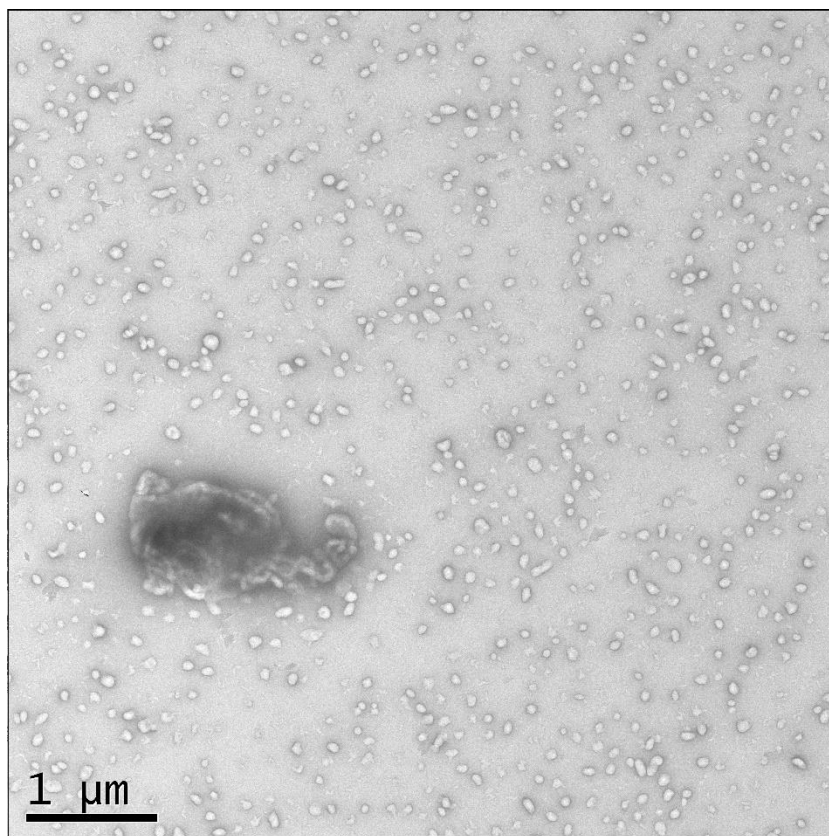


Figure 7.228: – TEM micrograph for SC PDMAm₅₀.b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 120 min)

t= 180 min

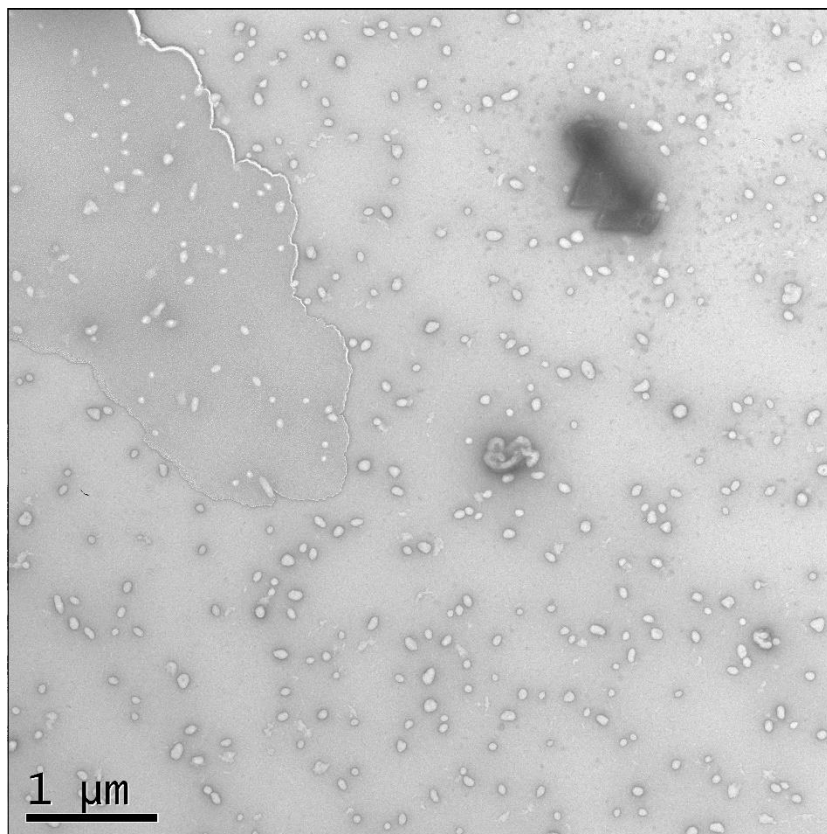


Figure 7.229: – TEM micrograph for SC PDMAM_{50.b}-PDAAM₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 180 min)

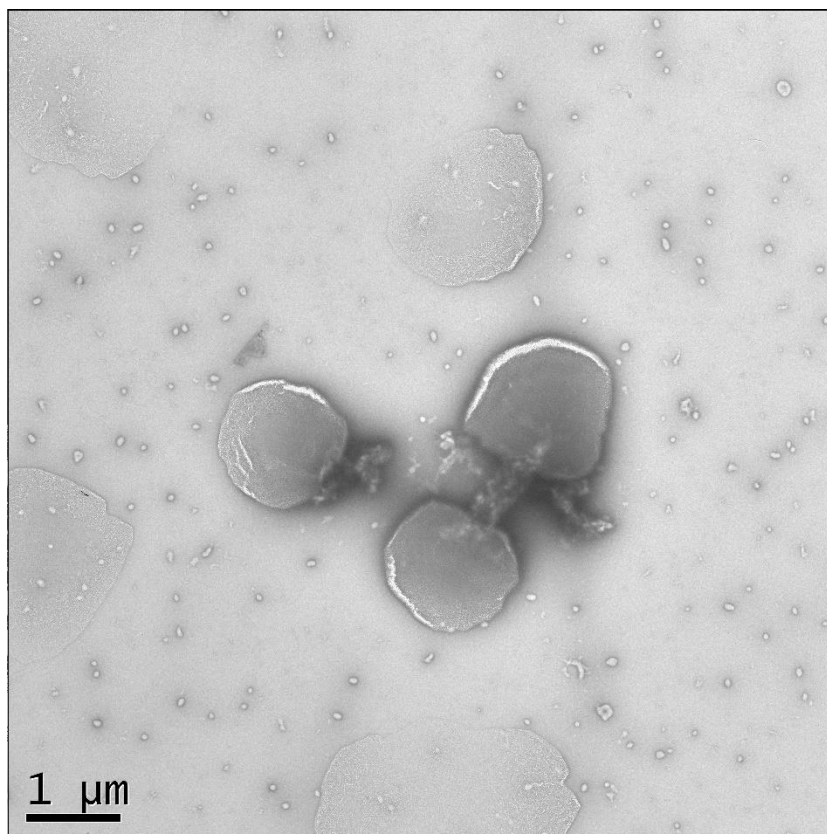


Figure 7.230: – TEM micrograph for SC PDMAm₅₀.b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 180 min)

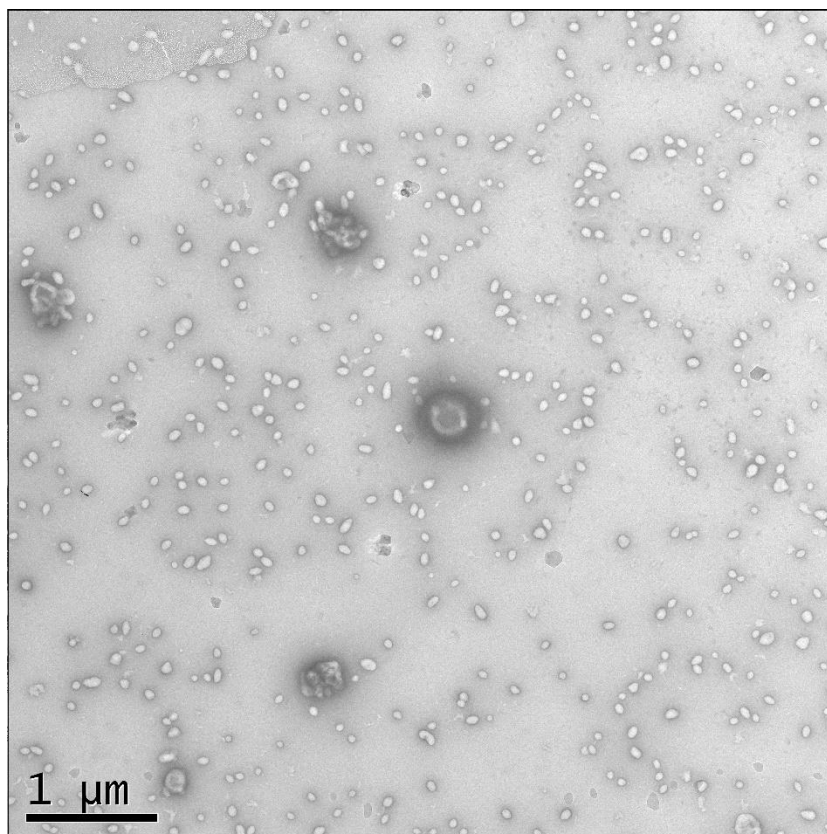


Figure 7.231: – TEM micrograph for SC PDMAM₅₀-b-PDAAM₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 180 min)

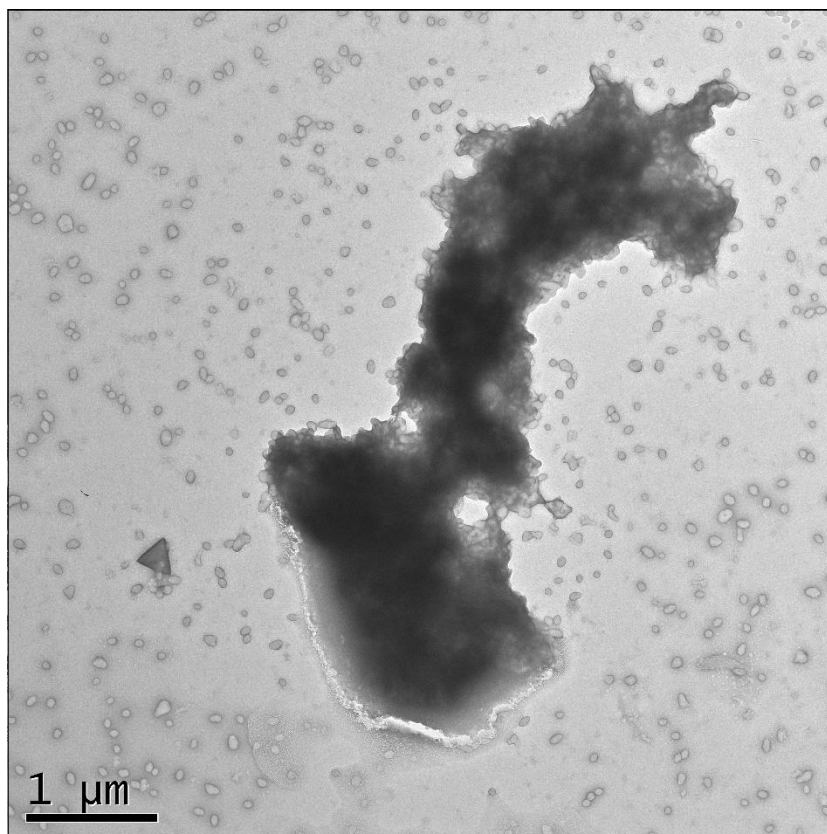


Figure 7.232: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 180 min)

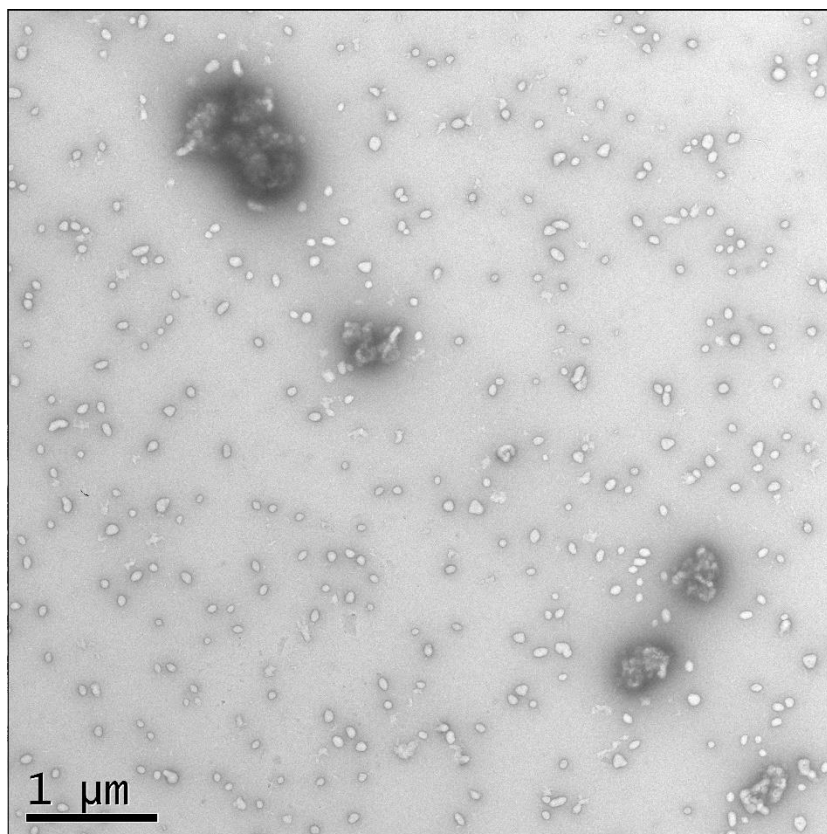


Figure 7.233: – TEM micrograph for SC PDMAm₅₀.b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 180 min)

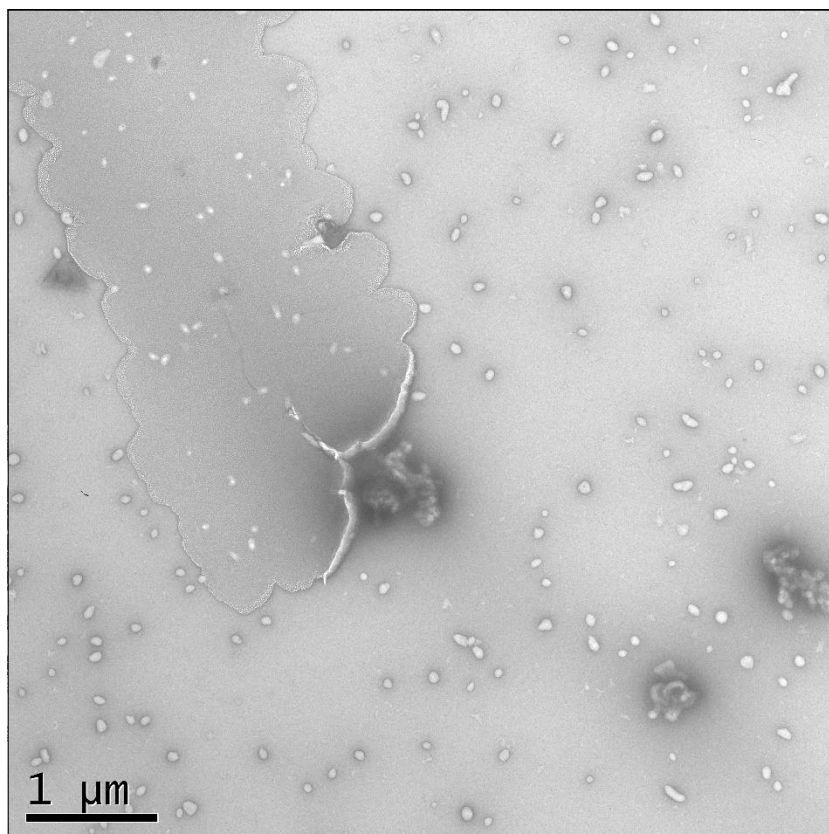


Figure 7.234: – TEM micrograph for SC PDMAm₅₀.b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 180 min)

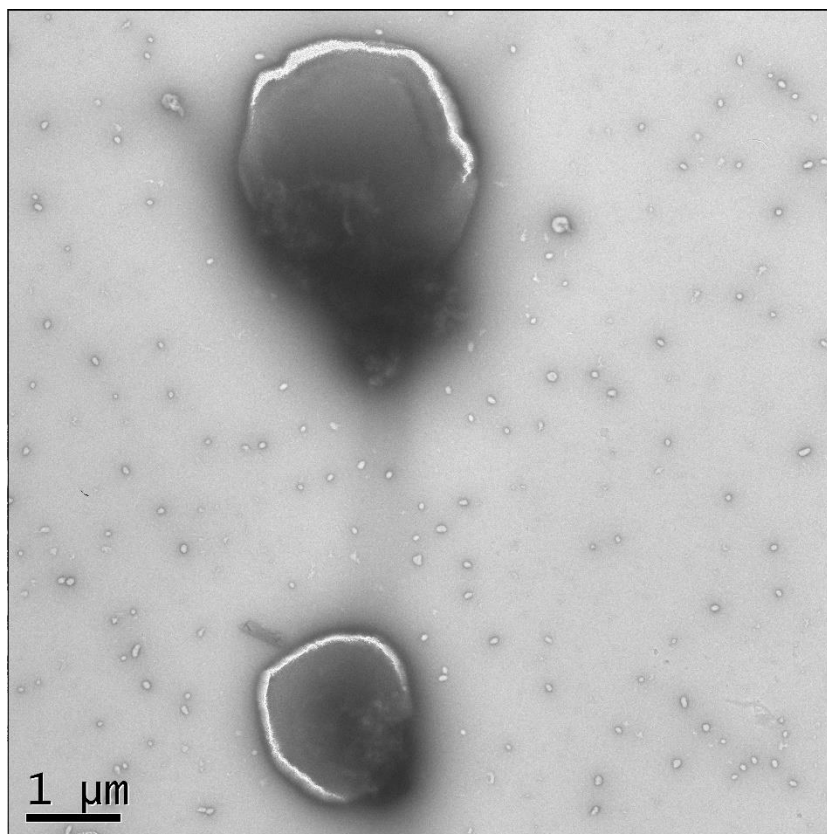


Figure 7.235: – TEM micrograph for SC PDMA_{m50}.b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 180 min)

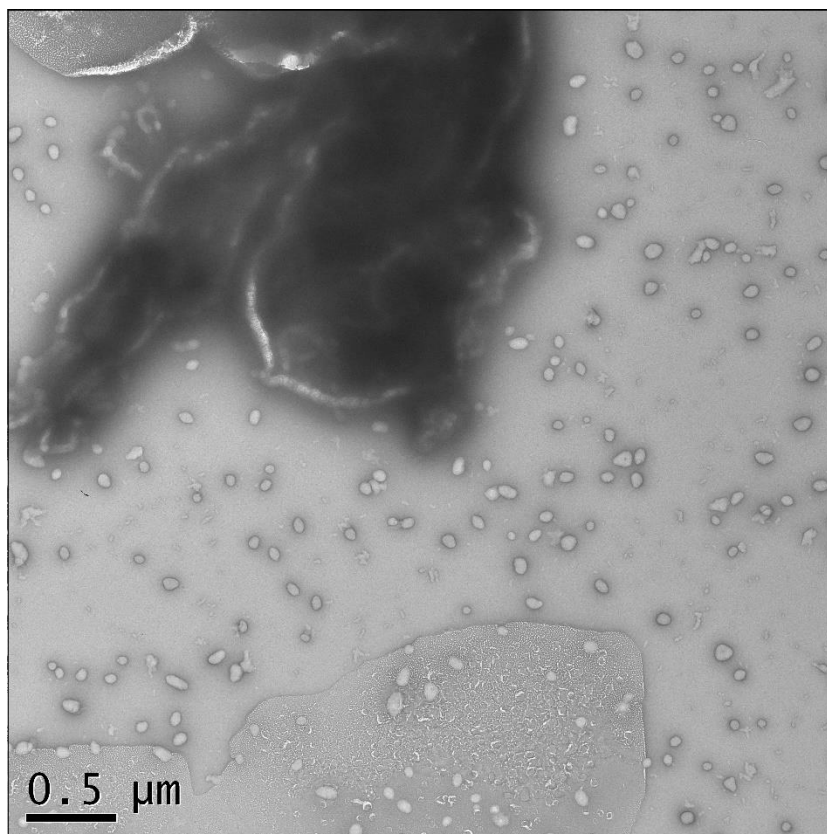


Figure 7.236: – TEM micrograph for SC PDMAm₅₀.b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 180 min)

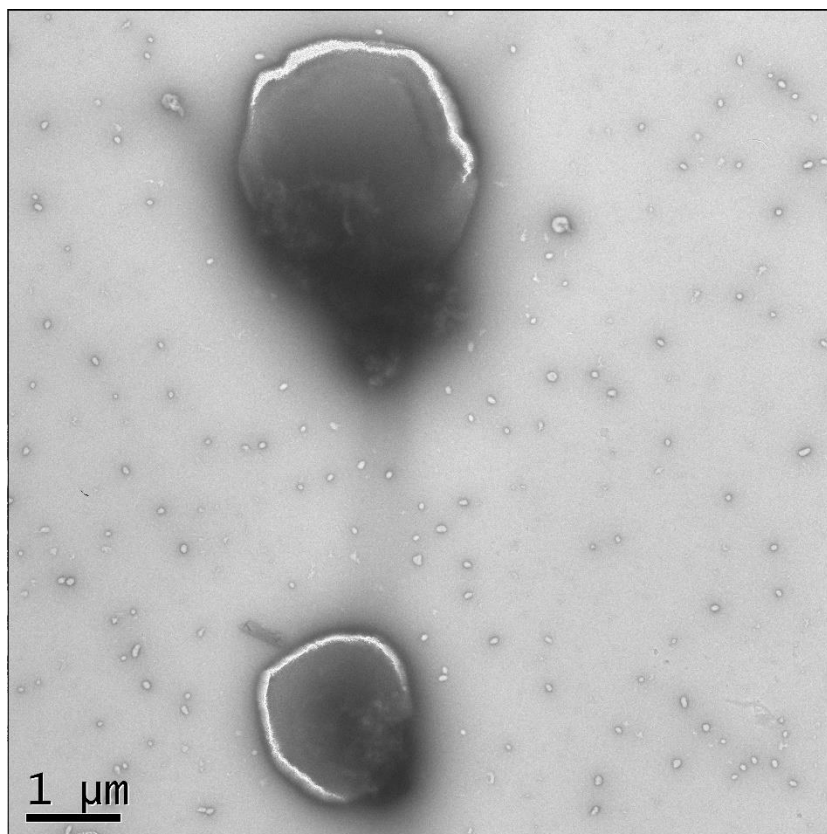


Figure 7.237: – TEM micrograph for SC PDMAm₅₀-b-PDAAm₃₀₀ nano-objects synthesised via SE at pH 7.5 with 500 mM NaCl (50% MeOH – Rapid Addition – t = 180 min)

Hybrid POPC-PDMAm₅₀-b-PDAAm₃₀₀ SE

SE of pure POPC with no polymer

t = 0 min, (5 min mixing pre-evaporation)

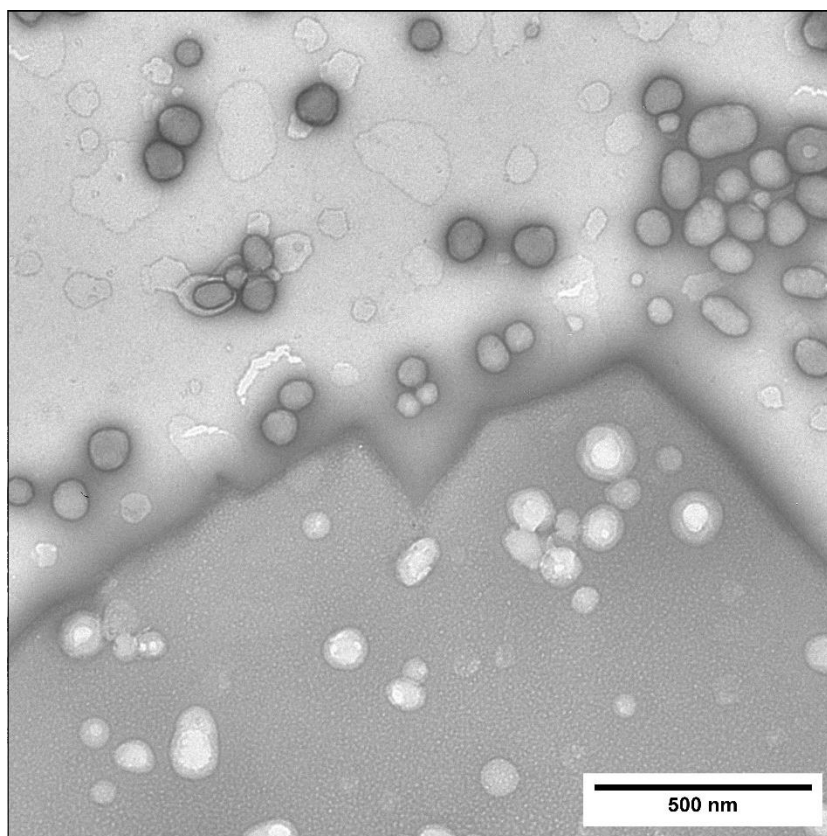


Figure 7.238: – TEM micrograph for POPC nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

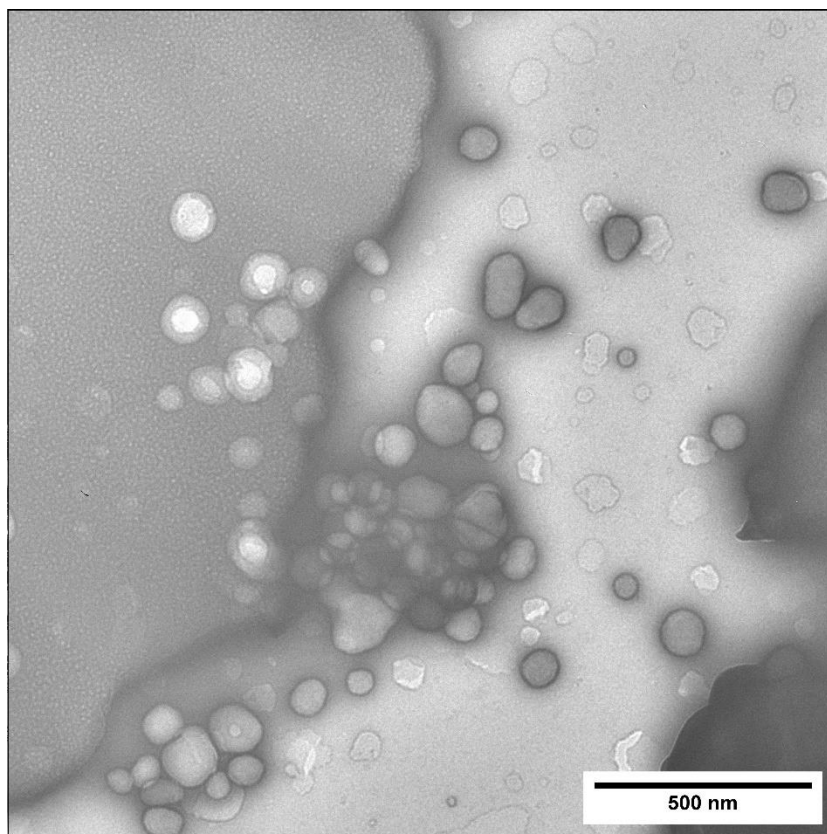


Figure 7.239 – TEM micrograph for POPC nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

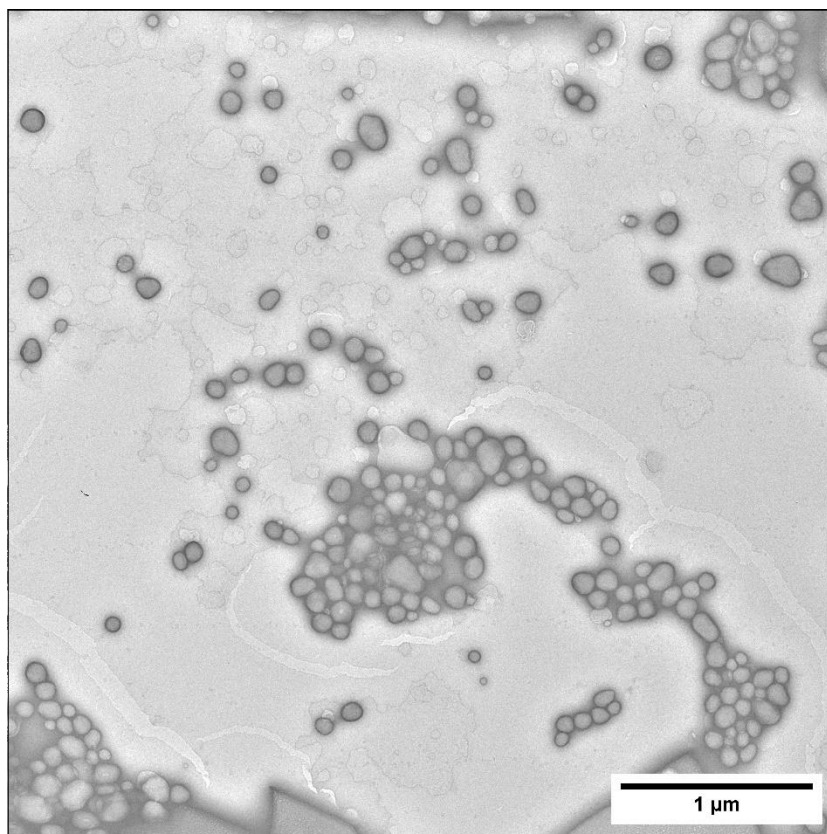


Figure 7.240: – TEM micrograph for POPC nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

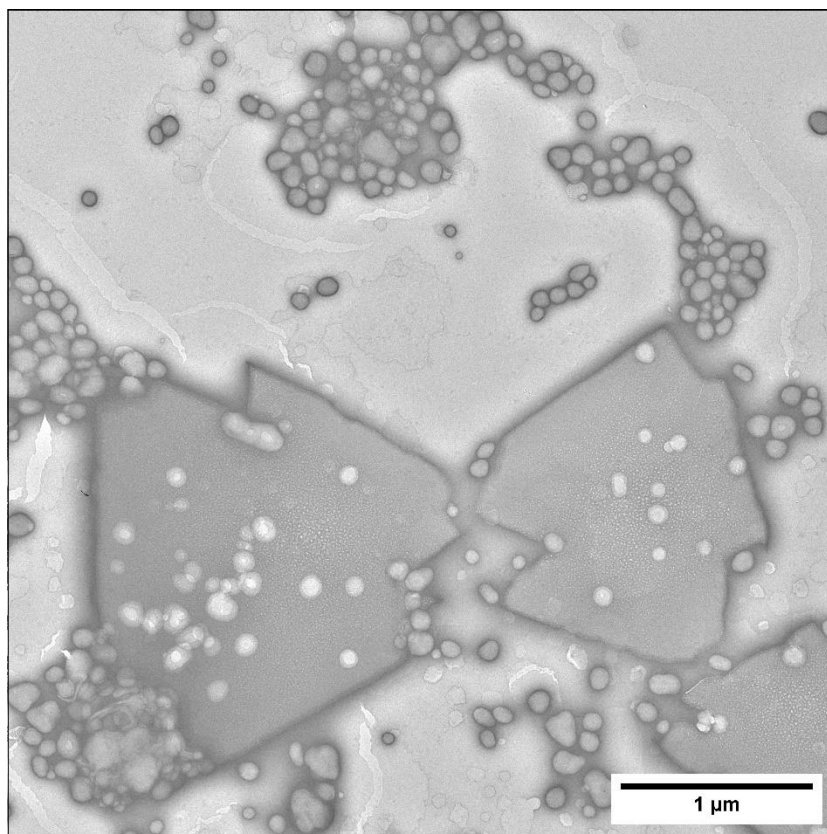


Figure 7.241: – TEM micrograph for POPC nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

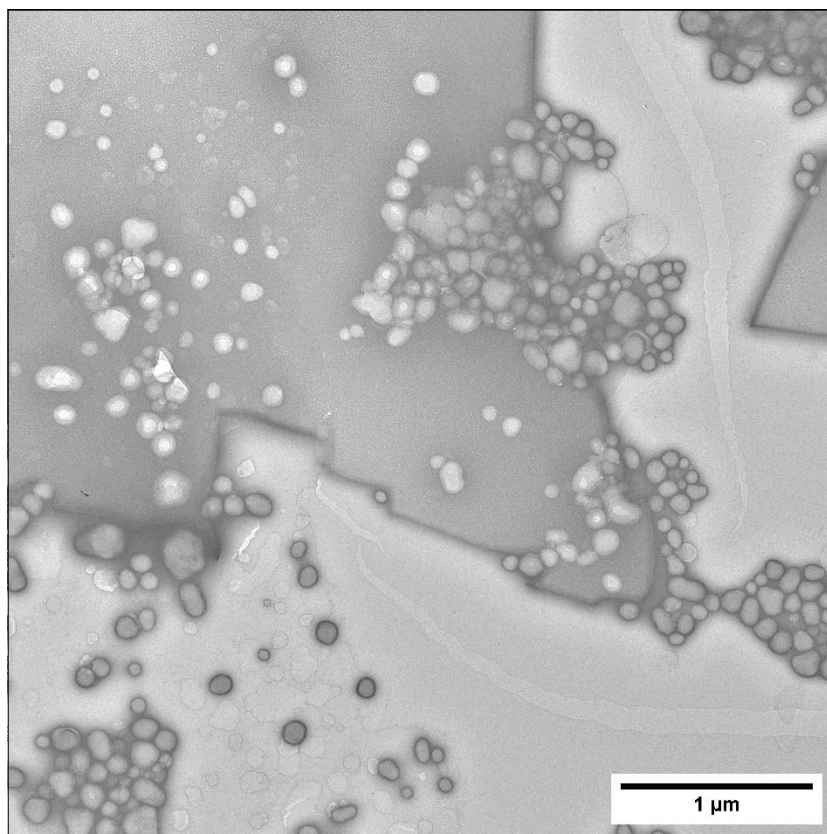


Figure 7.242: – TEM micrograph for POPC nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

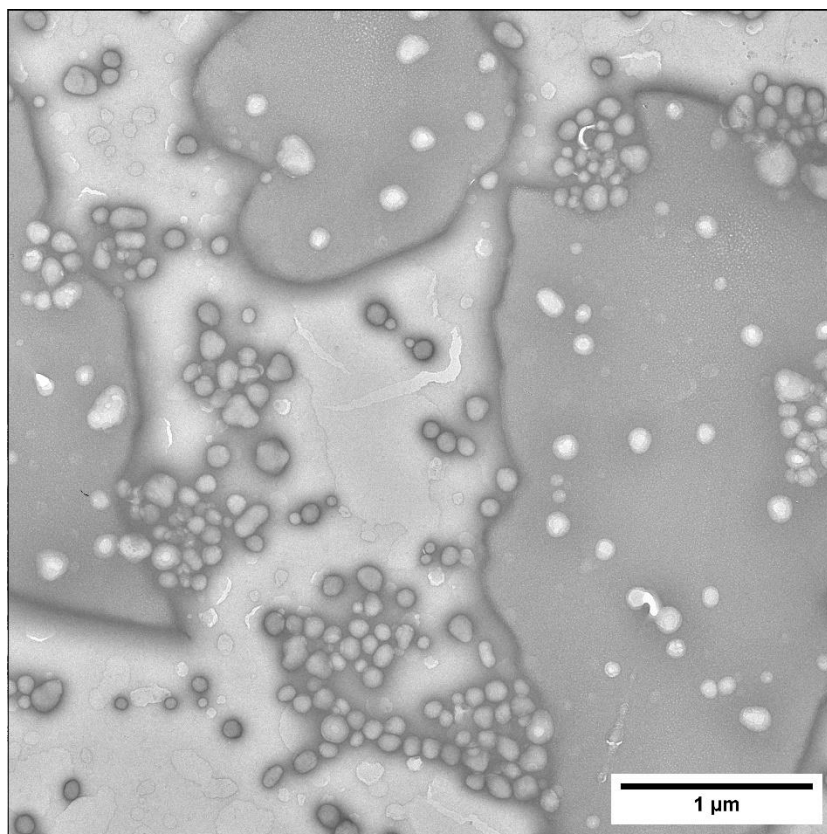


Figure 7.243: – TEM micrograph for POPC nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

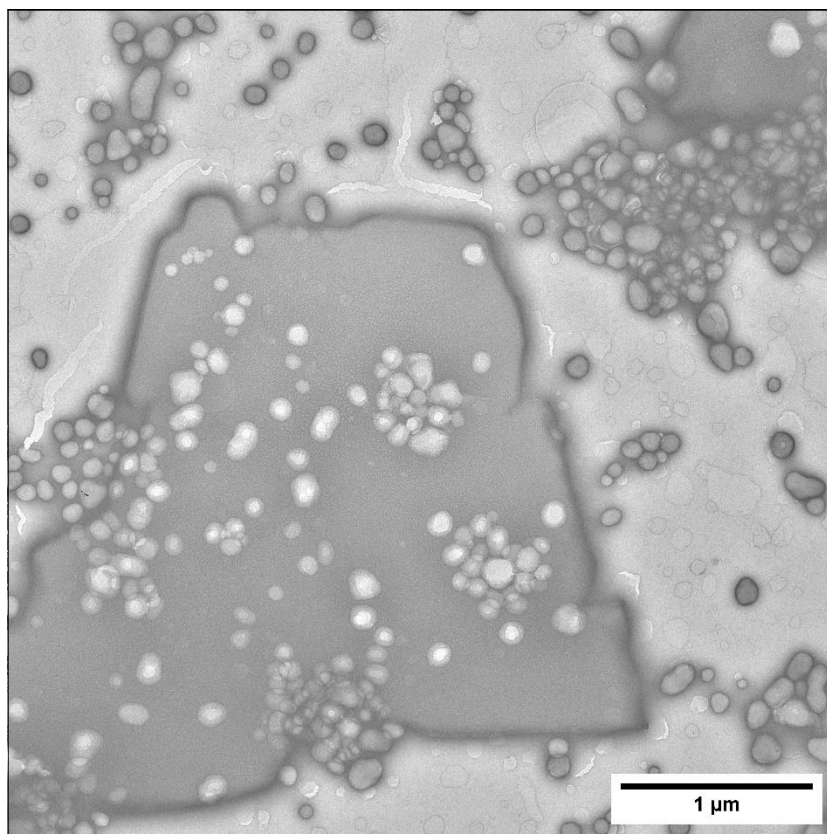


Figure 7.244: – TEM micrograph for POPC nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

t =30 min evaporation

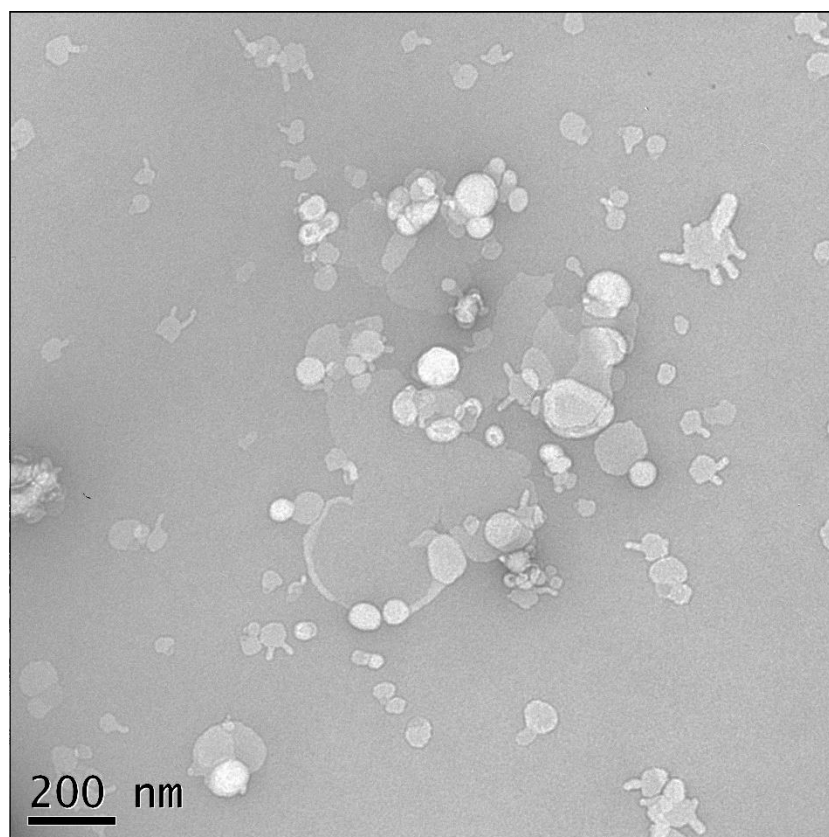


Figure 7.245: – TEM micrograph for POPC nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 30 min)

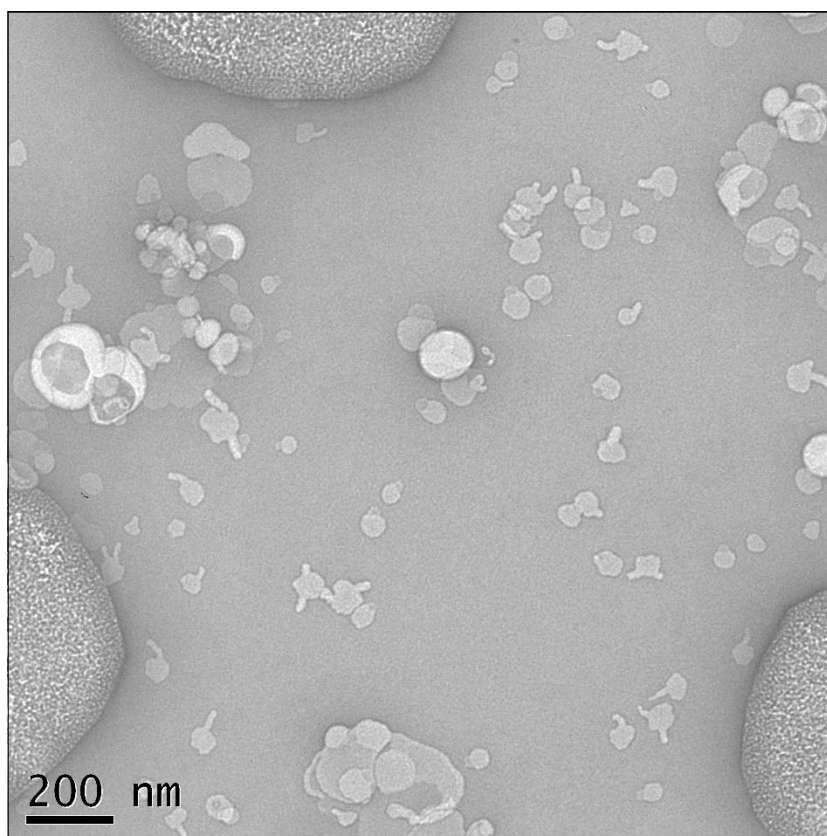


Figure 7.246: – TEM micrograph for POPC nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

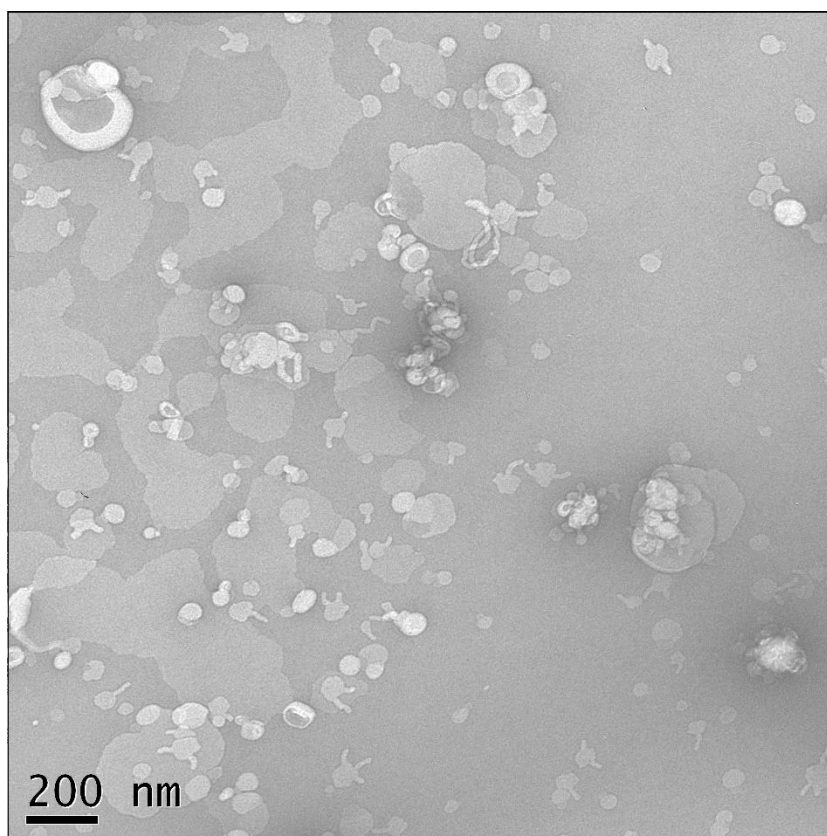


Figure 7.247: – TEM micrograph for POPC nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

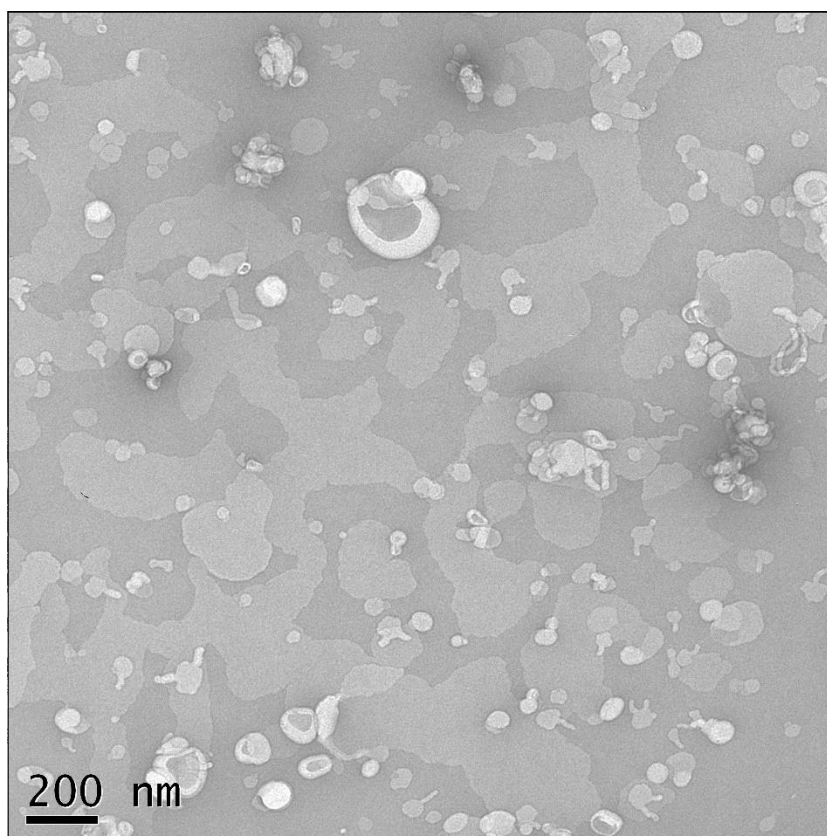


Figure 7.248: – TEM micrograph for POPC nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

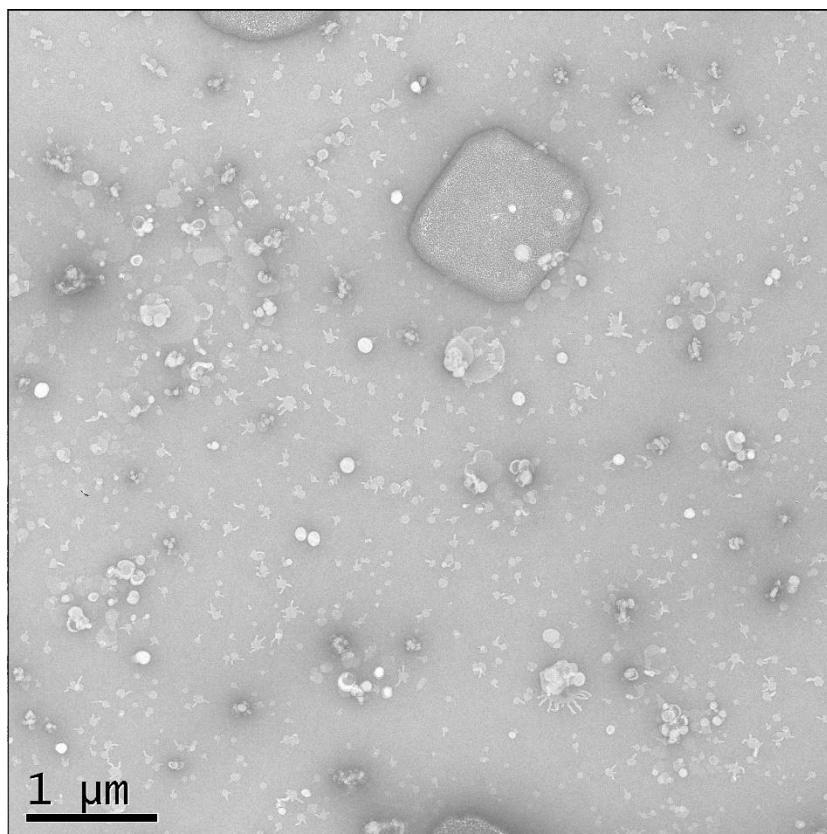


Figure 7.249: – TEM micrograph for POPC nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

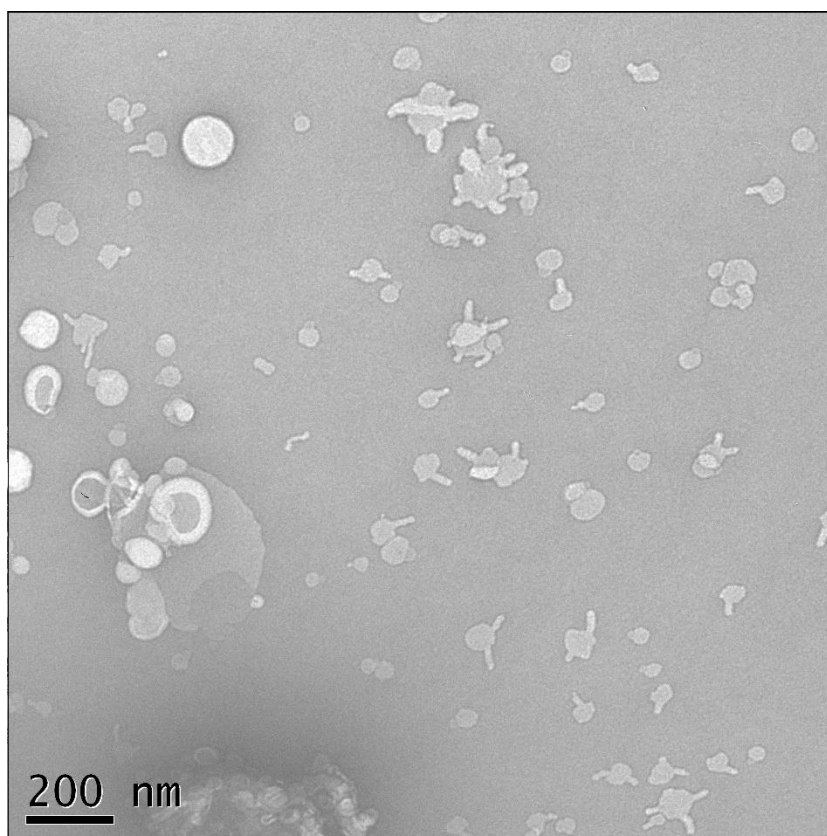


Figure 7.250: – TEM micrograph for POPC nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

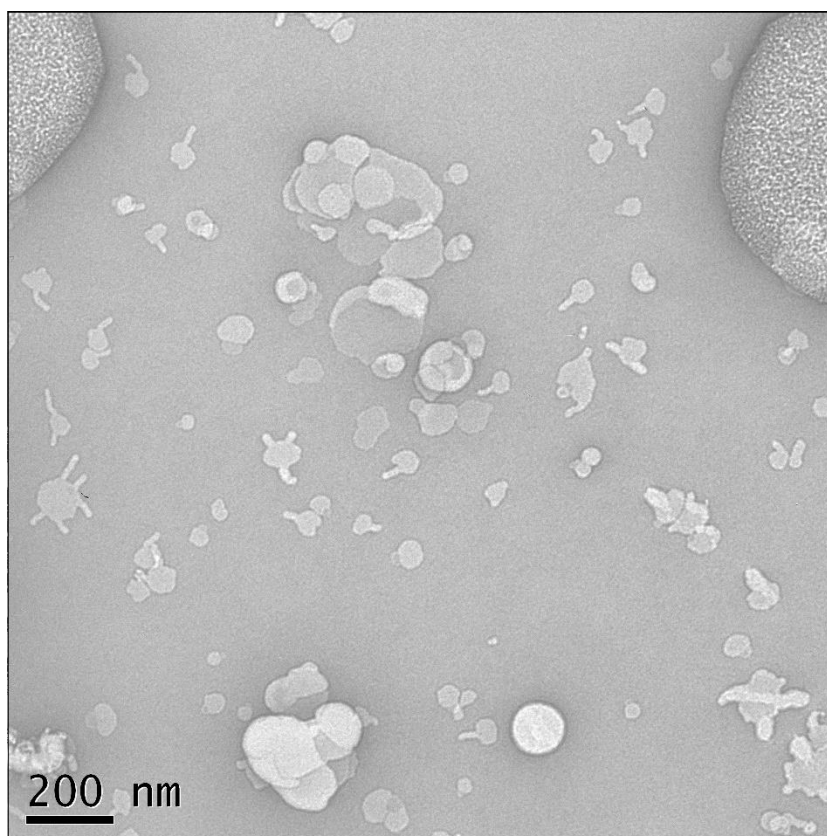


Figure 7.251: – TEM micrograph for POPC nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

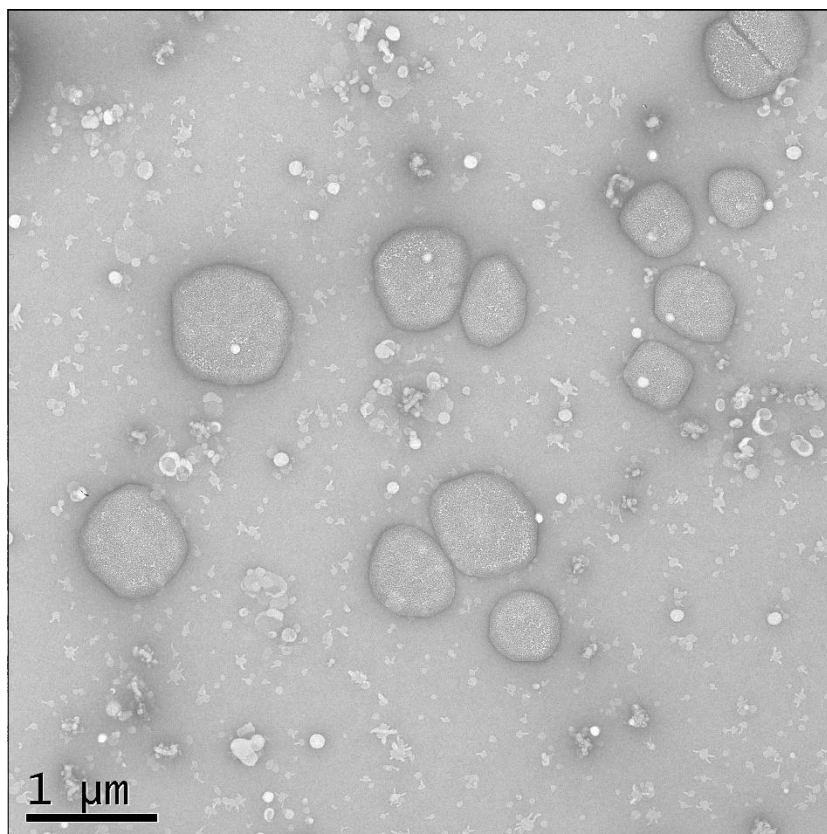


Figure 7.252: – TEM micrograph for POPC nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

75% POPC 25% Doubly charged PDMAm50PDAAm300

t=0 min

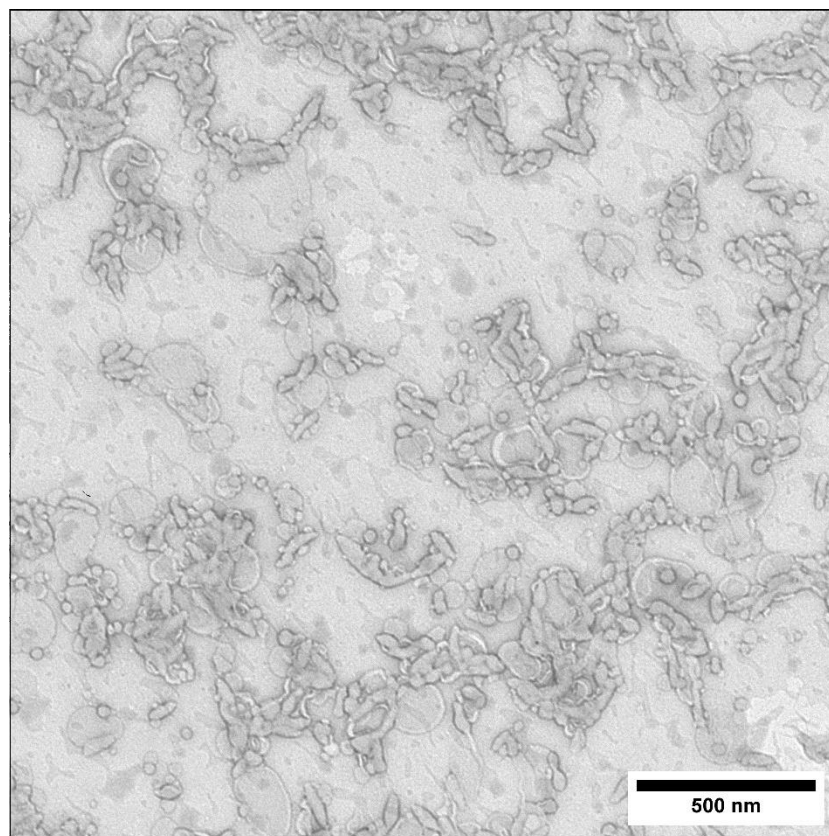


Figure 7.253 – TEM micrograph for POPC: DC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

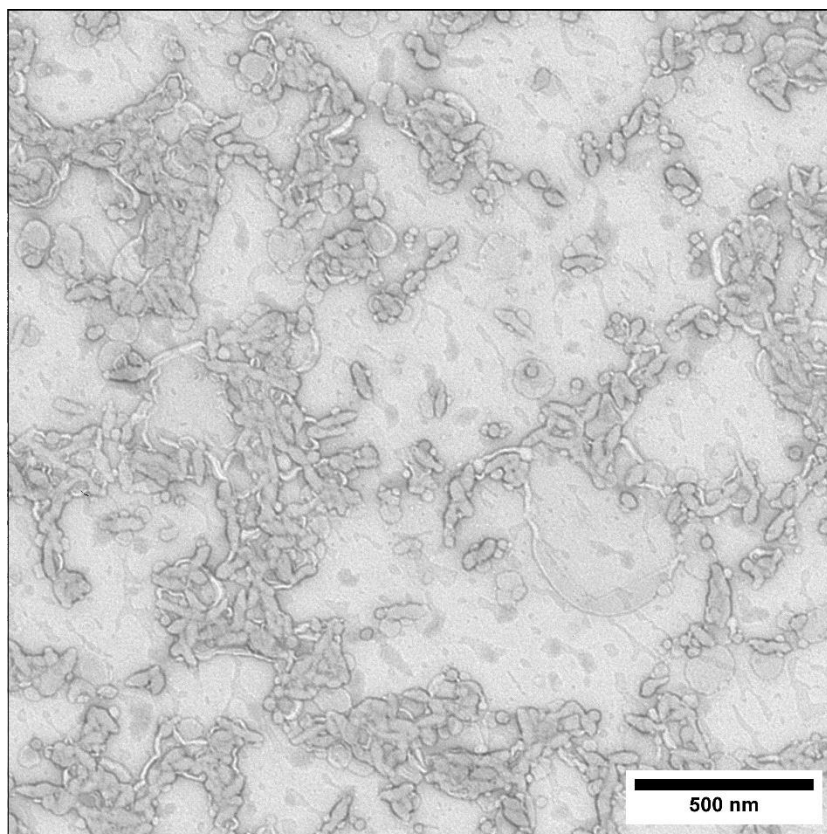


Figure 7.254: – TEM micrograph for POPC: DC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

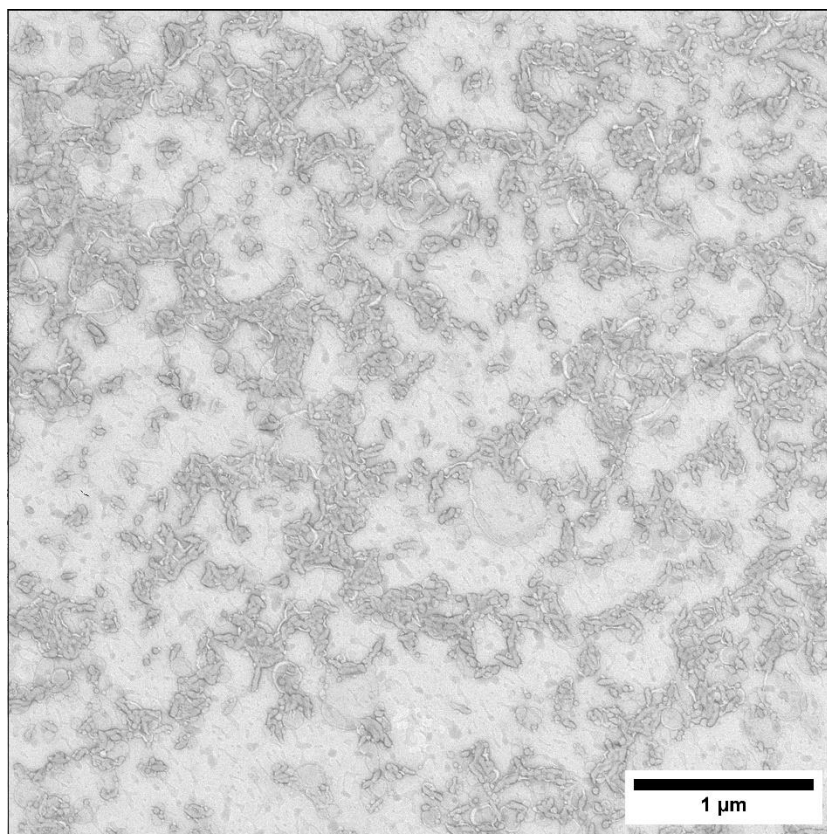


Figure 7.255: – TEM micrograph for POPC: DC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

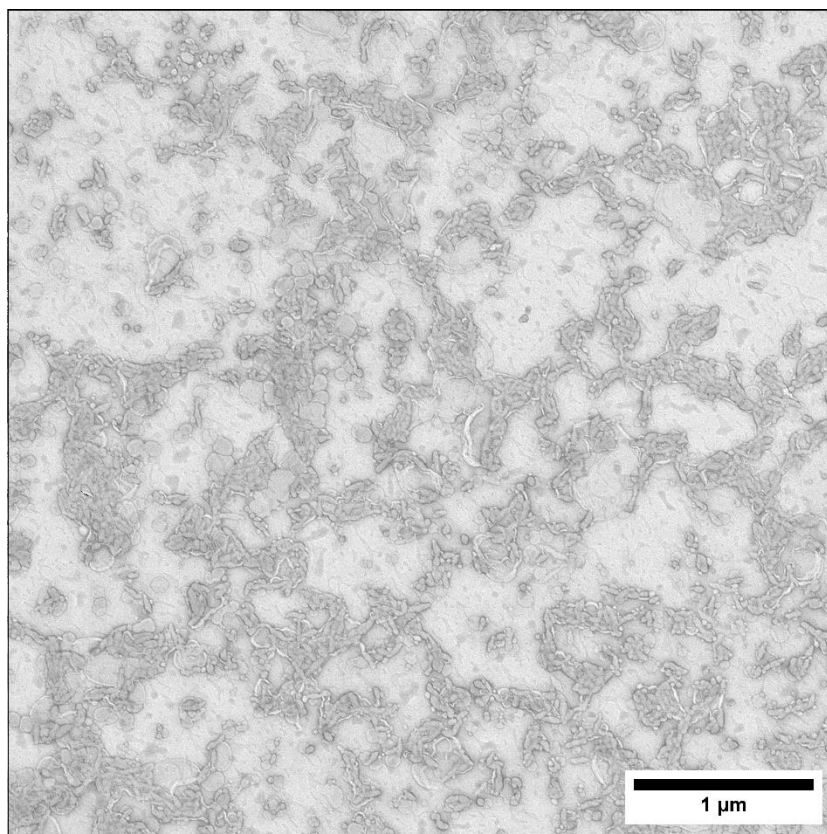


Figure 7.256: – TEM micrograph for POPC: DC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

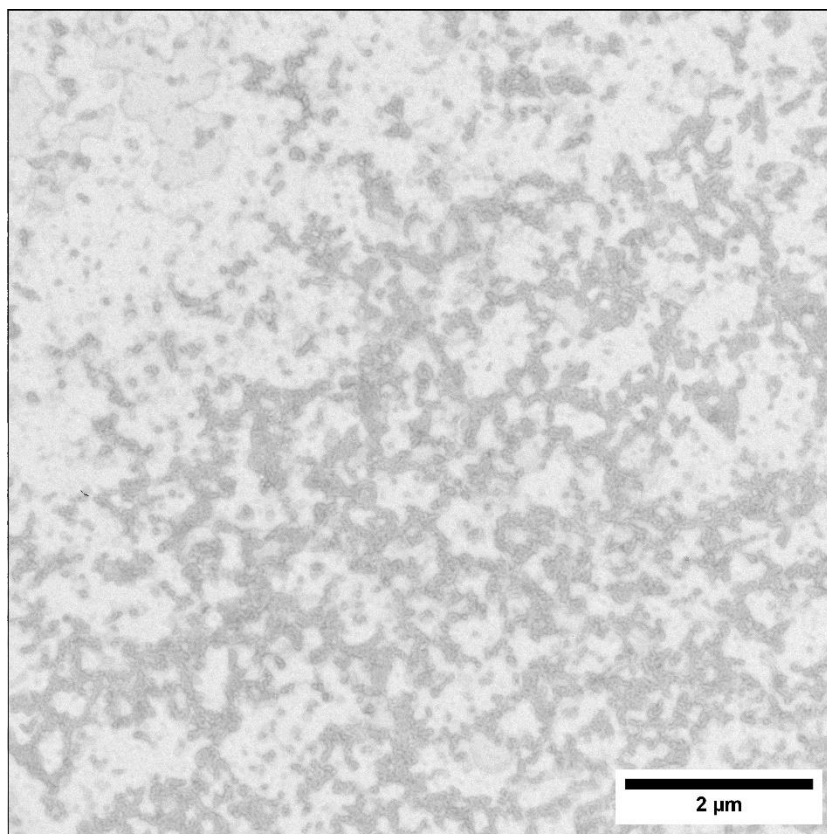


Figure 7.257: – TEM micrograph for POPC: DC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

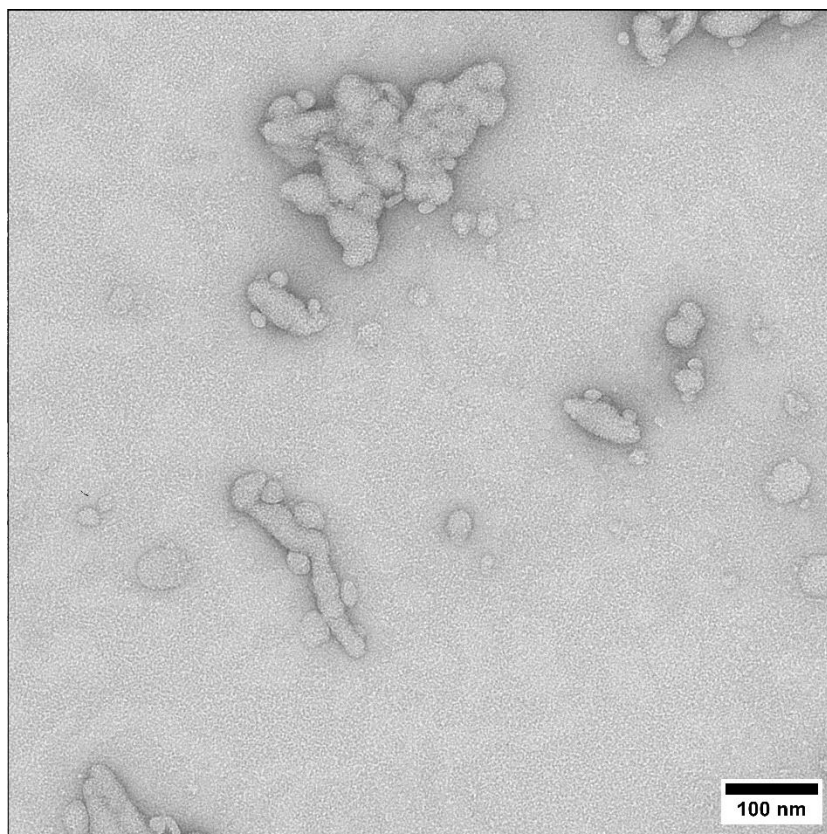


Figure 7.258: – TEM micrograph for POPC: DC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

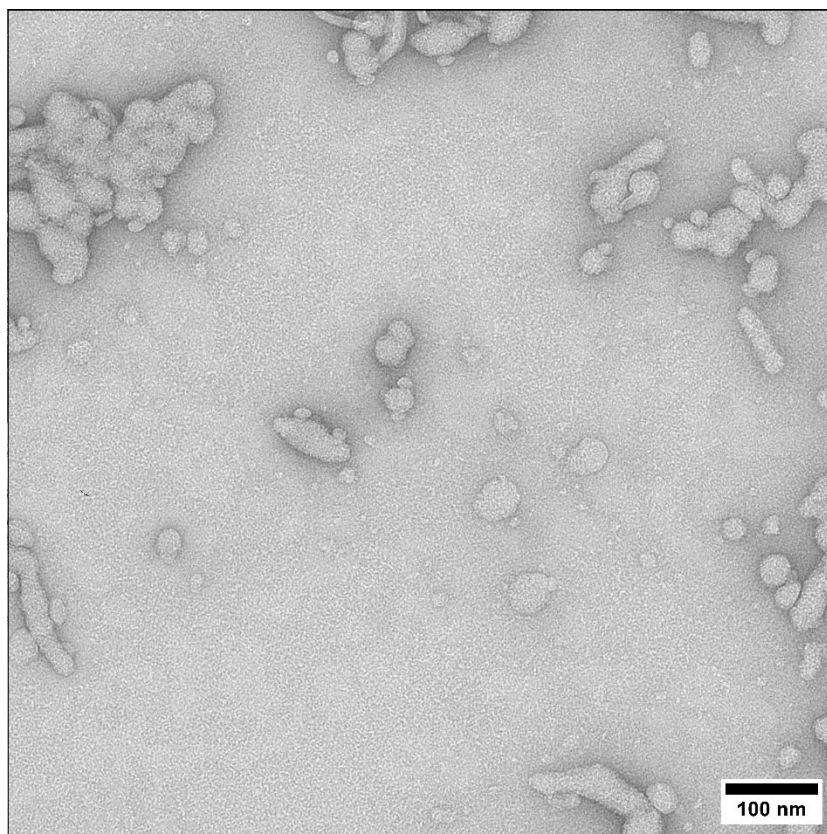


Figure 7.259: – TEM micrograph for POPC: DC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

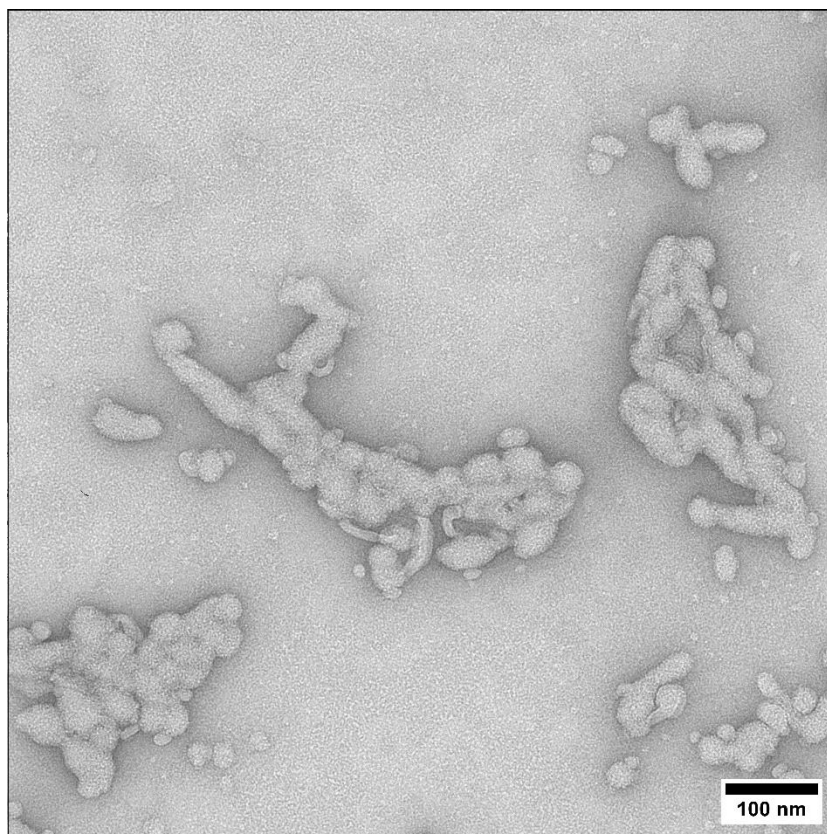


Figure 7.260: – TEM micrograph for POPC: DC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

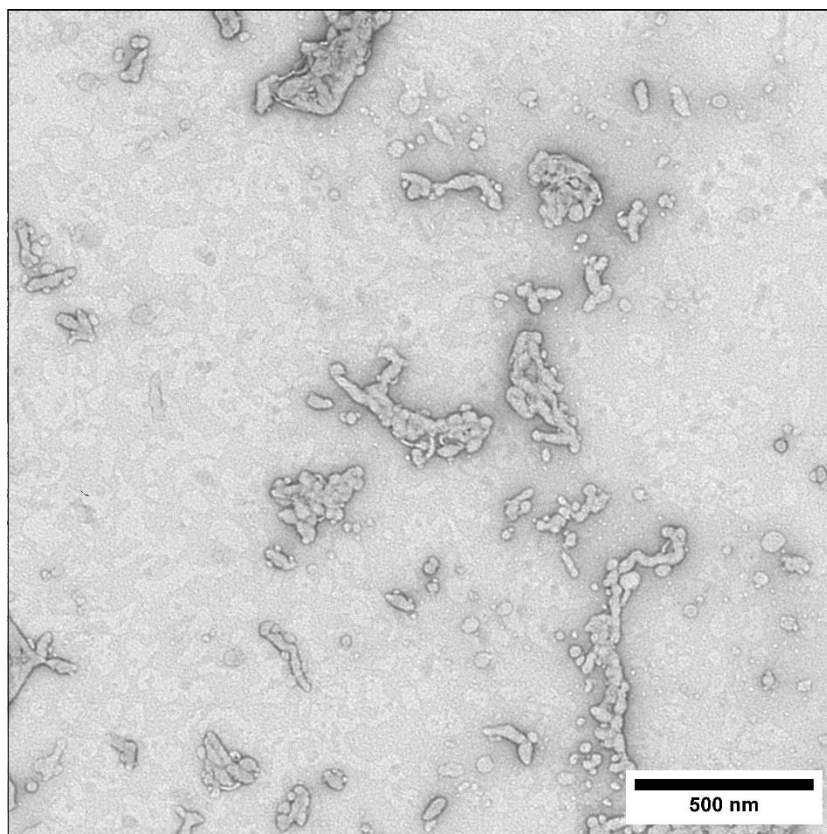


Figure 7.261: – TEM micrograph for POPC: DC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

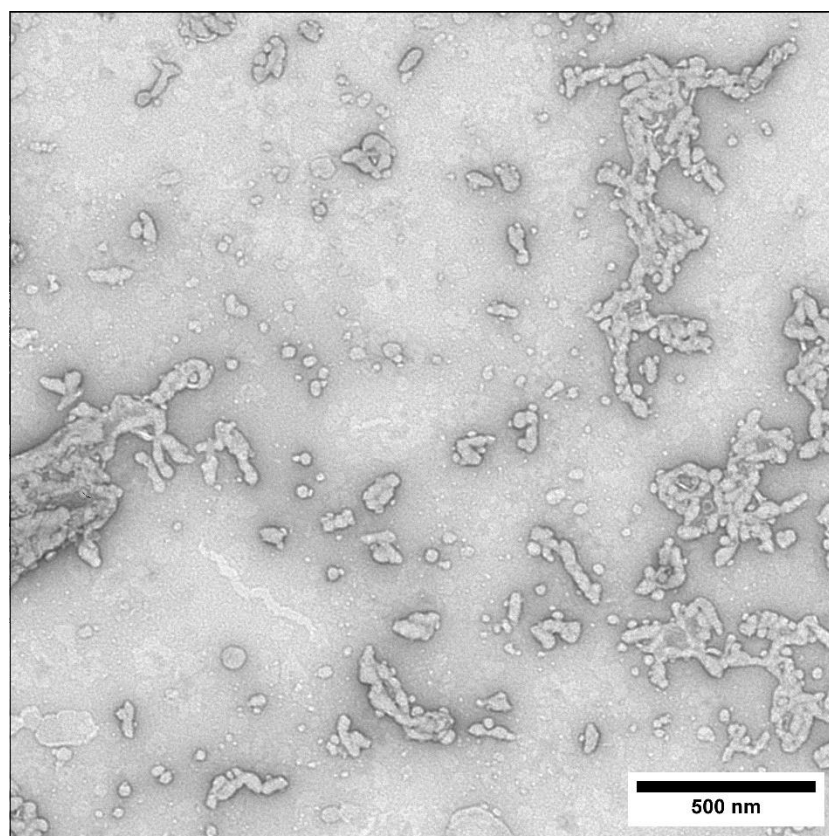


Figure 7.262: – TEM micrograph for POPC: DC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

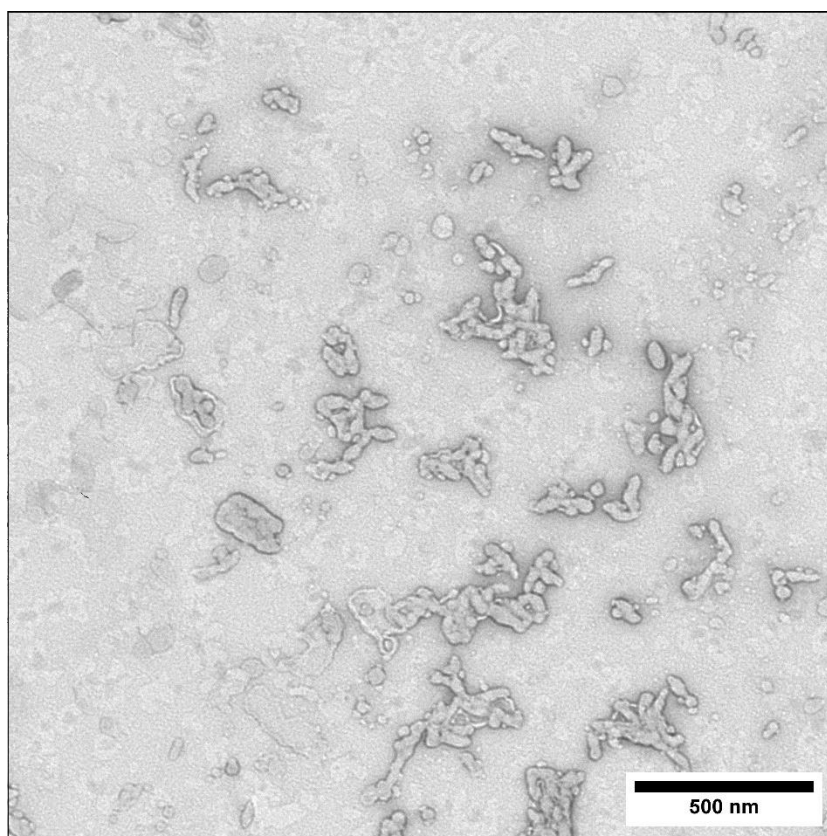


Figure 7.263: – TEM micrograph for POPC: DC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

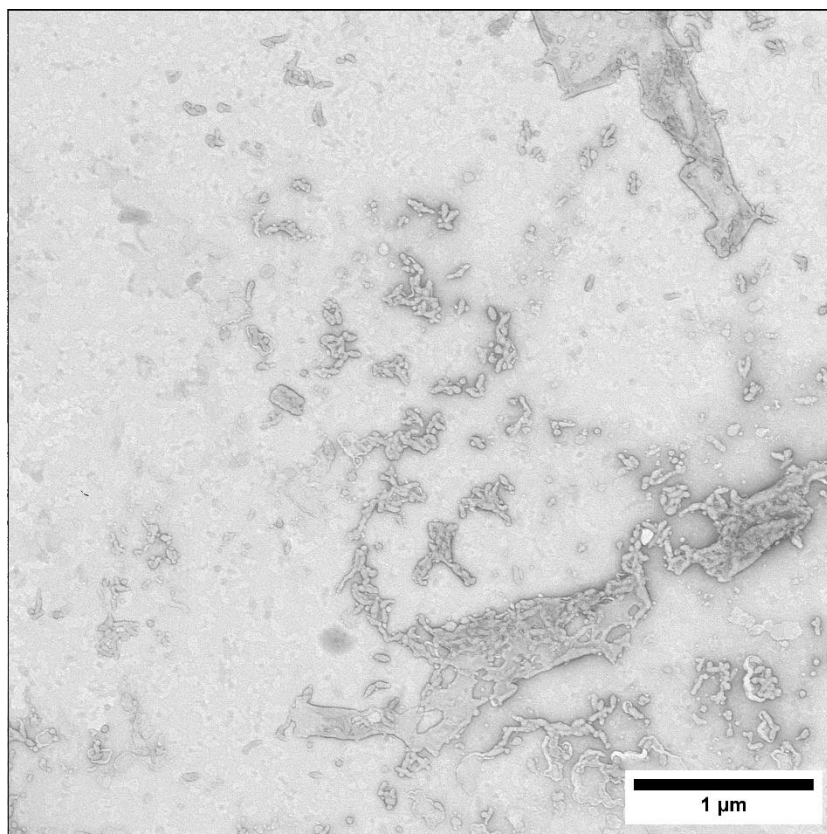


Figure 7.264: – TEM micrograph for POPC: DC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

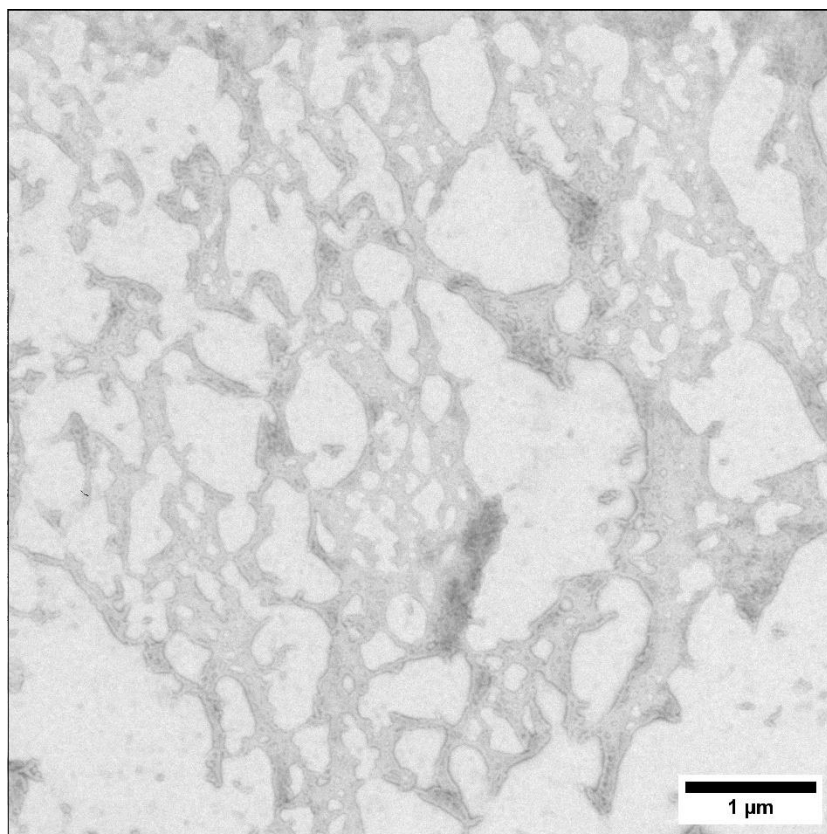


Figure 7.265: – TEM micrograph for POPC: DC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

t = 30 min

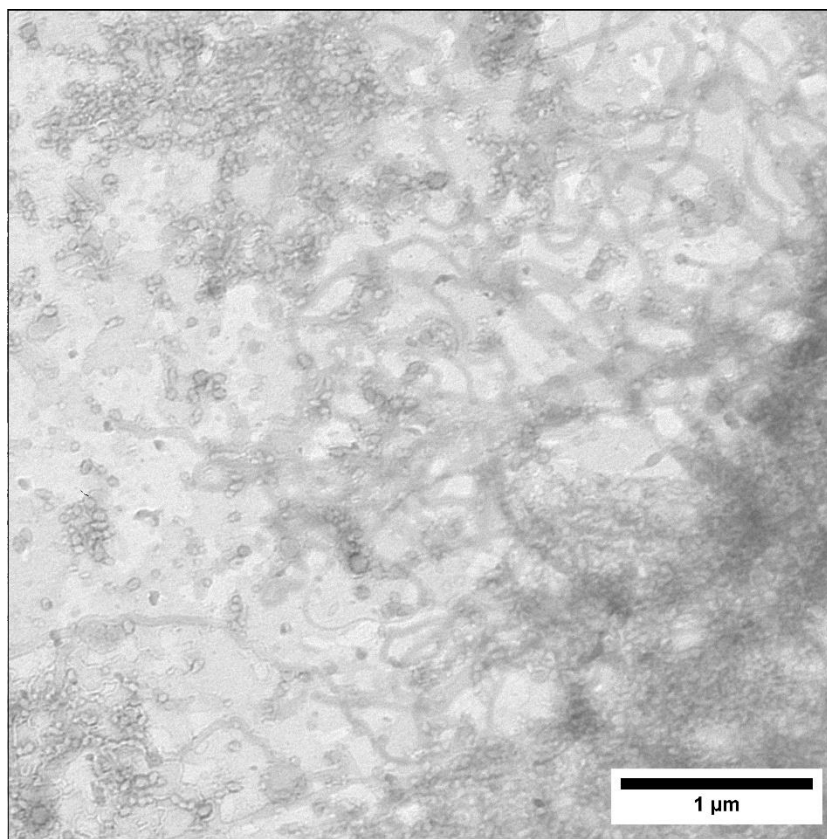


Figure 7.266: – TEM micrograph for POPC: DC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 30 min)

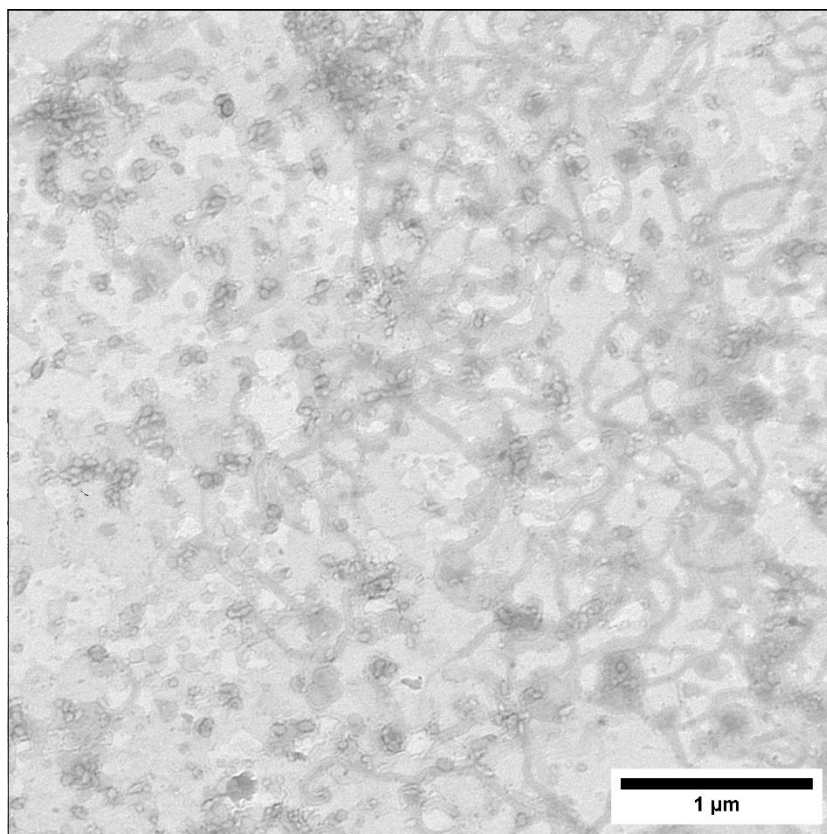


Figure 7.267: – TEM micrograph for POPC: DC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

75% POPC 25% Singly Charged PDMAm50PDAAm300

t=0 min

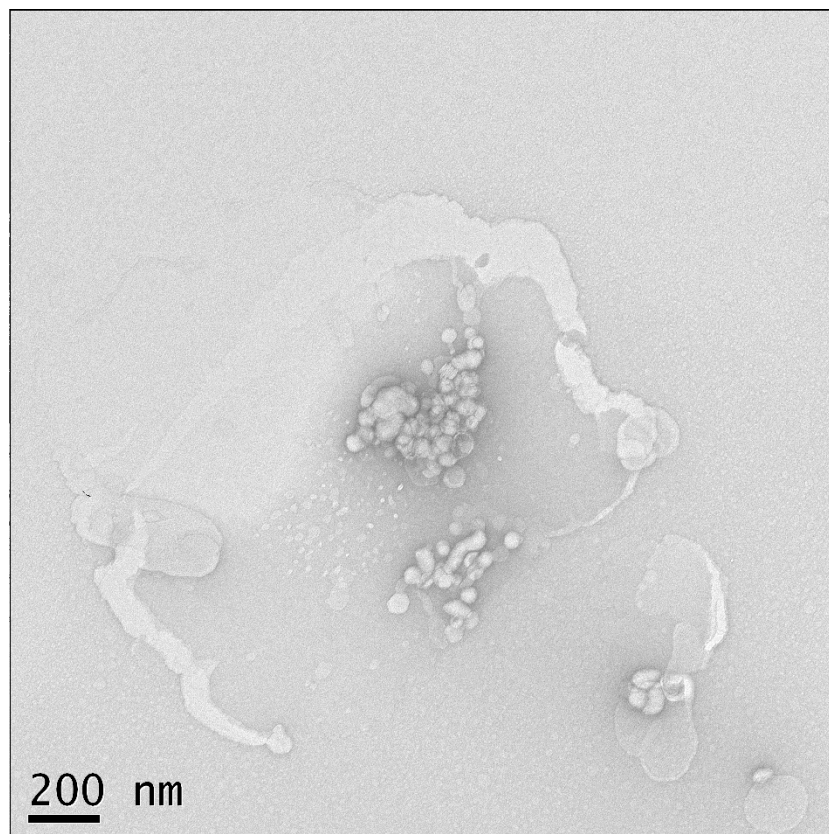


Figure 7.268: – TEM micrograph for POPC: SC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

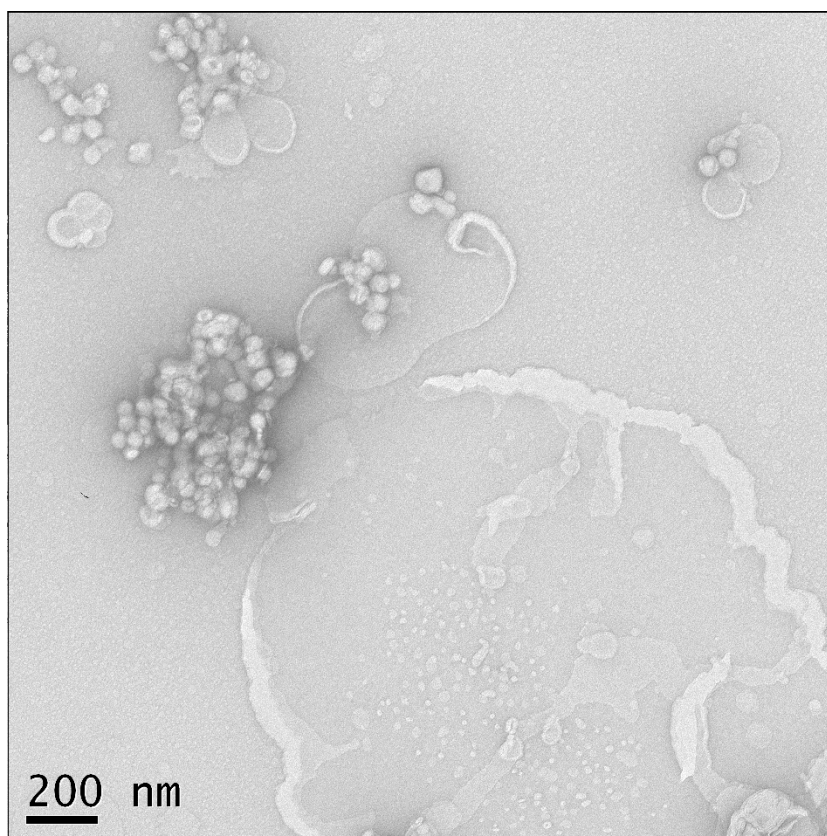


Figure 7.269: – TEM micrograph for POPC: SC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

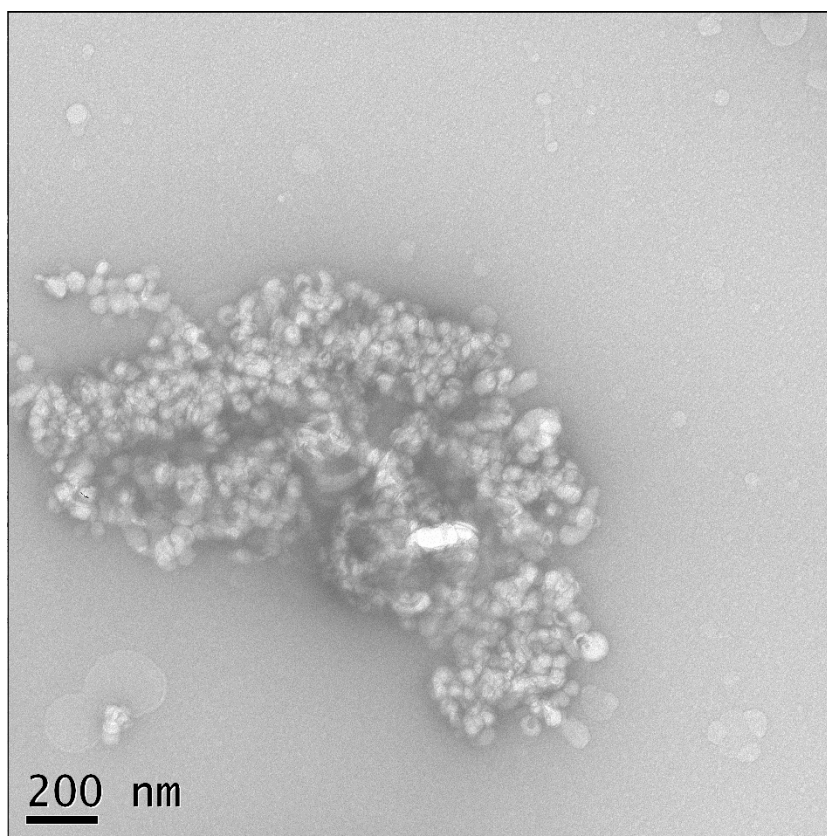


Figure 7.270: – TEM micrograph for POPC: SC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

75% POPC 25% Singly charged PDMAm50PDAAm300

t=30 min

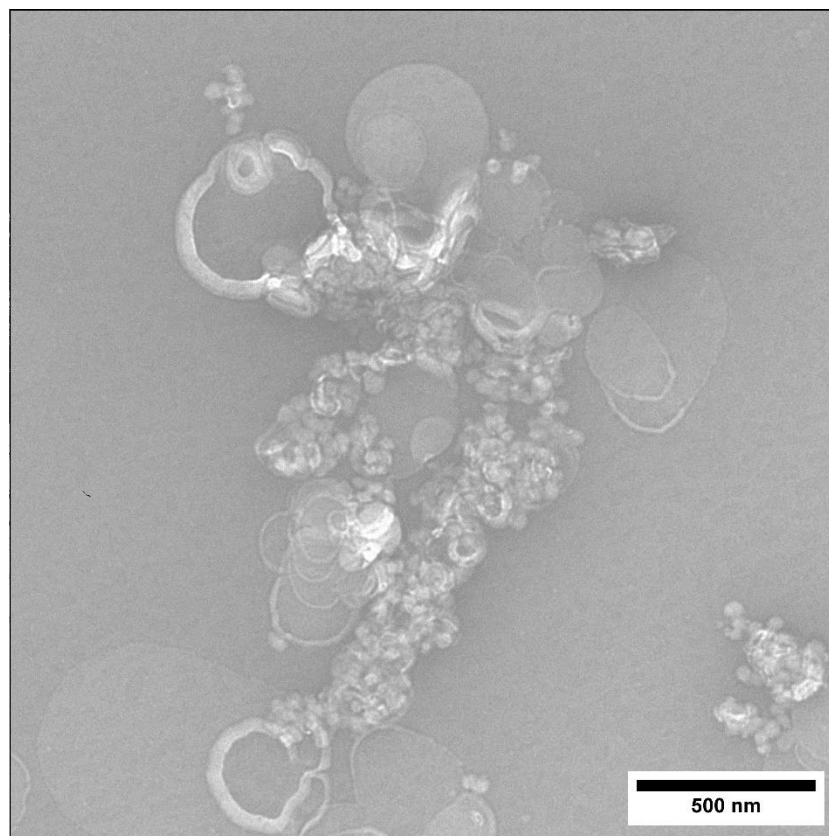


Figure 7.271: – TEM micrograph for POPC: SC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 30 min)

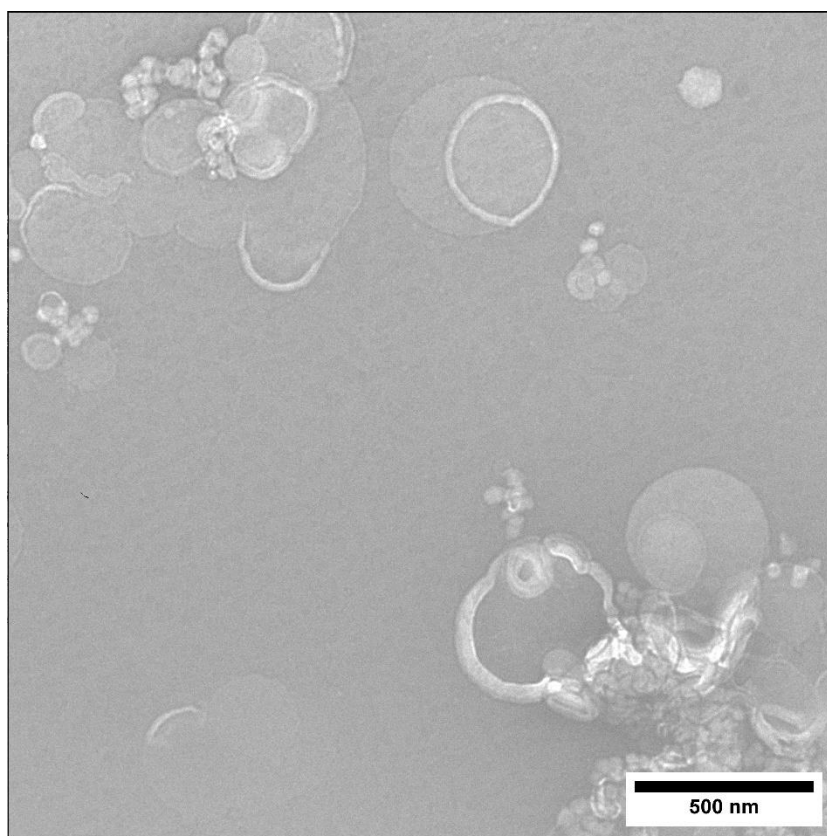


Figure 7.272: – TEM micrograph for POPC: SC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

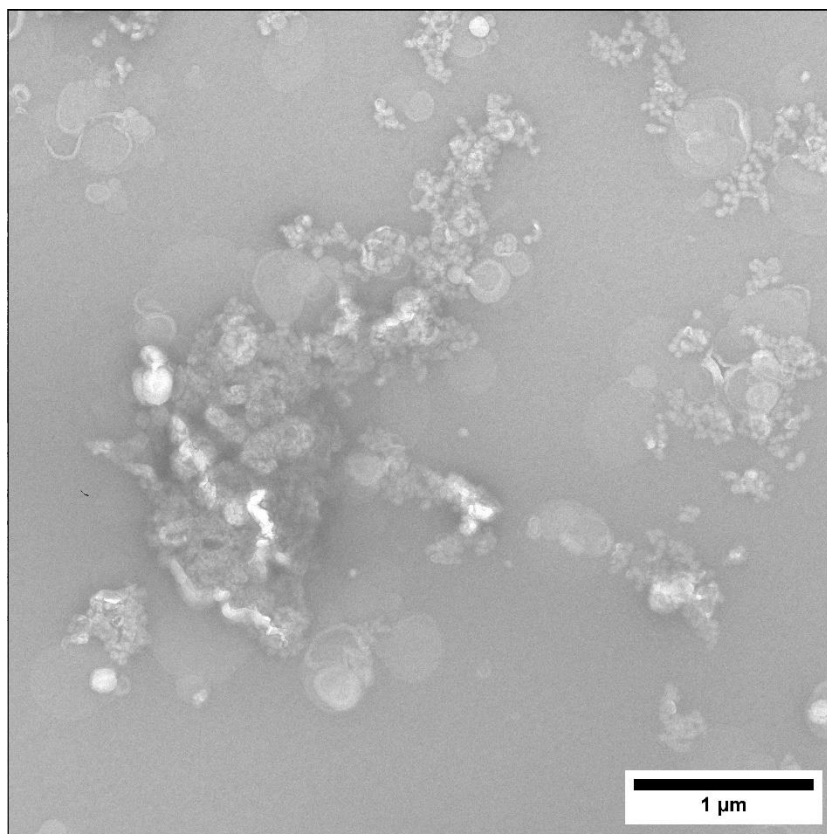


Figure 7.273: – TEM micrograph for POPC: SC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

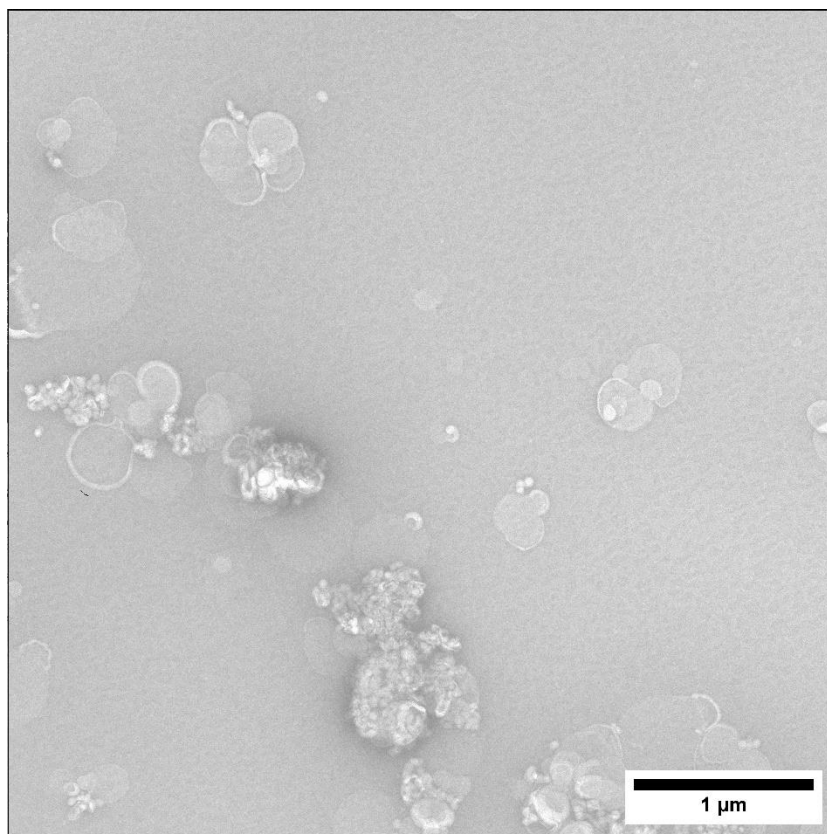


Figure 7.274: – TEM micrograph for POPC: SC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

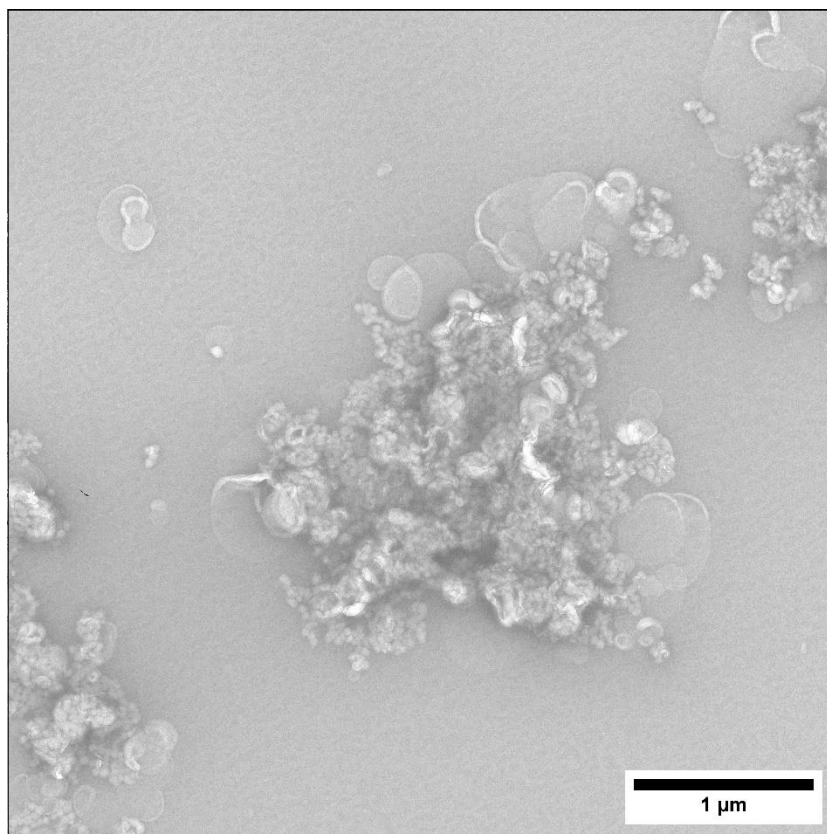


Figure 7.275: – TEM micrograph for POPC: SC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

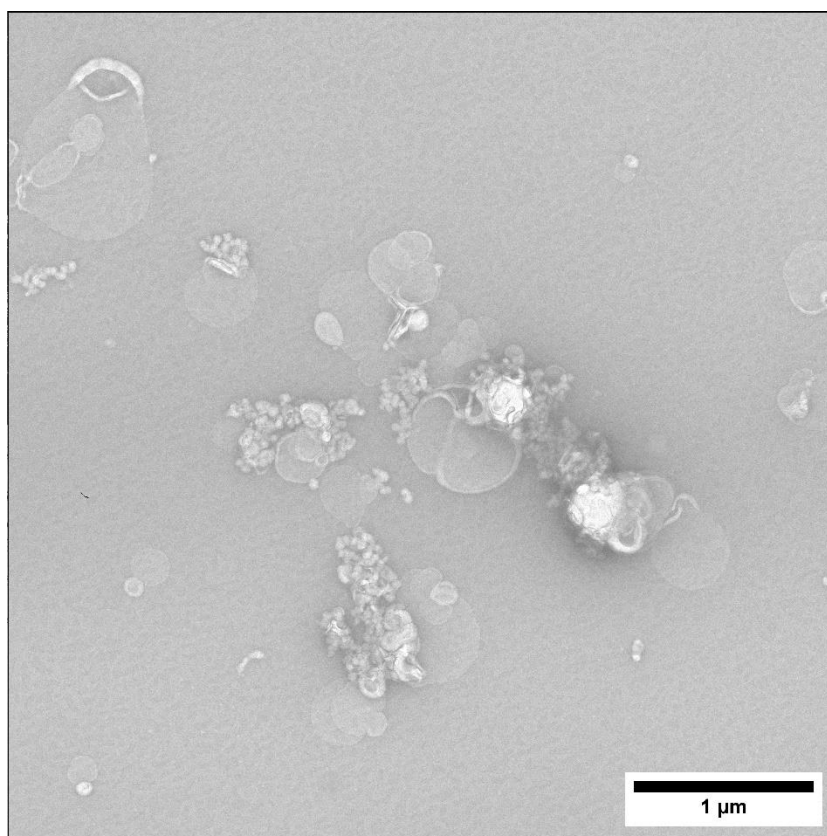


Figure 7.276: – TEM micrograph for POPC: SC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

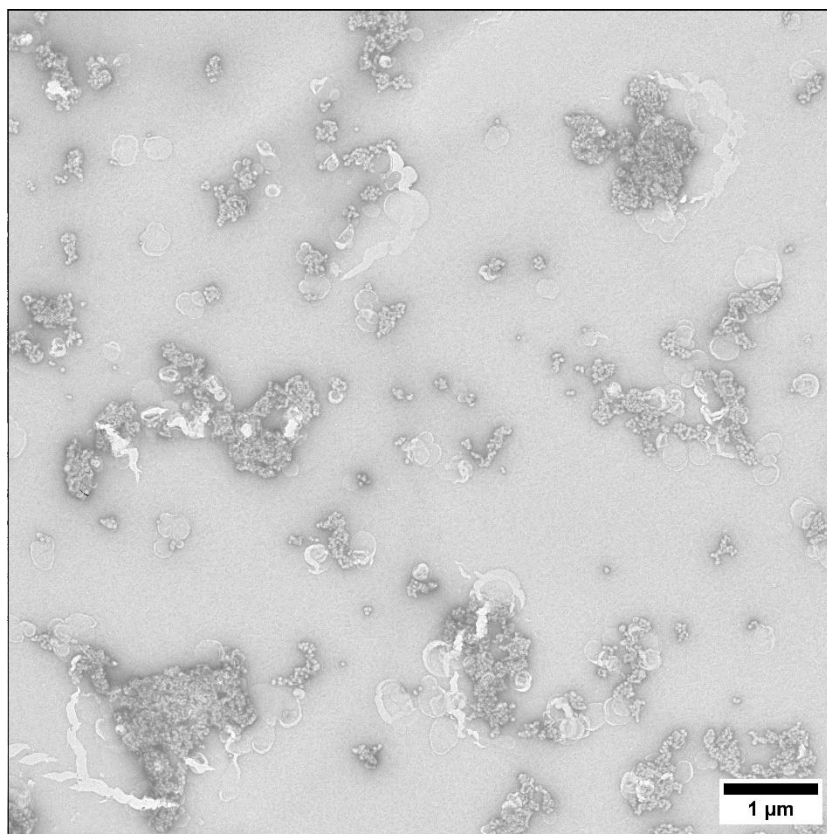


Figure 7.277: – TEM micrograph for POPC: SC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

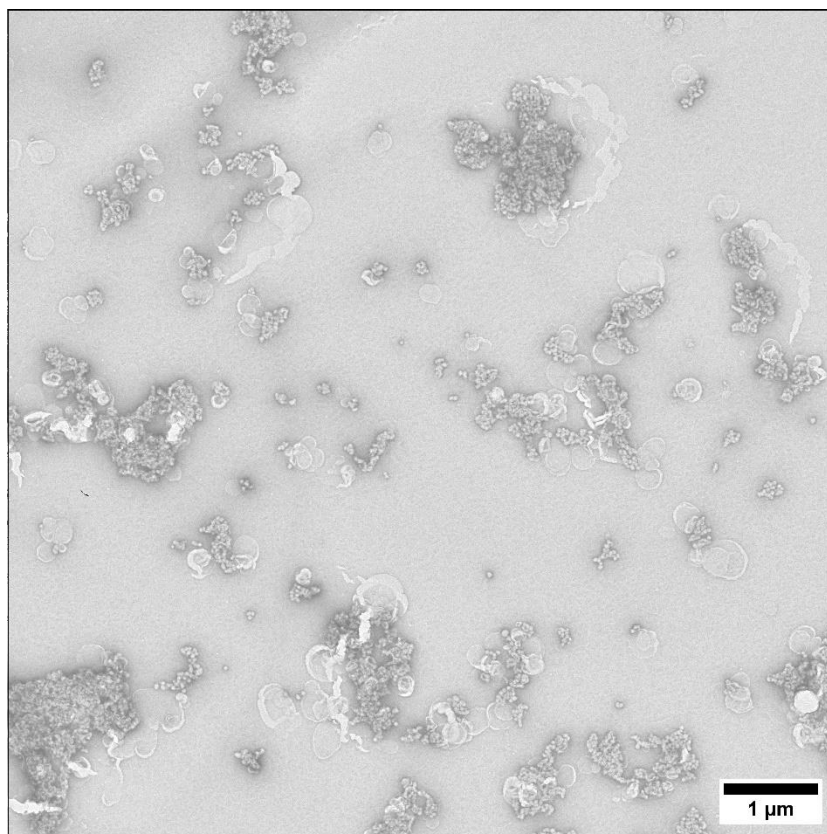


Figure 7.278: – TEM micrograph for POPC: SC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

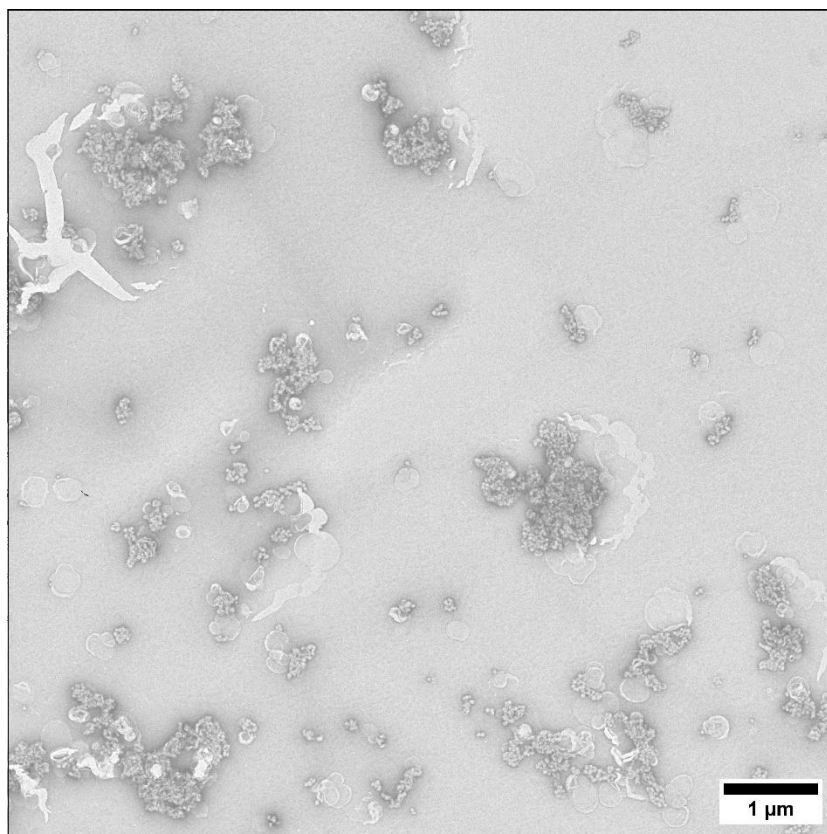


Figure 7.279: – TEM micrograph for POPC: SC BCP (75:25) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

50% POPC 50% Doubly charged PDMAm50PDAAm300

t= 0 min

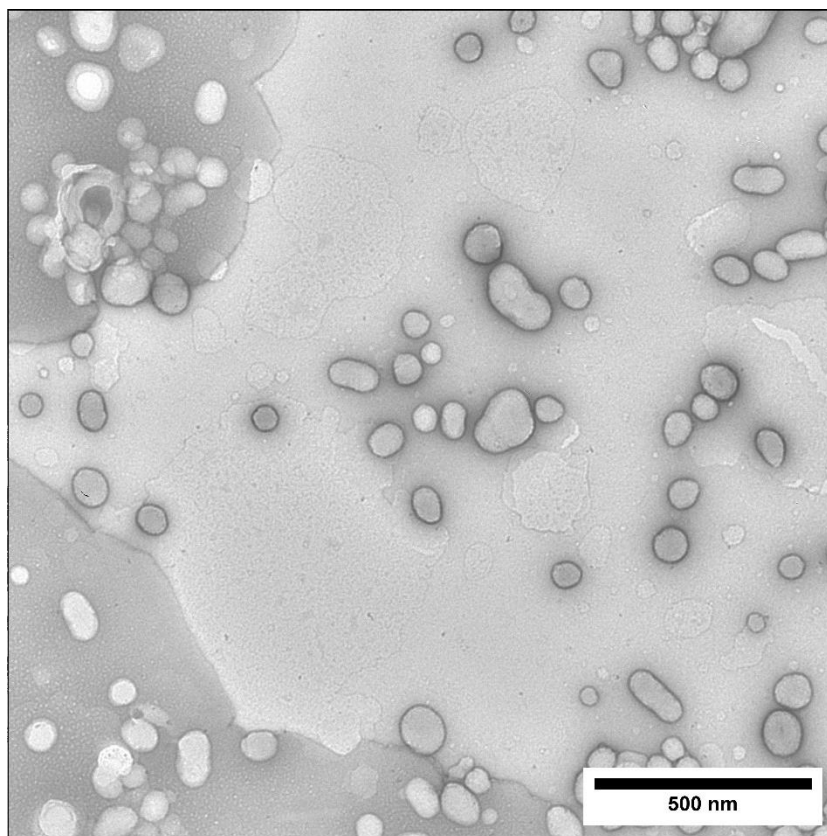


Figure 7.280: – TEM micrograph for POPC: DC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

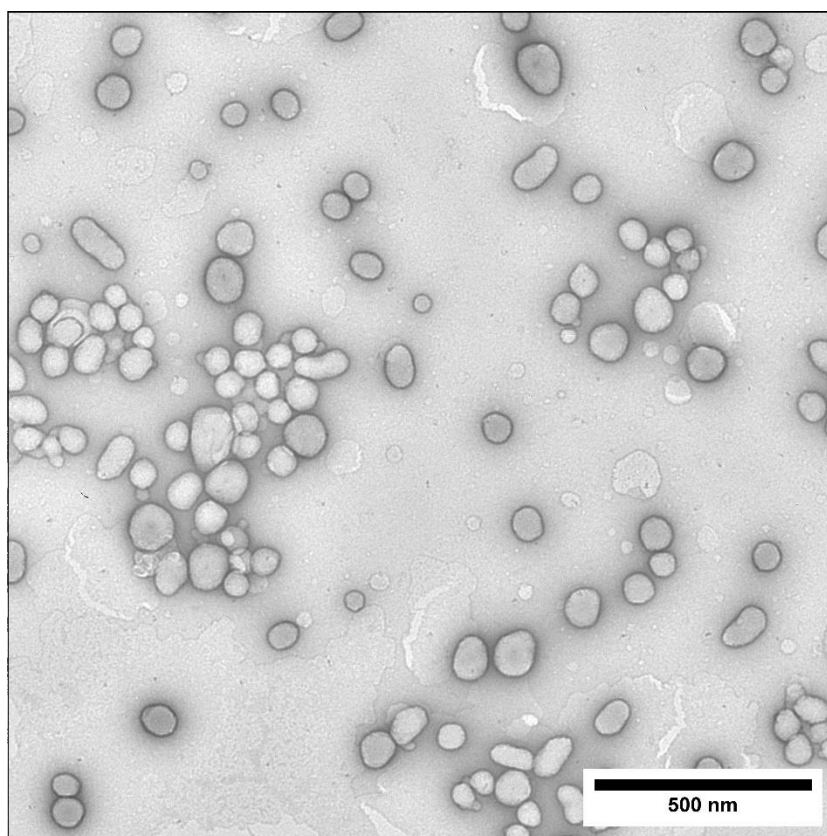


Figure 7.281: – TEM micrograph for POPC: DC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

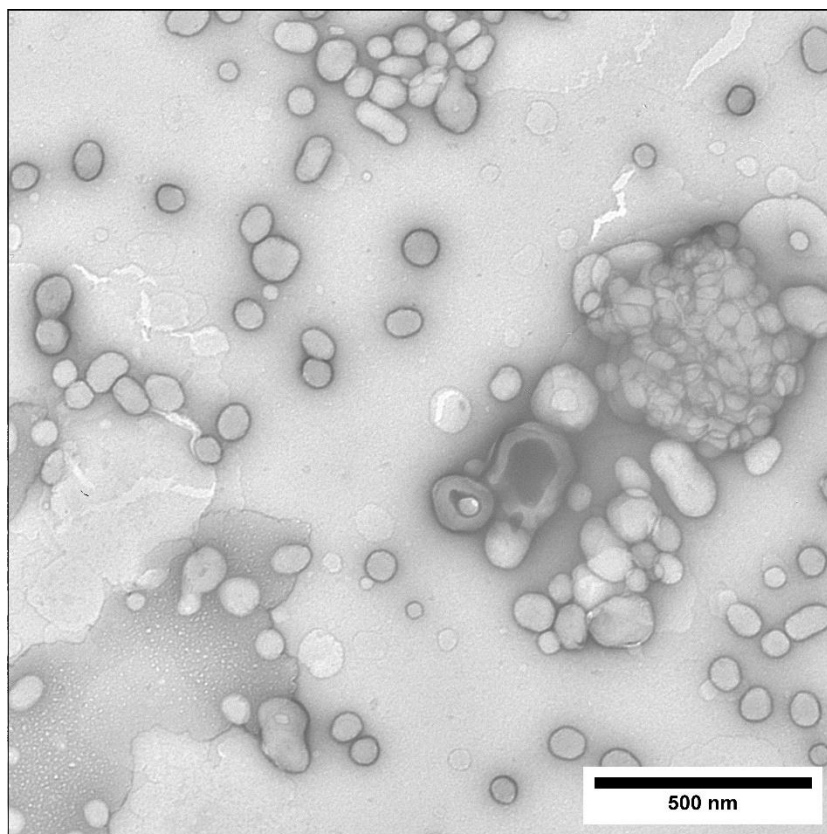


Figure 7.282: – TEM micrograph for POPC: DC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

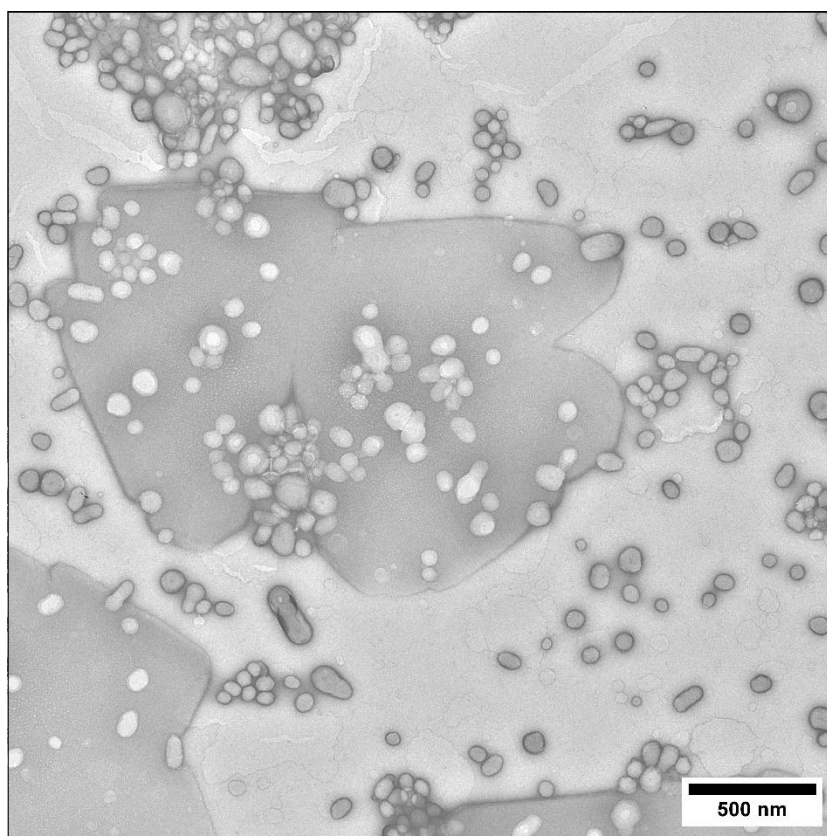


Figure 7.283: – TEM micrograph for POPC: DC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

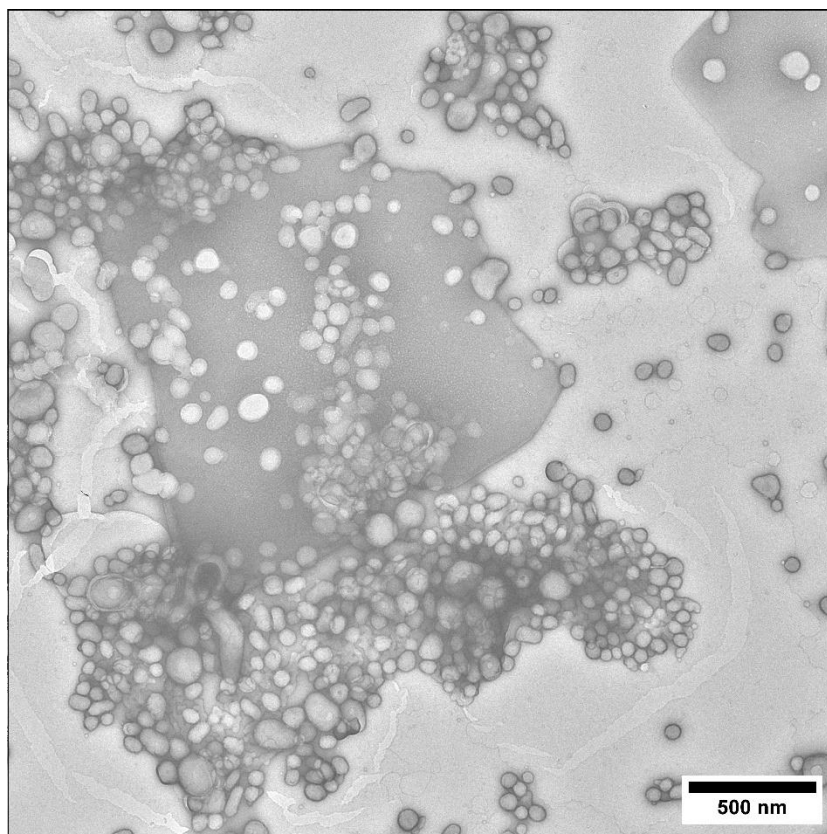


Figure 7.284: – TEM micrograph for POPC: DC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

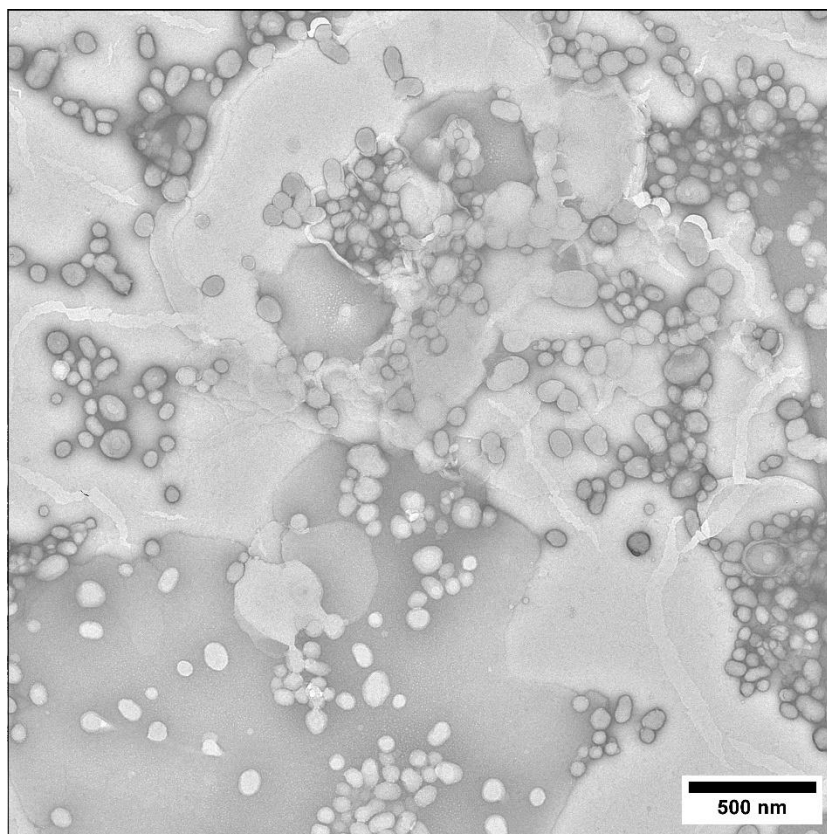


Figure 7.285: – TEM micrograph for POPC: DC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

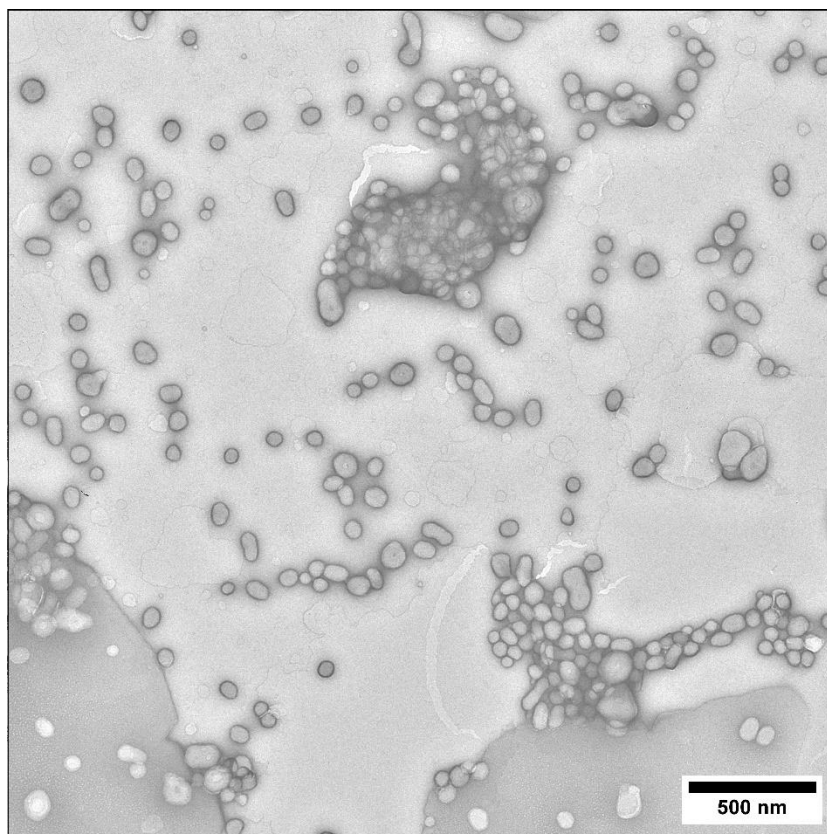


Figure 7.286 – TEM micrograph for POPC: DC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

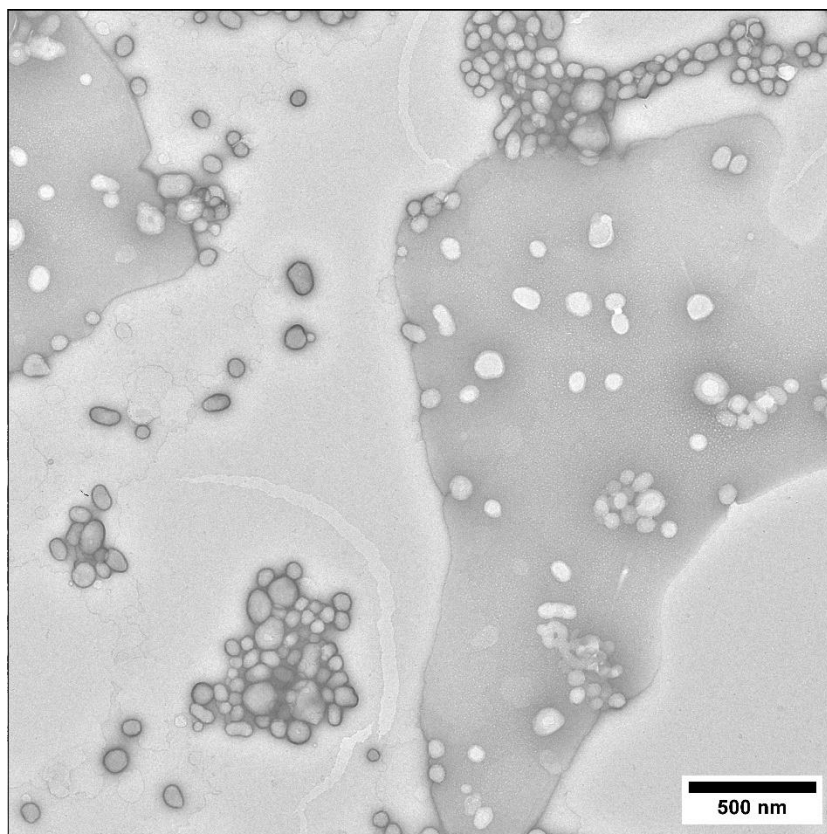


Figure 7.287: – TEM micrograph for POPC: DC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

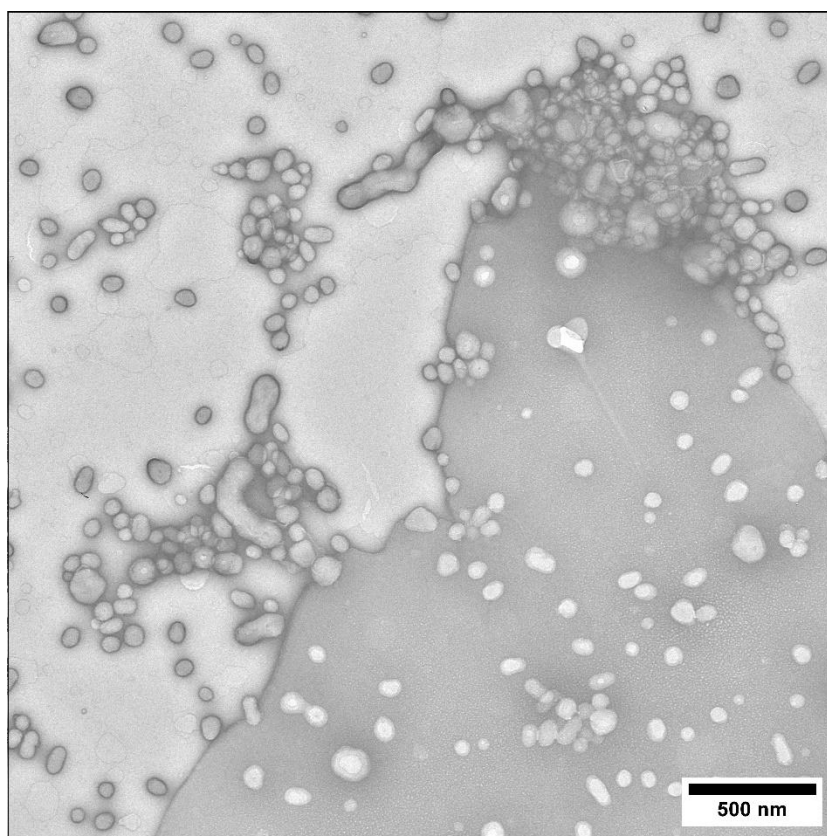


Figure 7.288: – TEM micrograph for POPC: DC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

50% POPC 50% Doubly charged PDMAm50PDAAm300

t= 30 min

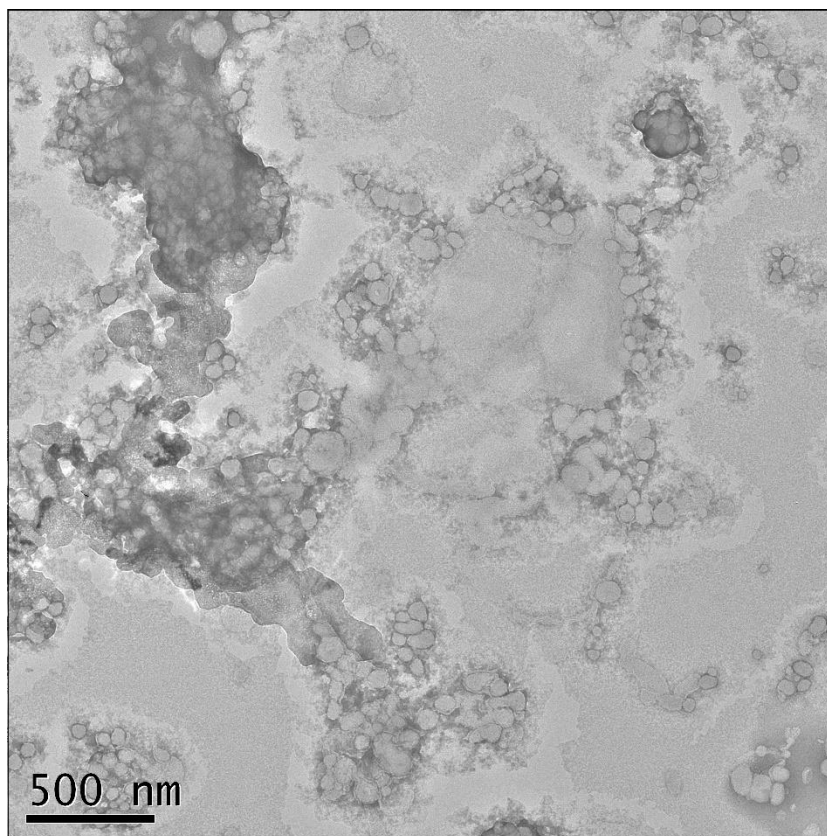


Figure 7.289: – TEM micrograph for POPC: DC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 30 min)

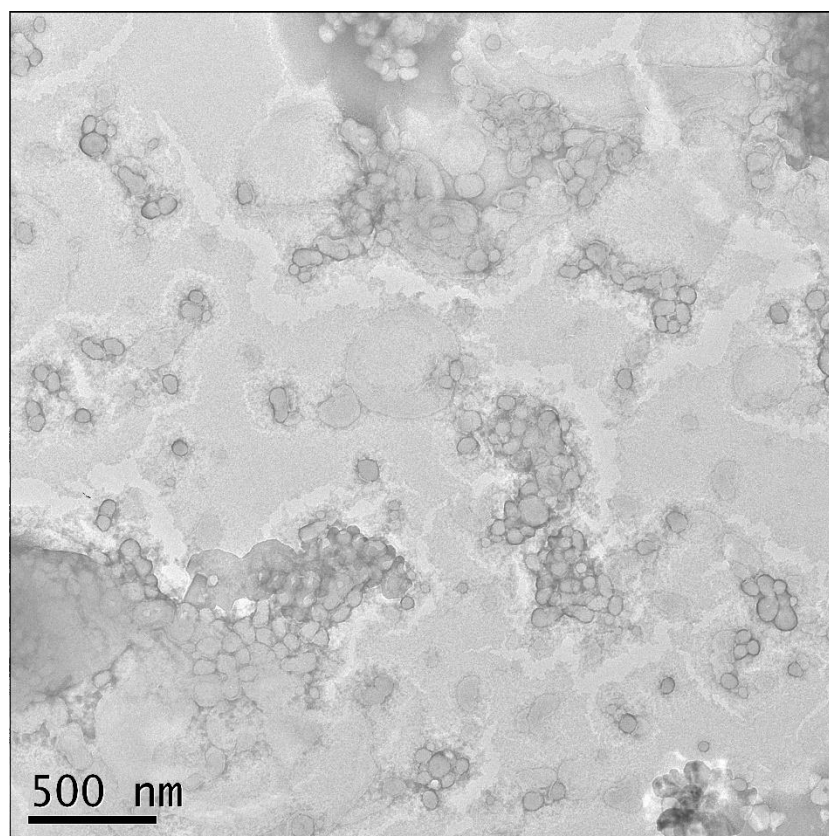


Figure 7.290 – TEM micrograph for POPC: DC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

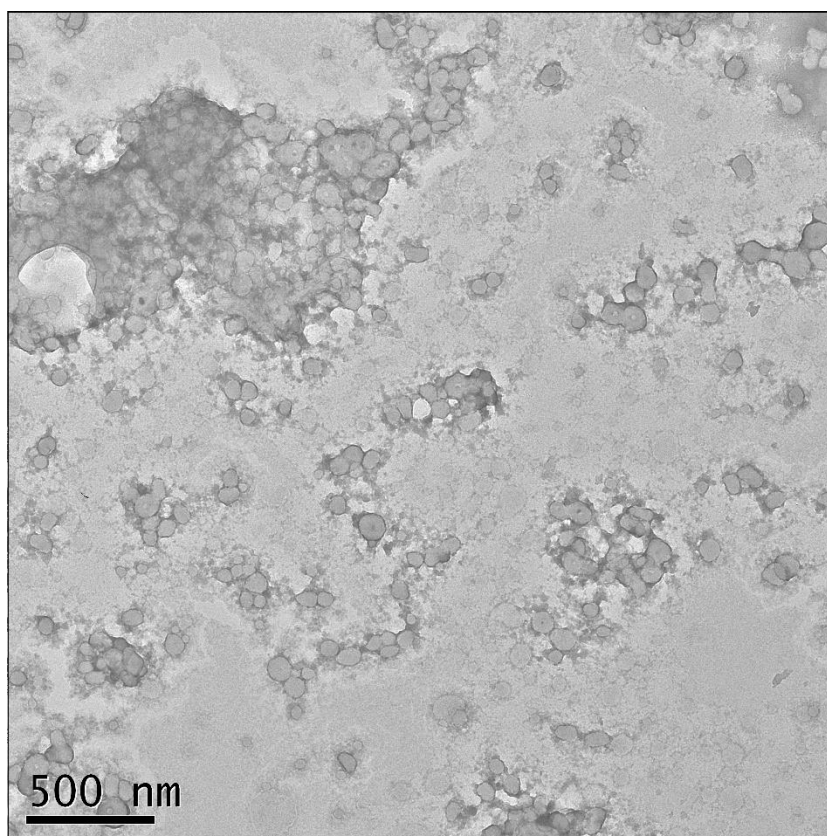


Figure 7.291: – TEM micrograph for POPC: DC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

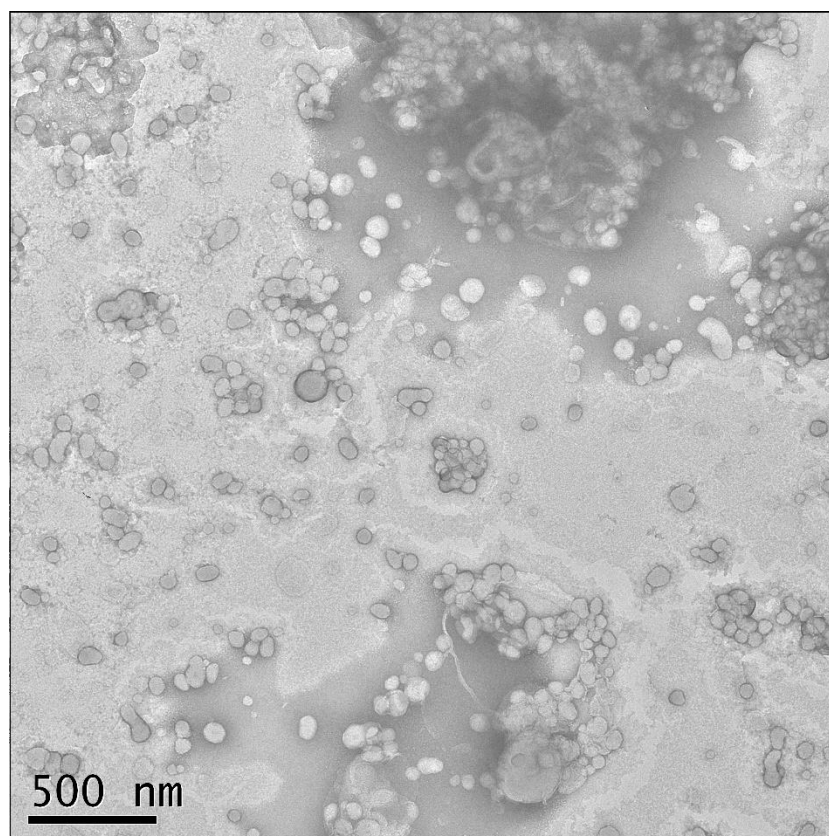


Figure 7.292: – TEM micrograph for POPC: DC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

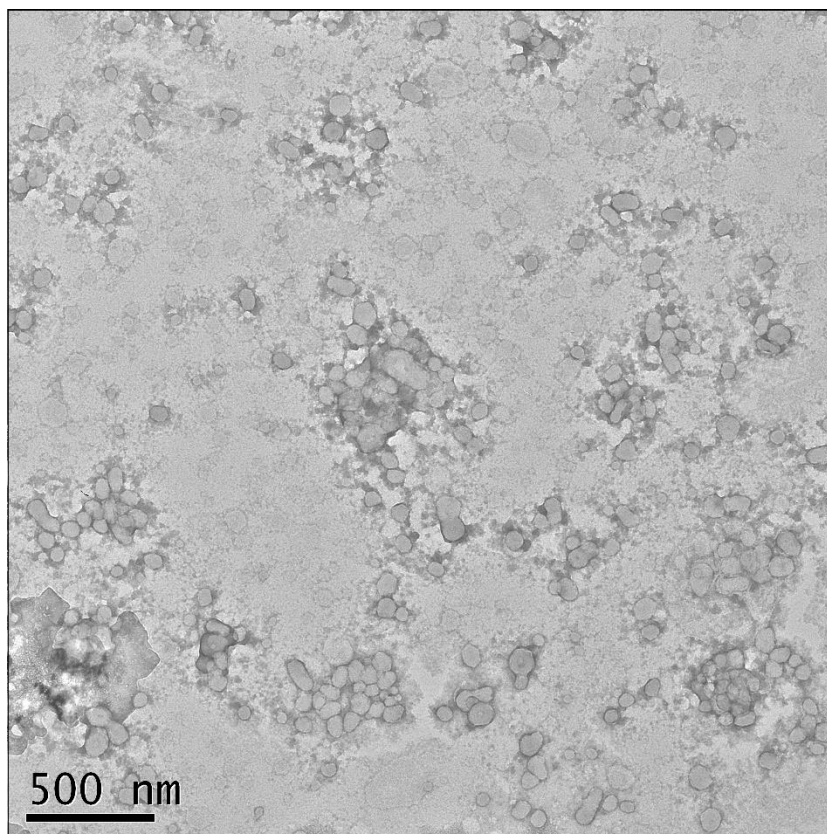


Figure 7.293: – TEM micrograph for POPC: DC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

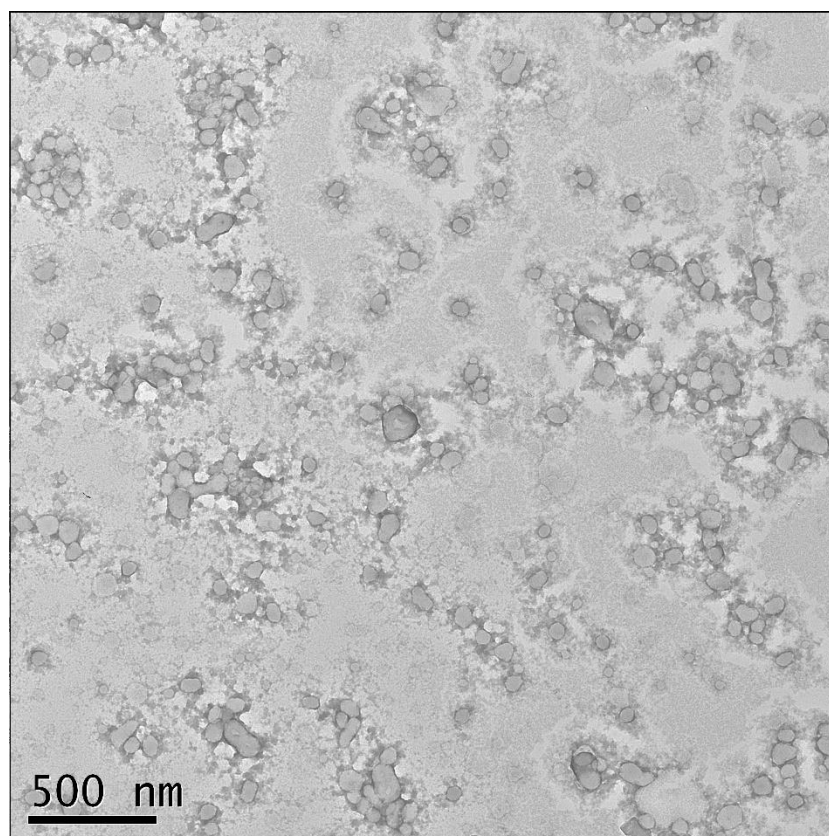


Figure 7.294: – TEM micrograph for POPC: DC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

50% POPC 50% singly charged PDMAm50PDAAm300

t= 0 min

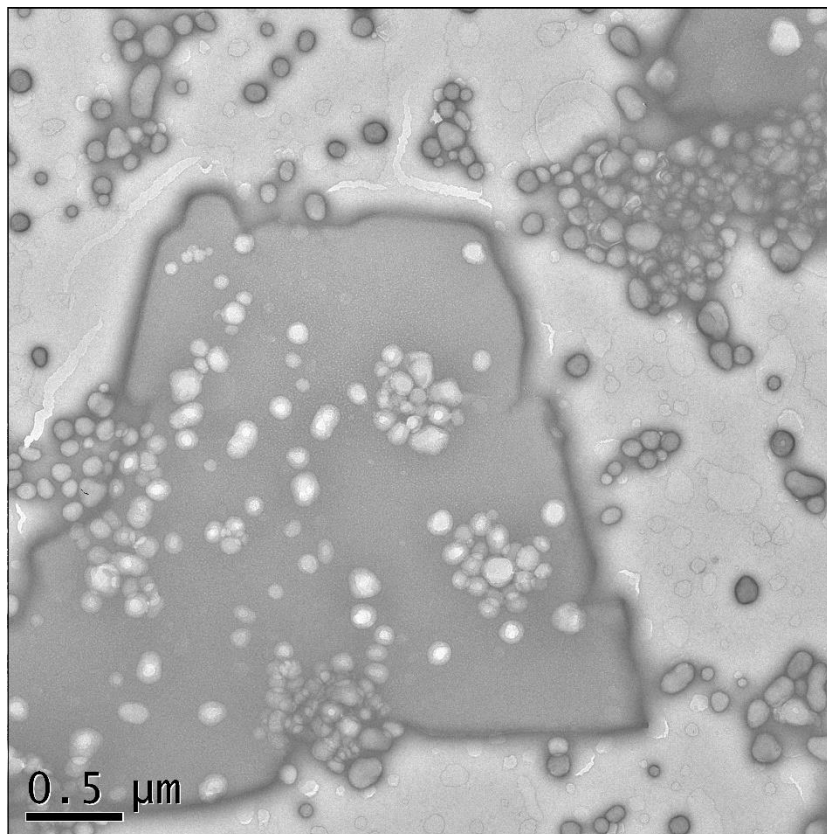


Figure 7.295: – TEM micrograph for POPC: SC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

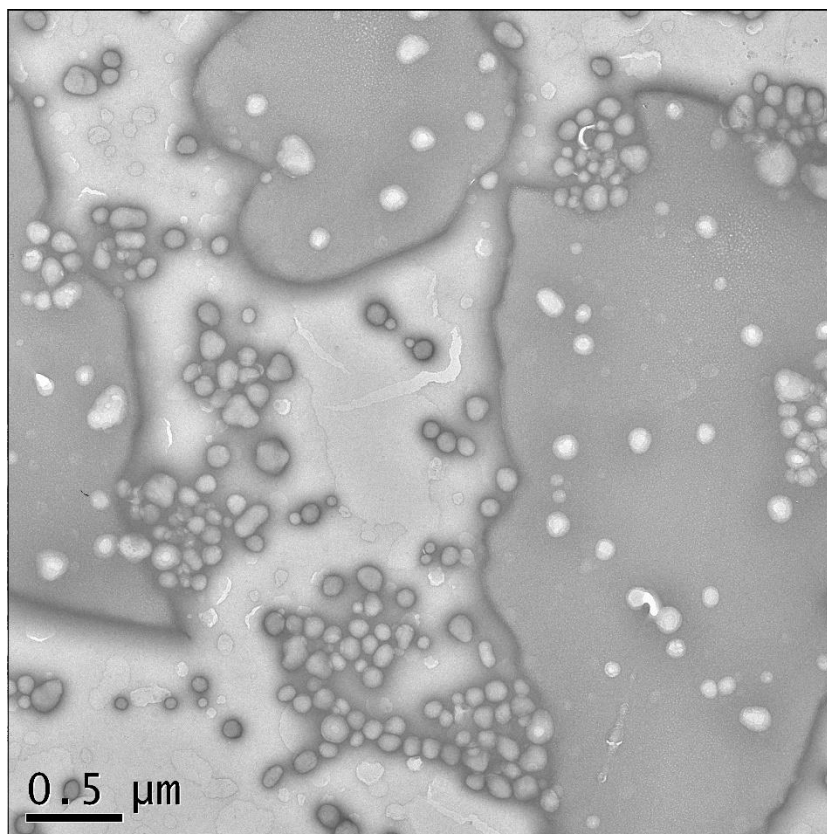


Figure 7.296: – TEM micrograph for POPC: SC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

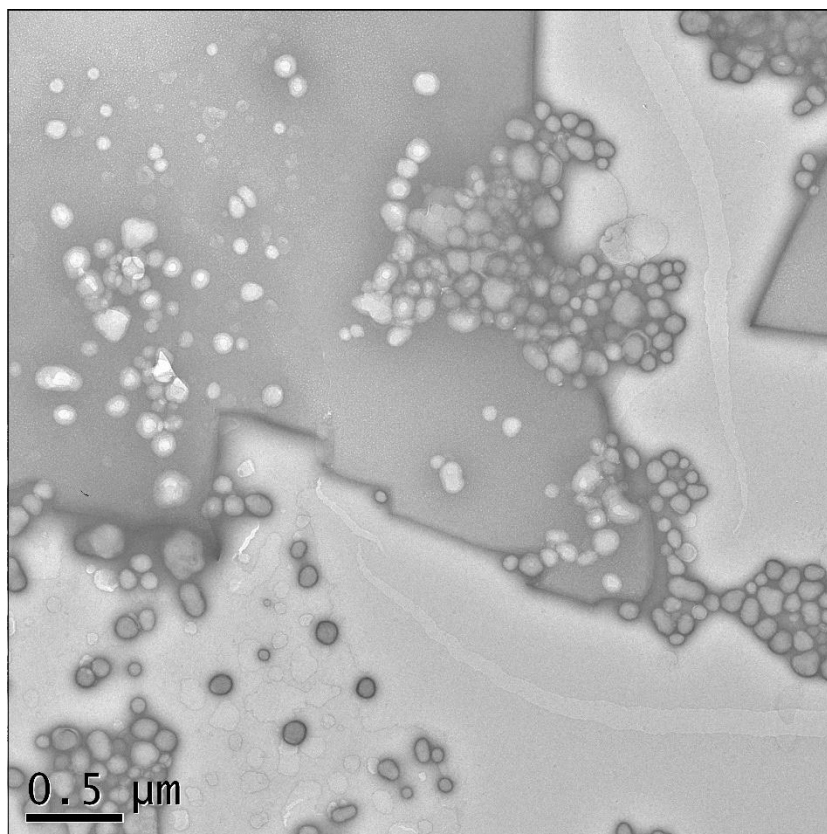


Figure 7.297: – TEM micrograph for POPC: SC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

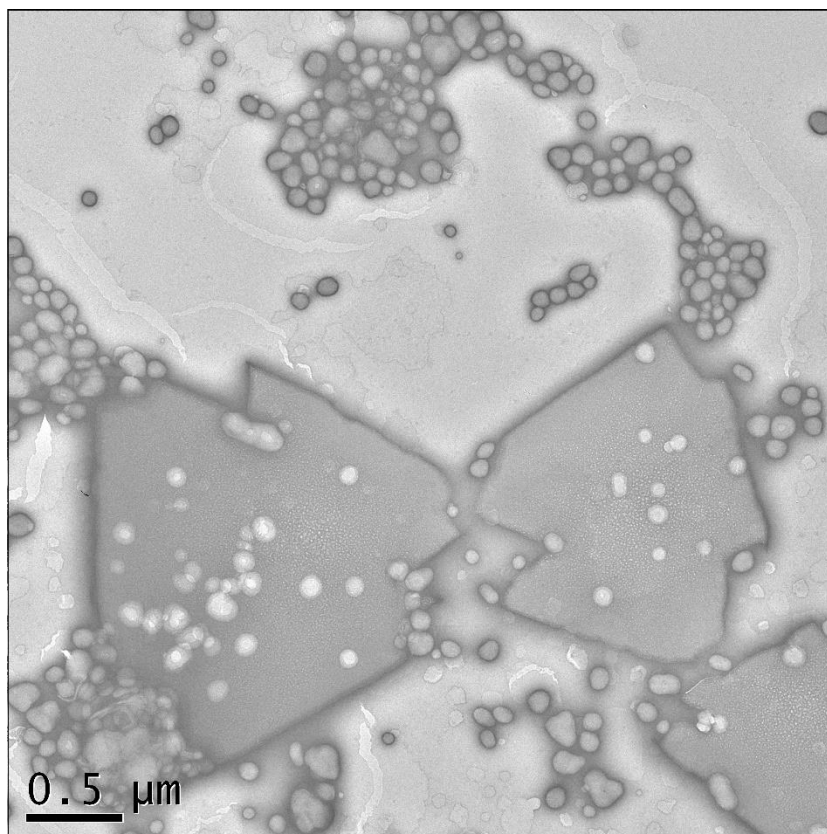


Figure 7.298: – TEM micrograph for POPC: SC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

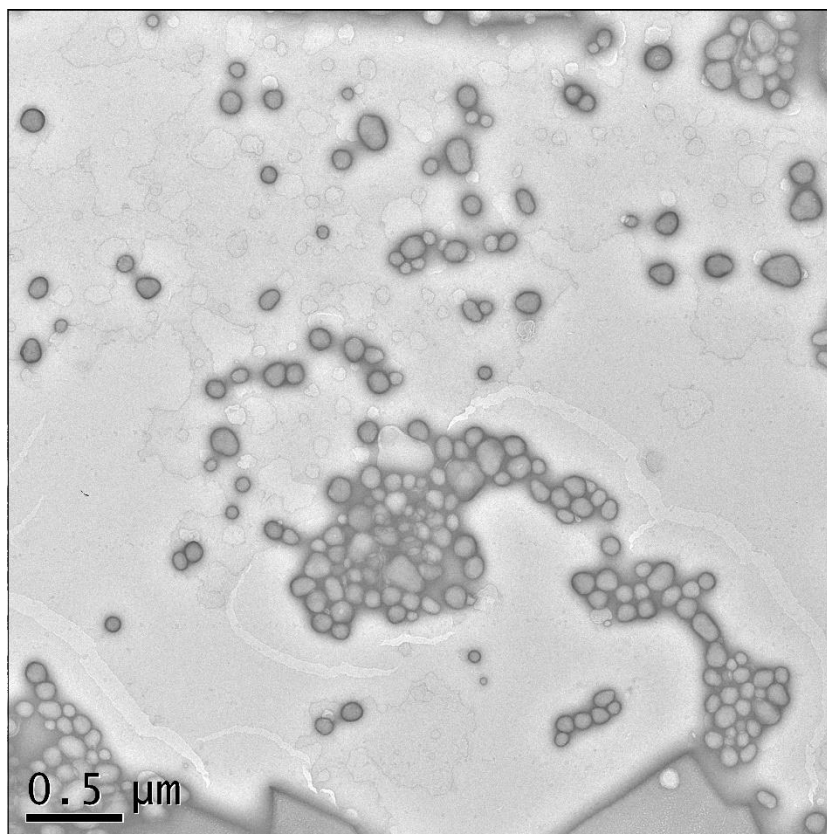


Figure 7.299: – TEM micrograph for POPC: SC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

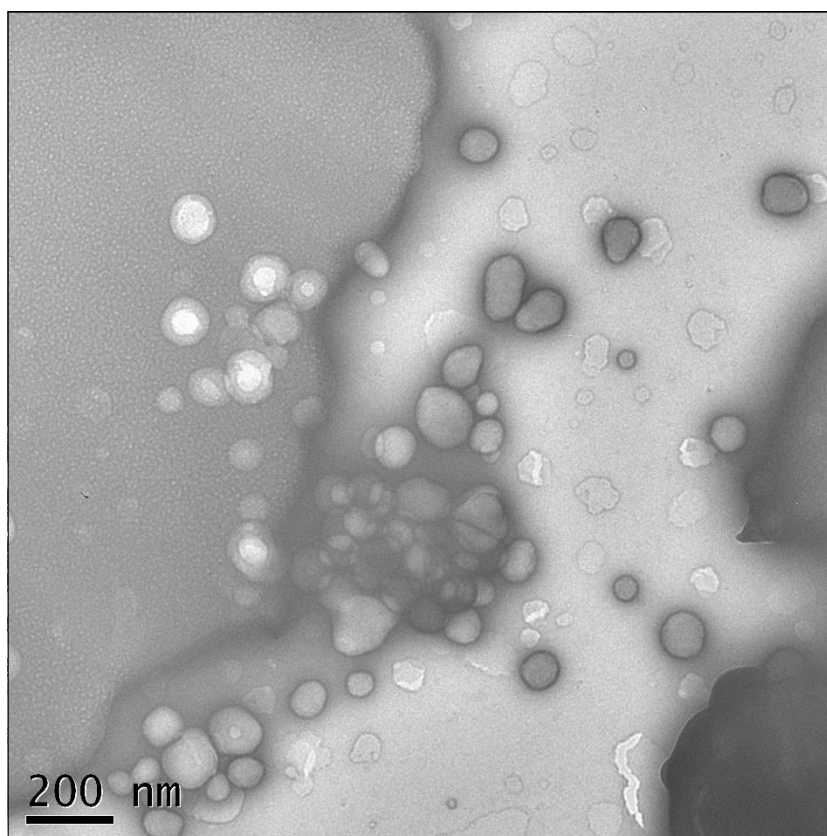


Figure 7.300: – TEM micrograph for POPC: SC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

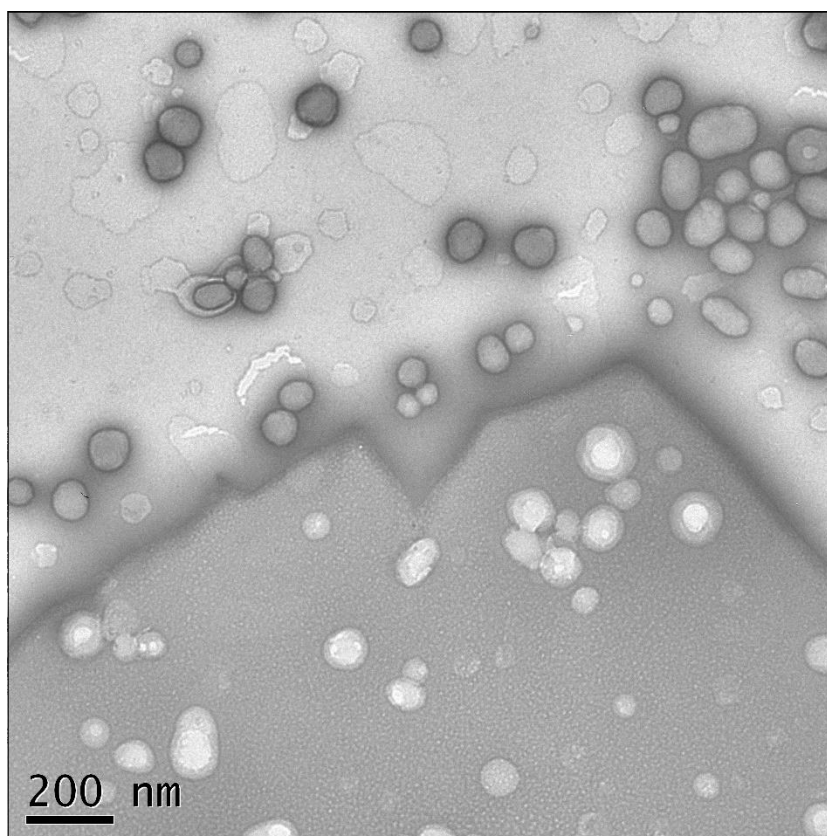


Figure 7.301: – TEM micrograph for POPC: SC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

50% POPC 50% singly charged PDMAm50PDAAm300

t= 30 min

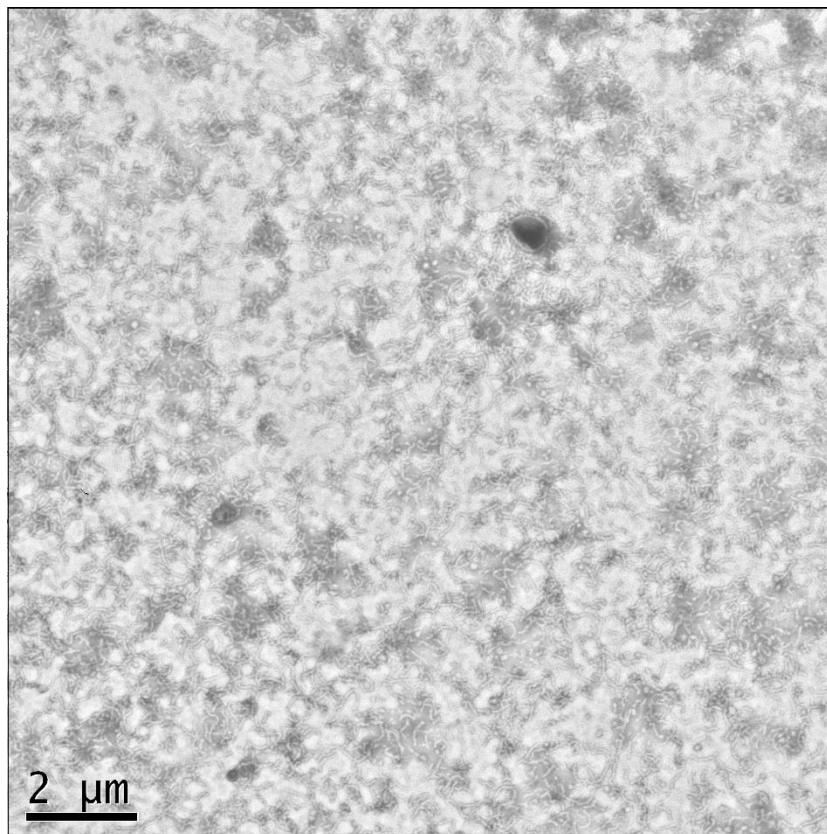


Figure 7.302: – TEM micrograph for POPC: SC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 30 min)

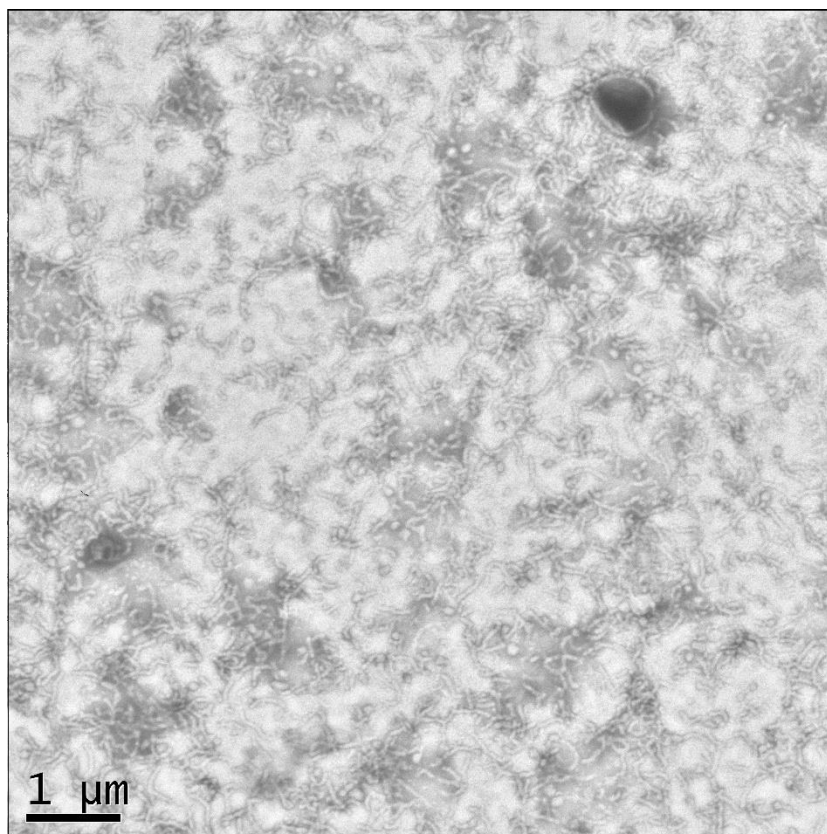


Figure 7.303: – TEM micrograph for POPC: SC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 30 min)

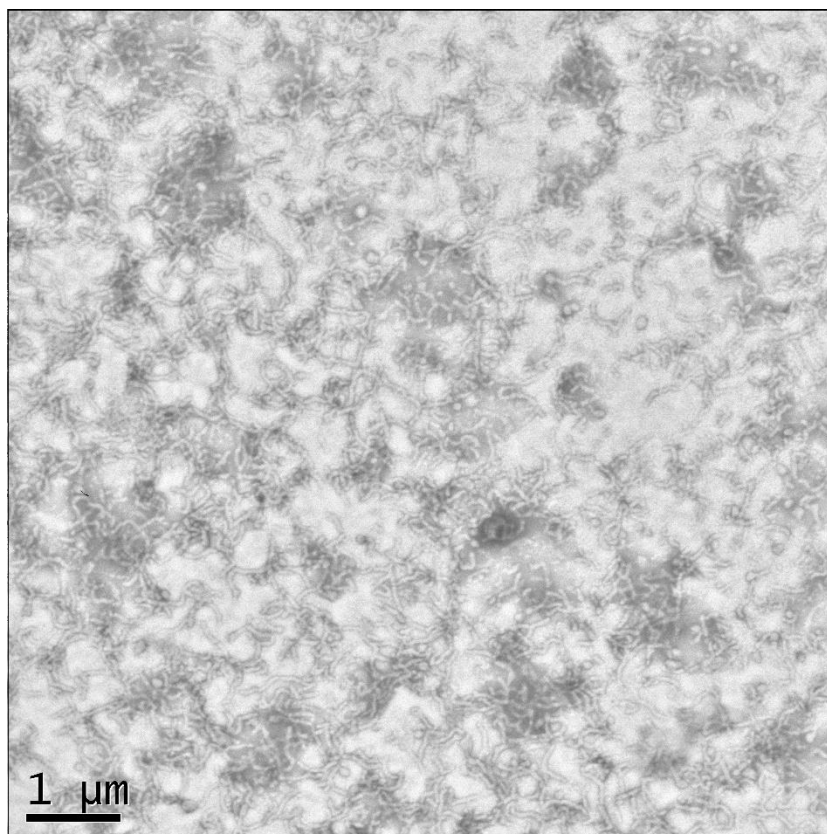


Figure 7.304: – TEM micrograph for POPC: SC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 30 min)

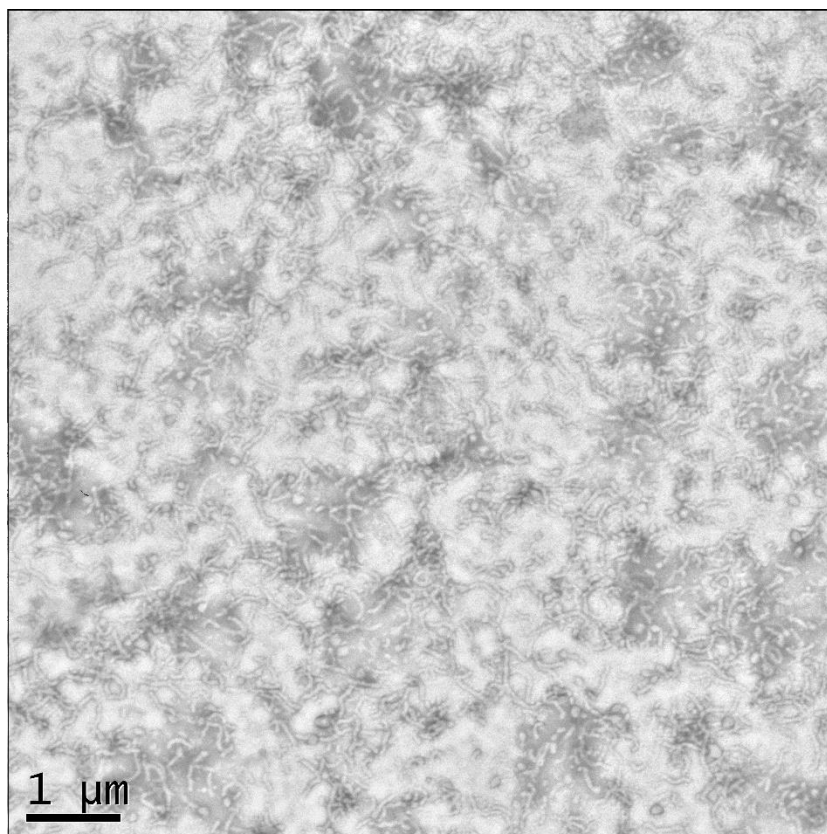


Figure 7.305: – TEM micrograph for POPC: SC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

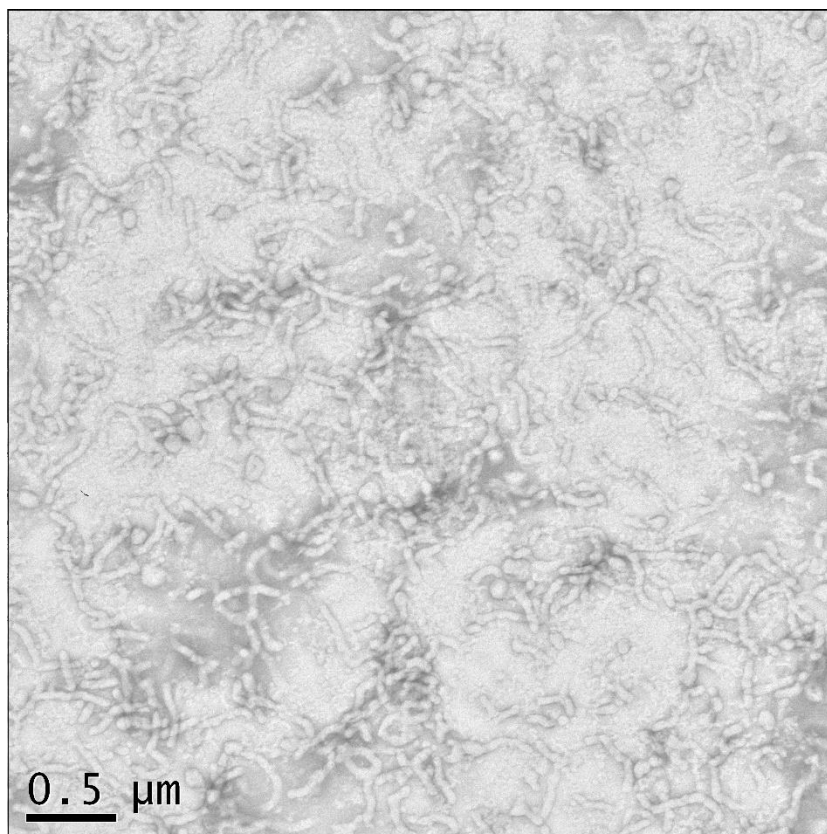


Figure 7.306: – TEM micrograph for POPC: SC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

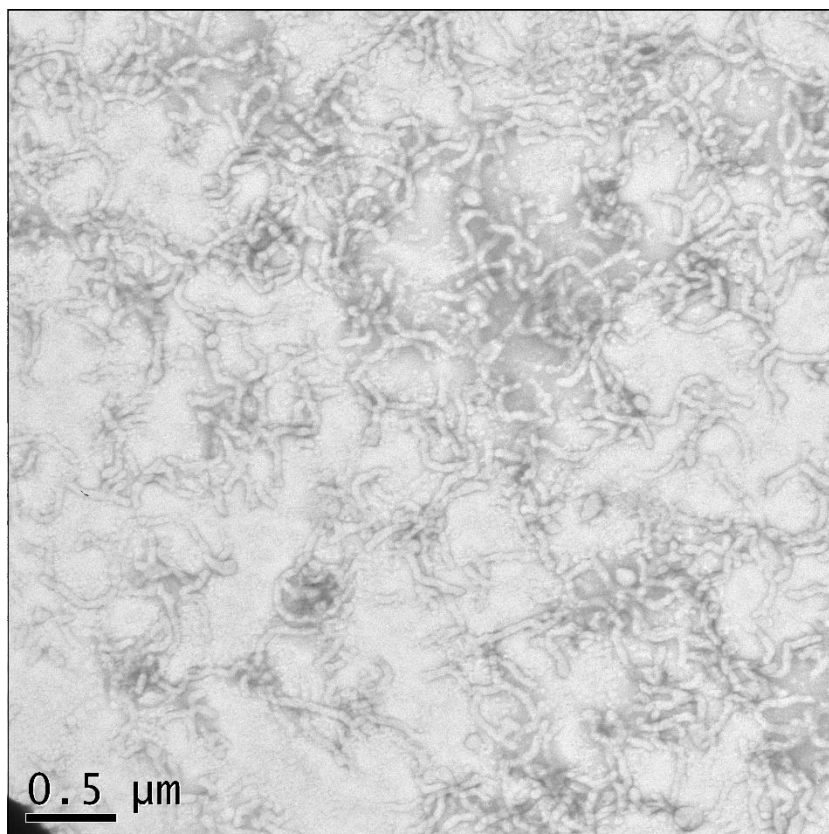


Figure 7.307: – TEM micrograph for POPC: SC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

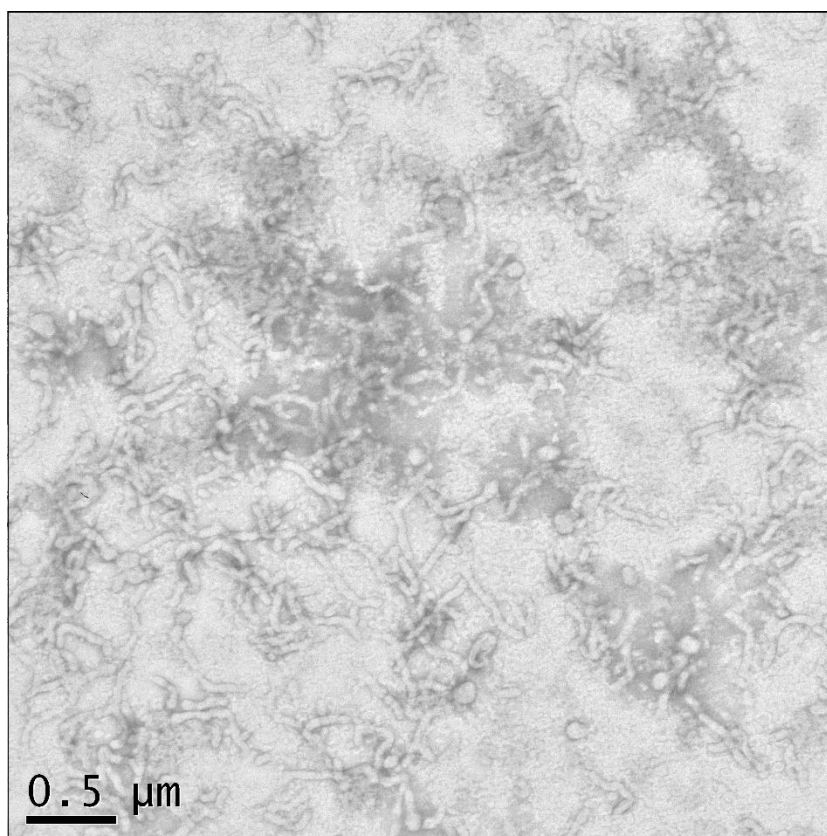


Figure 7.308 – TEM micrograph for POPC: SC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

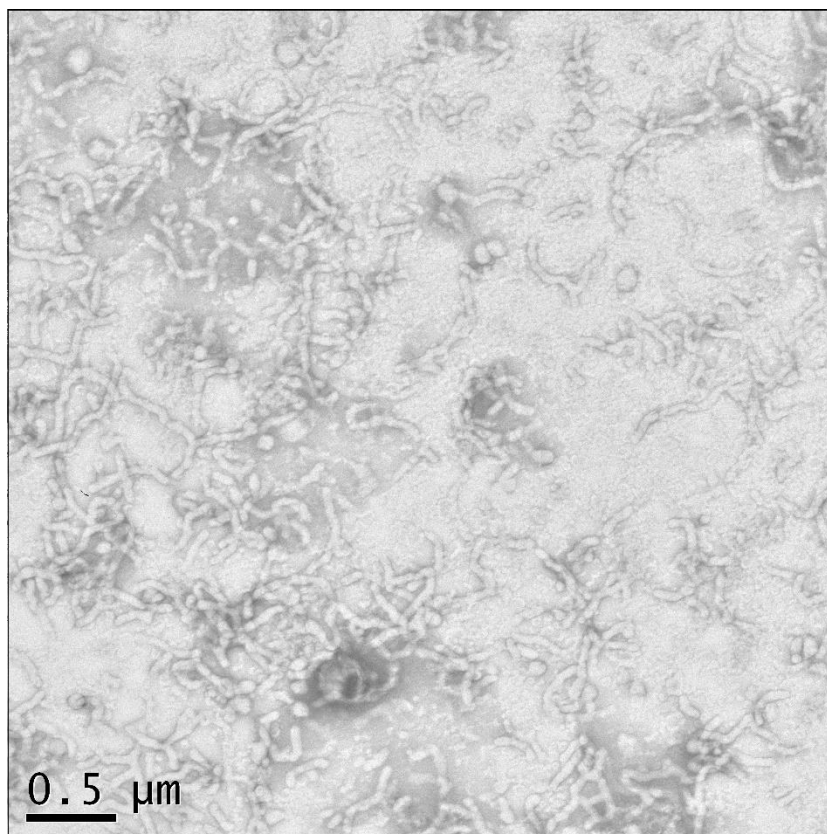


Figure 7.309 – TEM micrograph for POPC: SC BCP (50:50) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

25% POPC 75% Doubly Charged PDMAm50PDAAm300

t= 0 min

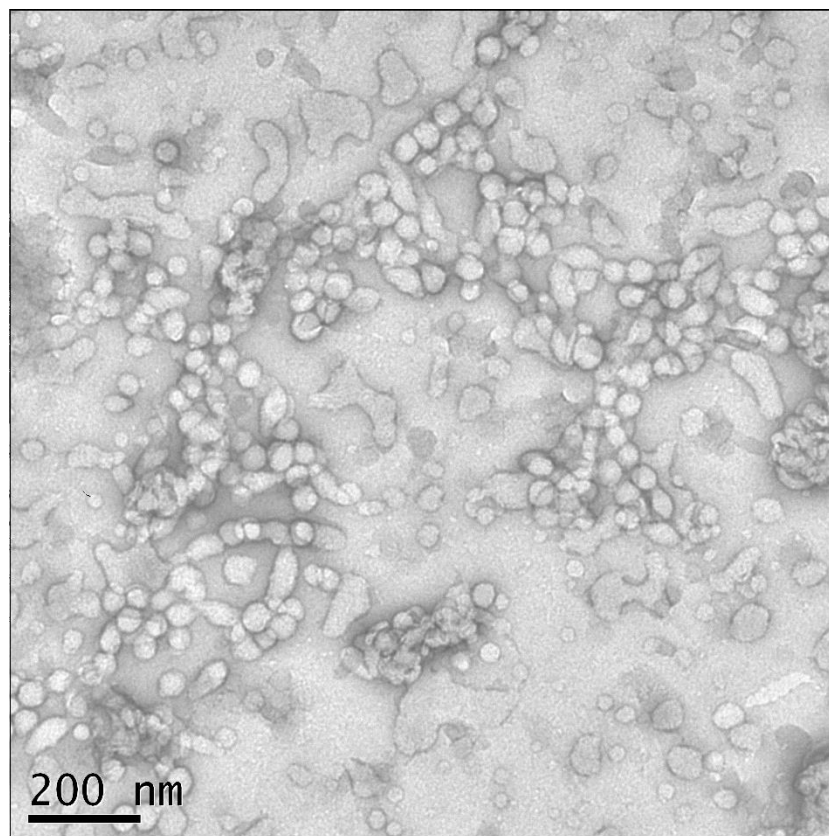


Figure 7.310: – TEM micrograph for POPC: DC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

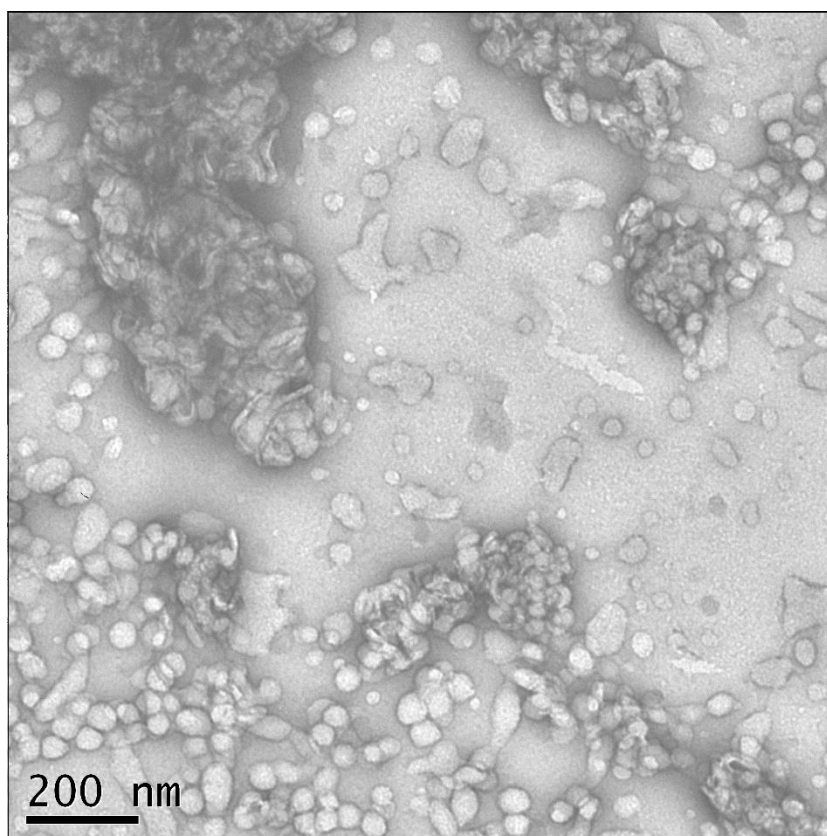


Figure 7.311: – TEM micrograph for POPC: DC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

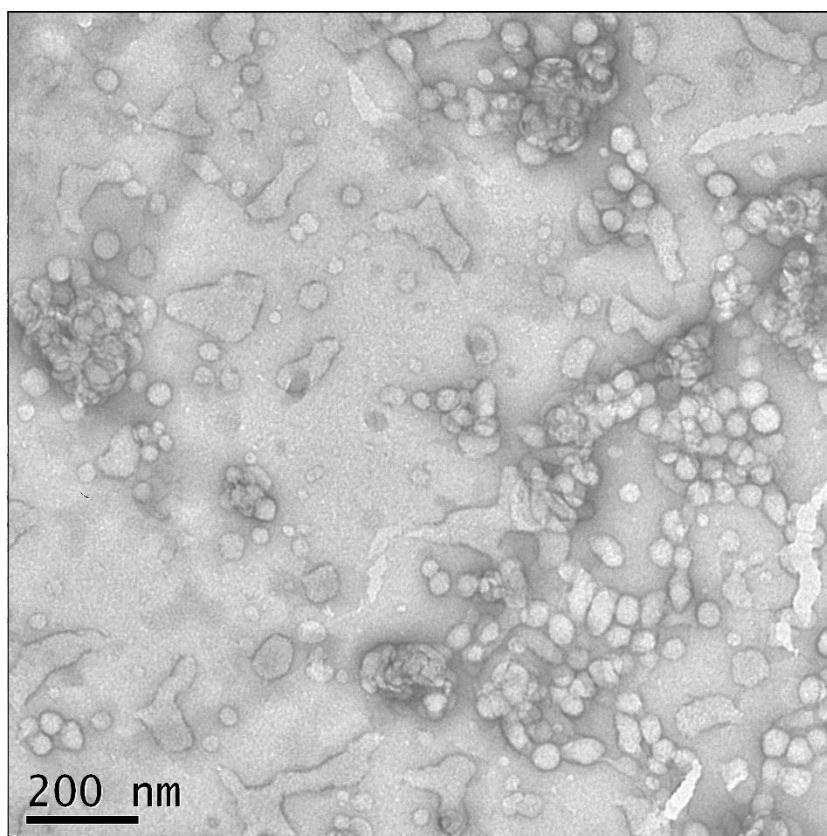


Figure 7.312: – TEM micrograph for POPC: DC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

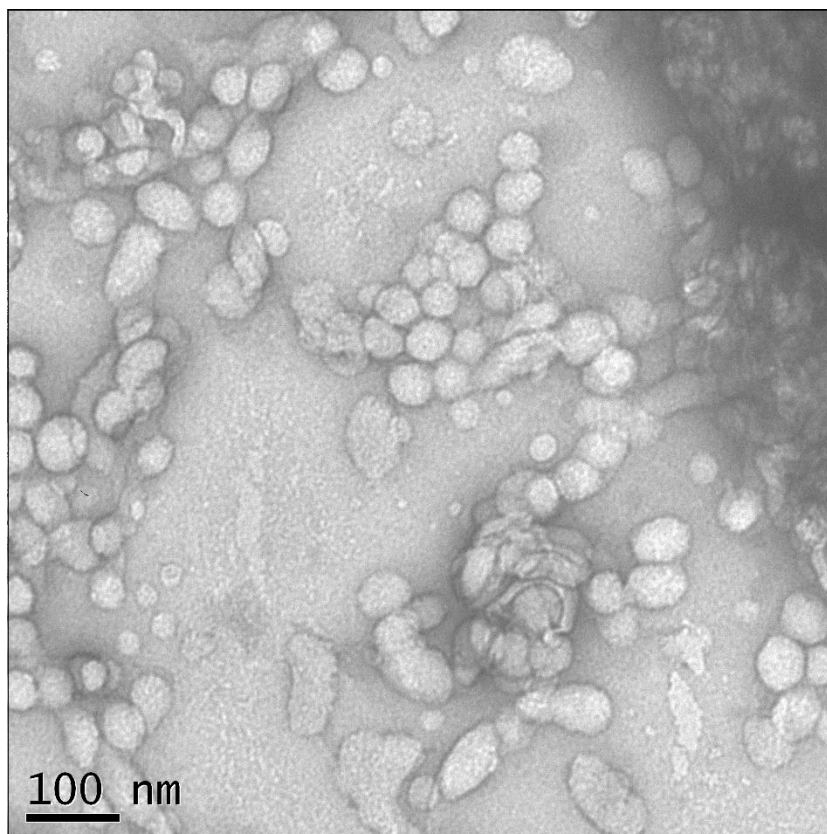


Figure 7.313: – TEM micrograph for POPC: DC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

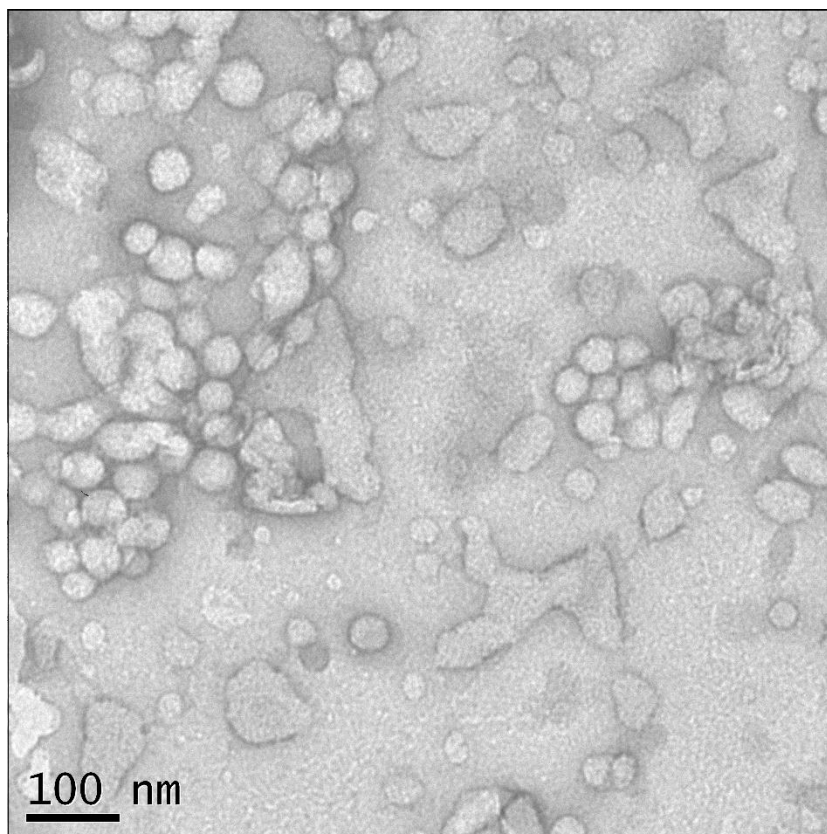


Figure 7.314: – TEM micrograph for POPC: DC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

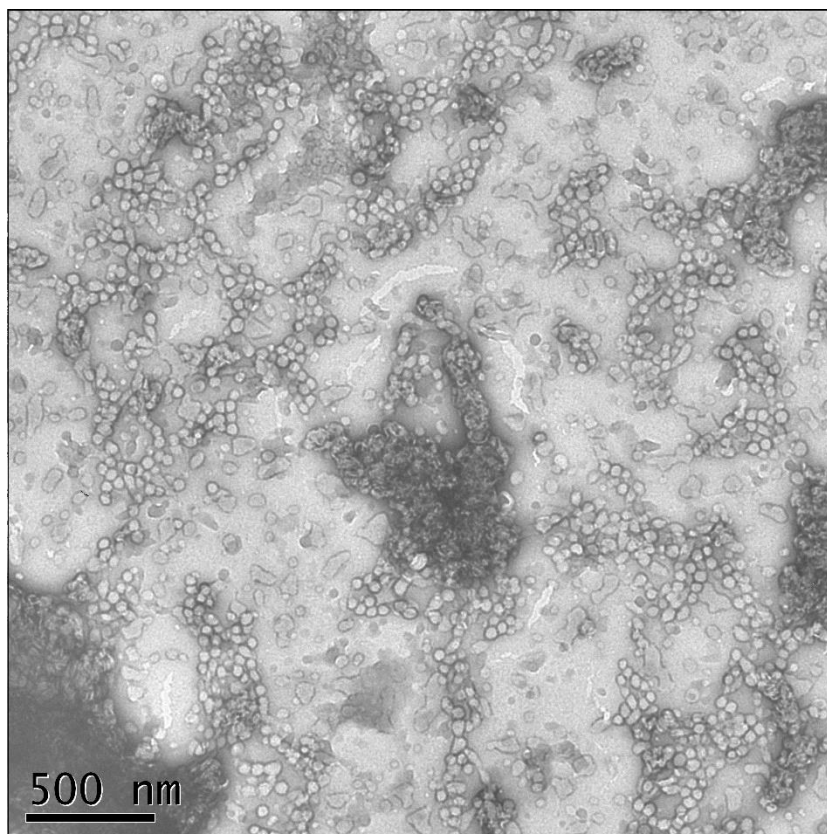


Figure 7.315: – TEM micrograph for POPC: DC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

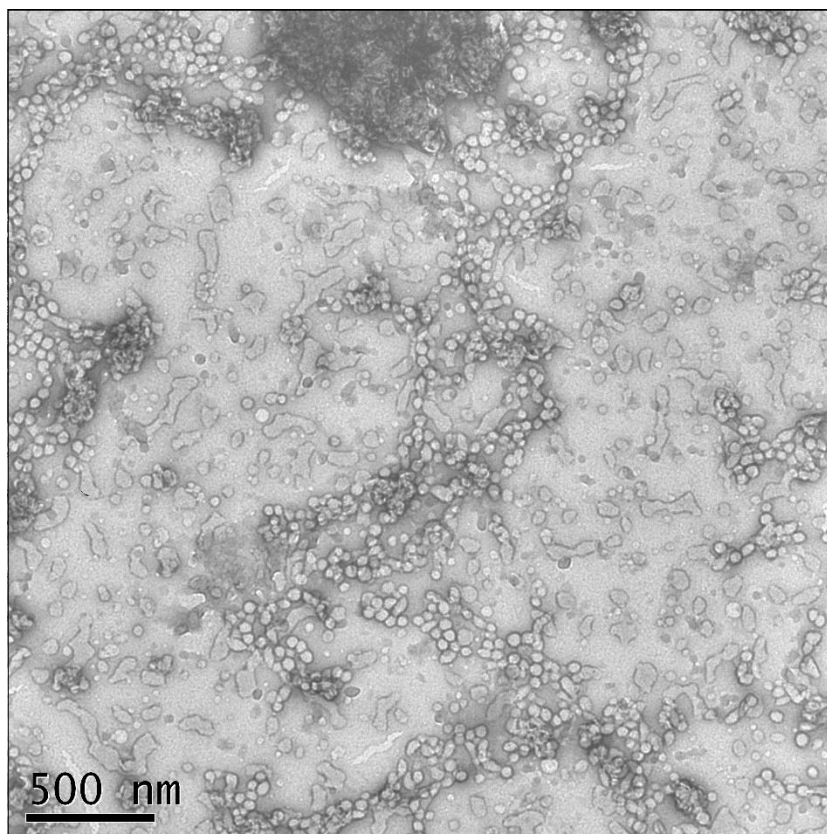


Figure 7.316: – TEM micrograph for POPC: DC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

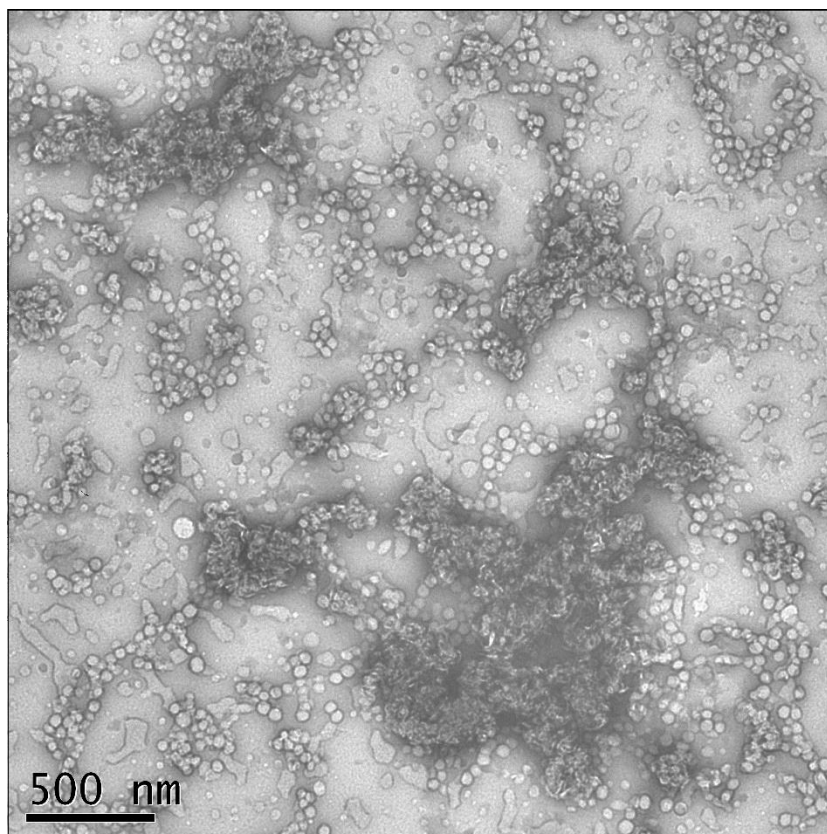


Figure 7.317: – TEM micrograph for POPC: DC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

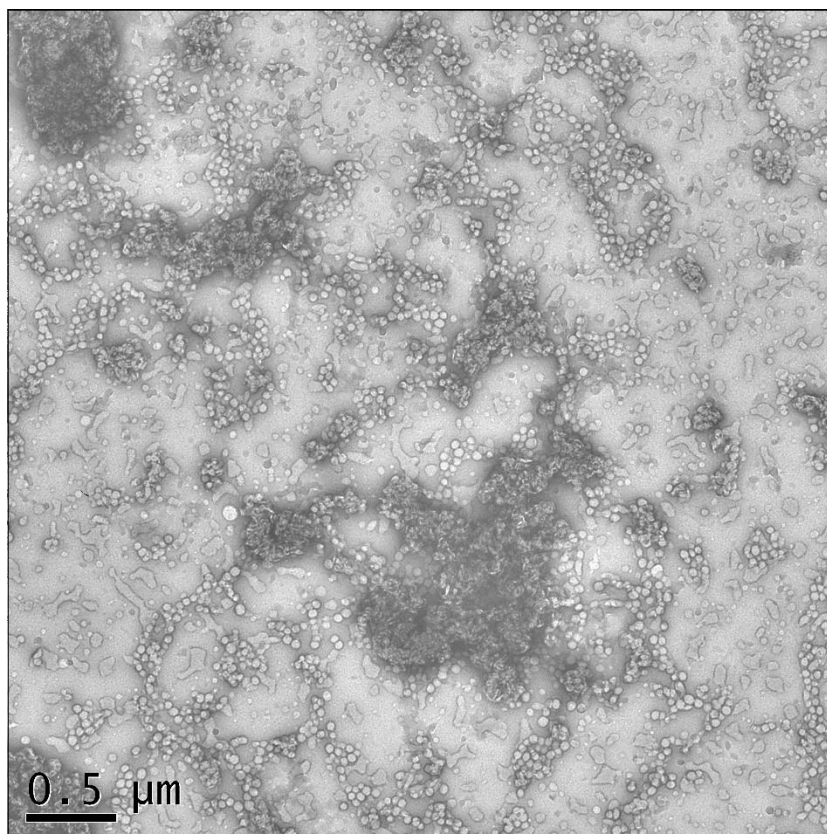


Figure 7.318: – TEM micrograph for POPC: DC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

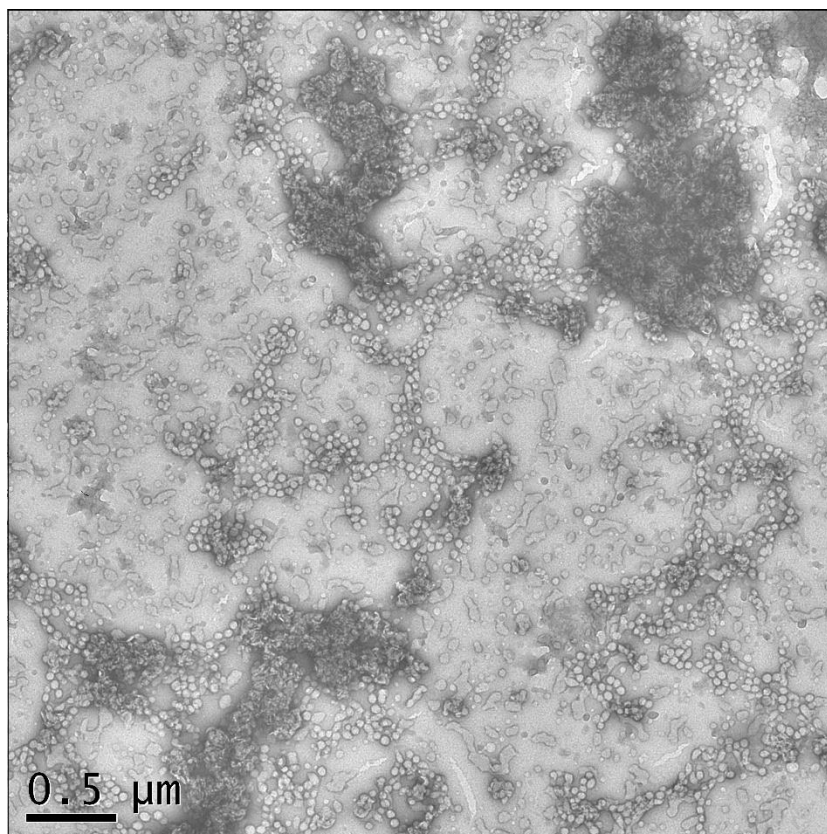


Figure 7.319: – TEM micrograph for POPC: DC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

25% POPC 75% Doubly charged PDMAm50PDAAm300

t= 30 min

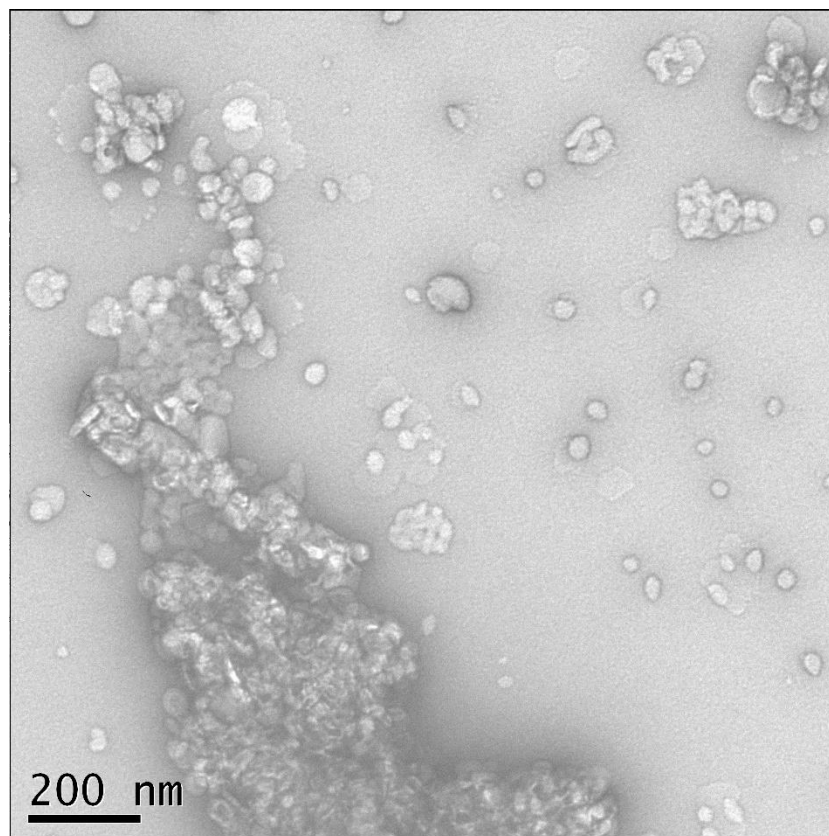


Figure 7.320: – TEM micrograph for POPC: DC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 30 min)

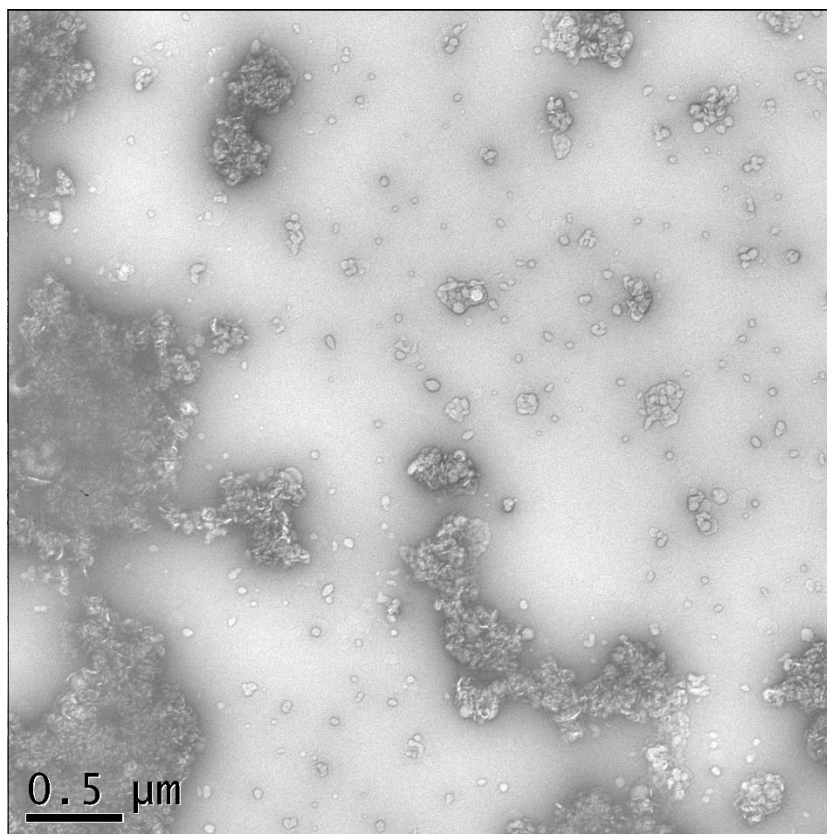


Figure 7.321: – TEM micrograph for POPC: DC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

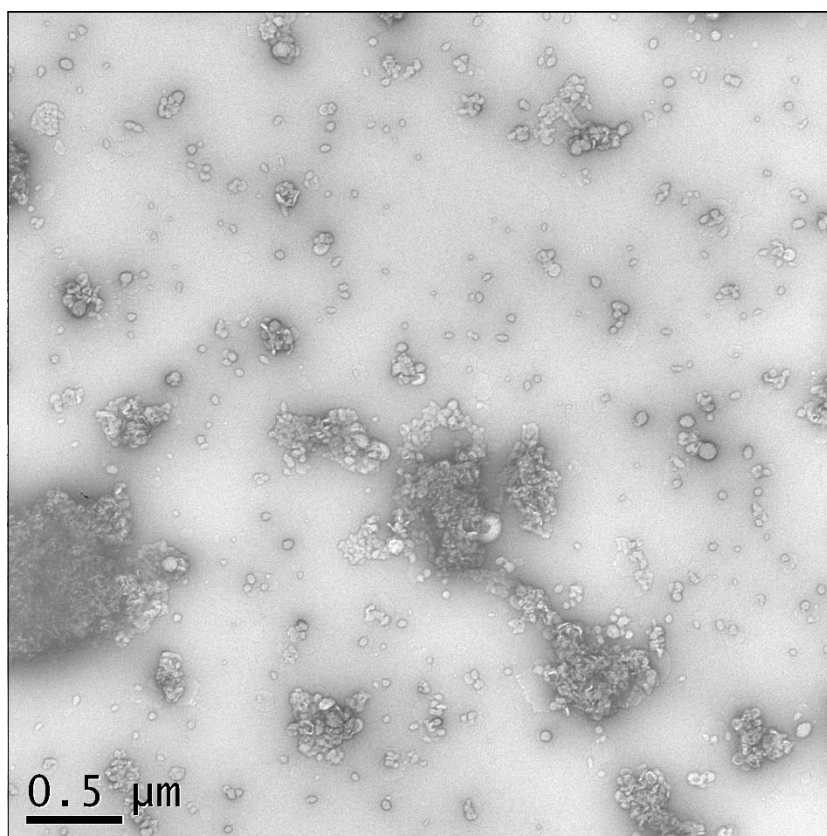


Figure 7.322: – TEM micrograph for POPC: DC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

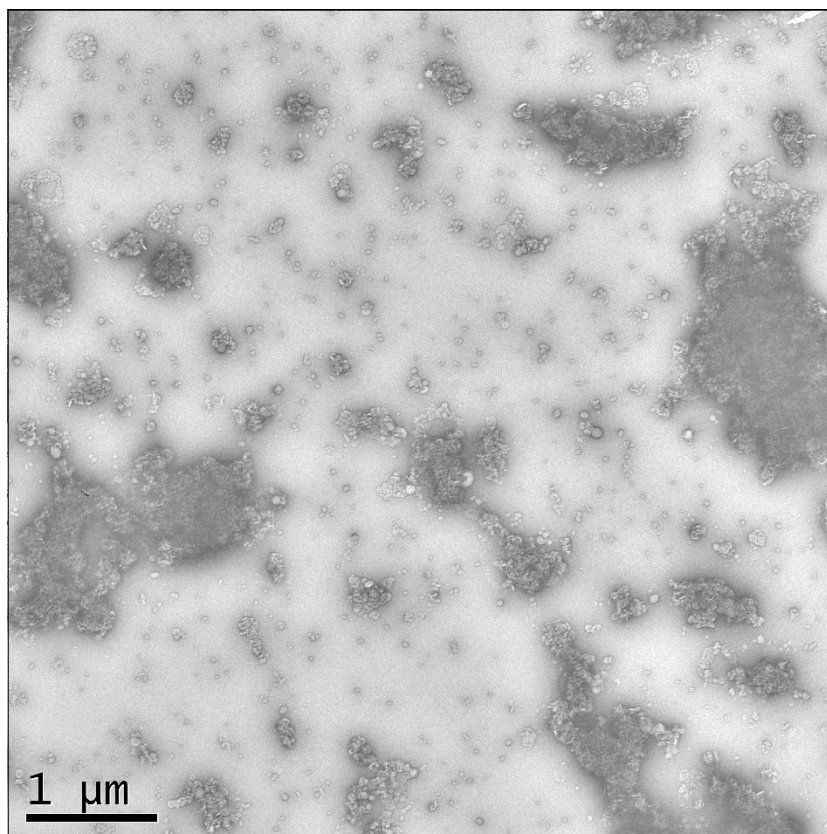


Figure 7.323: – TEM micrograph for POPC: DC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

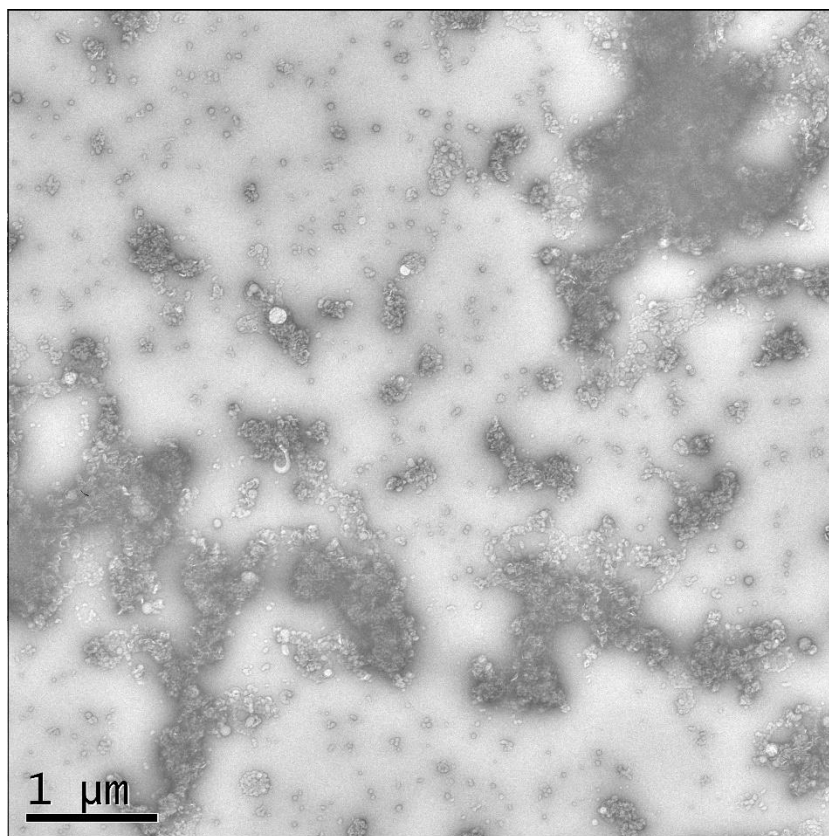


Figure 7.324: – TEM micrograph for POPC: DC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

25% POPC 75% singly charged PDMAm50PDAAm300

t= 0 min

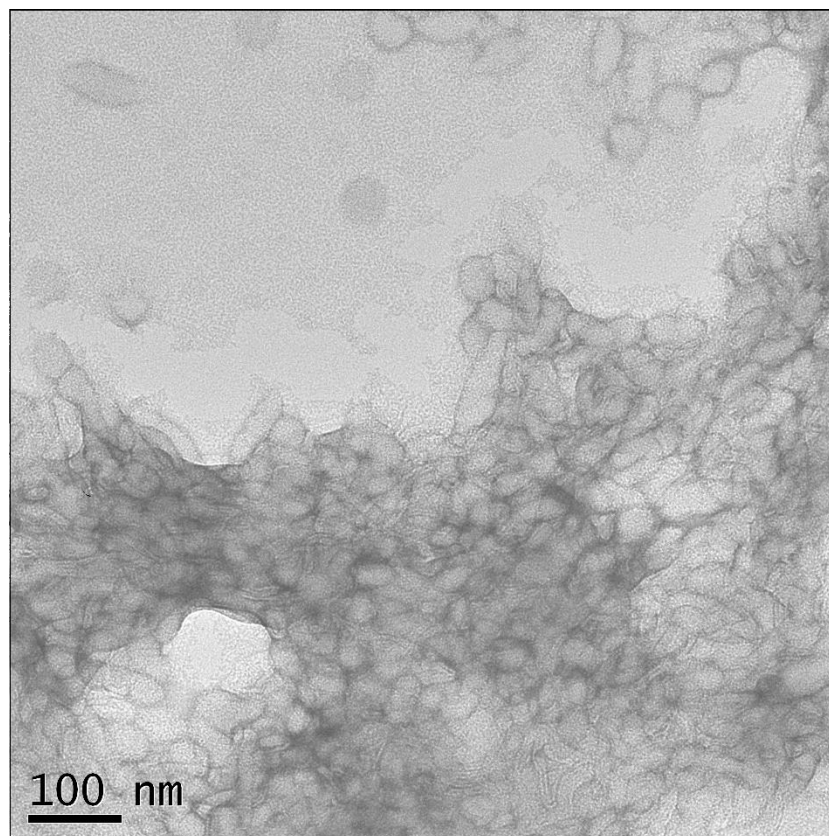


Figure 7.325: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 0 min)

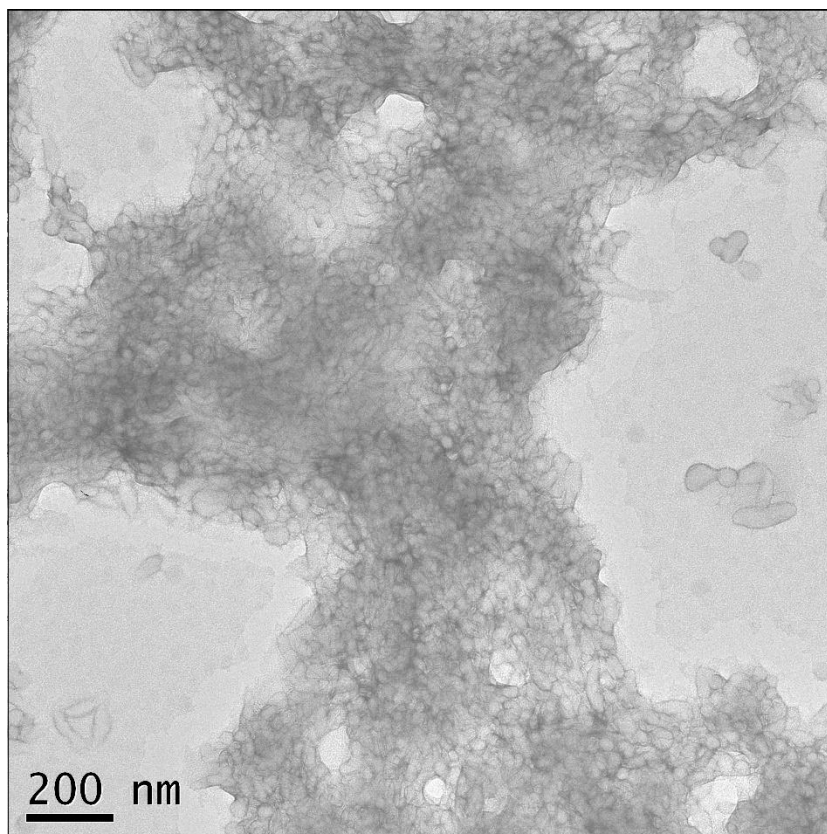


Figure 7.326: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

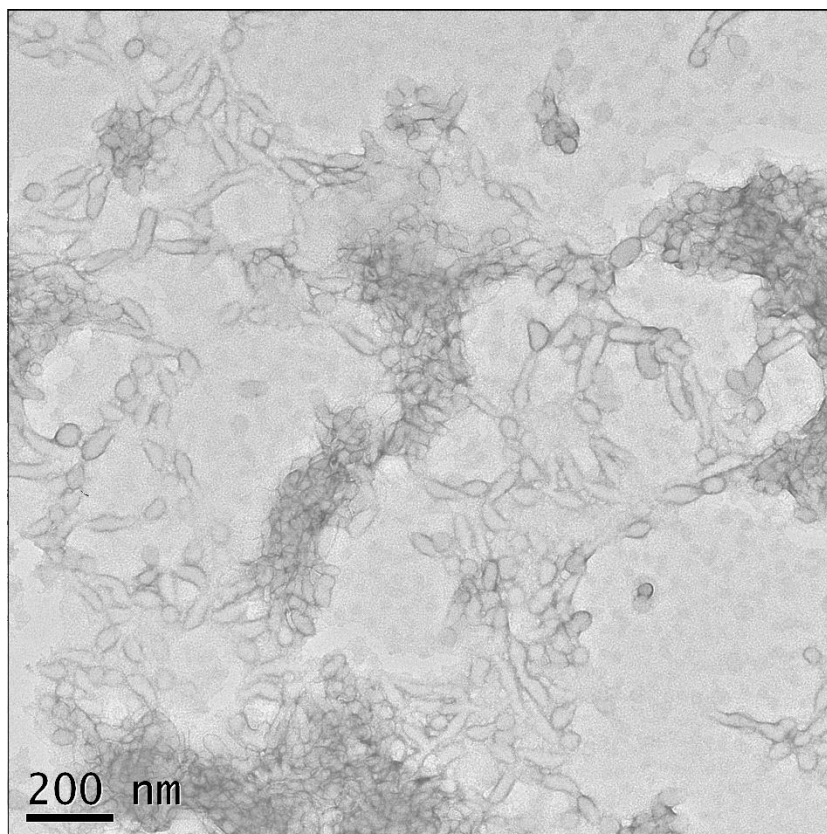


Figure 7.327: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

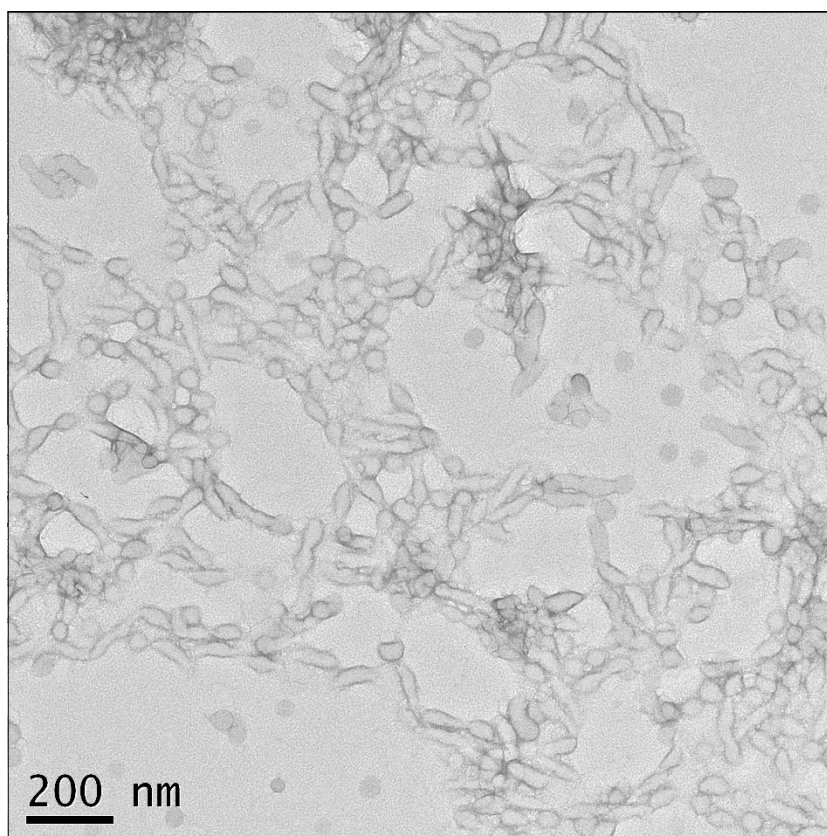


Figure 7.328: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

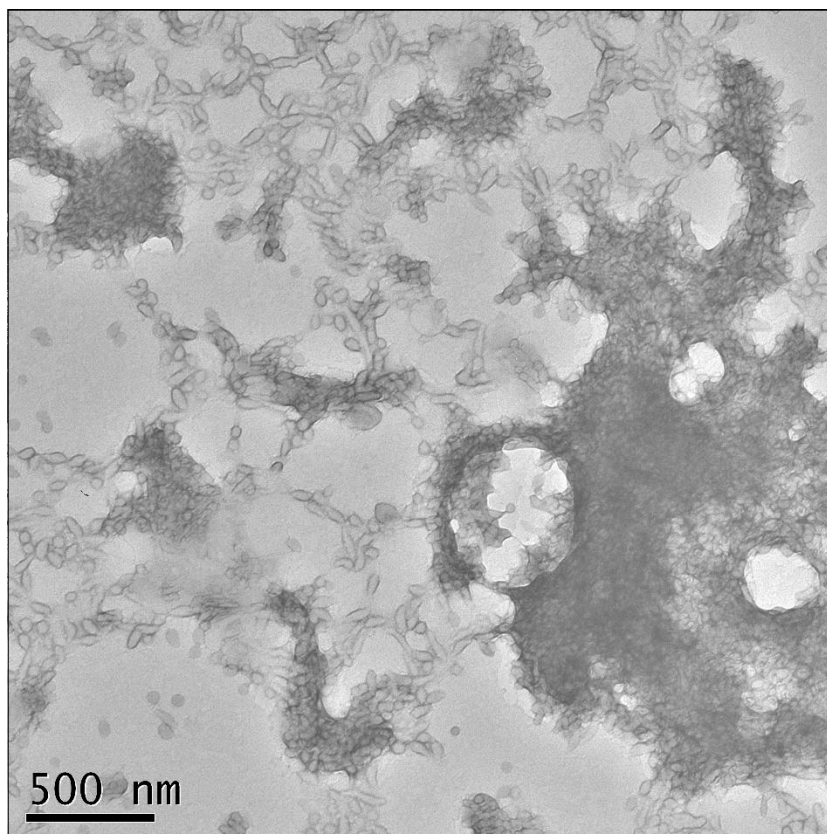


Figure 7.329: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

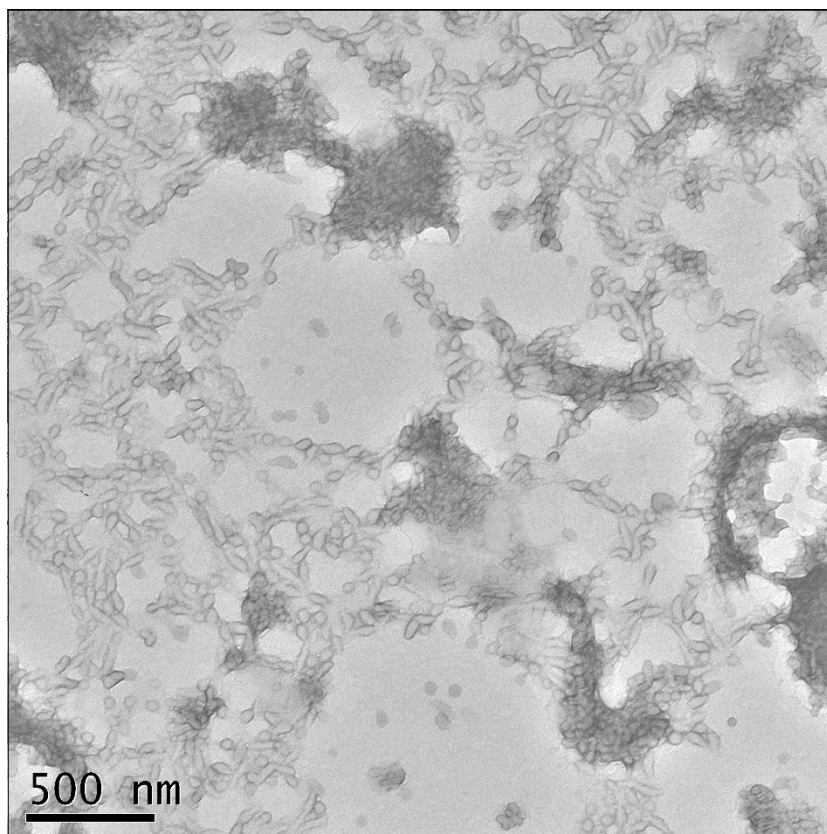


Figure 7.330: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

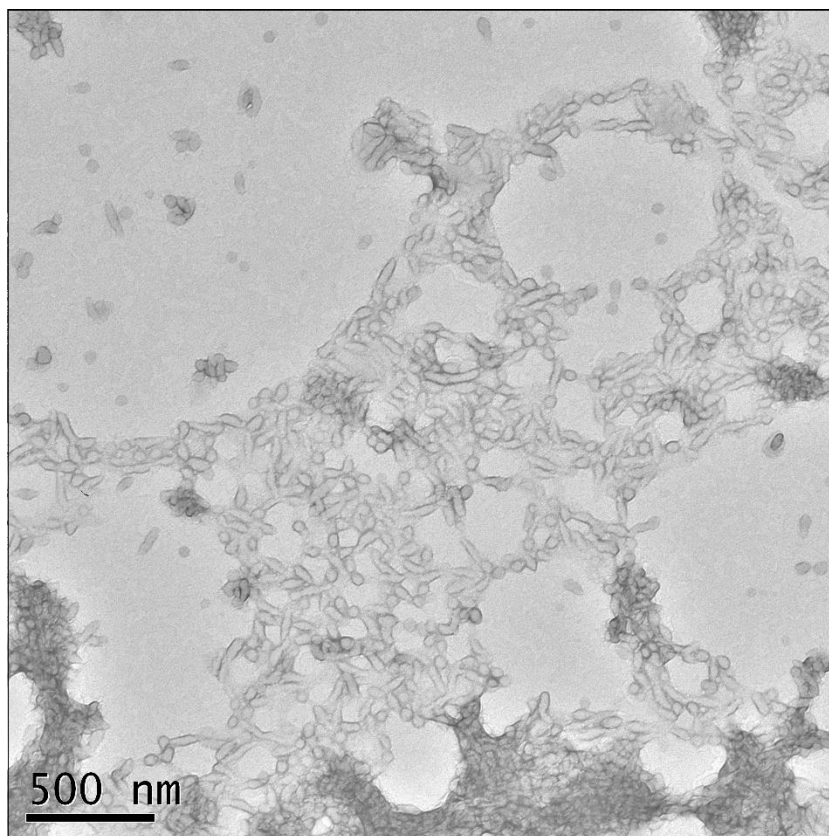


Figure 7.331: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

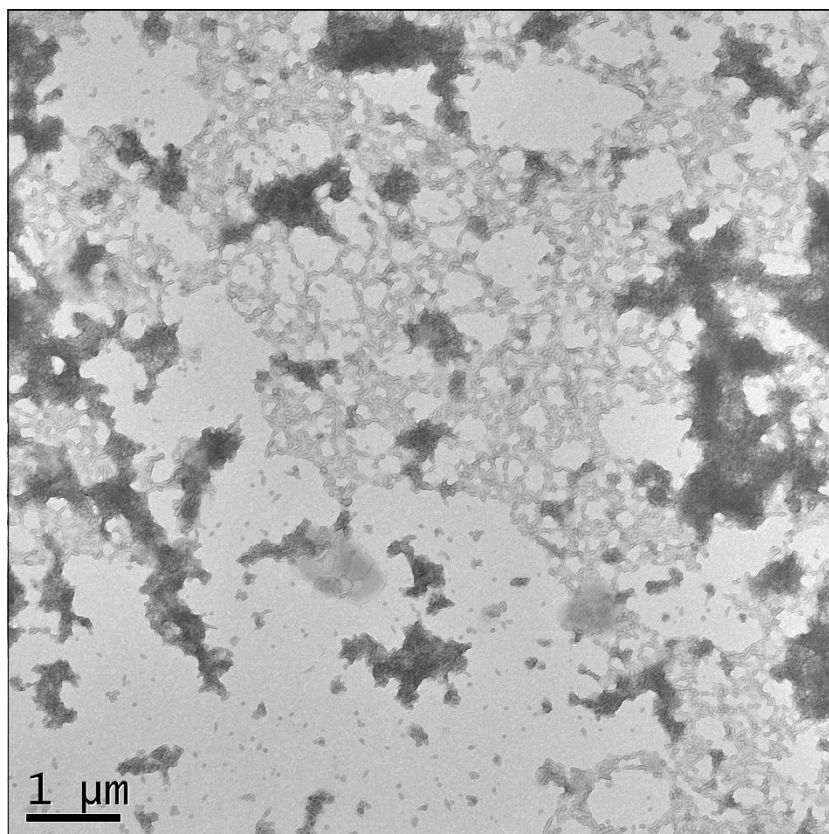


Figure 7.332: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

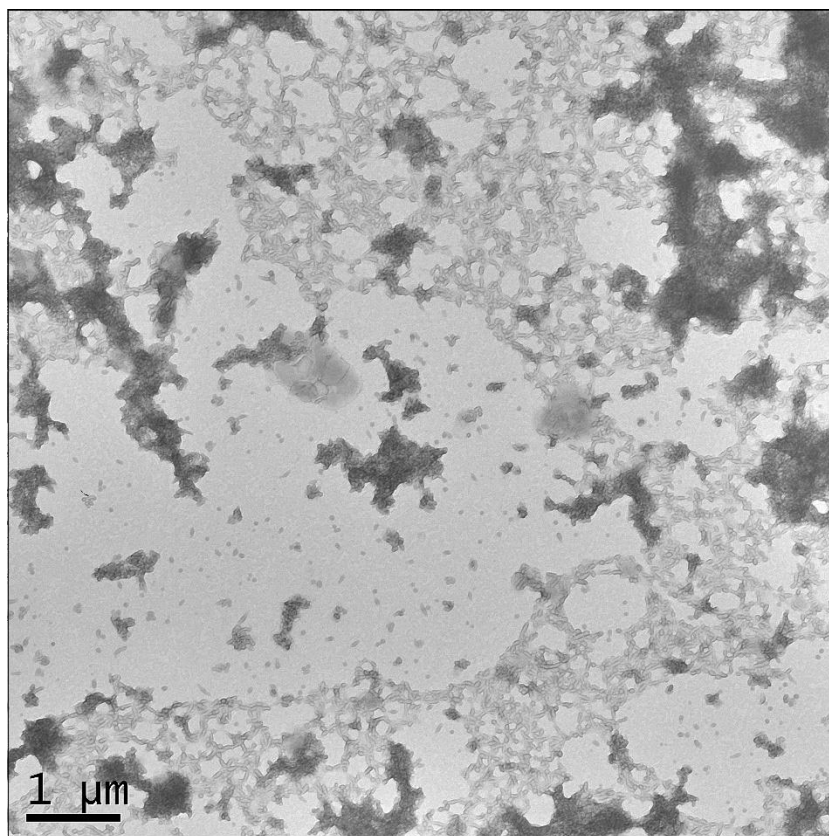


Figure 7.333: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

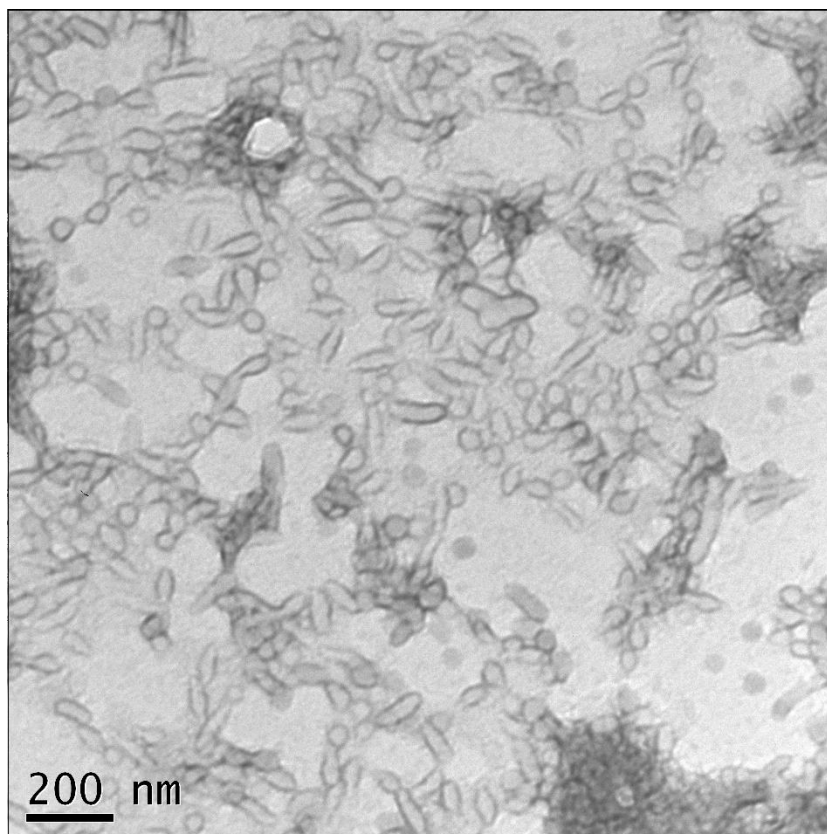


Figure 7.334: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

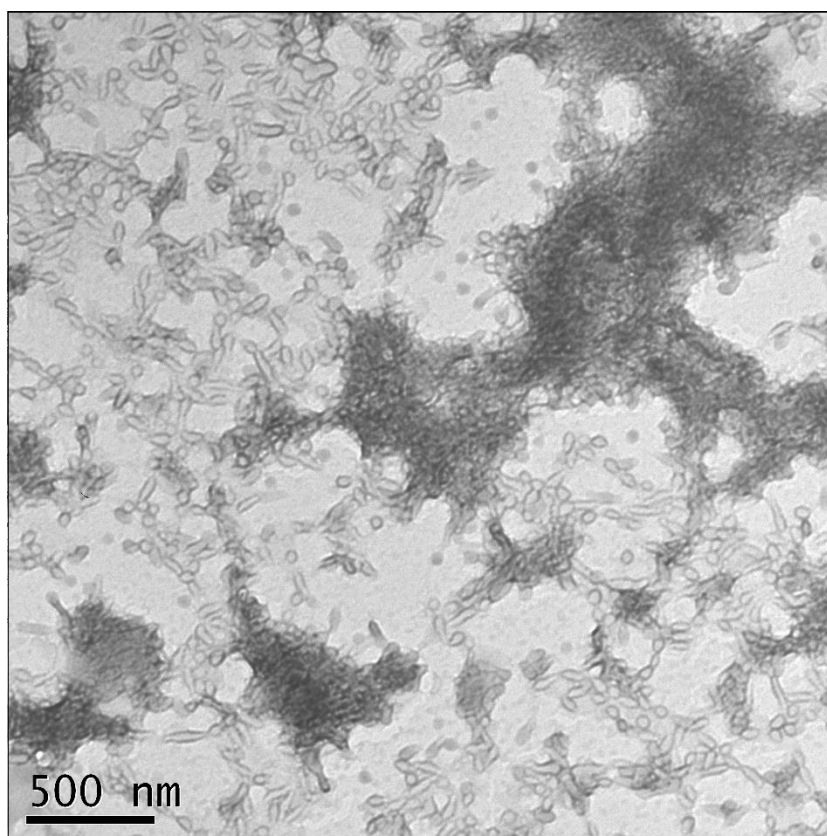


Figure 7.335: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

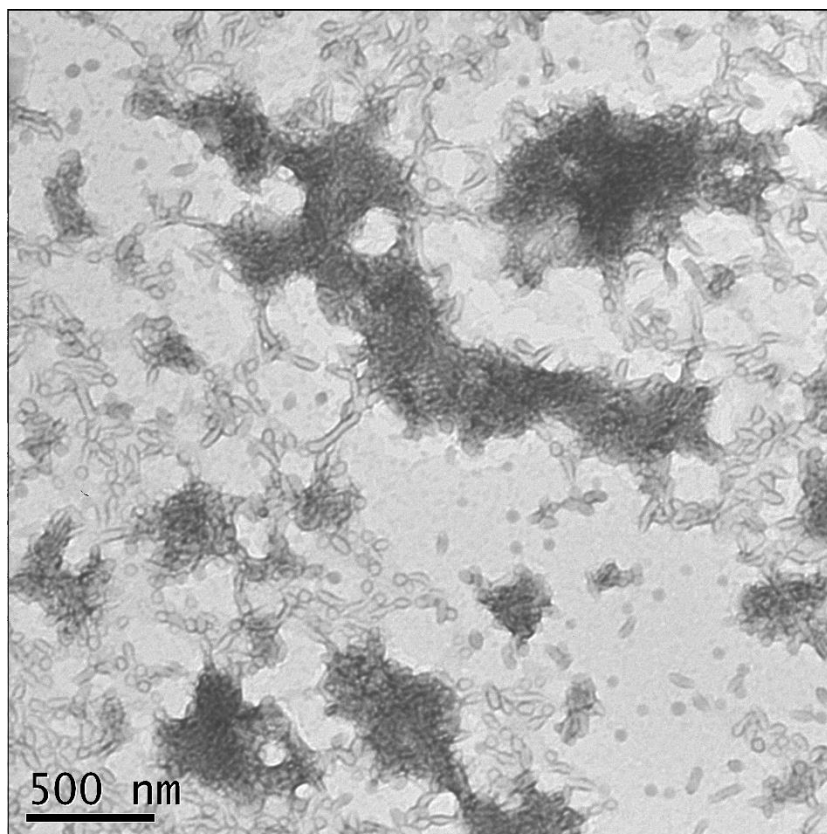


Figure 7.336: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

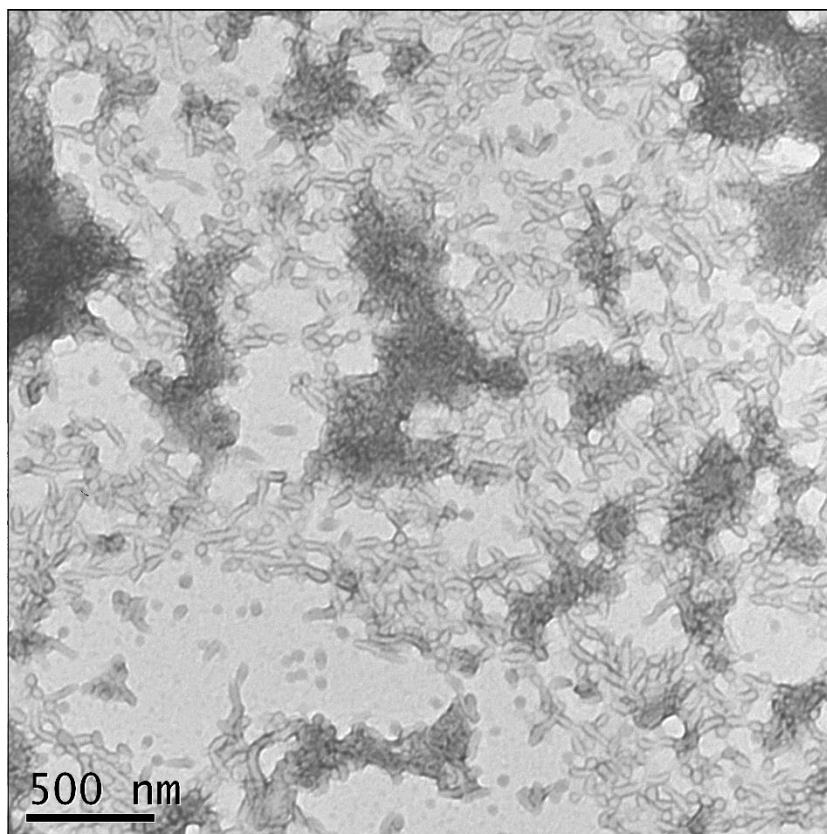


Figure 7.337: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 0$ min)

25% POPC 75% Singly charged PDMAm50PDAAm300

t= 30 min

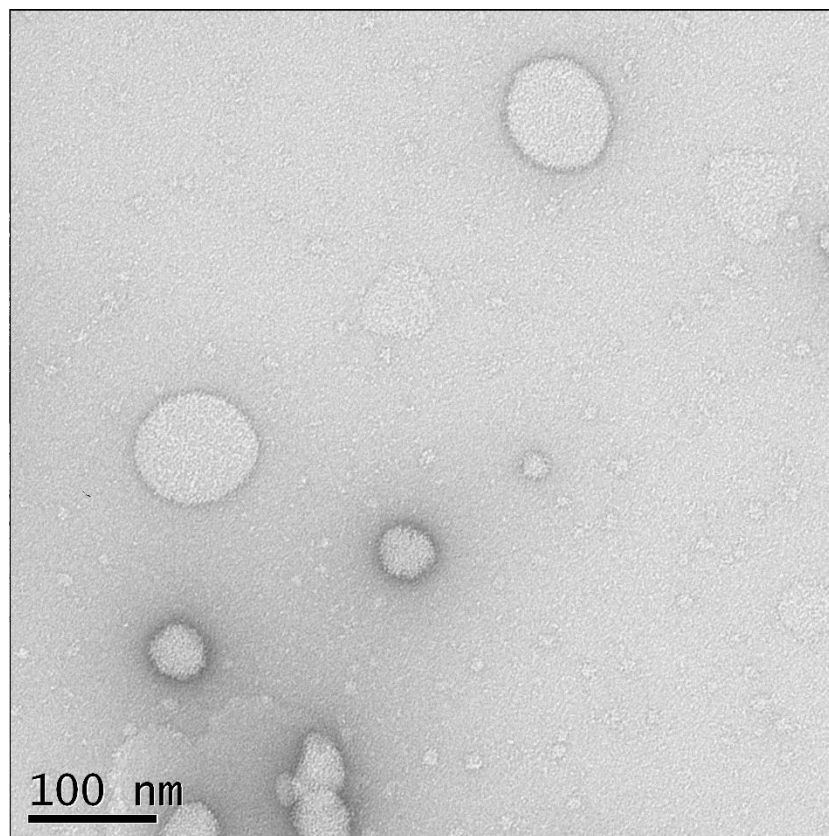


Figure 7.338: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – t = 30 min)

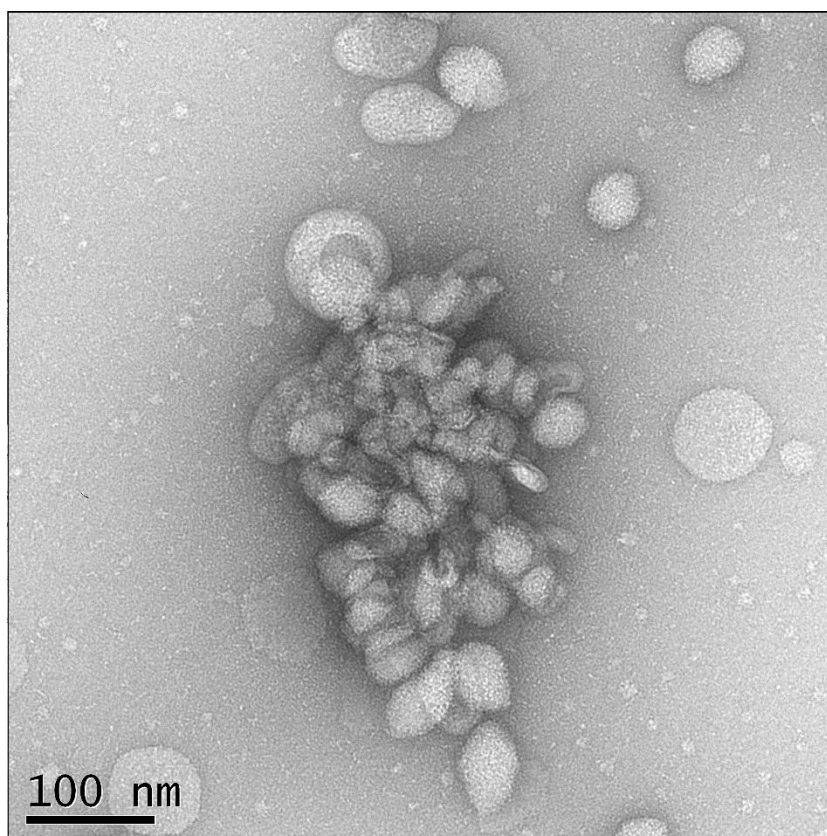


Figure 7.339: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

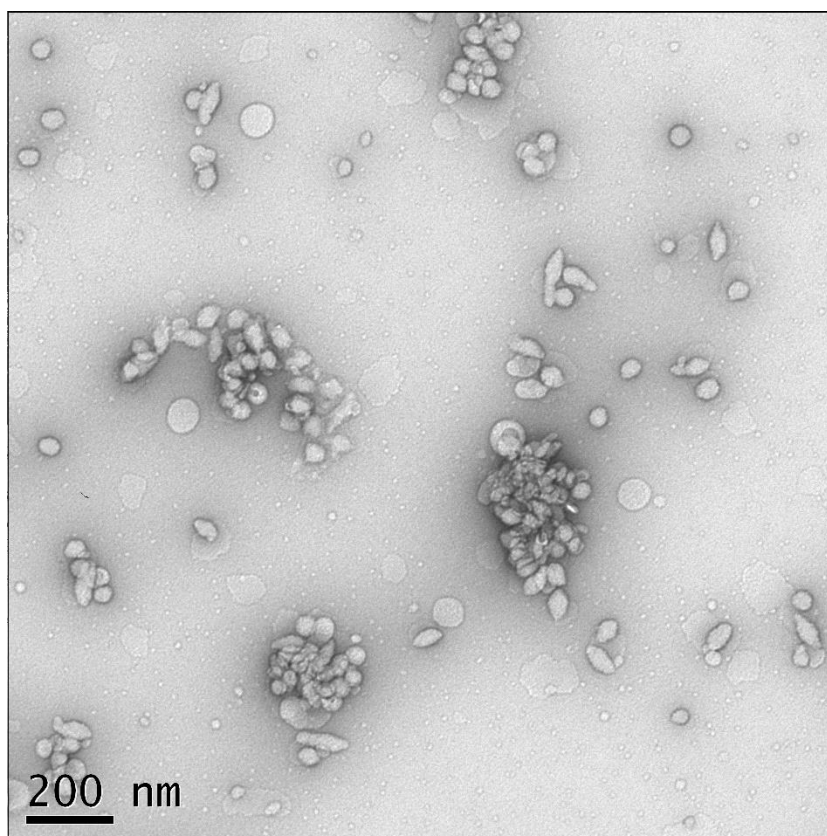


Figure 7.340: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

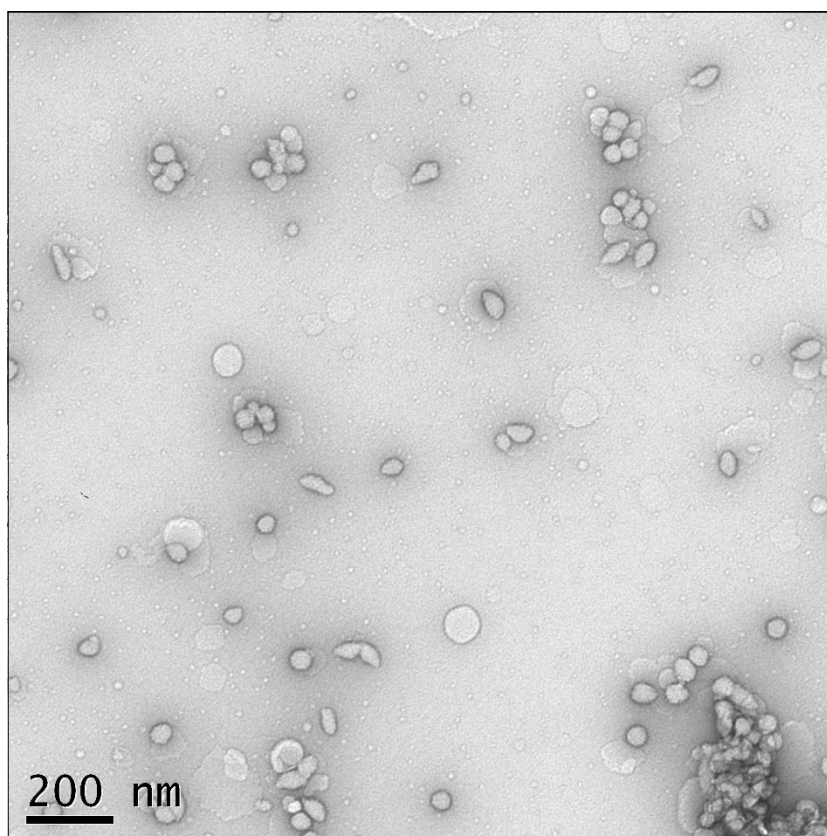


Figure 7.341: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

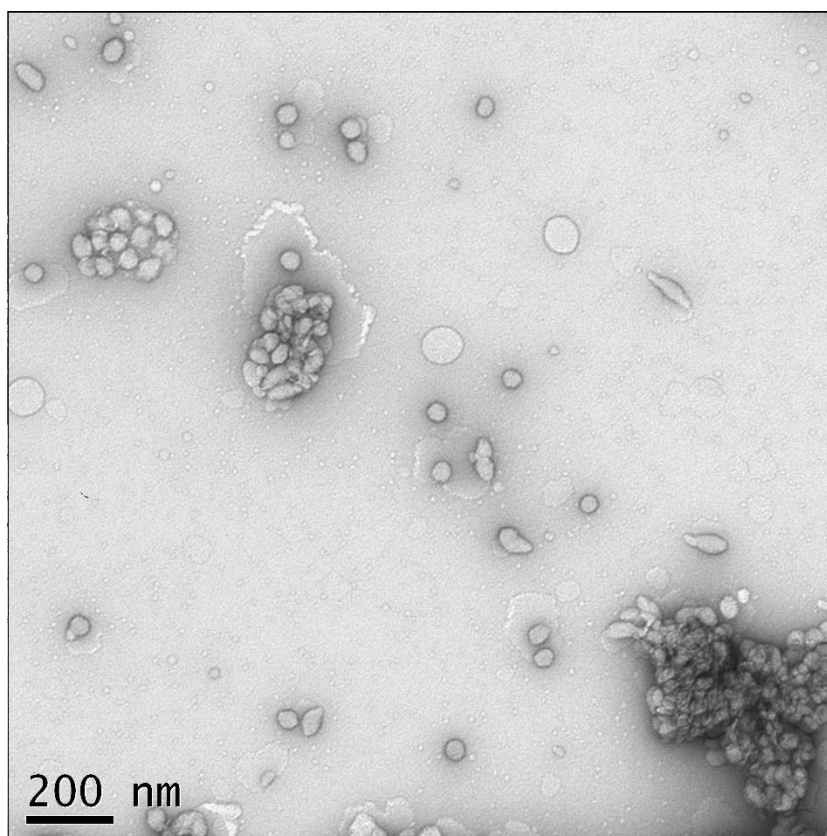


Figure 7.342: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

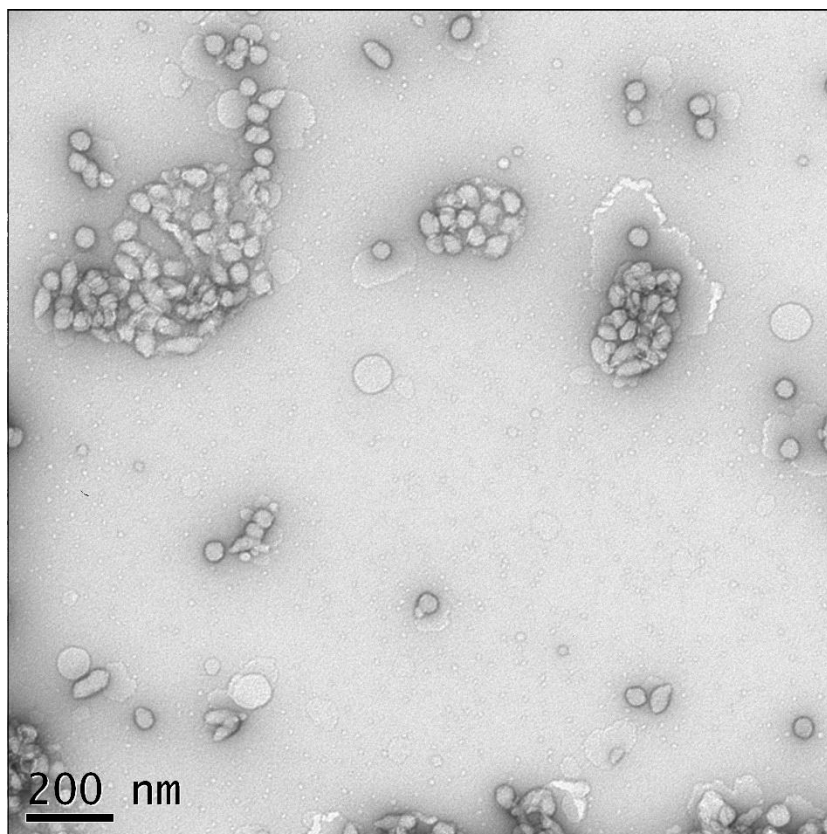


Figure 7.343: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

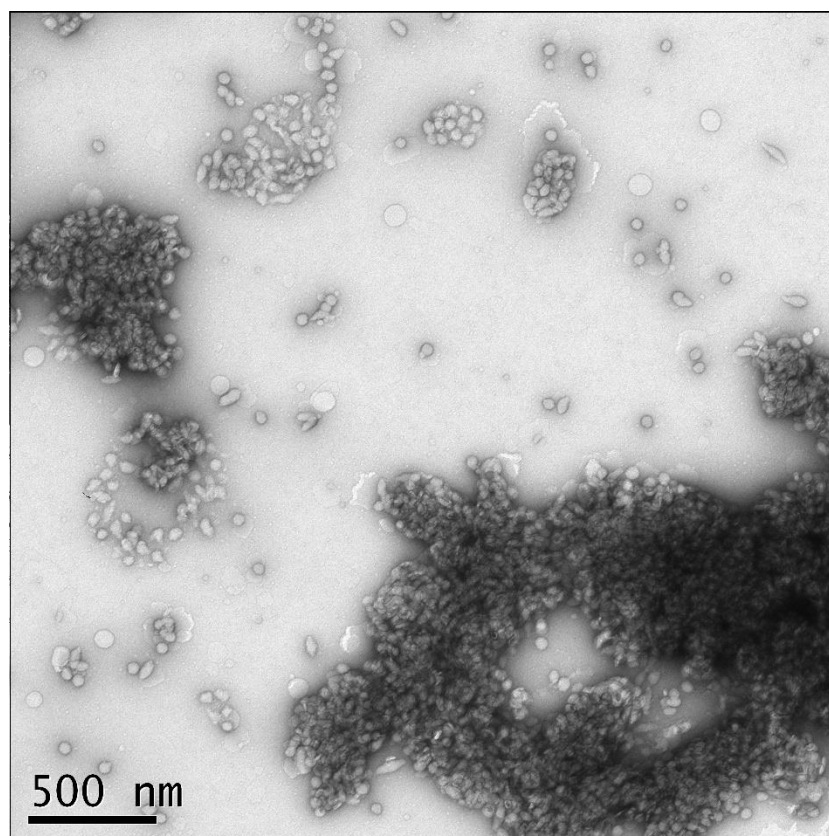


Figure 7.344: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

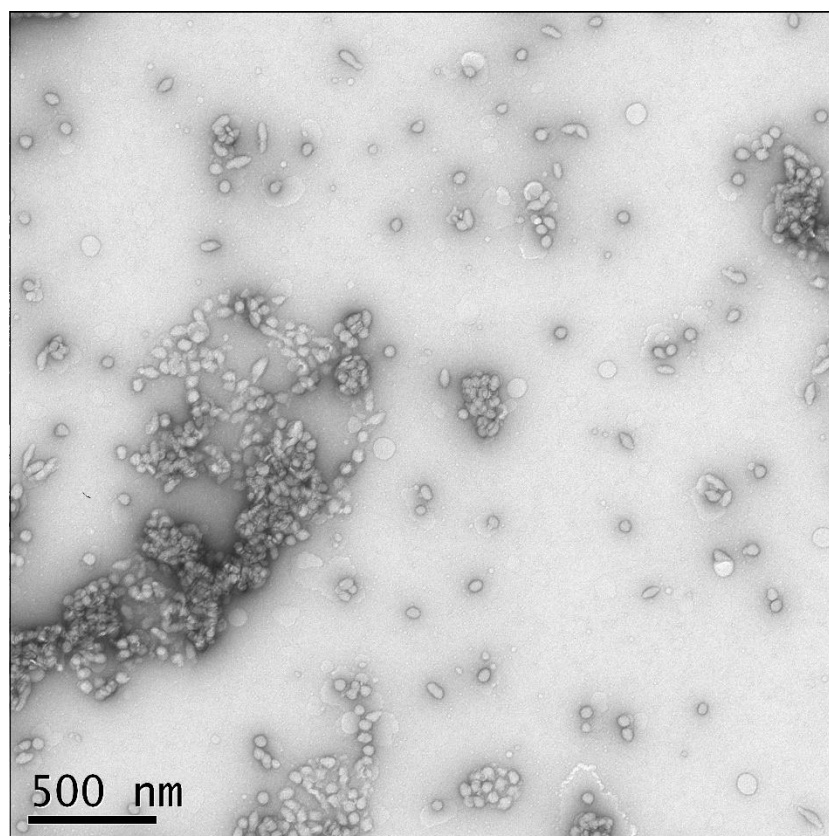


Figure 7.345: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

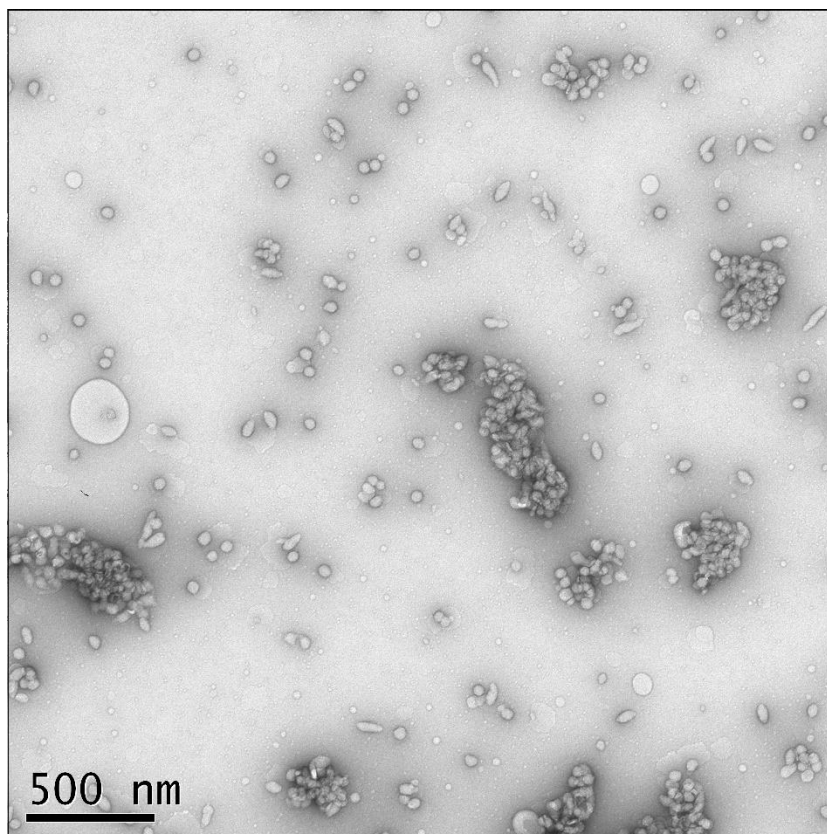


Figure 7.346: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

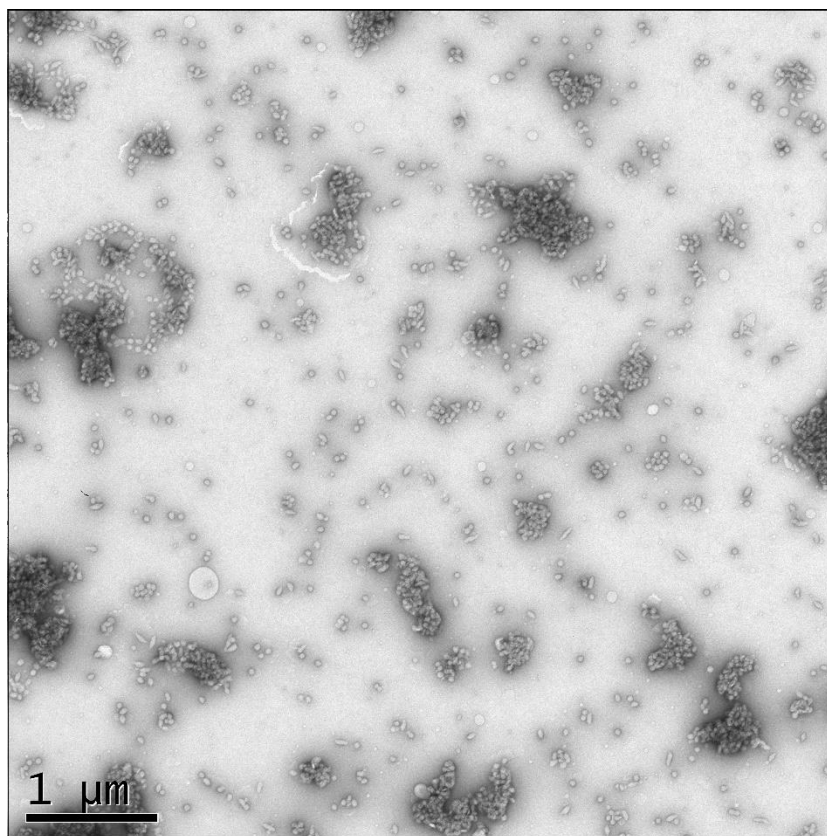


Figure 7.347: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

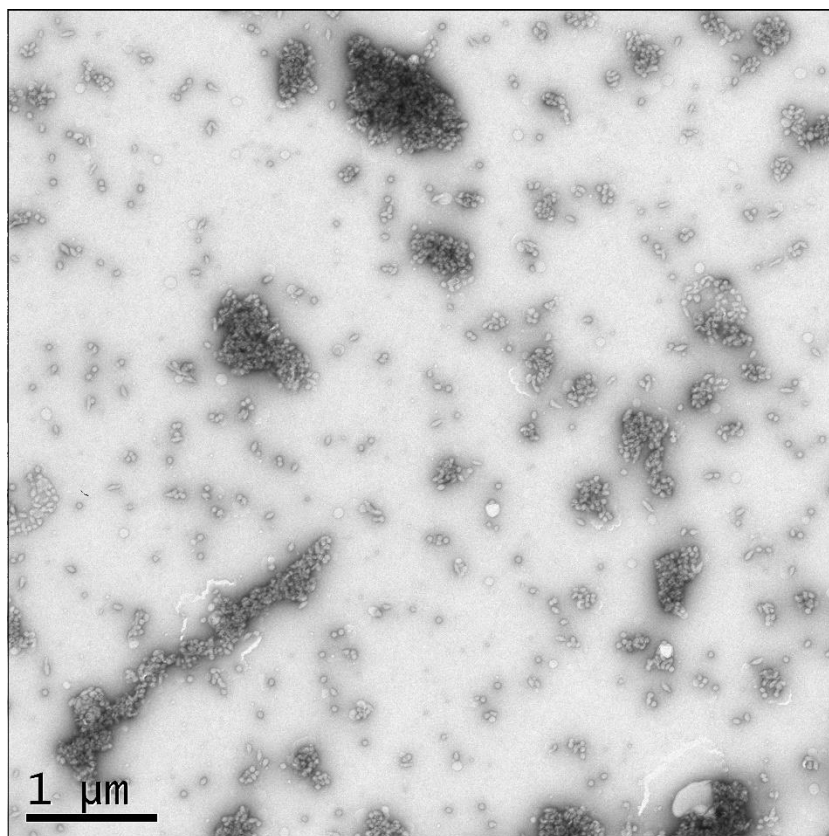


Figure 7.348: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

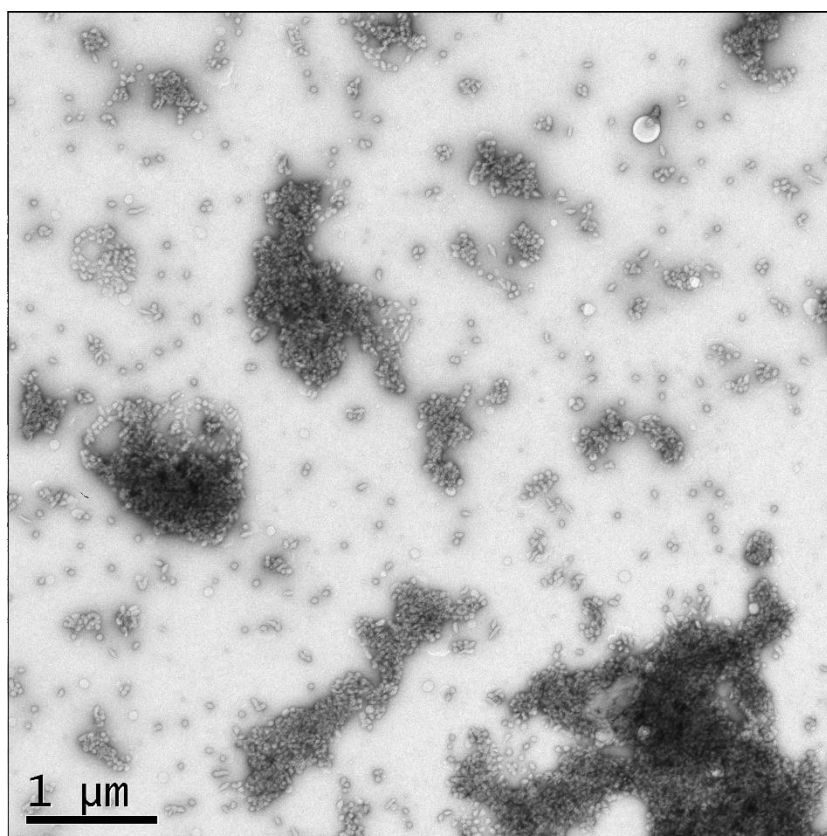


Figure 7.349: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)

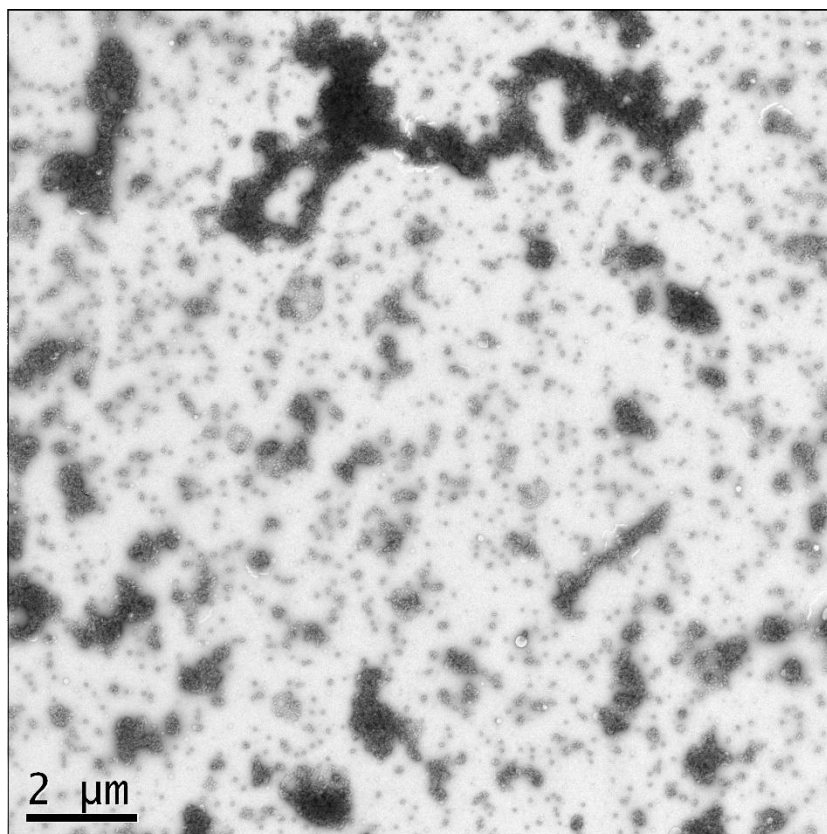


Figure 7.350: – TEM micrograph for POPC: SC BCP (25:75) nano-objects synthesised via SE at pH 7.5 with 140 mM NaCl (50% MeOH – Rapid Addition – $t = 30$ min)