

γ -cyclodextrin metal-organic frameworks for controlled release

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The candidate confirms that the work submitted is her own, except where work which has formed part of jointly-authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others. Details of the jointly-authored publications and the contributions of the candidate and the other authors to the work are outlined on the pages II and III.

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Further details of the jointly-authored publications and the contributions of the candidate and the other authors to the work are included below:

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Abstract

This thesis explored the use of γ -cyclodextrin metal-organic frameworks (γ -CD-MOFs) as a controlled-release delivery method for curcumin, a bioactive compound with significant pharmacological properties but low bioavailability due to low solubility and rapid degradation. The research aims to enhance the solubility, stability, and bioaccessibility of curcumin by utilizing the unique structural functional features of γ -CD-MOFs, which combine the porous nature of metal-organic frameworks with the biocompatibility of cyclodextrins.

The synthesis of γ -CD-MOFs was investigated after a thorough review that highlighted its distinct structural characteristics, biocompatibility, and potential as delivery vehicles for bioactive compounds. This demonstrated that the choice of solvent had a major impact on crystal morphology and that the porosity was essential to the functional performance of γ -CD-MOFs. Further investigation of the interaction between curcumin and γ -CD-MOFs indicated that curcumin may be present on crystal surfaces or between γ -CD pairs, in addition to the hydrophobic cavities of γ -CD-MOFs, affecting its release profile. Investigations into the synergistic effects of γ -CD-MOFs with surfactant micelles revealed that curcumin's apparent solubility and bioaccessibility were improved. However, when γ -CD-MOFs were incorporated into emulsion systems, it became clear that they were unstable in acidic gastric conditions. A promising delivery method for preventing γ -CD-MOFs from degrading was found to be capsule-based delivery, enabling prolonged release as well as enhanced intestinal bioaccessibility and absorption.

In summary, this thesis highlights the potential of γ -CD-MOFs as a versatile and effective delivery system for controlled release, offering a pathway to enhance the bioavailability of curcumin and potentially other poorly soluble bioactive compounds. These insights contributed to the growing body of knowledge on γ -CD-MOFs, paving the way for their application in pharmaceutical and functional food formulations.

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List of Abbreviations

$(\text{NH}_4)_2\text{CO}_3$	Ammonium carbonate
$(\gamma\text{-CD})_6$	Cavities of γ -CD-MOFs
A β	Amyloid-beta
AKT	Protein kinase B
AOAC	Association of Official Agricultural Chemists
ATM	Ataxia-telangiectasia mutated
BCS	Biopharmaceutical Classification System
BET	Brunauer-Emmett-Teller
C-H	Carbon-hydrogen bonds
C=C	Alkene bonds
C=O	Carbonyl group
C ₆₀	Fullerene
CaCl ₂	Calcium chloride
CaCl ₂ (H ₂ O) ₂	Calcium chloride dihydrate
Caco-2	Human colorectal adenocarcinoma cell line
CAGR	Compound annual growth rate
CD-MOFs	Cyclodextrin metal-organic frameworks
CDs	Cyclodextrins
CMC	Critical micelle concentration
c-Myc	Cellular myelocytomatosis oncogene
CoQ10	Coenzyme Q10
COVID-19	Coronavirus disease 2019
CPMC	Critical premicelle concentration
CTAB	Cetyltrimethylammonium bromide
Cu-K α	Copper K-alpha radiation
CUR	Curcumin
Cur- γ -CD-MOFs	Curcumin-loaded γ -cyclodextrin metal-organic frameworks
CVDs	Cardiovascular disease
DAD	Diode array detector
dH ₂ O	Deionized water
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid

DOX	Doxorubicin
EE	Encapsulation efficiency
FAO	Food and Agriculture Organization
FCCM	First critical micelle concentration
FDA	Food and Drug Administration
FFAs	Free fatty acids
FTIR	Fourier transform infrared spectroscopy
GOPOD	Glucose oxidase/peroxidase
GSH	Glutathione
h	Hours
HCl	Hydrochloric acid
HCT 116	Human colorectal carcinoma cell line
HepG2	Human hepatocellular carcinoma cell line
HLB	hydrophile-lipophile
HPLC	High-performance liquid chromatography
HuH-7	Human hepatocyte-derived carcinoma cell line
IGF-1	Growth factor-1
K ⁺	Potassium ion
KCl	Potassium chloride
KH ₂ PO ₄	Potassium phosphate monobasic
KOH	Potassium hydroxide
LC	Loading capacity
LDL	Low-density lipoprotein
MAPK	Mitogen-activated protein kinase
MgCl ₂ (H ₂ O) ₆	Magnesium chloride hexahydrate
min	Minutes
MOFs	Metal-organic frameworks
Mw	Molecular weight
N ₂	Nitrogen
Na ₂ C ₂ O ₄	Sodium oxalate
NaCas	Sodium caseinate
NaCl	Sodium chloride
N _{agg}	Aggregation number
NaHCO ₃	Bicarbonate of soda

NaOH	Sodium hydroxide
NDDs	Neurodegenerative disease
NF- κ B	Nuclear factor-kappaB
NMR	Nuclear magnetic resonance
NO	Nitric oxide
NOS	Nitric oxide synthases
O/W	Oil-in-water
-OH	Hydroxyl groups
PAA	Polyacrylic acid
PDF	Pair distribution function
PDI	Polydispersity index
PEG	Polyethylene glycol
PFA	Paraformaldehyde
pI	Isoelectric point
pK _a	Acid dissociation constant
PKC	Protein kinase C
PI3K	Phosphatidylinositol 3-kinase
PSD	Particle size distribution
PXRD	Powder X-ray diffraction
ROS	Reactive oxygen species
S/O/O	Solid-in-oil-in-oil
SCMC	Secondary critical micelle concentration
SEM	Scanning electron microscopy
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
SLS	Static light scattering
SNPs	Starch nanoparticles
SOD	Superoxide Dismutase
SSF	Simulated salivary fluid
TEER	Transepithelial electrical resistance
UHPLC	Ultra high-performance liquid chromatography
UV	Ultraviolet
Vis	Visible
WHO	World Health Organization

W/O

Water-in-oil

 γ -CD-MOFs γ -cyclodextrin metal-organic frameworks

List of Symbols

π	Pi
2θ	Two-theta angle
α	Alpha
β	Beta
d_{43}	Volume mean diameter
ζ	Zeta
λ	Wavelength

Chapter 1

General Introduction

1.1 Objectives

Bioactive compounds offer numerous health benefits and play a key role in preventing diseases and metabolic disorders, as demonstrated in various pharmacological studies (Câmara et al., 2021; Bergonzi et al., 2022). They exhibit antioxidant, anti-allergic, anti-inflammatory, antimicrobial, anticarcinogenic, and antimutagenic properties which are vital for human health. Additionally, these compounds were also found to have anti-aging effects, making them valuable in the cosmetic industry. A report by Grand View Research, Inc. states that the bioactive ingredients market was valued at USD 173.22 billion in 2022 and is expected to grow at a 7.9% compound annual growth rate (CAGR), reaching USD 317.9 billion by 2030 (Anon, n.d.). This growth is fuelled by increasing demand for functional foods, dietary supplements, and personal care products with health-promoting properties. Moreover, the rapid spread of COVID-19 (coronavirus disease 2019) pandemic led to a frantic search for new therapies, thereby propelling the research, development, and market growth of immune-boosting food bioactives (Galanakis et al., 2020; Quiles et al., 2020). The market's expansion highlights the growing importance of bioactive compounds in improving human health.

One major limitation to the widespread use of bioactive compounds is their instability and loss of activity, particularly in food applications, as many studies confirming their benefits are conducted under controlled conditions (Pai et al., 2022). Most bioactive substances are known to degrade during processing and storage, leading to the loss of their bioactivity and potential health benefits (Cao et al., 2021). Some bioactives are poorly absorbed and have reduced or low bioavailability in the gastrointestinal tract. Individual variability also plays a crucial role in determining their nutritional and therapeutic effects. Factors such as differences in absorption, metabolism, age, gender, and lifestyle can

lead to varying outcomes when these compounds are consumed by different individuals within a population.

Curcumin, the bioactive polyphenolic compound in turmeric (*Curcuma longa*), has attracted considerable attention for its pharmacological and potentially therapeutic effects such as anti-inflammatory, antioxidant, anticancer, and antibacterial activities (Gupta et al., 2012; Kocaadam and Şanlıer, 2017; Pagano et al., 2018). However, its clinical application is significantly hindered by its poor aqueous solubility (approximately 0.6 µg/mL), low bioavailability and rapid metabolism, often requiring the use of high oral doses exceeding 1 g per day to achieve potential therapeutic effect (Anand et al., 2007; Górnicka et al., 2023). In a clinical study conducted by Lao et al. (2006), curcumin was administered at doses ranging from 500 mg to 12 g/day, with minimal toxicity of mild gastrointestinal irritation such as nausea and diarrhoea reported at doses up to 8 g/day. Despite such high intake, plasma levels of free curcumin were barely detectable due to extensive first-pass metabolism and rapid systemic clearance. Following a regulatory toxicological assessment, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established an acceptable daily intake (ADI) for curcumin of 3 mg/kg body weight/day, based on a no-observed-adverse-effect level (NOAEL) of 250-320 mg/kg body weight/day derived from long-term animal studies (World Health Organization, 2004). These safety thresholds were also supported by the European Food Safety Authority (EFSA) in its re-evaluation of curcumin as a food additive (European Food Safety Authority, 2014). However, the discrepancy between therapeutic dosing and systemic availability highlights the need for improved delivery strategies.

With growing demand, advances such as encapsulation are driving innovation where an active substance is temporarily entrapped within a shell-forming material. Encapsulation strategies show good potential and versatility as carriers in retaining bioavailability, enhancing stability, reducing side effects while facilitating the controlled release of bioactive compounds for targeted delivery (Alu'datt et al., 2022). Hence, it has been extensively researched and widely implemented in various sectors such as pharmaceuticals, cosmetics, analytical chemistry, and food industries (Figueroa-Robles et al., 2021; Reis et al., 2022; Yan and Kim, 2024).

The primary objective of this research is to gain a better understanding of γ -cyclodextrin metal-organic frameworks (γ -CD-MOFs), a biocompatible, porous, “cage-like” particles, as an encapsulation strategy. The inclusion complex formed by γ -CD-MOFs with bioactive compounds are reported to improve the solubility and bioavailability of poorly soluble drugs, enhance their stability against degradation, mask unpleasant odours, and allows for sustained or controlled release (Abuçafy et al., 2018; Roy and Stoddart, 2021; Yang et al., 2024). While previous studies have shown the potential of γ -CD-MOFs in encapsulating curcumin, the underlying mechanism in improvements in solubility and bioaccessibility remain insufficiently understood (Moussa et al., 2016; Chen et al., 2021; Sun et al., 2021). Additionally, the aqueous stability of γ -CD-MOFs poses a significant challenge to their application, necessitating strategies to main structural integrity and support the prolonged solubilization of curcumin during digestion and absorption. Hence, the three main research objectives identified are: (1) to compare synthesis solvents of γ -CD-MOFs for a more food-grade application; (2) to assess the possible synergistic effect of γ -CD-MOFs with micelles in enhancing bioaccessibility; and (3) to evaluate the bioaccessibility and absorption of curcumin post-encapsulation in γ -CD-MOFs following *in vitro* digestion and *ex vivo* absorption studies. The bioavailability and absorption of bioactives encapsulated in γ -CD-MOFs are also compared to a traditional coarse emulsion. The hypothesis being tested is that γ -CD-MOFs could work synergistically with micelles to improve solubility of curcumin, and also significantly boost its bioaccessibility and absorption. The overall experimental workflow is illustrated in Figure 1-1.

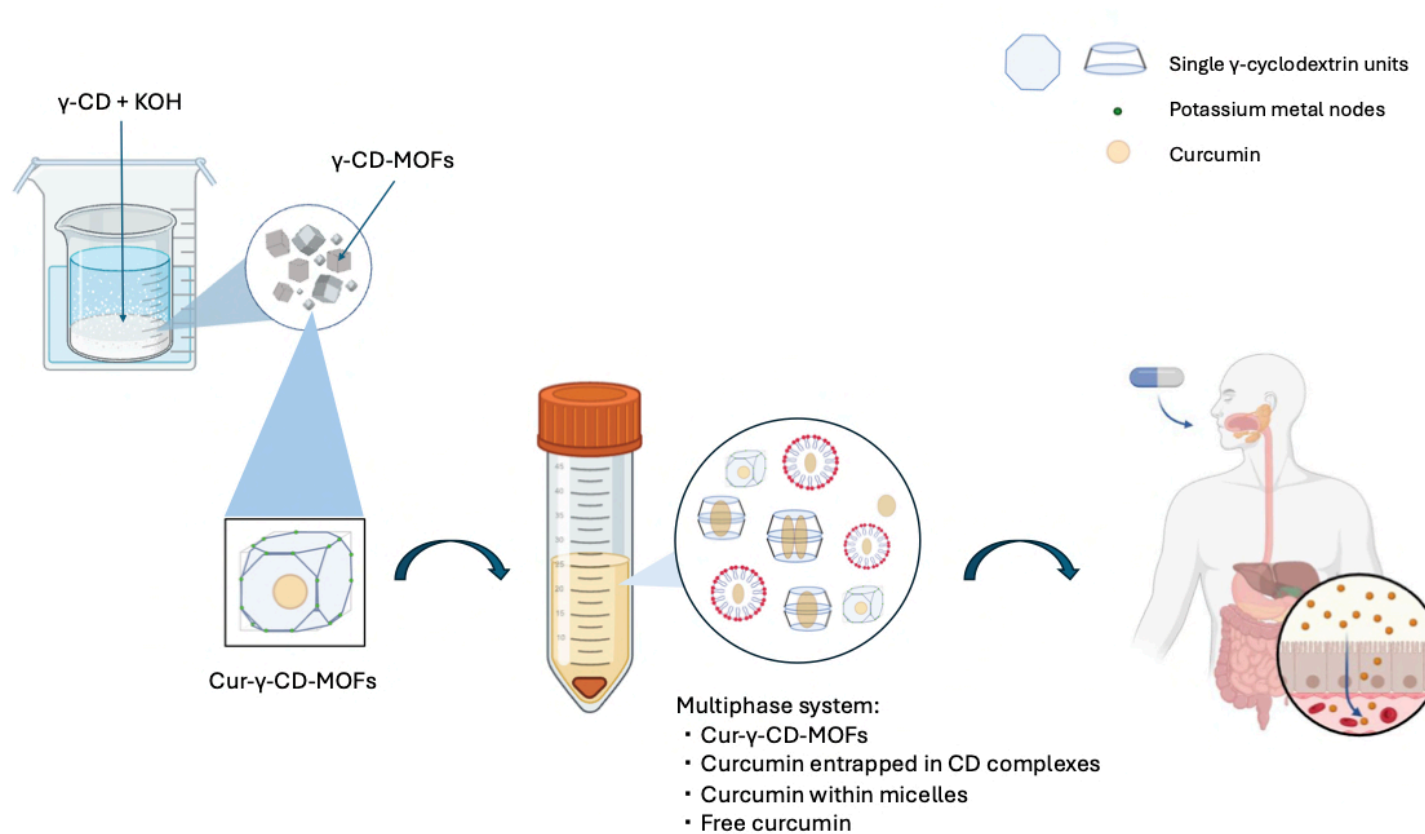


Figure 1-1 Schematic representation of encapsulation and delivery of curcumin within γ -CD-MOFs, illustrating structural components of γ -CD units coordinated with potassium ions, curcumin inclusion within MOF matrix and micelles and behaviour during digestion and potential intestinal absorption.

1.2 Bioactive compounds

Naturally present compounds in food that exert a certain bioactive effect on the human body beyond the basic nutritional properties are referred to as food bioactive compounds (Teodoro, 2019). Such compounds include polyphenols, phytosterols, terpenoids, polysaccharides, saponins, carotenoids and tocopherols, glucosinolates, triterpenes, and alkaloids, capsaicinoids, peptides, and polyunsaturated fatty acids (Câmara et al., 2021; Pai et al., 2022). The wide range of food bioactive compounds are mostly secondary metabolites produced by plants, and provide varying functionality in plant: competition, defence, attraction, and signalling (Anjali et al., 2023).

Extensive experimental studies on food bioactive compounds and the resulting evidence showed a positive biological impact on human health with protective effects against non-communicable diseases, metabolic and neurogenerative disorders. The general protective mechanism used by these compounds are said to be antioxidative protection, protection against deoxyribonucleic acid (DNA) damage, improved immunity and hormone modulation (Carbonell-Capella et al., 2014; Samtiya et al., 2021). Nevertheless, the amount and effect of bioactive naturally present in foods, mainly in fruits, vegetables, whole grains, oils, marine products, medicinal plants, and herbal plants, may differ as a result of the origin, environmental condition, cultivation, storage, and any food processing it has undergone (Andre et al., 2007). As a result, content and composition of bioactive compounds in plant foods are difficult to compare.

1.2.1 Polyphenols

Phenolic acids and polyphenols are categorized as phenolic compounds, the main class of secondary plant metabolites. Synthesis of these compounds is not possible in the human body and thus they are largely obtained from food. Molecularly, these compounds are found to have phenolic features, which can be associated with different organic acids and carbohydrates. With the presence of one or more aromatic rings with one or more hydroxyl groups in their chemical structure, a large and diverse group of polyphenols have been identified (Minatel et al., 2017; Truzzi et al., 2021). About 8000 variants of

dietary polyphenols naturally found in common fruits and vegetables have been characterized based on their number of phenolic rings, and the four main subclasses are flavonoids, phenolic acids, lignans, and stilbenes (Li et al., 2023). Other subclasses that are not included among these are tannins, alkylphenols, curcuminoids, furanocoumarins, tyrosols, and so on (Lacroix et al., 2018; Bertelli et al., 2021). A summary of classes and representative compounds of polyphenols is depicted in Figure 1-2.

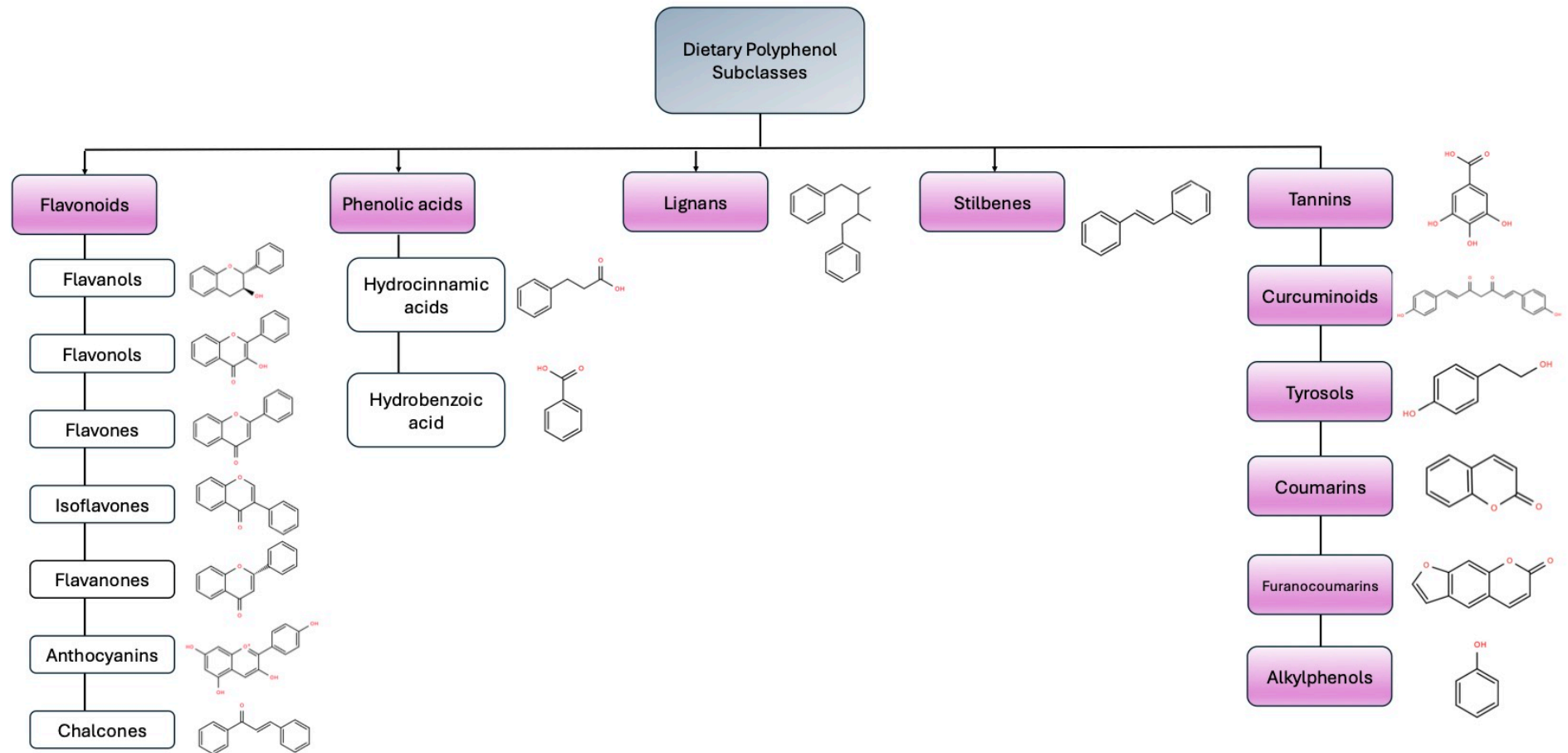


Figure 1-2 Dietary polyphenols and their main subclasses and structural characteristics.

1.2.2 Biological impact of polyphenols

Over the past decades, extensive research on polyphenols has revealed their significant biological activities and health benefits, particularly in the prevention and treatment of chronic illnesses associated with oxidative stress, such as age-related pathologies, cardiovascular diseases (CVDs), neurodegenerative diseases (NDDs), type 2 diabetes mellitus, and several forms of cancer (Daglia, 2020; Vaher et al., 2005). The bioactivities are largely attributed to the antioxidative, antimicrobial, and anti-inflammatory activities of polyphenols.

Cancer is a disease characterized by the uncontrolled division of abnormal cells, which can interfere with normal bodily functions and potentially spread throughout the body (Estrela et al., 2017). The excessive formation of reactive oxygen species (ROS) is believed to be the cause of cellular mutagenesis, a process that may be influenced by environmental factors, hereditary factors and/or lifestyle choice (Nitti et al., 2022; Zhao et al., 2023). In healthy individuals, cellular antioxidant mechanism maintains a balance between ROS production and elimination to maintain cell signalling and redox homeostasis. However, immune responses against pathogens and inflammation can disrupt this balance, leading to the overproduction of ROS. Excessive ROS can accelerate cell proliferation through mutations in DNA, ultimately contributing to the formation of carcinomas (Thyagarajan et al., 2020). Various literature suggests that many polyphenols can exert antioxidant effects against carcinogenesis and promote normal cellular function. The chemopreventive effects of polyphenols have been demonstrated via numerous mechanisms, including antiproliferation, antioxidation, estrogenic/antiestrogenic activity, induction of cell cycle arrest of apoptosis, induction of detoxification enzymes, regulation of the host immune system, and modulation of cellular signalling (Pandey and Rizvi, 2009). The anticancer activities of some phenolic compounds are summarized in Table 1-1.

Table 1-1 Anticancer activities of polyphenols.

Polyphenol Class	Compound	Effects
Stilbenes	Resveratrol	Induced DNA double-strand breaks via topoisomerase II poisoning, activated ATM kinase, and p53-dependent apoptosis in HCT-116 cells (Demoulin et al., 2015)
Flavonoids	Epigallocatechin-3-gallate (EGCG)	Suppress proliferation of HepG2 and HuH-7 cells by downregulating β -catenin signalling, leading to decreased expression of cyclin D1 and c-Myc, and induces G1 cell cycle arrest (Du et al., 2012)
	Quercetin	Induces apoptosis and cell cycle arrest in various cancer cell line through modulation of oxidative stress, inhibition of PI3K/AKT, MAPK, and PKC pathways, and alteration of intracellular redox status (Dajas, 2012)
	Theaflavins, thearubigins	Inhibit proliferation and increase apoptosis in Du 145 prostate carcinoma cells by blocking insulin like growth factor-1 (IGF-1) induced progression of cell proliferation (Sharma and Rao, 2009)
	Anthocyanins/anthocyanidins (e.g., cyanidin)	Inhibit tumour angiogenesis, metastasis, and growth in murine colorectal and breast cancer models (Lin et al., 2017)
Curcuminoids	Curcumin	Curcumin derivatives induce mitotic spindle defects, G2/M arrest, and subsequent p53/p21-dependent G1/S arrest in HCT 116 cells, as well as DNA double-strand breaks and a dual-phase cell cycle block (Basile et al., 2009)
Phenolic acids	Gallic acid	Dysregulation of cell cycle through downregulation of G-quadruplex-containing genes in leukaemia/colorectal lines, induces DNA damage and suppress DNA repair (Liu et al., 2013; Sanchez-Martin et al., 2022)

Cardiovascular diseases (CVDs), one of the leading causes of death worldwide, are associated with risk factors that include both non-modifiable factors (e.g., age, gender, and hereditary conditions) and modifiable factors (e.g., cholesterol levels, obesity, hypertension, type 2 diabetes mellitus, diet, smoking, stress, etc.) (Caleja et al., 2017). Due to their vasodilatory, antiplatelet, blood pressure-lowering, and low-density lipoprotein (LDL) cholesterol-reducing properties, the cardioprotective effects of polyphenols have been extensively studied (Khurana et al., 2013; Sharifi-Rad et al., 2021; Li et al., 2023). Epidemiological and experimental studies also provided strong evidence that a diet rich in polyphenol content (i.e. a Mediterranean diet) is associated with lower rates of mortality and morbidity from coronary heart disease (De Pergola and D'Alessandro, 2018). Resveratrol, in particular, has been shown to reduce hyperlipemia and oxidative stress, key risk factors of atherosclerosis, through its hydroxyl and hydroperoxyl radical scavenging (Mazzone et al., 2013). Additionally, *in vivo* studies have demonstrated that oral administration of polyphenols in cases of overweight and obesity-related CVDs promotes the growth of beneficial gut microbiota, resulting in the reduction of fat mass, liver steatosis, body weight and improvements in glucose metabolism, thus lowering the risk of CVDs (Alves-Santos et al., 2020; Li et al., 2023).

Beyond their antioxidant capacity, polyphenols also modulate inflammatory redox signalling pathways, which plays a critical role in vascular inflammation. In the presence of free radicals, nitric oxide (NO), a key intravascular anti-thrombotic factor, can trigger an inflammatory response if converted to peroxynitrite. Studies suggest that polyphenols are able to inhibit NO release by suppressing the expression and activity of nitric oxide synthases (NOS) (Li et al., 2014). Furthermore, the I-kappaB/nuclear factor-kappaB (NF- κ B) pathway, a major intracellular inflammatory pathway, is implicated in many vascular inflammatory responses. Inhibition of the pathway is thought to be a useful strategy for inflammatory treatments (Virdis and Schiffrin, 2003). Polyphenols are shown to be able to modulate mitogen-activated protein kinase (MAPK) pathway, influencing the activation cascade and subsequent downstream effects, and consequently affecting the production of required

transcription factors for NF- κ B activities (Rahman et al., 2006; Arias-Sánchez et al., 2023).

Diabetes mellitus is one of the most common metabolic diseases and includes type I diabetes mellitus, type II diabetes mellitus, and gestational diabetes. It is characterized by elevated levels of blood glucose and over time will lead to serious impact to the heart, blood vessels, eyes, kidneys and nerves. According to the review published by Cho et al. (2018), it was estimated that 451 million people had diabetes in 2017, and by 2045, this number is expected to increase to 693 million. Thus, the prevention and treatment of diabetes is necessary and urgent. Generally, diabetes is managed by hyperglycaemic drugs and insulin therapy, but long-term usage of these drugs are usually accompanied with side effects, which could include hepatotoxicity, kidney damage, respiratory tract infection to name a few (Seetaloo et al., 2019). Recently, accumulated evidence suggests that dietary polyphenols may be pivotal for the prevention and treatment of diabetes (Sun et al., 2020). It is reported that dietary polyphenols exhibit hypoglycaemic effects in a multi-pathway manner, for example, through the inhibition of digestive enzymes, protection and improvement of pancreatic β cell functions, attenuation of oxidative stress, balance of secretion and synthesis of insulin, modulation of glucose transport, improvement of intestinal microflora, etc. (Choudhury et al., 2018; Xiao, 2022; Li et al., 2023). A higher intake of dietary polyphenols tends to indicate the lowering of diabetes risk. However, the relationship between structure and activity and its hypoglycaemic effects and antidiabetic activity remains unclear due to limited data on the concentrations or dosage, bioaccessibility, bioavailability, and metabolism. Although the co-administration of polyphenols with conventional medicines may further complicate the therapeutic effects, development of drugs derived from dietary polyphenols for diabetes management could be made possible in the future.

As life expectancy increases, so does the prevalence of age-related diseases, particularly NDDs, which are more common in individuals over 65 years of age. An impaired central nervous system (i.e. inhibited of neuron regeneration and significant increase in neuronal loss) is associated with NDDs such as Alzheimer's, Parkinson's, multiple sclerosis, among others (Câmara et al., 2021). Oxidative stress has been identified as a major cause to the

neurodegeneration, as it leads to the oxidation of macromolecules like proteins, DNA and lipids, ultimately resulting in cell death (Balu et al., 2006). Although there are no known cures for NDDs to date, current treatments aim to slow neuronal degeneration. Due to the notable antioxidant activity of polyphenols, several studies have explored their use in combating NDDs, as summarized in Table 1-2.

Table 1-2 Neuroprotective activities of polyphenols.

Polyphenol Class	Compound	Effects
Tannins	Proanthocyanidins	Reduced age-related oxidative DNA damage in spinal cord and brain regions of aged rats; improved antioxidant status (Balu et al., 2006)
Flavonoids	Catechin	Improved cognitive function and restored antioxidant enzyme activity (SOD, CAT) in 6-OHDA-induced Parkinson's disease rat model; reduced dopaminergic neuronal loss (Josiah et al., 2022)
	Rutin	Improved memory and reduced oligomeric A β level, oxidative stress, and neuroinflammation in Alzheimer's disease model mice (Xu et al., 2014)
	Mixed anthocyanins	Improved verbal fluency and memory in older adults with dementia in clinical trial (Kent et al., 2017)
Curcuminoids	Curcumin	Reduced neurological symptoms and restored myelination in a mouse model of multiple sclerosis (ELBini-Dhouib et al., 2022)

In addition to their well-established antioxidant activity, many phenolic compounds are shown to exhibit significant antibacterial, antifungal and antiviral activity. The antimicrobial activity of polyphenols has been studied against a wide range of microorganisms, with flavon-3-ols, flavonols, and tannins demonstrating a broad spectrum and higher antimicrobial activity in comparison to other polyphenols (Daglia, 2012). Research suggests that

polyphenols exert antibacterial effects through three primary mechanisms: direct bactericidal action, reduction of bacterial virulence or pathogenicity, and enhancement of the effectiveness of antibiotic (Barbieri et al., 2017; Liu et al., 2023). As infectious diseases rise and antibiotic resistance becomes a growing concern, polyphenols are increasingly recognized for their potential to enhance antibiotic effectiveness, reduce necessary dosages, and mitigate adverse reactions (Jassim and Naji, 2003; Mikłasińska-Majdanik et al., 2018; Murthy et al., 2021). Moreover, the antimicrobial properties of polyphenols suggest their potential application to be developed as natural food preservatives and innovative therapies to combat antimicrobial resistance and replacing the need of synthetic preservatives in the food industry.

1.2.3 Bioaccessibility and bioavailability

The release of active ingredients from ingested food in an accessible form in the gastrointestinal tract for luminal absorption is known as bioaccessibility; bioavailability refers to the degree of absorption of the ingested active substance reaching the targeted site, usually the systemic circulation and is utilized by the body (Rodrigues et al., 2022; Stielow et al., 2023). Bioavailability and bioaccessibility of bioactive compounds are essential and play a critical role in the design and manufacture of medicines and functional foods (Fernández-García et al., 2009). Various factors, including food composition, chemical structures, processing measures and conditions, host-related factors, and possible anti-nutritional factors, may limit the efficacy of these active substances (Septembre-Malaterre et al., 2018; Bertelli et al., 2021). Post-consumption, Cosme et al. (2020) noted that among the liberation of compounds from the matrix, absorption, distribution, metabolism, and elimination phases, the absorption phase has been identified as one of the major rate-limiting factors in bioavailability. Generally, a very low percentage of ingested polyphenols maintain their original molecular structure due to pH variation in the gastrointestinal tract, hydrolytic reactions by enzymes, phase II metabolization processes (glucuronidation, sulfation and methylation), and enzymatic and catabolic action by intestinal microflora (Bertelli et al., 2021; Bešlo et al., 2023).

Polyphenols undergo both phases I and II drug pharmacokinetic metabolism. Ingested polyphenols are partially released in the mouth due to the mechanical disruption of food structure, and the increased surface area may enhance their bioaccessibility in the gut, increasing interactions with digestive enzymes and salivary proteins. Most polyphenols remain intact until reaching the stomach, where they are released from the food matrix due to hydrolysis by the acidic environment. Depending on their chemical structure, the acidic pH can either stabilize or cause decomposition of polyphenols (Friedman and Jürgens, 2000; Velderrain-Rodríguez et al., 2014). Generally, β -glucosidase enzymes in the small intestine convert polyphenols into their respective aglycones (phase I drug metabolism), which may pass from the gut lumen into the cytosol of enterocytes through passive diffusion or via protein carriers (e.g., P-glycoprotein) and co-transporters (e.g., sodium-glucose transporter) (Truzzi et al., 2021). Some polyphenols that are resistant to enzymatic digestion in the small intestine pass directly to the colon, where they are metabolized by colonic microflora into low molecular weight aglycones (Jaganath et al., 2006). Following absorption of aglycones into enterocytes or colon cells, they are transported via the portal vein to the liver, where they undergo further conjugation, yielding O-glucuronides and O-sulphates (phase II drug metabolism). These compounds may be excreted into bile and re-enter the small intestine, repeating the metabolic cycle. Finally, the resulting phenolic conjugates are transported into the bloodstream by plasma proteins and excreted.

Dryden et al. (2006) stated that although polyphenols can be absorbed through the gastrointestinal tract, evidence suggests that luminal concentrations of polyphenols may be higher than that of plasma concentrations due to poor intestinal absorption or extensive metabolism by colonic flora, resulting in low bioavailability. The solubility and lipophilicity of each class of polyphenols differ due to their unique chemical structures, which influence the rate and degree of absorption of phenolic metabolites into plasma circulation (Truzzi et al., 2021). Consequently, metabolic processes in the human body often result in phenolic concentrations detected in plasma and urine not being directly related to the ingested phenolic content. Instead, these concentrations depend on their metabolic forms as it leads to a change

in chemical structure and the subsequent lipophilicity and solubility characteristics which allows entry into the blood circulation (Bento-Silva et al., 2020; Bešlo et al., 2023). Thus, polyphenols are typically found in their conjugated forms in plasma circulation. Due to the multitude of factors governing the bioaccessibility and bioavailability, *in vivo* and *in vitro* studies may contradict clinically observed effects of polyphenols and the study of biological activities of polyphenols remain a challenge.

1.2.4 Biopharmaceutical Classification System (BCS)

The Biopharmaceutical Classification System (BCS) to classify bioactive compounds and active pharmaceutical ingredients with consideration to their water solubility and intestinal permeability influencing bioavailability was proposed by Amidon et al. (1995). The Food and Drug Administration (FDA) has approved the use of the BCS system to avoid or reduce *in vivo* tests through the defined limitations of dissolution, solubility and permeability (Dima et al., 2020; Truzzi et al., 2021). The guidelines highlighted that (i) drugs of high solubility does not limit dissolution nor its absorption, (ii) the high permeability of a drug ensures the drug's complete absorption during the limited transit time through the small intestine, and (iii) the gastric emptying process is the rate-limiting step for absorption of highly soluble and permeable drug due to rapid dissolution (Tubic-Grozdanis et al., 2008; Polli et al., 2008). The compound is considered to be highly soluble if its release is immediate at highest dosage in a volume of 250 mL (or less) of aqueous environment at a temperature of $37 \pm 1^\circ\text{C}$ and corresponding pH ranges to the gastrointestinal system and considered to have low solubility if solubility requires volume exceeding 250 mL (Ku, 2008). The compound is also classed to have high permeability by the BCS system if the intestinal absorption rate is at least 90% when compared to its absorption rate administered intravenously at the same dosage. Hence, the system divides drugs or bioactive compounds into four categories based on the solubility and permeability (Figure 1-3): Class I – high permeability, high solubility; Class II – high permeability, low solubility; Class III – low permeability, high solubility; Class IV – low permeability, low solubility (McClements, 2015a).

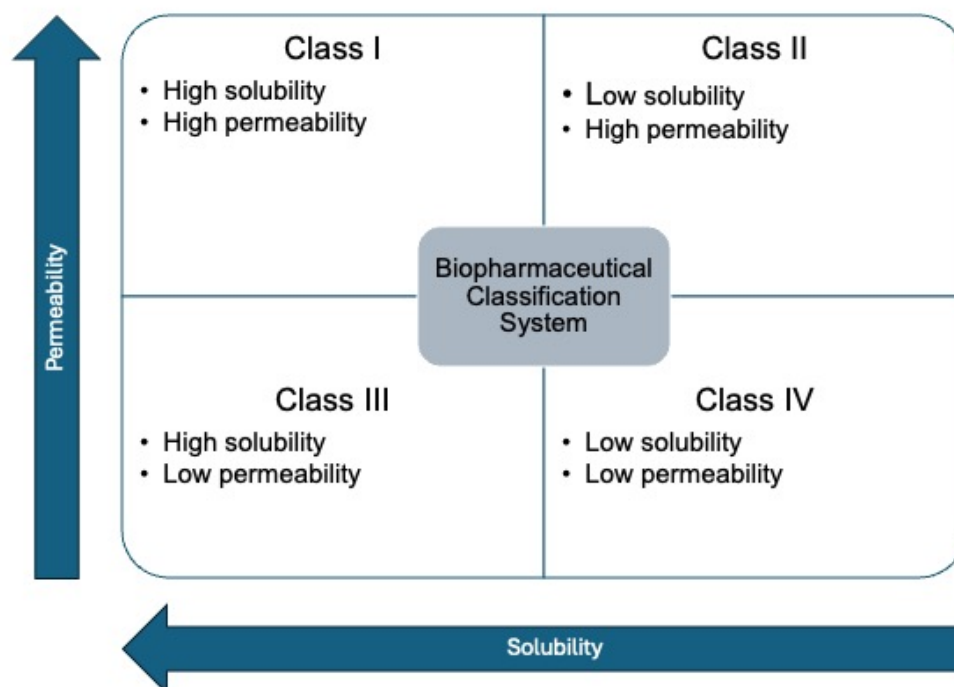


Figure 1-3 Biopharmaceutics Classification System (BCS).

Although the system was originally purposed for orally administered drugs, it has proved useful for classification of bioactive compounds in food, for example, polyphenols. Polyphenols are generally belonging to BCS Classes II and IV due to their described solubility and permeability with limitations on their potential health benefits (Kaur and Kaur, 2014). It is suggested that to improve the bioavailability of the Class II and IV compounds, techniques to improve solubility or emulsifying delivery systems could be applied (Cao et al., 2021; Polia et al., 2022).

1.3 Encapsulation systems

With the growing interest in utilizing bioactive compounds as nutraceuticals and overcoming barriers of human metabolism and absorption, various delivery systems have been studied to improve the poor physicochemical stability and bioavailability, and their subsequent limited applications for controlled release (Zabot et al., 2022; Alu'datt et al., 2022). To address these limitations, encapsulation techniques have been extensively employed in the food industry for functional food manufacturing, processing, and product innovation (Timilsena et al., 2020). The technique involves entrapping or

incorporating an active core material into a matrix of an inert material, allowing the contents to be released at controlled rates under specific conditions. Numerous encapsulation technologies have been explored, tailored to the active ingredient, properties of encapsulating matrix, desired product characteristics, and intended use. They are primarily applied to mask unpleasant odours and colour, ease handling, extend shelf-life, control the release and increase the bioaccessibility and bioavailability of encapsulated bioactive compounds (Domínguez et al., 2021).

The entrapped substance is referred to as the active agent, core material or internal phase, whereas the surrounding material is known as the coating, outer shell, carrier material, external phase or matrix (Alu'datt et al., 2022). The core material in applications within the food industry often consists of bioactive compounds extracted from natural food sources, and the coatings could be a wide range of polysaccharide, proteins and lipids, with particle sizes ranging from a few nanometres to millimetres post-encapsulation (Zuidam and Shimon, 2010; Zabet et al., 2022).

1.3.1 Encapsulation technologies

The encapsulation techniques can be classified into three main categories: physical-mechanical, physical-chemical, and chemical methods (Řepka et al., 2023). To determine the appropriate encapsulation technique for use, one should consider the efficiency, desired particle size, nature of active ingredient, stability, release mechanism, the sensitivity to surrounding medium for release, and economic viability.

1.3.1.1 Physical-mechanical methods

As most active compounds are formulated in the liquid form such as solutions, suspensions, or emulsions, many encapsulation technologies require drying methods, such as spray-drying, spray-chilling, spray-cooling, and fluidized bed coating. Spray-drying is widely used in the food industry, typically using a hydrophilic polymer and a hydrophobic active compound core in an emulsion, solution or suspension sprayed at high feed velocity into a hot chamber. Following the removal of water, it yields a coated spherical dry active ingredient of micro-scale that is more stable to moisture, oxygen, light and other environmental stressors (Gharsallaoui et al., 2007). Fluidized bed

coating process involves fluidizing the core material followed by coating with a prepared solution, without the use of high temperature. This technique is often applied as a secondary coating to enhance the stability of already encapsulated products (Dewettinck and Huyghebaert, 1999; Timilsena et al., 2020). Spray-chilling and spray-cooling is the process of solidifying an atomized liquid spray of lipid polymer/bioactives solution into particles via cooling or chilling, rather than by the evaporation of solvent (Oxley, 2012). Upon cooling, instant solidification takes place, thus yielding spherical and free-flowing microcapsule powders that are insoluble in water.

Highly dense encapsulated products can be produced using an extrusion-based encapsulation method. The bioactive core and coating agent are pumped separately through a concentric orifice in opposing directions while being coextruded by a rotating shaft, and the centrifugal force propelling the extruder causes the end-product to shatter into tiny particles (Alu'datt et al., 2022). Examples of extrusion techniques include coaxial air flow extrusion, electrostatic extrusion, vibrating jet/nozzle extrusion, spinning disk atomization and jet cutting extrusion. Particles ranging from 0.2 to 5 mm in size are produced, and the mild conditions allow for encapsulation of bioactives under anaerobic and aerobic conditions, as well as heat-sensitive products and living cells (Rodríguez et al., 2016). However, the method has a relatively lower production capacity, and the size of products may be considered too bulky for certain food applications.

Electrospinning is a cost-effective, versatile and scalable method of encapsulation. It involves the incorporation of core materials into appropriate carriers through an electrohydrodynamic process (Xue et al., 2019; Noore et al., 2021). High-voltage electricity is passed through polymeric fluid with electric charges, thereby generating a jet from a liquid droplet, which then stretches and solidifies into nanofibers. The technique could be operated at ambient conditions to produce micro or nanostructures in dried form in a one-step process. It is reported that the electrospun nanofibers showed improved thermal stability during subsequent thermal processing of bioactive compounds due to the low processing temperatures (Munteanu and Vasile, 2021).

1.3.1.2 Physical-chemical methods

The coacervation process relies on the partial desolvation of a homogeneous polymer solution, followed by the deposition of the developed coacervate phase around the suspended or emulsified active compound, forming microencapsulated products (Gouin, 2004; Jyothi et al., 2010). Coacervation is divided into two categories: simple coacervation and complex coacervation. The mechanisms are similar, but the phase separation differs: simple coacervation involves the addition of a desolvation agent, whereas complex coacervation involves interactions between two oppositely charged polymers. This technique yields high payload and efficiency with good controlled or sustained release properties, but it has a narrow range of pH and ionic strength stability, thereby limiting the variety of polymers that can be used as shell material (Trifkovic et al., 2016).

Colloidal delivery systems, such as emulsions, are widely applied in the food industry and could be considered as physical-chemical or chemical methods of encapsulation (Alu'datt et al., 2022). Fundamentally, emulsions consist of two immiscible liquids, typically oil and water, with one being dispersed as small spherical droplets in the other (Lu et al., 2016). The type of emulsion can be classified based on their relative spatial distribution of the oil and aqueous phase (McClements, 2015b). A system with oil droplets dispersed in an aqueous phase is called an oil-in-water (O/W) emulsion, and the inverse is called a water-in-oil (W/O) emulsion. A variety of emulsions with desirable structures and properties have been developed for encapsulation and delivery of bioactive compounds. Alternative emulsion systems, such as double or multilayer emulsions coated with layer(s) of polymers, have also been proposed (McClements et al., 2007). These systems rely on polymer interactions (i.e., electrostatic forces) which enhance their stability.

Liposome entrapment is considered a popular method used for drug delivery and nutraceutical industry (Timilsena et al., 2020). Liposomes are colloidal particles composed of lipid bilayers encapsulating aqueous spaces, formed through hydrophilic-hydrophobic interaction between phospholipids and water molecules (Fang and Bhandari, 2010). Due to their amphiphilic nature, liposomes can be utilized to entrap, deliver and release water-soluble, lipid-

soluble and amphiphilic materials. They have been applied in the delivery of vaccines, hormones, enzymes, vitamins and other sensitive nutraceuticals (Shade, 2016). Liposome delivery systems have been reported to have good controlled release properties and enhanced bioactivity and bioavailability due to their stability (Lu et al., 2016).

1.3.1.3 Chemical methods

Polymerization is a chemical reaction that produces larger molecules based on a combination of smaller molecules (i.e. monomers) (Noore et al., 2021). This technique involves the deposition of formed polymers onto the surface of particles in a solution which forms a coating layer. The reaction is dependent on pressure and temperature conditions, and the use of a catalyst. Various methods of polymerisation include *in situ*, emulsion, bulk and interfacial polymerisation (Tawiah et al., 2022). Polymerization is particularly effective for creating robust encapsulation systems tailored to specific applications.

In contrast to polymerization, encapsulation via molecular inclusion relies on the unique properties of cyclodextrins (CDs) to entrap bioactive compounds. Among the naturally occurring cyclic oligosaccharides, α -, β -, and γ -cyclodextrin, β -cyclodextrin is the most used due to its reasonably sized cavity and simplicity and economical production (Song et al., 2009; Timilsena et al., 2020). CDs have a hydrophilic exterior and hydrophobic interior, where bioactive cores are entrapped through hydrophobic interactions (Christaki et al., 2023). Due to their structural characteristics, CDs are considered a good medium for delivering small molecules of high hydrophobicity. Evidence has also suggested that CDs are able to improve the water solubility of polyphenols and phytochemicals, enhancing and their bioactivity and bioavailability, thus expanding their potential for application in the food industry (Lu et al., 2016; Trifkovic et al., 2016). Although CDs were initially studied and applied as drug delivery systems, their characteristics have expanded their use in various food matrices, as well as indirect applications in active food packaging (Christaki et al., 2023).

1.4 Controlled release

As the conventional drug delivery system is eliminated from the body very quickly, the dose is not well maintained within the therapeutic window, thus resulting in a sub-therapeutic response (Adepu and Ramakrishna, 2021). Conversely, the controlled release of bioactives in the gastrointestinal system allows for a single dosage in which the concentration is maintained for an extended time within the therapeutic window. This in turn has a great importance in the bioavailability and its potential health benefits.

Understanding encapsulation mechanisms, the interactions between active compounds and matrices, and the stability of the active ingredients is crucial, as these factors directly influence the release behaviour of the encapsulating compounds. The term “release” refers to the process by which bioactives are transported out of their delivery system. This can occur in two primary ways: immediate release or modified release (Boostani and Jafari, 2020). Immediate release involves the rapid delivery of bioactives shortly after administration. In contrast, modified release is designed to either prolong the release over an extended period or target a specific site within the body (Adepu and Ramakrishna, 2021). Controlled release systems utilize modified release strategies, where bioactives are released in a sustained manner over time to enhance stability, safety and therapeutic efficacy. With an understanding of the release mechanisms (see Table 1-3) and release profiles, various systems can be fabricated or designed for optimal control and performance.

Table 1-3 Release mechanisms of controlled release systems.

Mechanism	Principle
Diffusion	Random movement of bioactive molecules through a potential, chemical, or concentration gradient from inside to outside the carriers, governed by solubility and permeability (Fredenberg et al., 2011)
Dissolution	Bioactive compounds are released as the carrier dissolves in a compatible environment, governed by solubility (Wang and Shmeis, 2006; McClements, 2014)
Erosion	The carrier degrades (on the surface or throughout), releasing the bioactives without transport events, influenced by the carrier's properties and surrounding conditions (McClements, 2014)
Swelling	Absorption of solvent causes the encapsulation system to swell, causing release, governed by rate of swelling and diffusion (Siepmann et al., 2011; McClements, 2014)
Osmosis	Water absorption creates osmotic pressure, driving bioactive transport through osmotic pumping (Srikonda et al., 2006; Boostani and Jafari, 2020)
Degradation	Bioactives are released as the polymer carrier degrades, often through chain scission or biological breakdown (McClements, 2014)
Fragmentation	Environment factors (pressure, shearing, pH, enzymatic activity, etc.) cause the carrier to crack, releasing bioactives (Boostani and Jafari, 2020)

Release profiles describe how encapsulated compounds are released from their carrier, often represented on concentration-time curves (Boostani and Jafari, 2021). The five known release profiles are burst release, sustained release, delayed release, triggered (or stimuli responsive) release, and targeted release.

Burst release is characterized by the disorganized or uncontrolled leakage of encapsulated bioactives during the initial release phase, with approximately 30–60% of the bioactives released (Flores and Kong, 2017; Bhattacharjee, 2021). This profile is considered typical, though it may be unfavourable for systems requiring constant release, it is satisfactory when rapid release of the compounds improves bioactive functionality.

A sustained release profile, or “long acting” release, occurs when the release of a specific bioactive is reduced twofold when compared to the immediate or conventional release profiles (Borah et al., 2023). In a delayed release system, bioactives are released after a lag time, allowing for control over the release site (Perrie and Rades, 2012). This can protect the bioactive in the stomach and release it in the small or large intestine. Both sustained and delayed release profiles are commonly utilized in controlled release systems as the plasma concentration of bioactives could be stabilized at a constant level (Boostani and Jafari, 2021).

The triggered (or stimuli responsive) release profile refers to the burst release of bioactives in response to changes in surrounding stimuli. These stimuli can either be internal (e.g., enzymes, pH, glutathione (GSH), ROS, temperature) or external (e.g., light, ultrasound, magnetic or electrical fields, mechanical triggers) (Alarcón et al., 2005; McClements, 2014; Thang et al., 2023). Boostani and Jafari (2020) suggested that externally triggered release could be beneficial in active packaging systems.

A targeted release profile allows bioactives to be released at or near their site of physiological activity, potentially with immediate or extended release properties (Tewabe et al., 2021). Targeting can be achieved through passive designs, such as the use of ligands attached to encapsulation systems that bind to specific receptors for delivery (Perrie and Rades, 2012).

Several parameters influence controlled release systems and their release profile, namely bioactive component characteristics, carrier or matrix properties, gastrointestinal conditions, and external environmental factors.

The initial load of bioactives and their molecular size affect diffusion and release rates, with smaller molecules and higher bioactive loads generally resulting in faster release. This release rate may also be governed by the solubility and partition coefficient of a bioactive component. According to Boostani and Jafari (2020), decreasing the solubility of an entrapped bioactive compound alters the partition coefficient and causes a change in release profile and bioavailability. The authors also reported carrier-bioactive and/or bioactive-bioactive interactions influences the release rate as intense attractive forces create a slower release rate whereas intense repulsive forces

induce a faster release; conjugation or complexation with the matrix system would slow the release or even cause unsatisfactory or inadequate release of bioactive.

Factors associated with carrier/matrix properties can be classified into two groups: intrinsic and physical properties. Intrinsic properties include physical state of phases, charge of emulsion droplets, and the composition and rheology of phases (Patra et al., 2018). For example, changes in the physical state of a solid lipid carrier system (from solid to liquid) through melting can trigger bioactive release (Boostani and Jafari, 2021). McClements (2014) and Vlachopoulos et al. (2022) agree that carriers with higher molecular weight or longer chains decomposes more slowly and may exhibit lower permeability, leading to extended-release profile. Examples of physical properties include carrier's size, shape, structure, and porosity. Larger nanoparticles favour a slower rate of release due to slower degradation and lower diffusion rate of entrapped bioactive within the particle, however large particle-sized emulsions may destabilize oil and water phases (Fredenberg et al., 2011; McClements, 2014; Boostani and Jafari, 2020). Pore size, relating to the degree of cross-linking, is also a crucial factor. Higher cross-linking is said to slow diffusion by reducing water absorption through created pores and degree of swelling may be slowed and hindered as compared to larger pores of greater swelling tendency and intensified release rates (Mikušová and Mikuš, 2021).

Encapsulation carriers may undergo significant changes during digestion due to physical forces, enzymatic or chemical decomposition (e.g., ionic strength and pH), and interactions with phospholipids (McClements, 2018). Therefore, depending on the site of release, carriers can be chosen or engineered to resist digestive conditions, protecting bioactives without becoming indigestible.

External environmental stimuli, such as pH changes, temperature, and presence of ions, also influence release behaviour (Thang et al., 2023). Temperature-sensitive systems such as polymers or hydrogels have been exploited for drug delivery (Grassi et al., 2005; Zhao et al., 2020). At physiological temperature (35-38°C), these systems may swell or shrink with temperature changes, and increased temperatures (40°C) can accelerate

chemical reactions, affecting pore formation or closure (Oh et al., 2004; Fuciños et al., 2016). In pH sensitive matrices, the release mechanisms are associated with the acid or basic protonation relating to the acid dissociation constant (pK_a) values of carboxylic acid and amine groups present. Flores et al. (2017) suggested that the mechanism may be due to the glass to rubbery transition when there is a change in pH. However, extreme pH conditions may also be detrimental to an encapsulation system in which degradation or reconfiguration could occur. The osmotic pressure may be affected in the presence of ions such as sodium chloride, which in turn influences the swelling capacity of an encapsulated system and subsequently influences the bioactive release (Rizwan et al., 2017).

It is suggested that the main mechanisms involved in the controlled release of encapsulated material are diffusion and controlled degradation, solvent choice, as well as the combined effects of pH, temperature and pressure (Desai and Park, 2005). Often, several mechanisms are generally at play, where a single mechanism may be dominant, or a combination of mechanisms may be governing the release at the same time. In some conditions, the carrier material may remain intact and is permeable for the release of bioactive compounds and is later degraded as release progresses.

In conclusion, understanding the release mechanisms, interactions, and stability of bioactives (i.e., polyphenols) is essential for optimizing their controlled delivery in biological systems. Polyphenols, like many bioactives, face challenges in bioavailability and bioaccessibility due to their instability, rapid metabolism, and poor solubility, which limits their therapeutic potential. Encapsulation systems offer a solution by protecting these compounds from degradation and enabling their controlled release. With an understanding of the mechanisms of release from encapsulation systems, tailored solutions can be provided to tackle these challenges by offering precise control over the release profile and enhanced stability of encapsulated bioactive compounds. This chapter lays the groundwork for exploring innovative encapsulation systems in overcoming the limitations of bioactives and advancing their applications in health and functional food industries.

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Chapter 2

An Overview of Cyclodextrin Metal-Organic Frameworks (CD-MOFs)

2.1 Metal-organic frameworks (MOFs)

Metal-organic frameworks (MOFs) are highly ordered porous crystalline materials composed of organic ligands and metal ions. The self-assembly and formation of coordinative or covalent bonds between metal ion centers and bridging organic ligands build up the network structure of MOFs (Han et al., 2018b; Shen et al., 2020). Due to their rich geometry and connectivity the design and properties of MOFs can be dictated through the appropriate choice of ligands and linkers, enabling their adaptation for various applications (Islamoglu et al., 2017; Tibbetts and Kostakis, 2020). According to Rajkumar et al. (2019) metal ions used for the synthesis of MOFs include transition metals, p-block elements, alkali metals, alkaline earth metals, lanthanides, and actinides. The diverse organic linkers that have been used include carboxylate, phosphonate, sulfonate, pyridyl, imidazolate, and azolate functional groups. In addition to their high tunability, MOFs exhibit high porosity, surface area and thermal stability, making them promising candidates for numerous applications and the focus of much research. These include uses in the food industry for bioactive delivery, monitoring, preservation, hygiene and packaging purposes (Roy and Stoddart, 2021). Other non-food applications include gas adsorption and storage, separation and purification, catalysis and energy storage (Dummert et al., 2022).

However, conventional MOFs face limitations due to their non-recyclable process and high toxicity due to the use of synthetic components or chemical reagents. Thus, they are considered undesirable for use in drug delivery and other bio-related applications, posing a hurdle to their practical use (Roy and Stoddart, 2021; Shen et al., 2021). To address this challenge, greener and more biocompatible methods for synthesising MOFs have been explored. These methods utilize nontoxic metal ions (e.g. Ca, K, Ti) and naturally occurring building blocks such as amino acids, peptides, and carbohydrates

as organic ligand linkers (Horcajada et al., 2012). For example, Manton et al. (2008) reported on the first peptide-based MOF, constructed using oligovaline peptides as organic linkers coordinating with calcium centers. Reducing unfavourable factors in MOF synthesis has broadened their applications in the food industry and nanomedicine, particularly for drug loading and delivery.

2.2 Cyclodextrins and their applications

The well-recognized edible material, carbohydrate, is regarded as a unique candidate for drug-carrier preparation due to its biocompatibility and biodegradability. Carbohydrates have been utilized as nanocarriers in various forms, including covalently or ionically cross-linked polysaccharide-based nanosponges, self-assemblies, dendrimers, nanocapsules, hydrogels, micelles, and multilayered polysaccharide vesicles (Qiu et al., 2020b). However, the inherent asymmetry of carbohydrates as building units is not conducive for the formation of highly porous crystallized frameworks and thus a strategy to adopt cyclic oligosaccharide cyclodextrin (CD) was proposed.

CD, a naturally occurring oligosaccharide, is enzymatically produced from starch and can be mass-produced from *Bacillus amylobactor* culture medium grown on starch (Xu et al., 2019; Roy and Stoddart, 2021). CD is composed of D-glucopyranose units linked by α -1,4 bonds, forming “cage” molecules with a truncated cone shape (Tian et al., 2020). It exists in three main forms: α -, β -, and γ -CD, containing 6, 7, and 8 glucopyranose units, respectively (Figure 2-1), with identical depths of ~ 0.8 nm and inner diameters of 0.57, 0.78, and 0.95 nm (Jansook et al., 2018; Qiu et al., 2020b). The glucopyranosyl units are located at the C-2 and C-3 positions on the secondary face of CD, while the hydroxymethyl groups at the C-6 position are orientated with ring oxygen atoms toward the primary face of CD (Roy and Stoddart, 2021).

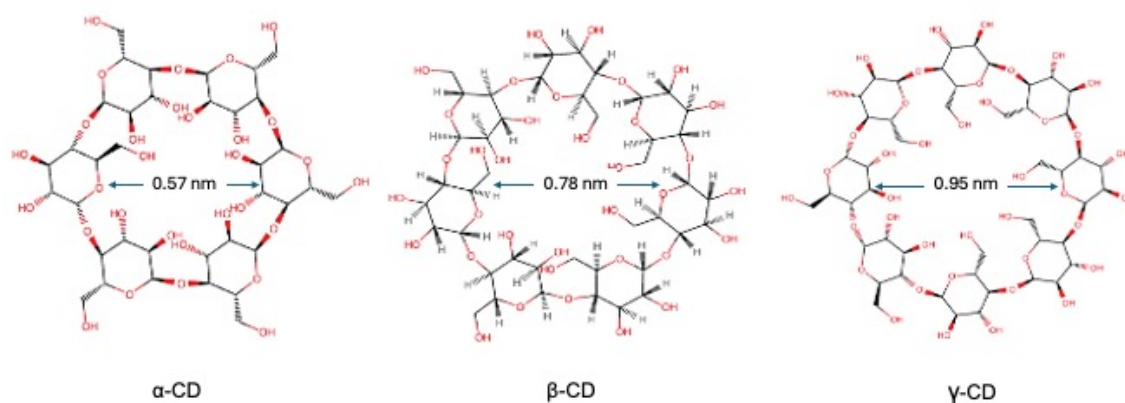


Figure 2-1 Structural representation of three types of CDs with diameters of their respective cavities.

The presence of hydroxyl (-OH) groups contributes to the hydrophilic nature of CD's external surface, while the pyranose groups within the repeating segments results in the hydrophobicity of its internal cavity (Topuz and Uyar, 2024). Additionally, Szejtli (1998) reported that the nonbonding electron pairs of the glycosidic oxygen bridges, pointing toward the cavity of CD molecule, impart a Lewis base character. This unique structure enables CDs to combine with many inorganic and organic molecules, forming host-guest inclusion complexes through multiple interactions (i.e. van der Waals forces, hydrogen bonding, and electrostatic interactions) (Yang et al., 2024). Depending on the size of CDs cavities, they can encapsulate guest molecules of varied sizes with different stoichiometry (see Figure 2-2). CDs have been widely used for the encapsulation and solubilisation of lipophilic substances, especially within the field of drug delivery systems. Studies have shown that CD-based inclusion complexes improve the stability, solubility and bioavailability of the loaded drug (Jansook et al., 2018; Dummert et al., 2022). However, the low drug-loading capacity and burst release have been identified as drawbacks which limited their use (Han et al., 2018b).

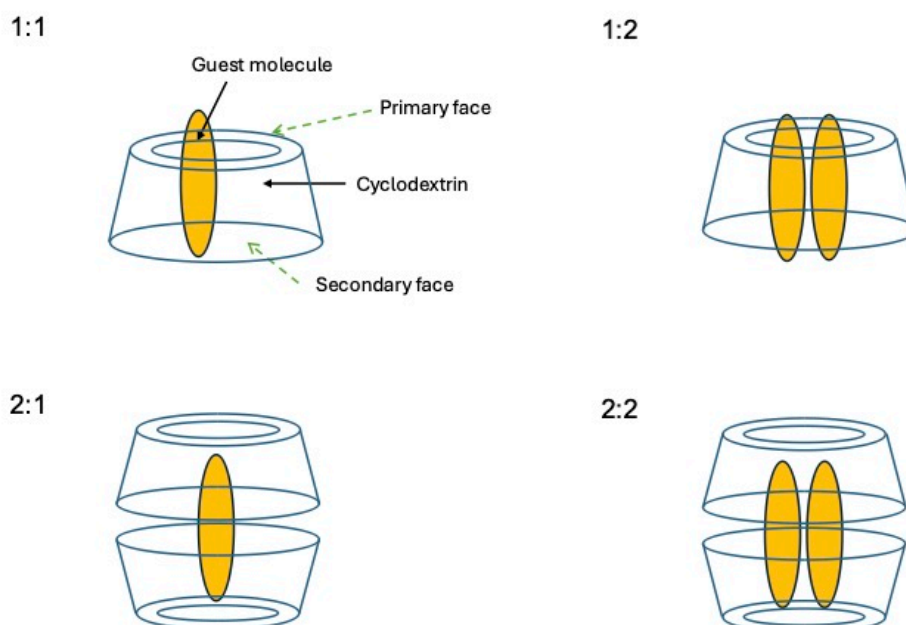


Figure 2-2 Stoichiometry of CD inclusion complexes.

Structural differences between α -, β -, and γ -CD led to the variation in their aqueous solubility (Dhiman and Bhatia, 2020; Dummert et al., 2022). The hydrolysis rate of different types of CDs follows the order: β -CD < α -CD < γ -CD (Gidwani and Vyas, 2015). Due to the reduced flexibility from the formation of an intramolecular hydrogen bond belt between -OH groups, β -CD was found to be least soluble. It has been suggested that the distorted position of one of the glucopyranose units in α -CD allows formation of only 4 intramolecular hydrogen bonds, compared to 6 in γ -CD, resulting in an incomplete hydrogen bond belt and better water solubility. The increased flexibility of γ -CD was also found to further enhance its aqueous solubility.

To improve the aqueous solubility of CDs, particularly β -CD, functional groups have been introduced to modify the -OH groups (Topuz and Uyar, 2024). It was suggested that, like starch, CDs can be reacted with various reagents to produce water-soluble CD derivatives (Christaki et al., 2023). For example, methyl groups can be added through O-methylation, while acetylation introduces hydroxypropyl groups. As a result, these modifications have broadened their adaptability for diverse applications.

Despite the biocompatibility, CDs can pose some toxicity concerns under certain conditions. α -, β -, and γ -CD are listed as “Generally Recognized As

Safe (GRAS)” by the US Food and Drug Administration (FDA), with no restrictions on the consumption of α and γ -CD while the oral intake of β -CD is limited (Christaki et al., 2023). According to Ferreira et al. (2022), the toxicity associated with CDs at elevated doses arises from enhanced membrane fluidity, invagination and subsequent cell lysis. A review of γ -CD safety data conducted by Munro et al. (2004) found it to be well-tolerated and elicits no toxicological effects. The reviewed toxicity studies included standard genotoxicity tests, oral and intravenous administration rat and dogs in subchronic studies lasting up to a year, and embryotoxicity and teratogenicity tests in rats and rabbits. However, CDs have been reported to induce haemolytic effects on human erythrocytes and cytotoxicity in P388 cells (Irie et al., 1982). Notably, γ -CD requires a higher concentration (16 mM) to induce haemolysis as compared to α -CD (6 mM) and β -CD (3 mM), largely due to the rapid and complete digestion of γ -CD.

In vitro and *in vivo* studies have demonstrated that, unlike α -CD and β -CD, γ -CD is hydrolysed by α -amylase (human or porcine pancreatic amylase) and metabolized like starch or linear dextrans, yielding maltose, maltotriose, and traces of glucose. As a result, γ -CD is not detected in the digesta (Lumholdt et al., 2012; Jansook and Loftsson, 2022). Conversely, α -, β -CD and their derivatives are said to be primarily digested by colonic bacteria (Christaki et al., 2023). While CDs are generally considered safe at normal doses and have low bioavailability, their potential to cause toxicity at elevated concentrations or in certain applications means that careful consideration is needed for targeted use.

2.3 Cyclodextrin metal-organic frameworks (CD-MOFs)

Cyclodextrin metal-organic frameworks (CD-MOFs) have emerged as a novel class of porous, extended framework with robust crystallinity, permanent porosity, low cytotoxicity and good biocompatibility. As summarized in a review by Roy and Stoddart (2021), CD-MOFs can absorb a wide range of guest molecules, including gases, drugs, metal-based nanoclusters, and nanoparticles due to their amphiphilic nature. This capability has enabled their use in diverse applications such as molecular adsorption and separation,

purification, sensing and molecular recognition, serving as host template for nanoparticle synthesis, drug delivery systems, entrapment of highly reactive intermediates, catalyst stabilisation, and catalyst supports. Moreover, MOFs prepared using food-grade CD have shown no toxicity in human hepatocellular carcinoma (HepG2) and colorectal adenocarcinoma (Caco-2) cell lines, effectively addressing the safety issues traditionally associated with MOFs (Xu et al., 2019; Qiu et al., 2019). This makes CD-MOFs highly promising for expanded applications in the food and pharmaceutical industries with continued research and development.

The -OH groups of CD molecules coordinate with alkali and alkaline earth metals to form metal-organic backbones, with CD serving as organic linkers and metal ions acting as nodes in CD-MOFs (Lopez and Perez, 2023; Yang et al., 2024). Wei et al. (2012) reported that these units are connected by metal ions to form body-centered cubic structures, with interconnected CD cubes that assemble into extended three-dimensional crystalline arrays. Metal cations commonly used in CD-MOF formation include K^+ , Na^+ , Rb^+ , Cs^+ , Sr^+ , Pb^{2+} , and Fe^{3+} (Rajkumar et al., 2019). Moreover, the structure of CD-MOFs can be tailored by altering the type of CD, metal ions, metal source and synthesis methods (Si et al., 2024).

Since the successful synthesis of γ -CD-MOF reported in 2010, the synthesis and characterisation of α -CD-MOF and β -CD-MOF have also been explored (Smaldone et al., 2010; Roy and Stoddart, 2021). However, γ -CD-MOF is comparatively more extensively studied due to the more symmetrical structure of γ -CD, which contributes to its better stability.

2.3.1 Synthesis methods

Various synthetic methods have been developed for the preparation of CD-MOFs, with the vapor diffusion method being the most commonly used. Efforts have also been made to improve the processes to achieve homogenous size distribution and even reduce the size of crystals formed. Such alternative methods include hydrothermal/solvothermal method, microwave-assisted method, ultrasound and seed-mediated approaches.

The earliest pathway for synthesizing MOFs was the vapor diffusion method (Han et al., 2018b). The method was similarly used by Smaldone et al. (2010)

to produce the first edible MOFs – CD-MOFs. Depicted in Figure 2-3, the method involved reacting γ -CD and potassium hydroxide (KOH) in aqueous solution at ambient temperature and pressure, followed by methanol vapor diffusion into solution for 2-7 days. The resulting crystals typically range from 200-400 μm in diameter. However, the conventional vapor diffusion method is time-consuming and there is limited control over crystal size.

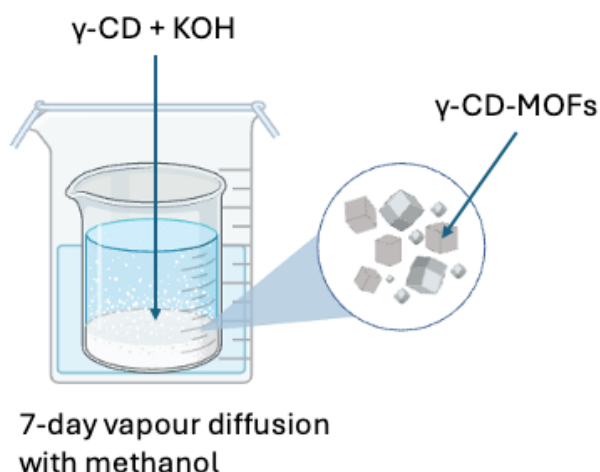


Figure 2-3 Synthesis of γ -CD-MOFs via the vapour diffusion method.

To address these limitations, Furukawa et al. (2012) introduced cetyltrimethylammonium bromide (CTAB) into the crystallization medium, producing smaller, homogenous, and monodisperse γ -CD-MOFs with diameters of 1-10 μm . Further refinements by Zhou et al. (2019) yielded nanoscale γ -CD-MOFs (200-300 nm) through the addition of methanol and CTAB. Liu et al. (2016) was able to reduce the reaction time to 6 hours by manipulating the reactant concentrations and ratios, temperatures, and surfactant concentrations. Despite CTAB's effectiveness in controlling crystal size and accelerating crystallization, its potential toxicity and difficulty in removal has made it unsuitable for drug delivery application. To address this, Zhang et al. (2018) substituted CTAB with polyethylene glycol 20000 (PEG 20000), synthesizing γ -CD-MOFs with sized between 2-5 μm . Conversely, Qiu et al. (2018a) proposed a "green" seed-mediated method using starch nanoparticles (SNPs) that rapidly produces crystals with a mean diameter of 2 μm while preserving their structure and morphology.

The hydrothermal/solvothermal method involves heating the reaction system in a confined space above the boiling points of the reactants (Qiu et al., 2020b; Yang et al., 2024). This approach has been shown to improve the thermal stability of CD-MOF backbone, making it a preferred alternative to the vapor diffusion method. For example, Lu et al. (2015) successfully synthesized a new chiral helical β -CD-MOF by reacting β -CD and sodium oxalate ($\text{Na}_2\text{C}_2\text{O}_4$) in a methanol-water solution at 160°C for 3 days. Similarly, Sha et al. (2016) synthesized a new α -CD-MOF featuring a chiral helical chain. This method has also facilitated the development of novel CD-MOFs species. Liu et al. (2017) used a novel template-induced approach to produce β -CD-MOFs of varying crystallinity and porosity, with the use of 1,2,3-triazole-4,5-dicarboxylic acid, methyl benzene sulfonic acid or ibuprofen molecule as templating agents. The addition of PEG 20000 was also employed in combination with this method to shorten reaction times (Han et al., 2018a; Zhang et al., 2019).

The microwave-assisted hydro/solvothermal method offers several advantages, including simplicity, rapid synthesis, cost effectiveness, environmental friendliness, and efficient, nonconventional heating that yields high output (Han et al., 2018b). The high-frequency magnetic field during the reaction facilitates molecular interactions, generating uniform and rapid heating that promotes chemical reactions. Sun et al. (2013) demonstrated the synthesis of γ -CD-MOFs within 10 minutes with better morphological control.

The ultrasound-assisted method is another effective approach for synthesizing γ -CD-MOFs. In this method, metal salts, organic ligands, and organic solvents are mixed in a reaction vessel under ultrasonic conditions (Si et al., 2024). This technique facilitates removal of gas from the reaction mixture, which enhances crystal formation, resulting in the production of uniformly smaller crystals within a significantly reduced crystallization time (Liu et al., 2013). Depending on the ultrasonic frequency applied, γ -CD-MOFs in the nanometer range can be synthesized within an hour (Qiu et al., 2019; Wei et al., 2021). Additionally, sonication inhibits crystal aggregation, further contributing to the formation of uniform, small-sized crystals.

2.3.2 Encapsulation methods of CD-MOFs

The encapsulation of molecules into biocompatible CD-MOFs via molecular inclusion encapsulation technology has been studied to improve the properties of encapsulated molecule and expand their applications. Various molecules have been incorporated in CD-MOFs using three main strategies: impregnation method, co-crystallization method, and grinding method.

The impregnation method, also known as the absorption method, is the most common method of incorporation with three main steps: (1) synthesizing CD-MOFs, (2) activating MOFs by removing solvents or ligands from the pores, and (3) incorporating guest molecules in CD-MOF using suitable solvents. Factors such as solvent type, pH, and encapsulation material significantly influence guest molecule loading and properties. For instance, Lv et al. (2017) found that neutralized CD-MOFs exhibit better thermal protection for sucralose due to reduced hydrolysis in an alkaline environment. Additionally, Hartlieb et al. (2017) reported that polar solvents enhanced the uptake of ibuprofen into CD-MOFs. Optimizing variables such as mass ratio, encapsulation time, and temperature can further improve drug molecule encapsulation (Han et al., 2018b).

The co-crystallization method is said to be a promising approach for drug encapsulation. In this method, drugs are introduced into the crystallization medium before CD-MOF formation. Unlike the impregnation method, where drug molecules must diffuse through preformed tight CD-MOF channels, this approach assembles CD-MOF:drug complexes directly, preserving crystallinity (Qiu et al., 2020b). This method has demonstrated improved interaction between drugs and CDs within the crystalline matrix, significantly enhancing the lansoprazole loading capacity of γ -CD-MOF (Li et al., 2017b). However, outcomes can vary, requiring careful control of stoichiometric compositions for reliable cocrystal formation. Moreover, the highly basic pH during CD-MOFs formation limits its applications to drugs stable and soluble in alkaline conditions.

A guest molecule/CD inclusion complexes was also successfully prepared via mechanical grinding. This method involves accurately weighing the guest molecule and CD-MOFs in stoichiometric ratio, grinding them with suitable

solvents for a set period at designated temperatures, rinsing and drying the product to a constant weight (Han et al., 2018b). Variables such as molar ratio, grinding time and temperature impact drug inclusion. Lu et al. (2015) successfully co-encapsulated two drugs, 5-fluorouracil and quercetin, into CD-MOFs using this method at 1:1:1 molar ratio, grinding for 1 hour with ethanol at room temperature. This method has also been associated with higher drug payload.

2.4 Modifications of γ -CD-MOFs

CD-MOFs readily dissolve in water, dissociating into their precursor metal salts and CD. Upon vapour diffusion with methanol, they recrystallize and reform into the original CD-MOF structure (Smaldone et al., 2010). However, their potential use in the biomedical field is limited due to their physical frailness and degradability in aqueous media, which leads to disassembly before reaching the target tissues or organs (Han et al., 2018b).

Like other MOFs, CD-MOFs can also be functionalized to provide tailored release performance and achieve other therapeutic effects. Many modifications target the active -OH groups to enhance the hydrolytic stability of CD-MOFs (Liu et al., 2017). According to Lopez and Perez (2023), the stability of MOFs is determined by intrinsic structural factors such as basicity, configuration, ligand hydrophobicity, the charge density of metal ions, and the coordination numbers of metal ions or clusters. Theoretically, modifying these factors (i.e. synthesize water-stable MOFs with strong metal coordination bonds and hydrophobic linkers) could yield chemical stable MOFs. However, this approach is limited by the complexity of multistep synthesis procedure (Zhou et al., 2019).

An alternative strategy is post-synthesis modification, which imparts hydrophobicity to the external surfaces of MOFs. This method improves hydrolytic stability while enhancing properties and functions suitable for various applications (Qian et al., 2017; Xie et al., 2020). For instance, Furukawa et al. (2012) created aqueous-media-stable cubic gel particles by cross-linking ethylene glycol diglycidyl ether to -OH groups in adjacent CDs within γ -CD-MOF supramolecular assemblies. However, the resulting

particles were not biodegradable in biological media, rendering them unsuitable for biomedical applications. Moreover, the process was complicated and time-consuming.

To maintain the nanometric size of γ -CD-MOFs, Li et al. (2016b) functional groups onto γ -CD-MOFs as a surface modification strategy. The incorporation of fullerene (C_{60}) into CD-MOFs matrices improved the hydrophobicity while preserving structural integrity. This carrier system was used to load Doxorubicin (DOX) and demonstrated sustained DOX release in phosphate buffer saline with a payload of 6.5 wt%. Interestingly, replacing C_{60} with tetrakis (4-hydroxyphenyl) porphyrin-conjugated C_{60} introduced fluorescent properties to the crystals, offering a novel idea to exploit *in vivo* fluorescence imaging for CD-MOFs (Nagai et al., 2018).

To improve moisture stability, Singh et al. (2017a) cross-linked diphenyl carbonate to the γ -CD units of γ -CD-MOFs, though this resulted in a loss of crystallinity. Subsequently the same group successfully surface-modified γ -CD-MOFs with cholesterol, producing a crystal that remained intact after 24 h of water treatment (Singh et al., 2017b). Cholesterol-shielded DOX showed good tolerance *in vivo*, with blood half-life of DOX increasing up to fourfold.

Ke et al. (2019a) observed that interactions between K^+ ions in MOFs and water molecules resulted in the collapse of the crystal's structure. To counter this, they induced the coordination of hydrogen sulfide (H_2S) with the K^+ ions, effectively attracting water molecules away from the metal centers of MOFs thus providing moisture resistance. Another approach incorporated γ -CD-MOF nanocrystals into biocompatible polymer (polyacrylic acid, PAA) using a solid in oil-in-oil (S/O/O) emulsification-solvent evaporation method (Li et al., 2017a). While the resulting composite microspheres exhibited enhanced aqueous stability and sustained drug release, their size exceed the micrometre range.

Aside from improving their aqueous stability, the modification of CD-MOFs can introduce a wide range of functions: slow release, release triggered by specific stimuli, and targeted release (Yang et al., 2024). Hence, the adaptability of CD-MOFs for biomedical and industrial applications could prove to be a

valuable strategy for enhancing the efficiency, specificity, and safety of drug delivery systems.

2.5 Applications of CD-MOFs

2.5.1 Native CD-MOFs applied for drug delivery

Many bioactive compounds exhibit strong antioxidative properties, but their poor stability and solubility hinder the delivery of its physiological benefits. The amphiphilicity of CD-MOFs is one of their most essential attributes. The hydrophobic cavity lined with carbon-hydrogen (C-H) groups offers non-polar interaction sites for various types of molecules, thus making them excellent carriers and storage systems for hydrophobic drugs, gases and also inorganic compounds (Szejtli, 1998; Xu et al., 2019; Yu et al., 2019; Chen et al., 2021b); the hydrophilic character attributed by the large number of hydroxyl groups on the exterior is said to enhance the solubility, permeability and bioavailability of various water insoluble drug (Brewster and Loftsson, 2007; Jansook and Loftsson, 2022).

Studies have shown that CD-MOF significantly improve the stability of bioactive compounds, especially polyphenols, generating increasing interest in their application. Hence, it was proposed that CD-MOFs may be utilized as carriers for bioactive compounds such as polyphenols, flavouring agents, lipids and other natural products (Blight et al., 2020; Wang et al., 2021; Liu et al., 2021).

To counter the low water solubility, Hu et al. (2021) encapsulated menthol in three kinds of CD-MOFs and concluded that β -CD-MOFs demonstrated significantly higher encapsulation efficiency (22.54%) compared to other materials. Similarly, the encapsulation of glycyrrhizic acid – a compound with anti-inflammatory and antibacterial properties, but poor water solubility – using γ -CD-MOFs protected the drug and showed no significant toxicity to Caco-2-cells (Qiu et al., 2019). A notable 1450-fold increase in the apparent solubility of insoluble folic acid (1.6 $\mu\text{g/mL}$) was achieved with γ -CD-MOF encapsulation using a ‘Ship-in-a-Bottle’ loading strategy (Xu et al., 2019). This improved solubility also enhanced the bioavailability of folic acid compared to its free form. Likewise, increased solubility of bioactive compounds such as curcumin,

ellagic acid, Coenzyme Q10 (CoQ10), anise leaf polyphenols, and resveratrol have also been reported with the use of CD-MOF as a carrier (Inoue et al., 2018; Qiu et al., 2020a; Zhou et al., 2020; Wang et al., 2021; Nyamba et al., 2021). Moreover, evidence suggests that CD-MOFs can be utilized in controlled-release systems, enabling continuous release and further improving bioavailability (Xu et al., 2019; Li et al., 2020a; Zhou et al., 2020).

Under neutral, alkaline or heated conditions, curcumin is known to degrade into feruloyl methane, ferulic acid and vanillin (Tønnesen et al., 2002). Post-encapsulation in γ -CD-MOFs, the stability of curcumin was shown to have significantly improved even at high pH (pH = 11.5) (Li et al., 2016a). Additionally, the porous structure of γ -CD-MOFs also enhanced the photostability and thermal stability of curcumin (Li et al., 2022).

Resveratrol is another compound prone to isomerization and deactivation under unfavourable light, temperature and pH conditions (Shen et al., 2021). Qiu et al. (2018b), demonstrated that loading resveratrol into nano and micro-scale CD-MOFs, synthesized via a seed-mediated method, improved its stability and enabled sustained release over 24 h. Similarly, stability improvements have been reported for inclusion compounds of epigallocatechin gallate, lansoprazole, ferulic acid, anise leaf polyphenols, and other compounds (Michida et al., 2015; Ke et al., 2019b; Li et al., 2020b; Wang et al., 2021).

Encapsulation efficiency and stability benefits extend beyond polyphenols. For instance, γ -CD-MOF encapsulated vitamin A palmitate with an efficiency of $9.77 \pm 0.24\%$. When compared to commercially available vitamin A powder, Zhang et al. (2018) reported an increase in half-life of vitamin A encapsulated in γ -CD-MOF by 1.6 times.

2.5.2 Encapsulation mechanism

Stereo compatibility between a guest molecule or active compound and encapsulant is a crucial factor in successful encapsulation. The size and shape of the guest molecule must align with the pores of MOFs, allowing it to be fixed within the host framework through various interactions, such as π - π interaction, hydrogen bonding, Coulombic forces, van der Waals forces, and coordinate bonds (Cai et al., 2019). The adsorption capacity of MOFs is

influenced by factors such as surface area, pore volume and structural characteristics. However, studies have shown that the crystallinity of some MOFs can remain unaffected by the presence of guest molecules, depending on the nature of the guest molecule, the MOF structure, and the experimental conditions (Moussa et al., 2016; Zhang et al., 2018; Xiong et al., 2019; Zhang et al., 2023).

Xu et al. (2019) highlighted that despite the high potential for CD-MOF as drug carriers, limited information is available regarding the precise drug incorporation mechanisms within their crystalline network. However, several hypotheses have been proposed based on experimental and computational studies.

For instance, ferulic acid is believed to be absorbed onto the walls of the (γ -CD)₆ (large cavity of MOF, Figure 2-4) pores network to form an inclusion complex. In contrast, the loading of ibuprofen has been associated with an anion exchange process where the free acid of ibuprofen is deprotonated by hydroxyl anions of CD-MOF (Michida et al., 2015; Hartlieb et al., 2017). Similarly, Moussa et al. (2016) reported that curcumin loading into CD-MOFs occurs via hydrogen bonding and adduct formation, involving interactions with both potassium ions and CDs.

Beyond their inherent cavities, CD-MOFs generate additional pores and channels through interconnection via metal ions, enabling further encapsulation applications. The dual-CD units (Figure 2-4) of CD-MOFs, also possessing amphiphilic characteristic, has also been identified as a loading mechanism for compounds such as sucralose, vitamin A palmitate, and folic acid (Lv et al., 2017; Zhang et al., 2018; Xu et al., 2019). For example, molecular dynamics simulations revealed that sucralose preferentially occupies the cavities formed by γ -CD pairs rather than the hydrophilic chamber surrounded by γ -CDs (Lv et al., 2017). The confined environment provides steric hindrances, stabilizing the compound and delaying its decomposition at elevated temperatures. It was presumed that due to the confinement of sucralose offering steric hindrances, CD-MOFs gave a stabilizing effect and delayed decomposition at elevated temperatures.

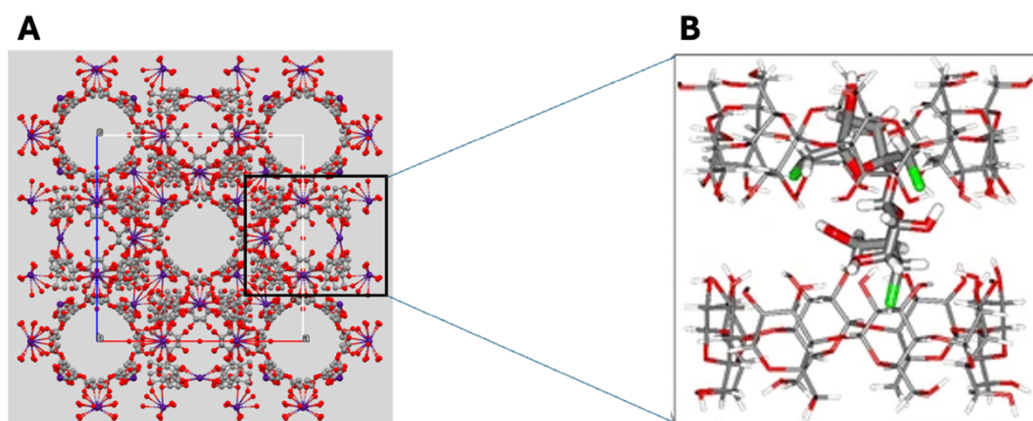


Figure 2-4 Molecular structure of (a) γ -CD-MOFs and (b) the γ -CD pairs (Lv et al., 2017).

Xu et al. (2019) also investigated the encapsulation mechanism of folic acid in CD-MOF using molecular docking and simulations. They proposed that folic acid is initially included within dual-CD units, followed by the formation of tiny nanoclusters inside the hydrophobic cavities and on the MOF's surface. Surface adsorption and hydrogen bonding are additional mechanisms that have been suggested for folic acid loading (Lv et al., 2017).

Studies on curcumin and isosteviol revealed concentration-dependent loading mechanisms. At low concentrations, these compounds preferentially form hydrogen bonds with hydroxyl groups of γ -CDs and occupy the hydrophobic voids of dual γ -CD units. At higher concentrations, they transition to occupying the larger cycloidal cavity within $(\gamma\text{-CD})_6$ units, forming nanoclusters via hydrophilic interactions (Chen et al., 2021a; Chen et al., 2022).

2.5.3 Release mechanism

Han et al. (2018b) suggested that drug release from drug-loaded CD-MOFs does not exhibit an obvious burst effect. This phenomenon may be attributed by the gradual collapse of the framework, driven by the cleavage of weak bonds between the drug and functional groups of the framework. Generally, active release is the main mode for MOFs, with molecular diffusion being the key mechanism (Lv et al., 2022). This hypothesis was supported by Liu et al. (2019) where the release mechanism for drugs in CD-MOF through a molecular simulation and modelling of prednisolone release from γ -CD-MOF

was studied. They reported that the ability of γ -CD-MOF to retain drugs within its porous network was related to the crystal size, in which diffusion was the limiting parameter. The release process was described as an isotropic continuous diffusion process.

In an evaluation of Nano-CD-MOFs as a carrier for curcumin, Chen et al. (2022) noted two distinct release phases: an initial sharp rise in the release rate, followed by a plateau or relatively slower releasing rate. Molecular docking data linked the sharp rise to the collapse of CD-MOFs, which released a significant amount of curcumin trapped within the MOF cavities. The subsequent slower release phase was attributed to the slow diffusion of entrapped curcumin within the voids of dual γ -CD units. The collapse is generally associated with the breakdown of the metal-ligand linkages (Ding et al., 2019).

The breakdown of these linkages occurs through a coordination competition at the metal center, where water molecules displace the metal-linker coordination bonds. This process is followed by a hydrolysis, which deprotonates the water molecules and ultimately leads to the complete breakdown of the metal-linker connections (Lopez and Perez, 2023). The severity of this breakdown or displacement is largely dependent on the characteristics of the metal cation (i.e. reactivity and lability), as well as the length and rigidity of the organic linkers.

Moisture variations also significantly influence the control of active substance release. Magri et al. (2021) reported that low moisture values inhibit drug release, whereas high moisture values (70-96%) promoted release. High moisture content can cause structural changes of CD-MOFs, leading to erosion of the encapsulant material. Consequently, the release mechanisms involved may involve drug desorption, diffusion from framework's pores, material erosion, or a combination of these processes.

CD-MOFs demonstrate exceptional potential for drug delivery, improving the stability, solubility, and bioavailability of various bioactive compounds. Their versatile encapsulation mechanisms and controlled-release properties make them suitable for a range of applications, from therapeutics to natural

products. However, further research is needed to fully understand their encapsulation and release mechanisms.

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Chapter 3

γ -Cyclodextrin Metal-Organic Frameworks: Do Solvents Make a Difference

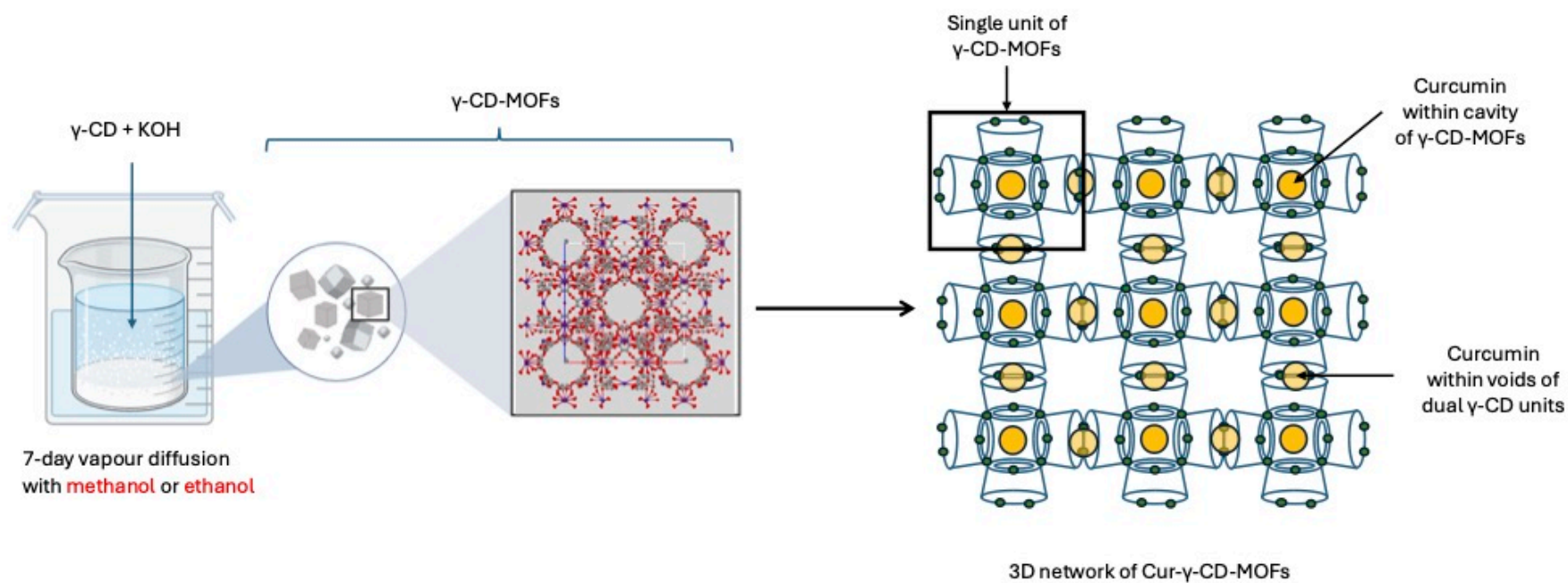


Figure 3-1 Graphical abstract of synthesis of γ -CD-MOFs and encapsulation of curcumin.

3.1 Abstract

Conventionally, methanol is the solvent of choice in the synthesis of γ -cyclodextrin metal-organic frameworks (γ -CD-MOFs), but using ethanol as a replacement could allow for a more food-grade synthesis condition. Therefore, the aim of the study was to compare the γ -CD-MOFs synthesized with both methanol and ethanol. The γ -CD-MOFs were characterized by scanning electron microscopy (SEM), surface area and pore measurement, Fourier transform infrared spectroscopy (FTIR) and powder X-ray diffraction (PXRD). The encapsulation efficiency (EE) and loading capacity (LC) of the γ -CD-MOFs were also determined for curcumin, using methanol, ethanol and a mixture of the two as encapsulation solvent. It was found that γ -CD-MOFs synthesized by methanol and ethanol do not differ greatly, the most significant difference being the larger crystal size of γ -CD-MOFs crystallized from ethanol. However, the change in solvent significantly influenced the EE and LC of the crystals. The higher solubility of curcumin in ethanol reduced interactions with the γ -CD-MOFs and resulted in lowered EE and LC. This suggests that different solvents should be used to deliberately manipulate the EE and LC of target compounds for better use of γ -CD-MOFs as their encapsulating and delivery agents.

3.2 Introduction

Metal-organic frameworks (MOFs) are known as highly ordered porous crystalline materials consisting of organic ligands and metal ions. Their high tunability, porosity, surface area, and thermal stability can be dictated to suit various applications (Islamoglu et al., 2017; Tibbetts and Kostakis, 2020; Yusuf et al., 2022). Such applications include gas storage, separation science, sensors, catalysts, and drug delivery (Li et al., 2012; He et al., 2019; Xu et al., 2019a; Benedetto et al., 2023). However, the process of synthesizing MOFs was reported to be non-recyclable and highly toxic depending on the type of synthetic components or chemical reagents used (Roy and Stoddart, 2021). To counter these limitations, a greener and more biocompatible method was investigated with the use of non-toxic alkali and alkaline metal ions in combination with organic ligand linkers of naturally occurring building blocks

of amino acids, peptides and carbohydrates (Shen et al., 2021). With the reduction in unfavourable factors in their synthesis, the applications of MOFs in the food industry and nanomedicine should be more promising.

Naturally occurring carbohydrate-based carriers, cyclodextrins (CDs) have been regarded as excellent candidates for drug-carrier preparations. In connection with this, more sustainable, edible CD-MOFs have been fabricated from γ -CDs and potassium ions via a vapour diffusion method (Smaldone et al., 2010). The γ -CD-MOFs have a three-dimensional microporous structure in which the primary (C-6) hydroxyl groups are linked to potassium ions. Following the successful synthesis of γ -CD-MOFs, α - and β -CD-MOFs were also investigated and characterized (Roy and Stoddart, 2021). However, γ -CD-MOFs have been more extensively studied due to the inherent symmetrical structure of γ -CD that gives the encapsulated compound better stability. Hence, γ -CD-MOFs have attracted growing interest as potential delivery systems for a wide range of bioactive molecules including curcumin, folic acid, resveratrol, glycyrrhizic acid, and more (Moussa et al., 2016; Xu et al., 2019b; Qiu et al., 2019; Qiu et al., 2020; Chen et al., 2021b; Sun et al., 2021; Jiang et al., 2022).

The success of various applications of γ -CD-MOFs has been said to be dependent on their crystal size since this would impact the interaction between the crystals and other molecules and their ability to be taken up by cells when being used as a delivery vehicle (Lu et al., 2012; Sadeh et al., 2023). The vapour diffusion method of preparing γ -CD-MOFs at ambient temperature requires a reaction time of 7 days. The extensive time results in high polydispersity and large sizes of the crystals obtained. Much research has thus focused on the development and optimisation of synthesis methods with shortened reaction time that produce more homogenous crystals with sizes within the micron or even nanometre range (Furukawa et al., 2012; Liu et al., 2016; Qiu et al., 2019; He et al., 2020; Chen et al., 2021b; Sadeh et al., 2023). Such methodologies require, for example, the addition of cetyltrimethylammonium bromide (CTAB) or polyethylene glycol (PEG 20,000), and/or manipulation of ratio of reactants, concentrations, and temperatures. This further complicates the synthesis method and the range of

parameters to control, whilst also introducing additional compounds that are not desirable to food.

Curcumin is a well-known natural bioactive polyphenolic compound. It has been reported to have numerous biological and pharmacological benefits, but applications of it have been limited due to its low bioavailability, generally attributed to its low water solubility and rapid metabolic conversion to inactive forms. Nevertheless, detection methods of curcumin have been well documented, and many methods have been explored attempting to improve its oral bioavailability (Araiza-Calahorra et al., 2018; Kotra et al., 2019; Stohs et al., 2020; Sabet et al., 2021; Tabanelli et al., 2021). Encapsulation of curcumin in γ -CD-MOFs has been shown to be possible although much remains to be understood about the interaction between the curcumin and the crystals and the effects of the solvents used to prepare the γ -CD-MOFs or load them with this polyphenol (Moussa et al., 2016; Chen et al., 2021b; Sun et al., 2021).

In this study, the effect of the variation of two solvents, methanol and ethanol, on the synthesis of γ -CD-MOFs and their encapsulation of curcumin was investigated. With the replacement of methanol with ethanol, the process of synthesis may be made to be more food-grade, which has not been highlighted before. It was hypothesized that this change in synthesis solvent, of differing polarity, may result in the variation of crystal polymorphs obtained via the conventional vapour diffusion method. Curcumin was then encapsulated in the γ -CD-MOFs obtained, via the impregnation method, again using different encapsulation solvents. A limited amount of data is available on ethanol-synthesized γ -CD-MOFs, but a proper comparison of γ -CD-MOFs obtained synthesized using methanol (via the vapour diffusion method) has not been carried out. In addition, the effect of a variation in encapsulation solvent has never been reported. The synthesized free γ -CD-MOFs and curcumin-loaded γ -CD-MOFs (Cur- γ -CD-MOFs) were characterized by SEM, surface area and pore measurement, FT-IR, and PXRD. To further demonstrate that the encapsulation solvent affects the EE and LC of γ -CD-MOFs, the encapsulation process was performed with pure methanol, pure ethanol, and mixed ratios of methanol to ethanol of 3:1, 1:1, and 1:3.

3.3 Materials and methods

3.3.1 Materials

γ -cyclodextrin (> 90% purity), methanol (HPLC-grade, >99.9% purity), and curcumin (from *Curcuma longa* (turmeric), powder, > 65% purity) were purchased from Sigma-Aldrich (Steinheim, Germany). Potassium hydroxide (pellets, ACS reagent, >85% purity) was purchased from Fisher Scientific (Loughborough, UK). Absolute ethanol (HPLC-grade, >99.8%) was purchased from VWR Chemicals (Rosny-sous-Bois, France). Pure deionised water (with a resistivity of not less than 18.4 M Ω cm) was used in all experiments from a Milli-Q IQ 7000 system (Merck Millipore, Darmstadt, Germany).

3.3.2 Synthesis of γ -CD-MOFs

The γ -CD-MOFs crystals were prepared via the conventional vapour diffusion method as described by Smaldone et al. (2010). Briefly, 1.30 g of γ -CD and 0.45 g of KOH (1:8 mole equivalent ratio of γ -CD to KOH) were dissolved in 20 mL of deionized water and magnetically stirred for 6 h at 600 rpm. The mixture was then filtered through a 0.45 μ m organic filter membrane and sealed in a beaker containing 50 mL of methanol to allow diffusion into the sample for 7 days. The procedure was repeated with substitution of methanol to ethanol for comparison.

An activation process was carried out to free the pores of the resulting crystals from any residual moisture entrapped during synthesis. The crystals were filtered, rinsed with the corresponding solvent used for synthesis (methanol/ethanol) to remove extra unlinked potassium ions, and air-dried at room temperature for 30 min. This was followed by soaking in methanol/ethanol for 3 days before being dried under vacuum at 30°C overnight. The crystals were either used immediately or stored in a desiccator.

3.3.3 Encapsulation of curcumin in γ -CD-MOFs

To study the inclusion of curcumin, an impregnation method was used. A 10:1 molar ratio of γ -CD-MOFs (0.3 g) to curcumin (0.01 g) were simultaneously dispersed and dissolved in 50 mL of methanol and stirred magnetically in the dark at 450 rpm for 3 h at room temperature. Subsequently, the mixture was

centrifuged (Rotina 380R, Hettich, Tuttlingen, Germany) at 5000 rpm for 30 min. The sediment was collected and washed twice with methanol to remove the unencapsulated fraction of curcumin, whether free in solution or adsorbed to the surface of γ -CD-MOFs. Finally, the Cur- γ -CD-MOFs were obtained by vacuum drying overnight at 30°C in the dark. The procedure was similarly repeated, replacing methanol with ethanol.

3.3.4 Characterization

3.3.4.1 Scanning electron microscopy (SEM)

Morphological characterization of γ -CD-MOFs was carried out using a SEM (Fei NanoSEM Nova 450, Thermo Fisher, Loughborough, UK) operating at 3 kV. The samples were prepared by mounting crystals on a SEM stub with adhesive copper tape and then sputter depositing a layer of iridium onto the sample's surface using a 208 HR Sputter Coater (Cressington Scientific Instruments, Watford, UK). Considering the high polydispersity of γ -CD-MOF crystals, the sizes of γ -CD-MOFs were measured with the Image Pro Plus 6.0 software (Media Cybernetics, Rockville, USA).

3.3.4.2 Surface area and pore volume measurements

The Brunauer-Emmett-Teller (BET) surface area, Langmuir surface area, and pore diameters of the activated γ -CD-MOF crystals was determined using an Accelerated Surface Area and Porosimetry System (ASAP 2020, Micromeritics, Tewkesbury, UK) equipped with nitrogen (N_2) gas. Approximately 100 mg of samples were added to the sample tubes and degassed under vacuum (10^{-5} Torr) at ambient temperature for 12 h.

3.3.4.3 Fourier transform infrared (FT-IR) spectroscopy

The FT-IR spectra of samples (curcumin, γ -CD, γ -CD-MOFs, and Cur- γ -CD-MOFs) were obtained using a Spectrum One FT-IR Spectrometer (PerkinElmer Instruments, Waltham, USA) with a Golden Gate Diamond attenuated total reflection (ATR) accessory (Specac, Orpington, UK). Samples were placed directly onto the ATR diamond crystal with a set pressure of 90 cN.m applied using a torque wrench. Each spectrum was recorded with an accumulation of 100 scans at resolution of 4 cm^{-1} across a range of $550 - 4000\text{ cm}^{-1}$.

3.3.4.4 Powder X-ray diffraction (PXRD)

The crystalline diffraction patterns of curcumin, γ -CD-MOFs, and Cur- γ -CD-MOFs were detected with a Bruker D8 Advance diffractometer (Bruker, Karlsruhe, Germany) at a tube voltage of 40 kV and tube current of 40 mA using Cu-K α radiation ($\lambda = 1.5418 \text{ \AA}$), utilising a 1.0 mm detector slit. The scans were carried out over a 2θ angle range of $3 - 45^\circ$ at 0.033° increments.

The observed intensities in the diffraction patterns were fitted using the program FullProf using the LeBail method where a general constant scale factor was used to match the observed intensities while adjusting the zero-error, lattice parameter, and the peak broadening parameters (Rodríguez-Carvajal, 1993). The background was adjusted with a cubic spline interpolation of a set of user-provided points. This ensured convergence of the fit within a few cycles. The 3D crystal structure is visualized in Mercury environment using the Crystallographic Information File (CIF) deposited on the Cambridge Structural Database (CCDC: 773708) by Smaldone et al. (2010). The same file was used to fit the diffraction patterns.

3.3.4.5 Encapsulation efficiency (EE) and loading capacity (LC)

The amount of unencapsulated curcumin in the supernatant collected as described in section 1.3.3 was calculated from a calibration curve using the SPECORD 210 Plus UV-Vis spectrophotometer (Analytik Jena, Jena, Germany) at 426 nm. Ratios of methanol to ethanol of 3:1, 1:1, and 1:3 were also investigated on their effect on encapsulation. The encapsulation efficiency (EE, %) and loading capacity (LC, %) of curcumin in inclusion complexes were subsequently determined by using the following equations (Chen et al., 2021b):

$$EE (\%) = \frac{\text{Amount of encapsulated curcumin}}{\text{Total amount of curcumin}} \times 100\% \quad (1)$$

$$LC (\%) = \frac{\text{Amount of encapsulated curcumin}}{\text{Total amount of curcumin and CD-MOFs}} \times 100\% \quad (2)$$

3.3.4.6 Statistical analysis

Data were collected in triplicate and the results were calculated and expressed as mean $\pm$ standard deviation where applicable. Statistical analysis was carried out using OriginPro 2023b (OriginLab, Northhampton, USA). Statistical significance was established at $p < 0.05$.

3.4 Results

3.4.1 Characterization

A conventional 7-day vapour diffusion method of synthesizing γ -CD-MOFs using methanol has reported a yield of approximately 85% (Moussa et al., 2016). In our study, with an identical methodology of synthesis, the average percentage yield was $78.40 \pm 0.79\%$. The use of ethanol as synthesis solvent has been adopted previously but the yield was not reported (Pan et al., 2022). The average yield of γ -CD-MOFs synthesized using ethanol in the current study was $75.53 \pm 3.53\%$: the average yields from both solvents were found to be not significantly different ($p > 0.05$).

The γ -CD-MOF crystals appeared to have similar, block-like morphologies for both solvents (Figure 3-2). Such structures were also observed by Tse et al. (2022) and Kathuria et al. (2019) as opposed to regular, clear cubic structures in various other reports (Smaldone et al., 2010; Chen et al., 2021b; Sun et al., 2021). Both synthesis solvents resulted in crystals with a wide range of sizes as evaluated from SEM images (Figure 3-2). Methanol-synthesized crystals had a size range of 35 – 450 μm , whereas the size of ethanol-synthesized crystals was between 60 μm and 460 μm , with the average particle size of the former $165 \pm 92 \mu\text{m}$ and the latter $259 \pm 96 \mu\text{m}$. Thus, the mean sizes were significantly different ($p < 0.05$). The results also agree with the size range of 40 – 500 μm obtained via the vapour diffusion method reported elsewhere (Smaldone et al., 2010).

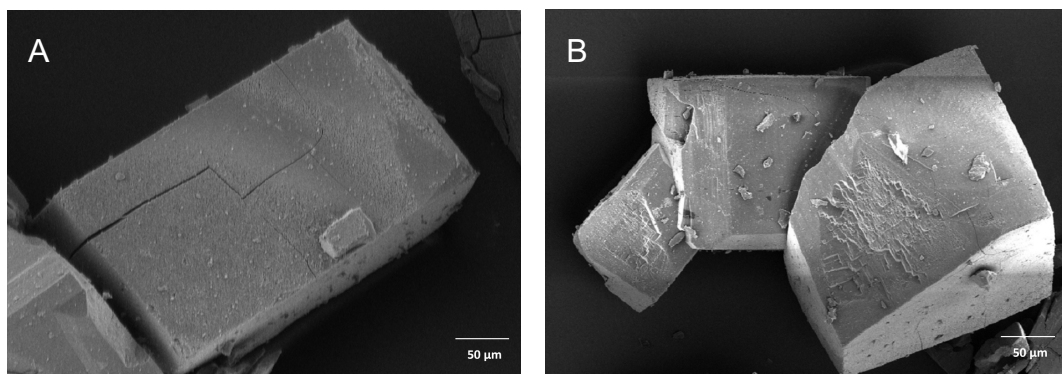


Figure 3-2 Scanning electron microscopy (SEM) morphology images of γ -cyclodextrin metal-organic frameworks (γ -CD-MOFs) prepared using different synthesis solvent: (a) methanol and (b) ethanol, respectively.

The surface properties (i.e., surface areas and pore diameters) of free and Cur- γ -CD-MOFs are summarized in Table 3-1. The measured pore sizes of approximately 18.5 Å of the free γ -CD-MOFs, regardless of synthesis solvent used, were typical of a mesoporous material. The porosity of the activated γ -CD-MOFs was also verified by measuring the N₂ gas adsorption of the samples, as shown in Figure 3-3. The steep increase in the low-pressure area of the isotherm is known to be distinctive to the porosity of MOFs. The Braunauer-Emmett-Teller (BET) surface area results of methanol-synthesized crystals were slightly higher than those of ethanol-synthesized but the two were not found to be significantly different ($p > 0.05$). The large reduction in the BET surface of post-encapsulation γ -CD-MOFs to approximately 1 m²/g is thought to be a result of curcumin occupying the pores of the crystals.

Table 3-1 Surface parameters and crystallite sizes obtained from Scherrer equation using 200 reflection of the γ -CD-MOFs prepared with varying synthesis solvent and Cur- γ -CD-MOFs prepared with varying encapsulation solvent.

Samples	BET surface area (m ² /g)	Langmuir surface area (m ² /g)	Pore diameters (Å)	Crystallite size (nm)
CD-MOF (M)	799.4 $\pm$ 14.6 ^a	813.7 $\pm$ 14.3 ^a	18.6 $\pm$ 0.1 ^a	398 $\pm$ 38
CD-MOF (E)	719.1 $\pm$ 13.4 ^a	848.1 $\pm$ 10.2 ^a	18.7 $\pm$ 0.2 ^a	750 $\pm$ 56
Cur-CD-MOF (M, m)	1.6 $\pm$ 0.4 ^b	-	-	372 $\pm$ 76
Cur-CD-MOF (M, e)	1.3 $\pm$ 0.2 ^b	-	-	-
Cur-CD-MOF (E, m)	1.7 $\pm$ 0.6 ^b	-	-	-
Cur-CD-MOF (E, e)	1.6 $\pm$ 0.5 ^b	-	-	270 $\pm$ 89

Letters in brackets: M or E indicates that the synthesis solvent used in preparation of γ -CD-MOFs was either methanol or ethanol; m or e indicates that solvent used for encapsulation was either methanol or ethanol. Values in the same column having different letters were significantly different ($p < 0.05$).

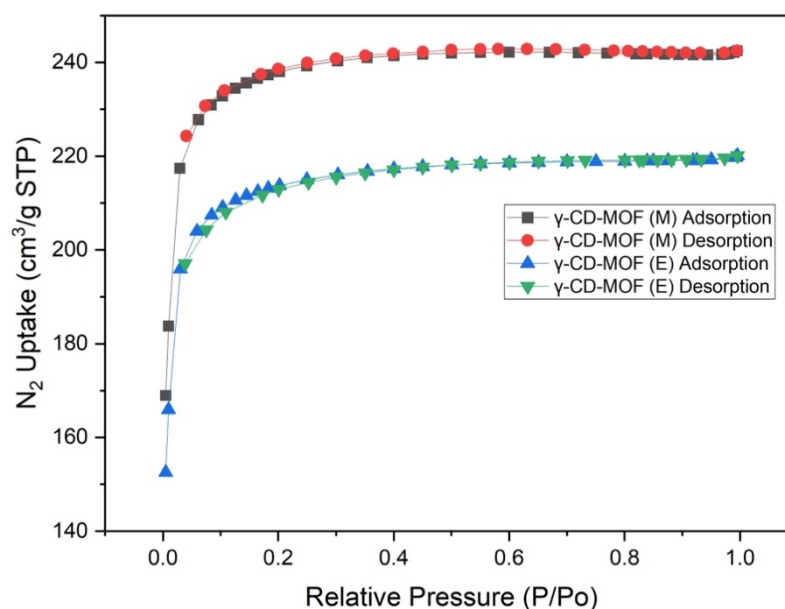


Figure 3-3 Nitrogen adsorption-desorption isotherms of activated free γ -CD-MOFs synthesized using either methanol (M) or ethanol (E).

The possible bonding between the functional groups of curcumin, free γ -CD-MOFs, and Cur- γ -CD-MOFs were examined by FT-IR spectroscopy (Figure 3-4). The comparison of FT-IR spectra of γ -CD, γ -CD-MOFs synthesized using methanol, and γ -CD-MOFs synthesized using ethanol is shown in Figure A-2. Dominant peaks observed in the FT-IR spectrum of curcumin at 3551 cm^{-1} , 1626 cm^{-1} , and 1602 cm^{-1} corresponded to -OH stretching, C=O stretching, and aromatic C=C stretching, respectively. Peaks expressed by free γ -CD-MOFs at approximately 3295 cm^{-1} and 1364 cm^{-1} showed a blue shift indicating that some interaction possibly occurred between curcumin and the γ -CD-MOFs post-encapsulation. A slight blue shift of -OH stretching vibration peak at 3295 cm^{-1} to approximately 3304 cm^{-1} , and the shifting -OH plane bending vibration peak from 1364 cm^{-1} to approximately 1407 cm^{-1} was observed in Cur- γ -CD-MOFs. This could be inferred as attractive interactions occurring between curcumin and γ -CD-MOFs.

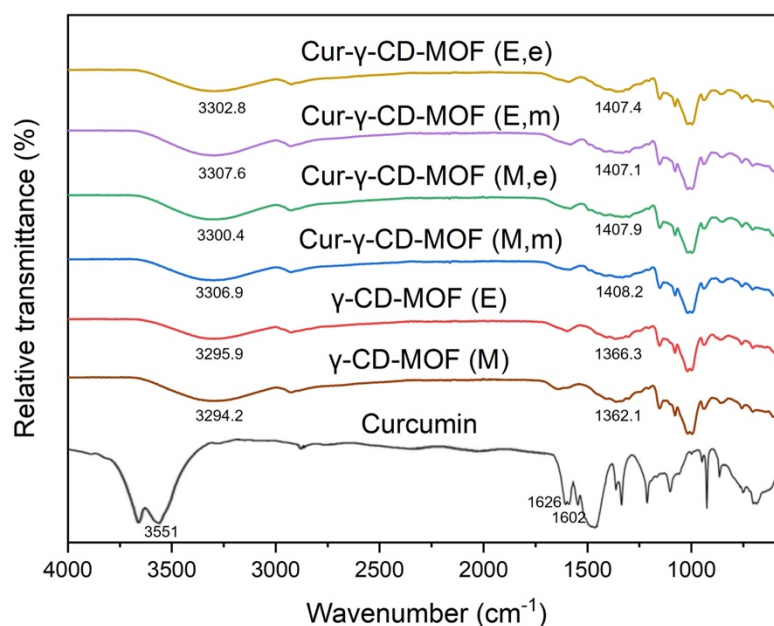


Figure 3-4 Fourier transform infrared spectroscopy (FT-IR) patterns of curcumin, free γ -CD-MOFs, and Cur- γ -CD-MOFs. (M) denotes crystals synthesized with methanol, and (E) with ethanol; (m) indicating that methanol was used as encapsulation solvent, and (e) as ethanol.

To confirm the crystallinity of the γ -CD-MOFs synthesized, PXRD of the crystals was performed. The PXRD patterns of all MOF samples as shown in Figure 3-5, including curcumin-loaded ones, showed a similar set of diffraction peaks, but differ from the diffraction pattern of pure curcumin. This observation indicates that curcumin molecules were incorporated within the γ -CD-MOFs. Detailed insights into the synthesized crystal structures were obtained by comparing diffraction patterns reported elsewhere (Smaldone et al., 2010; Singh et al., 2017; Shen et al., 2020; Chen et al., 2021b). The reflections observed at 2.87, 4.05, 4.97 and 5.73 (nm^{-1}) were identified as the characteristics 110, 200, 211 and 220 from $\text{Im}3\text{m}$ cubic structure (Figure 3-5). This structure was previously reported by Smaldone et al. (2010) for γ -CD-MOFs. The global fitting of PXRD patterns using the CIF file (CCDC 773708) compared well with the experimental data, thus confirming the presence of $\text{Im}3\text{m}$ cubic crystal structure (space group 229; see Figure 3-6a) in all the MOF samples. It should also be noted that as the results for the free unloaded γ -CD-MOFs did not show significant differences with the varying solvent, PXRD was only carried out on Cur- γ -CD-MOFs using pure methanol or ethanol as the encapsulation solvent, not mixtures of the two – see section 3.4.2 below.

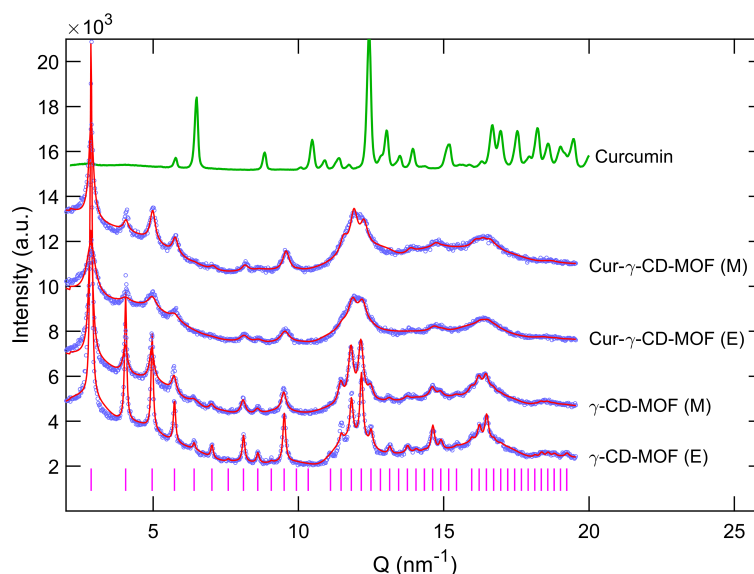


Figure 3-5 Powder X-ray diffraction patterns (PXRD) of γ -CD-MOFs synthesized with methanol (M) and ethanol (E) as synthesis solvent and Cur- γ -CD-MOFs using corresponding encapsulation solvent of methanol and ethanol. The experimental diffraction patterns of all MOF samples are shown in blue while their corresponding theoretical fitted curves are shown by solid red lines. The pink dashed lines show the 64 reflections from the Im3m cubic crystal structure (space group I432). The diffraction pattern from pure curcumin powder is shown in green line. The x-axis represents the scattering vector magnitude equivalent to $4\pi/\lambda \times \sin(\theta)$. The diffraction curves are shifted vertically for better clarity.

While the PXRD spectrum indicated that Im3m cubic crystal structure for all the synthesized MOF samples, the broadness and intensities appeared to vary from one sample to another, indicating variable crystallinity in different samples. Applying the Scherrer equation and using the broadness of 200 reflection, the crystallite sizes of all samples were estimated, and the results are summarized in Table 3-1 (Smilgies, 2009). For free unloaded samples, ethanol as the synthesis solvent seemed to promote the formation of larger γ -CD-MOFs compared to methanol; however, when curcumin was involved, larger crystals were obtained in the presence of methanol as encapsulation solvent. These nanoscale crystallite size variations by PXRD corresponded well with microscale crystal dimensions from SEM data. In addition, the global fitting of diffraction patterns resulted in the determination of the lattice parameter with high precision. While both methanol- and ethanol-synthesized γ -CD-MOFs showed similar lattice parameters of 31.04 and 31.01 (± 0.01) Å

respectively, the curcumin-loaded samples demonstrated a notable reduction in the lattice parameters, i.e., 30.72 and 30.88 (± 0.01) Å (see Figure 3-6b).

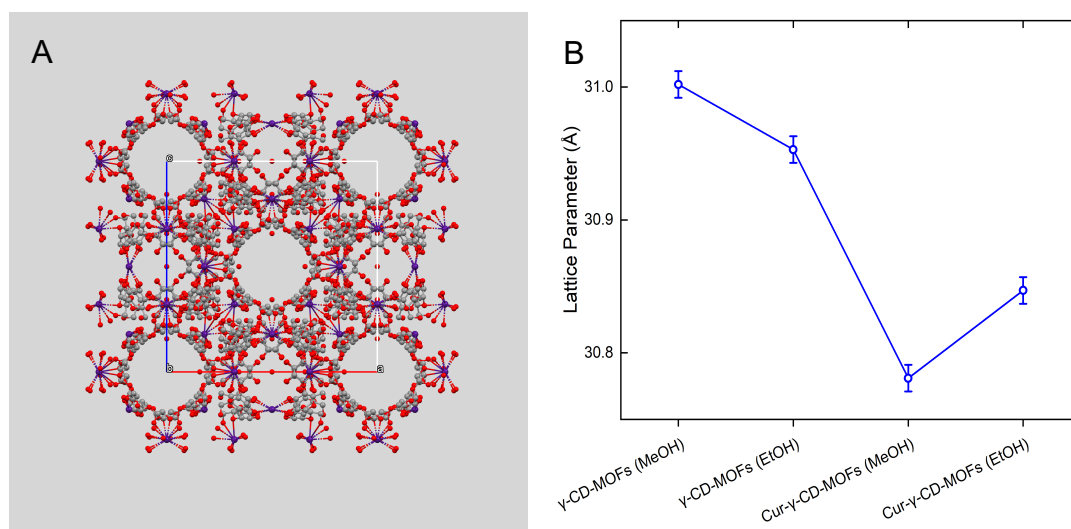


Figure 3-6 (A) Representation of crystal structure of γ -CD-MOFs observed in our study using methanol and ethanol as synthesis and encapsulation solvents in absence/presence of curcumin. All synthesized MOFs demonstrated an $Im\bar{3}m$ cubic symmetry ($I432$ space group). (B) Different lattice dimensions evaluated for samples synthesized and encapsulated with different solvents. Noticeable reduction in lattice parameter is observed for curcumin-loaded samples.

3.4.2 Encapsulation efficiency (EE) and loading capacity (LC)

To further investigate the effect of solvent on the EE and LC of γ -CD-MOFs, pure methanol, ethanol, and varying ratios of the two (methanol to ethanol = 3:1, 1:1, and 1:3) were used for the encapsulation of curcumin into the two types of crystals synthesized. Based on the results (Figure 3-7), a distinct advantage of methanol as an encapsulation solvent was observed. With methanol as the major encapsulating solvent, the EE was between 80 – 100%, regardless of the synthesis solvent used to prepare the γ -CD-MOFs. When ethanol dominated as the encapsulation solvent, a 10% reduction in EE was initially observed at a 1:3 methanol to ethanol ratio and further halved when pure ethanol was used. The LC of the γ -CD-MOFs behaved similarly where the presence of methanol resulted in a comparable LC of approximately 30% and halved with use of pure ethanol as encapsulation solvent. However, there

was no clear trend on whether the methanol- or ethanol-synthesized γ -CD-MOFs had superior EE and LC. Thus, it could be inferred that the encapsulation solvent had a greater impact on the EE and LC than the synthesis solvent.

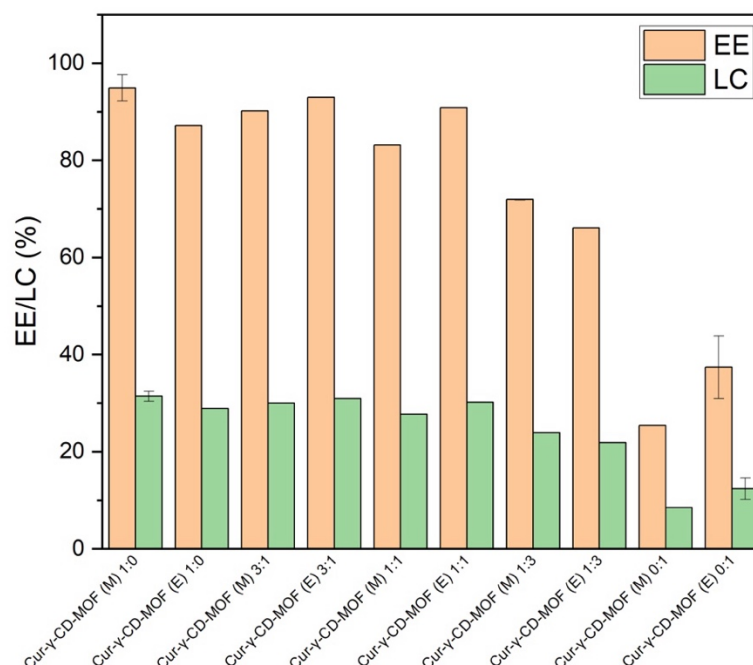


Figure 3-7 Encapsulation efficiency (EE) and loading capacity (LC) of methanol- and ethanol-synthesized γ -CD-MOFs in varying ratios of encapsulation solvents of methanol to ethanol at 1:0, 3:1, 1:1, 1:3, and 0:1.

3.5 Discussion

3.5.1 Characterization

The consistently cuboid shapes of γ -CD-MOFs have been attributed to the ability of γ -CDs and potassium ions to form coordinate bonds in the mixed solvent environment of water and alcohol (Rajkumar et al., 2019). In some cases, fine particles formed by the disintegration of γ -CD-MOFs may be caused by a neutralisation step in the procedure, using weak acids (Lv et al., 2017). Furthermore, it has been hypothesized that vacuum treatment and solvent evacuation processes may damage or collapse the structure of the crystals (He et al., 2020; Kim et al., 2023).

Larger crystals and slower crystal growth were observed here when ethanol was used as the synthesis solvent compared to methanol. Solution conditions

such as supersaturation, solvent type, and temperature are known to have significant effects on crystallization, particularly nucleation and crystal growth. Solvent molecules can be barriers to crystal formation since they are closely associated with prenucleation solute clusters in solution (Parambil et al., 2017). A more polar solvent of greater dielectric constant and miscibility with water promotes supersaturation conditions and interfacial interactions during γ -CD-MOFs formation, which generally induces quicker rates of nuclei formation and thereby results in more uniform crystals of reduced sizes (Neugebauer et al., 2018). Conversely, the use of a solvent with decreased polarity would tend to cause a decrease in growth rate. This was supported by a study conducted by Liu et al. (2016) where the use of ethanol as the synthesis solvent promoted formation of hexagonal, larger-sized crystals as compared to methanol. The authors also suggested that solvents with lower boiling point facilitated faster vapour diffusion, resulting in faster nuclei formation and rapid growth of smaller crystals.

Various BET surface area values have been reported, ranging from approximately 230 m²/g to 1800 m²/g depending, on the method by which the γ -CD-MOFs were synthesized (Moussa et al., 2016; Qiu et al., 2019; He et al., 2020; Chen et al., 2021a). The large deviation in BET surface areas has been said to be caused by incomplete solvent evacuation or collapse of the crystal structure during the evacuation process (Nelson et al., 2009). The results collected from the current study fall within the range reported and are similar to those reported by Chen et al. (2021b) and Lv et al. (2017), but lower than the theoretical BET surface area of 1030 m²/g (Smaldone et al., 2010). Theoretically, any or all available surface area should be occupied by the encapsulant to maximise the encapsulation and loading efficiency of the crystals, but complete occupancy is difficult to achieve (Matsuyama et al., 2014). The observed large reduction of BET surface area post-encapsulation has similarly reported elsewhere, though not as drastic as seen in the current study (Moussa et al., 2016; Chen et al., 2021a). For comparison, Chen et al. (2021b) reported a reduction in BET surface area from 669.63 m²/g to 117.25 m²/g and 539.05 m²/g to 132.03 m²/g at an encapsulation mass concentration of γ -CD-MOFs to curcumin at 0.5:1 and 1.5:1 respectively. This may be due to the further fragmentation or collapse of the γ -CD-MOFs as a result of having

undergone a second solvent evacuation and vacuum treatment to collect the Cur- γ -CD-MOFs. Some fragmentation can be seen in SEM images (Figure A-1).

The FT-IR spectrum of Cur- γ -CD-MOFs suggests the encapsulation solvent did not chemically change the structure of the γ -CD-MOFs. The slight shift in transmittance of curcumin-loaded γ -CD-MOFs compared to free γ -CD-MOFs from 3295 cm^{-1} to approximately 3304 cm^{-1} may be due to an interaction between the OH groups of the γ -CD-MOFs and those of curcumin. The vibration peak at 3551 cm^{-1} , attributed to the phenolic hydroxyl group of curcumin, was no longer detected in post-encapsulated samples, suggesting the possibility of a hydrogen bonding with this curcumin OH groups. A more distinct transmittance shift in this region in Cur- γ -CD-MOFs has also been reported previously (Moussa et al., 2016; Inoue et al., 2018; He et al., 2020). The next most prominent shift observed was from 1364 cm^{-1} in free γ -CD-MOFs to 1407 cm^{-1} in curcumin-loaded γ -CD-MOFs. This shift may be largely due to aromatic C=C and C=O stretching (benzene skeleton) of curcumin in a similar region. It has been said that benzene rings of curcumin may be trapped within the cavities of γ -CD-MOFs via van der Waals forces and hydrophobic interactions (Chen et al., 2021b). Although it was noted that the vibration peaks at approximately 1400 cm^{-1} attributed to the alkene CH_2 of curcumin, were no longer visible, these wavenumbers overlap with those of the free γ -CD-MOFs, so little can be concluded about any specific the interactions (Gunasekaran et al., 2008). Other studies have suggested that the disappearance of the peaks could indicate a complete inclusion of curcumin in γ -CD-MOFs. This has led to the hypothesis of curcumin being trapped in the hydrophobic region in between γ -CD pairs and cavities of γ -CD-MOF (Inoue et al., 2018; Zhang et al., 2019; Chen et al., 2021a). In addition, the broadness of the peaks of free γ -CD-MOFs and Cur- γ -CD-MOFs remains constant which suggests that interaction with curcumin does not affect the structure of γ -CD-MOFs. This was similarly inferred by Moussa et al. (2016) and Wang et al. (2022).

Based on the PXRD patterns of Cur- γ -CD-MOFs, it can be inferred that the encapsulation of curcumin into γ -CD-MOFs did not disrupt the crystalline structure of the CD-MOFs, with only a slight shift in the position of the peaks

(Figure A-3). This non-disruptive phenomenon of γ -CD-MOFs post encapsulation has also been reported elsewhere (Moussa et al., 2016; Chen et al., 2021b; He et al., 2023). However, the numerous sharp diffraction peaks observed in the PXRD spectrum of curcumin alone were no longer observed post encapsulation. This indicates that curcumin was successfully encapsulated into the γ -CD-MOFs, but also that the curcumin was encapsulated in an amorphous form (Yang et al., 2019; Guo et al., 2020; Sun et al., 2021). The slight shift in the diffraction peaks could be interpreted as a shrinkage in the lattice structure as a result of encapsulation (see Figure 3-6b), caused by attractive interactions and if so, probably hydrogen bonding, as suggested to some extent by the FTIR spectra. Lattice shrinkage on encapsulation was also observed by (Moussa et al., 2016). Such interactions have been further investigated by other researchers through molecular docking calculations. It was found that curcumin, when added at low concentrations, may preferentially occupy the hydrophobic voids of dual γ -CD units, stabilized by hydrogen bonds, followed by the formation of nanoclusters inside the large cavities of MOFs at higher concentrations (Chen et al., 2021a; Chen et al., 2022). This mechanism has also been reported in the encapsulation of folic acid and sucralose (Lv et al., 2017; Xu et al., 2019b). In our study, encapsulation was performed at a low molar ratio of γ -CD-MOFs to curcumin of 10:1 and so, hydrogen bonding between curcumin and γ -CD pairs probably dominated and this stronger interaction resulted in shrinkage of the lattice structure.

With the presented PXRD patterns, we could not identify the exact location of curcumin molecules within the $Im\bar{3}m$ crystal. The exact atomic locations can be obtained from pair distribution function (PDF) which can only be calculated from diffraction patterns that provide access to higher order reflections (e.g., 80 reflections) and distinctly resolved diffraction peaks.

3.5.2 Encapsulation efficiency and loading capacity

The effect of the encapsulation solvent on the EE and LC of the γ -CD-MOFs may be affected by the solubility of curcumin in methanol and ethanol. The polarity of curcumin is relatively weak due to its symmetrical structure, and it is known to be more soluble in organic solvents. It was reported that curcumin,

at a constant temperature (298.15 K), has better solubility in ethanol (mole fraction 0.074) compared to methanol (mole fraction 0.050) (Cui et al., 2021). Therefore, it is reasonable to assume that curcumin more preferably interacted with ethanol as solvent rather than the hydrophobic cavity of γ -CD-MOFs, compared to methanol. It therefore makes sense that increasing polarity of the solvent by mixing or replacement with methanol would allow better entrapment of curcumin into the cavity of γ -CD-MOFs, as observed.

In contrast, it appears that the synthesis solvent (i.e., ethanol or methanol) used to prepare the γ -CD-MOFs had little effect on the EE and LC. It is noted that the BET surface areas and therefore potential curcumin adsorption capacities of either γ -CD-MOFs were more or less the same and that post-encapsulation both types of crystal gave the same large reduction in BET surface area to $1\text{m}^2/\text{g}$. However, the low EE and LC values measured for encapsulation via ethanol seem to contradict this. It is possible that the cavity space was occupied by both ethanol and curcumin, since curcumin has a higher affinity for ethanol than methanol. Inclusion of ethanol in γ -CD-MOFs has also been shown to be possible by Kathuria et al. (2019). Thus, the combined effect of encapsulation of both ethanol and curcumin plus possible fragmentation of crystals may be the cause of low BET surface area also observed on loading curcumin into crystals via ethanol.

In consequence, the results therefore highlight the possible need of improving the solvent evacuation process. Control of the pressure, time and temperature profiles would be key to keeping fragmentation of γ -CD-MOFs to a minimum. The use of equipment that with more sophisticated controls would be more beneficial than a vacuum oven with manual temperature control. Different encapsulation solvents should also be further investigated to improve EE and LC.

3.6 Conclusions

In this investigation, a comparison between the solvents, methanol and ethanol, in the synthesis of γ -CD-MOFs was made. It was found that the only significant difference the solvent made was the larger crystal size with ethanol. This is largely due to the lower polarity of this solvent compared to methanol

and its effects on the rate of nuclei formation and uniformity of the crystals formed. The crystalline structures and BET determined surface areas of both sets of γ -CD-MOFs appeared to be the same. This suggested that the preferable use of ethanol as a solvent could be used to prepare γ -CD-MOFs rather than the more usual methanol. However, variation of encapsulation solvent made a significant difference in entrapping curcumin into the cavities of γ -CD-MOFs: the use of methanol promoted the encapsulation process. When ethanol dominated as the encapsulation solvent, a drastic drop in EE and LC was observed, due to the higher solubility of curcumin in ethanol which allowed for less interaction with the hydrophobic cavities of the crystals. Hence, different solvents should be further investigated to deliberately manipulate the EE and LC of target compounds for better use of γ -CD-MOFs as their encapsulating and delivery agents.

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Chapter 4

Enhancement of Curcumin Bioaccessibility: An Assessment of Possible Synergistic Effect of γ -Cyclodextrin Metal-Organic Frameworks with Micelles

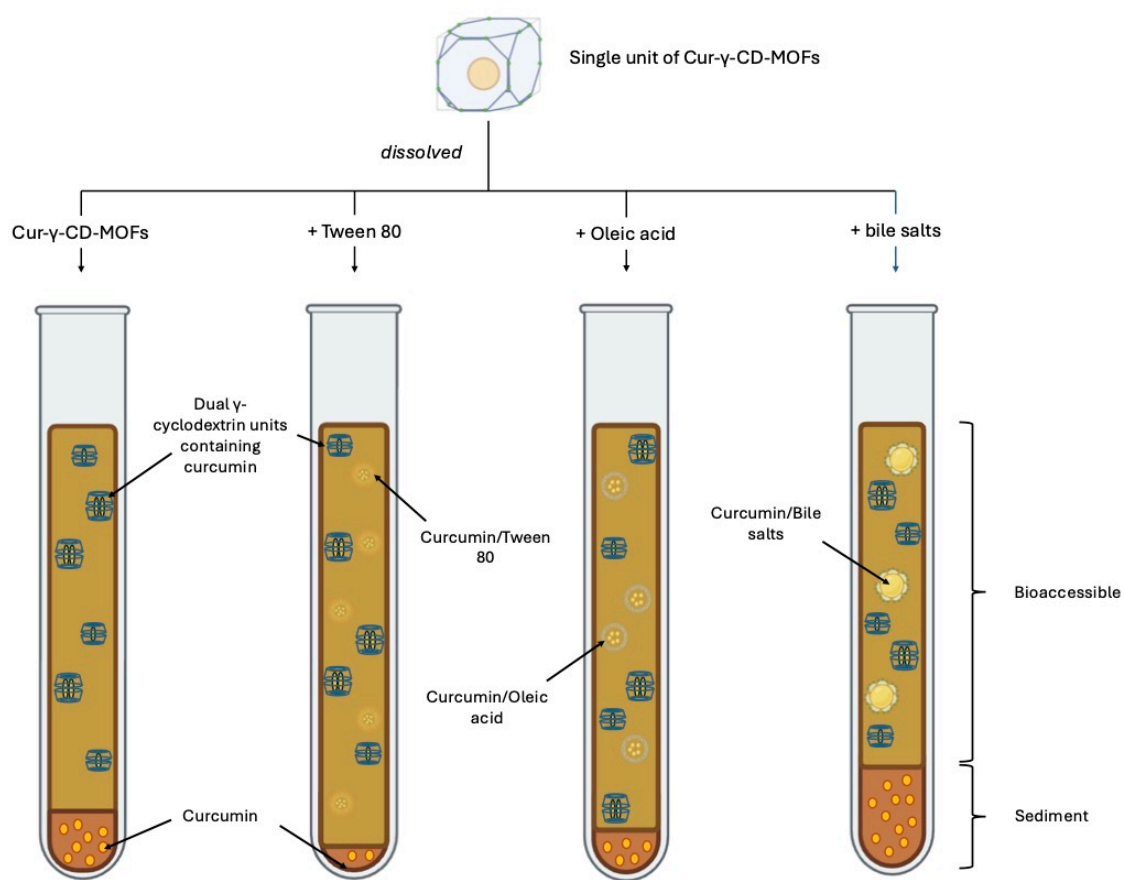


Figure 4-1 Graphical abstract of enhancement of curcumin bioaccessibility by γ -CD-MOFs and when dispersed in combination with Tween 80, oleic acid and bile salt micelles.

4.1 Abstract

Biocompatible γ -cyclodextrin metal-organic frameworks (γ -CD-MOFs) have been reported to improve the apparent solubility and bioavailability of encapsulated bioactive compounds, including curcumin, potentially enabling sustained delivery. However, disintegration of γ -CD-MOFs may “release” encapsulated curcumin within the cavity in its insoluble crystallized form. This study evaluated whether the presence of micelles (at a concentration above curcumin’s maximum solubility) would be able to take up the “released” curcumin from γ -CD-MOFs to further increase its apparent solubility. Release in the presence of a micellar phase was compared to curcumin encapsulated within γ -CD-MOFs alone. Results demonstrated that Tween 80 solubilized higher curcumin concentrations compared to oleic acid and bile salts. Dispersed in combination of Cur- γ -CD-MOFs, it was found that Tween 80 and oleic acid can improve the apparent solubility of curcumin, 5x and 1.5x higher than in the absence of these micelles respectively, whereas bovine bile salts exhibited a negative effect, due to a displacement of curcumin from within cavity of γ -CD-MOFs. Moreover, it was also found that Tween 80 was less affected by pH and salt concentrations due to its nonionic nature. *In vitro* digestion (via the INFOGEST protocol) revealed an 8-fold increase in apparent solubility of curcumin with bile salts and a 53-fold when combining of bile salts and γ -CD-MOFs, achieving bioaccessibility of 2% and 16%, respectively. In conclusion, this research revealed the evaluation of γ -CD-MOFs’ functionality for delivery and controlled release, with the possibility of an emulsion system boosting the fraction of curcumin that would potentially be bioavailable.

4.2 Introduction

Cyclodextrin (CD) combined with alkali metals can be used to form metal-organic frameworks (CD-MOFs), with an edible CD-MOFs being investigated with the use of γ -CD and potassium ions via a vapor diffusion method (Smaldone et al., 2010). γ -CD-MOFs have attracted growing interest as promising candidate drug carriers due to their non-toxicity, tunability, loading efficiency, and reported sustained and controlled drug release (Shen et al., 2021; Si et al., 2024). Several studies have

shown that various bioactive compounds and drugs can be successfully encapsulated in γ -CD-MOFs, such as curcumin, ibuprofen, folic acid, quercetin, lansoprazole, and many more (Roy and Stoddart, 2021; Oh et al., 2023).

After 'activation' (removal of any excess solvent) from γ -CD-MOFs they remain permanently porous, which enables them to encapsulate and convert drugs from a crystal state to an amorphous state. This can contribute to the improved solubility and bioavailability of insoluble drugs (Li et al., 2017; Zhou et al., 2020). Several modelling studies as well as Fourier transform infrared spectra (FT-IR) have suggested that not only does curcumin sit within the hydrophobic cavity of γ -CD-MOFs but also exists in between the γ -CD pairs (He et al., 2019; Chen et al., 2021; Zhou et al., 2021; Oh et al., 2023). According to Lopez and Perez (2023), in the presence of water, the γ -CD-MOFs degrades in two stages. Firstly, water molecules displace the metal-linker coordination bonds of MOFs. Secondly, a hydrolysis process occurs which deprotonates the coordinated water molecule, resulting in the breakdown of metal-linker coordination and a hydroxide ion is left attached to the metal center to preserve the charge balance. This supported our previous study where the improved apparent solubility was inferred to be due to the interaction between curcumin and γ -CD pairs because the γ -CD-MOFs disintegrate in an aqueous environment, whereas the "release" of trapped curcumin from within the cavity leads to its insoluble crystallized form (Oh et al., 2023).

Surface active agents (surfactants) are amphipathic compounds that are extensively used in a wide variety of applications. Examples include enhancing drug solubility, stabilizing emulsions, lubrication, cleaning and detergency (Park and Choi, 2006; Kralova and Sjöblom, 2009; Züge et al., 2013; Tung et al., 2021). Most applications are highly reliant on the formation of micelles at and above the critical micelle concentration (CMC). The CMC is defined as the concentration at which the surfactant starts to aggregate or self-associate and form discrete structures (micelles) typically no more than 2 to 3x the dimensions of the individual surfactant molecules (Shi et al., 2011; Vinothini and Rajan, 2019). The formation of micelles affects the physical properties of surfactant solutions (e.g., conductivity, density, viscosity, osmotic coefficient, water activity, and surfactant activity) (Paddon-Jones et al., 2001; Abdul Rub et al., 2022; Shadloo et al., 2022).

Extensive studies have been conducted on micellization. In fact, two stages of aggregation are sometimes observed: the formation of premicellar aggregates (the critical premicelle concentration, CPMC) at a concentration below the classic single CMC (sometimes now referred to as the first critical micelle concentration, FCMC). At higher surfactant concentrations the initial spherical-like micelles can grow to form spherocylindrical or ellipsoidal micelles, sometimes at a specific concentration termed the secondary critical micelle concentration, or SCMC (Shi et al., 2011; Shadloo et al., 2023). However, micellization depends very much on the nature of the surfactant, the environmental conditions plus the presence of impurities and the molecular weight (Mw) distribution of commercially relevant surfactants.

Micelles have also been extensively studied and reported to be promising candidates as drug delivery carriers. This could include targeted delivery, enhanced solubility and improved permeability and retention (Saha et al., 2023). However, the lack of stability in certain physiological conditions may limit their effectiveness. Hence, in this study, we investigated the capability of micelles formed from different surfactants to take up a bioactive material released from γ -CD-MOFs as they disintegrate, which has not been highlighted before. Furthermore, to our knowledge, this enhanced effect of micelles working with γ -CD-MOFs to enhance the bioaccessibility of a model payload – curcumin – is the first to be reported.

4.3 Materials and methods

4.3.1 Materials

Tween 80 was purchased from Cambridge Bioscience (Cambridge, UK). Tween 60, curcumin (from *Curcuma longa* (turmeric), powder, >65% purity), and γ -cyclodextrin (γ -CD, >90% purity), purchased from Sigma-Aldrich Company Ltd (Dorset, UK). Oleic acid (technical grade, 90%), sodium chloride (NaCl), potassium hydroxide (KOH, pellets, ACS reagent, >85% purity), hydrochloric acid (HCl, analytical reagent grade, S.G. 1.18, ~37%) and sodium hydroxide (NaOH, analytical reagent grade, pellets) was purchased from Thermo Fisher Scientific (Loughborough, UK). Pepsin (from porcine gastric mucosa, lyophilised powder,

500 U mg⁻¹), pancreatin (from porcine pancreas, 8 USP) and bovine bile (dried, unfractionated) were purchased from Sigma-Aldrich Company Ltd. HPLC grade methanol (>99.9%), acetonitrile (>99.9%), ethyl acetate (>99.7%), curcumin, demethoxycurcumin, and bisdemethoxycurcumin were purchased from Sigma Aldrich Company Ltd. Formic acid (for mass spectrometry) was purchased from Honeywell (Seelze, Germany). All solutions were prepared with pure deionized water (with a resistivity of not less than 18.4MΩ cm at 25°C) (Merck Millipore, Darmstadt, Germany).

4.3.2 Preparation of micelles

A 1.5 mM Tween 80 solution (100x CMC (Hait and Moulik, 2001; Krstonošić et al., 2019)) was prepared in water. Curcumin was first dissolved in methanol then added into Tween 80 solution at molar ratio of Tween 80:curcumin at 50:1, 10:1, 5:1 and 3:1. This was similarly prepared for Tween 60 (1.67 mM) (Feng et al., 2022), oleic acid (0.6 mM) (Grippio et al., 2021) and bovine bile salts (10mM). These solutions were allowed to stand for an hour before any measurements were recorded.

4.3.2.1 Particle size and polydispersity index

The particle size distribution (PSD) and polydispersity index (PDI) were determined for each micellar solution and after addition of curcumin at each molar ratio concentration using a dynamic light scattering instrument at 25°C (Zetasizer Nano ZS series, Malvern Instruments, Worcestershire, UK). The particle size measurement was repeated on samples of micellar solution containing increasing curcumin concentration after being filtered through a 0.45 µm organic filter membrane.

4.3.2.2 Concentration of dispersed curcumin

Micellar solutions containing curcumin were all centrifuged at 5,000 rpm for 20 minutes (Rotina 380R, Hettich, Tuttlingen, Germany). Discarding the supernatant, the sediment was collected and dissolved in methanol. The concentration of undispersed curcumin was calculated from a calibration curve using the SPECORD 210 Plus UV-Vis spectrophotometer (Analytik Jena, Jena, Germany) at 426 nm. The concentration of curcumin or apparent micellar-solubilized curcumin was calculated by difference.

4.3.3 Effect of pH and presence of salt on micelles

As per 4.3.2.2, it was determined that the maximal solubilization of curcumin was at molar concentration of micelles:curcumin at 5:1. Micellar solutions were prepared as described in 4.3.2 for Tween 80 and oleic acid either in the absence of NaCl or in the presence of 0.1M NaCl or 1.0M NaCl. The pH was then adjusted to pH 2 or pH 7 using 1.0M HCl or NaOH solution. The particle size and concentration of dispersed curcumin was similarly measured as per 4.3.2.1. and 4.3.2.2.

4.3.4 Effect of micelles in combination with Cur- γ -CD-MOFs

4.3.4.1 Preparation of γ -CD-MOFs and encapsulation

The preparation of γ -CD-MOFs and their use for encapsulating curcumin were conducted following established methods. γ -CD-MOF crystals were prepared via the conventional vapor diffusion method as described by Smaldone et al. (2010). Briefly, the process involved dissolving γ -CD and KOH in deionized water, filtering, and allowing methanol diffusion into the sample for 7 days. Following an activation process to remove residual moisture entrapped during synthesis, the crystals were dried under vacuum at 30°C overnight (Genevac EZ-2 Plus Evaporating System, Marshall Scientific, New Hampshire, USA).

Curcumin was encapsulated using an impregnation method, in which a 10:1 molar ratio of γ -CD-MOFs to curcumin was simultaneously dispersed and dissolved in methanol. Subsequently, the mixture was centrifuged, washed with methanol to remove unencapsulated fraction of curcumin, and then dried overnight under vacuum.

Full details are given in a previous publication by Oh et al. (2023).

4.3.4.2 Concentration of dispersed curcumin

Micellar solutions were prepared of Tween 80, oleic acid and bovine bile salts each at each of their respective 100x CMC concentrations. 10 mg of Cur- γ -CD-MOFs was dispersed in 5 mL of each micellar solution and allowed to stand for 1 h. The solutions were then centrifuged, and the sediments were collected. The concentration of dispersed curcumin in solution was then similarly recorded as per 4.3.2.2.

4.3.5 *In vitro* gastrointestinal digestion (static model)

A static digestion model was used in the *in vitro* digestion experiments with slight variation from the protocol published by Brodkorb et al. (2019), omitting the oral and gastric phases as it was assumed that the intended method of delivery (capsule form) would remain intact until it reaches the intestinal phase. The volumes of curcumin suspensions and Cur- γ -CD-MOFs were measured to each make up a curcumin starting concentration of 2 mg/mL. The samples were mixed with 8.5 mL of simulated intestinal fluid (SIF), consisting of 6.8 mM potassium chloride (KCl), 0.8 mM potassium phosphate monobasic (KH_2PO_4), 85 mM bicarbonate of soda (NaHCO_3), 38.4 mM sodium chloride (NaCl), and 0.33 mM magnesium chloride hexahydrate ($\text{MgCl}_2(\text{H}_2\text{O})_6$), 40 μL of 0.3M calcium chloride (CaCl_2), as well as 2.5 mL of bile (10 mM) and 5.0 mL pancreatin solution (100 U/mL trypsin activity) that were made up in SIF electrolyte stock solution. The *in vitro* intestinal digestion was carried out for 2 h at pH 7 and 37°C under agitation using a shaking incubator (Incu-Shake MAXI, SciQuip Ltd, Shropshire, UK) at 115 rpm.

4.3.6 Bioaccessibility of curcumin

The bioaccessibility of curcumin was determined for both samples at the end of the 2 h of *in vitro* digestion. The digesta obtained at the end of the digestion process was centrifuged at $13,000 \times g$ for 15 min at 4°C. Solubilized curcumin was assumed to be in the micellar phase, within the supernatant fraction. Curcumin was extracted from the supernatant with ethyl acetate, repeated three times. The ethyl acetate was evaporated off in vacuum and extracted curcumin was redispersed in acetonitrile. The concentration of curcumin was analyzed using ultra high-performance liquid chromatography (UHPLC) analysis. An Agilent 1260 Infinity II LC system coupled with DAD detector was used for the analysis. The measurement wavelength was 425 nm, and the separation column was Ascentis Express C18 (150 mm $\times$ 4.6 mm, 2.7 μm) fitted with a Phenomenex guard column. The mobile phase A was a 0.1% aqueous solution of formic acid, and the mobile phase B was 0.1% formic acid in acetonitrile, with a flow rate of 1 mL/min and maximum pressure limit at 400 bars. The gradient elution program was: 0 min, 82% of A; 10.8-16.8 min: 68% of A; 16.8-19.2 min: 40% of A; 19.2-21.7 min: 0% of A; 21.7-23.8min: 82% of A. The column temperature was

maintained at 35°C. The injection volume was 10 µL, and external curcuminoid standards (bisdemethoxycurcumin, demethoxycurcumin and curcumin) were used for quantitative analysis. A calibration curve was prepared with standard curcuminoids in acetonitrile in concentrations ranging from 0.1 µM to 20.0 µM. Bioaccessibility of curcuminoids were calculated by dividing the detected amount of total solubilised curcuminoids by the initial amount of curcumin the sample.

$$\text{Bioaccessibility (\%)} = C_{\text{micelle}} / C_{\text{initial}} \times 100 \quad (3)$$

Here, C_{micelle} was the concentration of curcumin solubilized in the simulated intestinal phase, C_{initial} was the concentration of curcumin initially added before digestion.

4.3.7 Statistical analysis

Statistical analysis was carried out using OriginPro 2024 (OriginLab, Northampton, USA) and the statistical significance was taken at $p < 0.05$. Data were collected in triplicate. Results in tables are expressed as mean $\pm$ standard deviation where applicable and error bars in figures represent standard deviation.

4.4 Results and discussion

Based on the mean particle size in the micellar solutions (Table 4-1), the size of micelles increases in the sequence of Tween 60 < Tween 80 < oleic acid < bovine bile salts. The PDI values suggest that the Tween 80 and oleic acid micelles were fairly homogeneous, but Tween 60 and bovine bile salts formed highly polydisperse micelles.

Table 4-1 Average hydrodynamic diameter and polydispersity index (PDI) measured from samples at 100x critical micelle concentrations (CMC).

Sample	Hydrodynamic Diameter (nm)	Polydispersity Index (PDI)
Tween 60	7.0 $\pm$ 5.1	0.72 $\pm$ 0.25
Tween 80	10.7 $\pm$ 3.0	0.16 $\pm$ 0.06
Oleic acid	85.6 $\pm$ 23.5	0.44 $\pm$ 0.09
Bovine bile salts	219.0 $\pm$ 24.8	0.94 $\pm$ 0.08

The measurements were carried out at 100x the accepted CMC concentrations. The CMC can be determined by various methods such as surface tension, conductivity, viscosity, light scattering, fluorimetry, calorimetry, spectrophotometry, and nuclear magnetic resonance (NMR) spectroscopy (Hait and Moulik, 2001; Shi et al., 2011; Szymczyk et al., 2018). Unfortunately, the CMC values obtained via different methods often disagree. The CMC values reported for Tween 60, Tween 80 and oleic acid are 0.0167 mM, 0.015 mM and 6 μ M, respectively (Hait and Moulik, 2001; Krstonošić et al., 2019; Grippo et al., 2021; Feng et al., 2022). The complexity of bovine bile, which contains various bile acids, makes it particularly difficult to specify a single CMC concentration. It was suggested that the CMC could range between 0.9 – 18 mM, resulting in varying micellar proportions for different bile acids and hence no clear consensus could be made on the CMC concentration (Parker et al., 2014; Naumann-Gola et al., 2019). Therefore, in this study a nominal CMC values of 10 mM was assumed.

The particle sizes reported in Table 4-1 for Tween 60, Tween 80 and oleic acid coincided within the range of previously reported values of the corresponding micelle sizes (Salentinig et al., 2010; Pisárčik et al., 2013; Bide et al., 2021). Studies have shown that Tween 60 is known to form a bimodal size distribution, with small micelles of 7 nm diameter plus larger aggregates of 60 – 120 nm, which contribute to higher PDI values (Pisárčik et al., 2013; Lohmann et al., 2022). The larger apparent values of the micelle size and PDI for the bile acid mixture are presumably a result of the complexity of the mixture, each individual bile acid have its own CMC and aggregation number (N_{agg}). It is known that bile salts do not orient themselves like classic amphiphiles – they have a facial structure based on a tetracyclic steroid ring, with polar hydroxyl groups orientating to the concave side and a hydrophobic convex side (Madenci and Egelhaaf, 2010). This tends to result in low N_{agg} so that it is debatable whether they should be termed micelles at all, but rather surfactant aggregates. The large apparent size observed here suggests that these mixed aggregates maybe be even larger and more complex in structure.

Table 4-2 Average hydrodynamic diameter and polydispersity index (PDI) measured from samples with addition of curcumin at 100x critical micelle concentrations (CMC).

Sample	Molar Concentration Ratio	Hydrodynamic Diameter (nm)	Polydispersity Index (PDI)
Tween 60	50:1	89.6 ± 114.2	0.79 ± 2.94
	10:1	466.5 ± 576.5	0.97 ± 0.64
	5:1	352.7 ± 427.1	1.00
	3:1	624.0 ± 776.8	1.00
Tween 80	50:1	13.7 ± 0.8	0.19 ± 0.01
	10:1	14.6 ± 1.1	0.20 ± 0.08
	5:1	15.9 ± 2.4	0.25 ± 0.02
	3:1	16.2 ± 0.1	0.30 ± 0.01
Oleic acid	50:1	103.2 ± 18.2	0.43 ± 0.07
	10:1	141.7 ± 23.5	0.42 ± 0.06
	5:1	288.6 ± 13.3	0.12 ± 0.11
	3:1	379.1 ± 9.6	0.63 ± 0.25
Bovine bile salts	50:1	169.1 ± 12.9	0.84 ± 0.02
	10:1	188.1 ± 23.5	0.94 ± 0.05
	5:1	205.6 ± 5.6	0.86 ± 0.04
	3:1	309.3 ± 22.3	0.93 ± 0.06

The particle size measurement was repeated with the addition of curcumin (Table 4-2). Following the addition of curcumin into the micellar environment, the micelles increased in average particle size. Samples of tween 80, oleic acid and bovine bile salts showed a gradual increase in average particle size as the molar concentration of curcumin was increased – the sizes showed a 50-60% increase in average particle size. The size distribution curves, pre- and post-addition of curcumin, are provided in the appendices (Figure B-1) and these confirm the large PDI values. This is attributed to curcumin occupying the hydrophobic core

which increased the N_{agg} value, resulting in a higher packing density and increased particle size of micelles. This has been observed in many other studies (Deng et al., 2016; Kuru et al., 2021; Huang et al., 2024). It is worth noting that due to the bimodal size distribution of Tween 60 micelles, a clear trend in particle size distribution could not be identified with the gradual increase in curcumin concentration (Figure B-1a). This resulted in a highly polydisperse system ($PDI = 1$), even at low concentrations of curcumin. Hence, Tween 60 was not considered for further experiments.

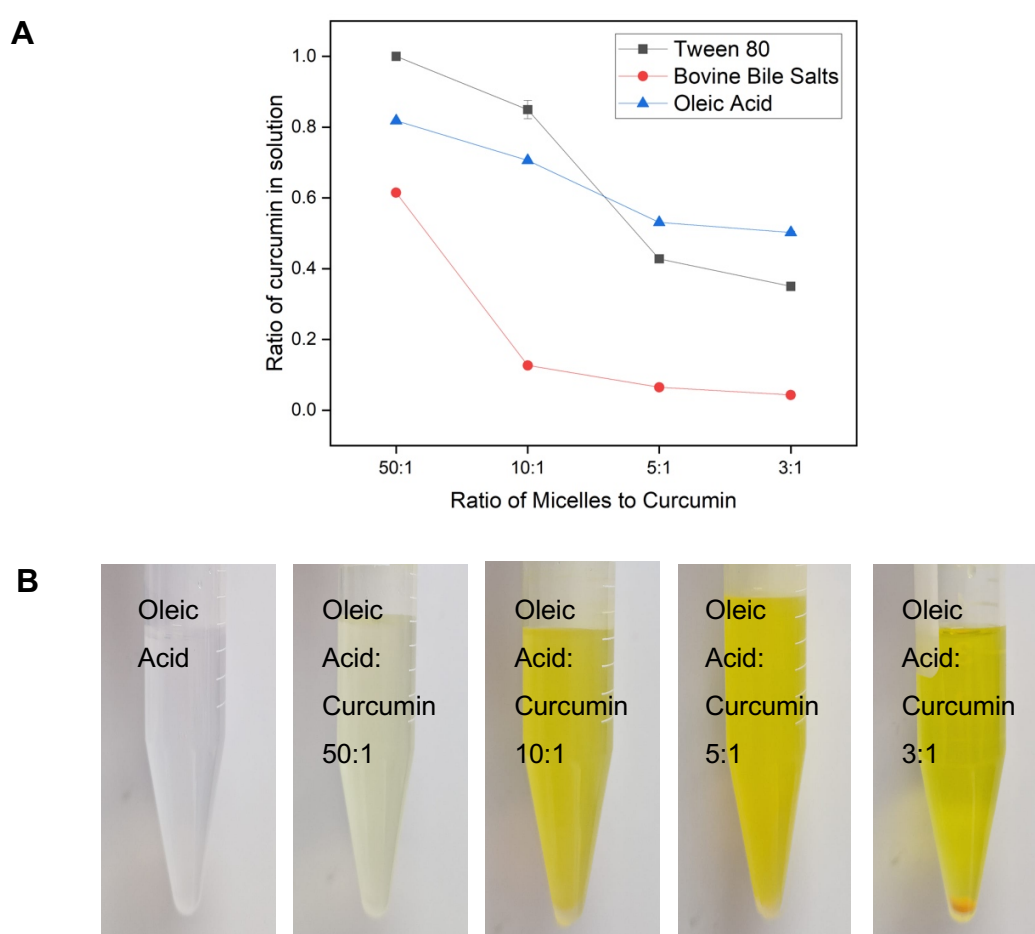


Figure 4-2 (a) Fraction of curcumin measured in solution (dissolved, not as particles) based on molar ratio of respective micelles at 100x CMC concentrations, and (b) images of oleic acid micelles with curcumin at increasing molar concentration of 50:0, 10:1, 5:1, and 3:1.

As shown in Figure 4-2a, the three micellar systems Tween 80, oleic acid and bovine bile salts showed micelles taking up curcumin, with a maximal solubility at molar ratio of micelles:curcumin at 5:1. All 3 systems have shown the capability

of taking up curcumin where the apparently solubilized fraction is retained in the supernatant. Similarly, as the concentration of curcumin added to these systems of constant micellar concentration increases, a limit in their capability to solubilize curcumin is seen. Any further addition of curcumin into the micellar systems resulted in the formation of curcumin sediments. This was also apparent visually (Figure 4-2b). With increasing concentration of curcumin, the intensity of the distinct yellow colour of curcumin increases, whilst visible sedimentation of curcumin was observed at a higher molar ratio of 3:1. Although not shown, tween 80 and bovine bile salts had similar visual characteristics when curcumin was added, with the only difference being the varied intensity of the yellow colour due to their differing ability of solubilizing curcumin.

The capability of a certain type of micelle to solubilize curcumin will be limited by a combination of factors, but most especially the N_{agg} . The value of N_{agg} will determine the number of micelles available for solubilization within the system, their size and therefore ability to accommodate curcumin molecules into their structure. Hence, an inverse relationship between N_{agg} and number of micelles is expected (Kuru et al., 2021). The balance between N_{agg} and the number of micelles is crucial for optimizing solubilization (Medoš et al., 2020). Moreover, the specific interactions (hydrogen bonding, van der Waals interactions, polarity of micelle core) between curcumin and the micelle structure add to the complexity of the relationship.

As the molar concentration of curcumin increases, the micellar system of a finite number of micelles approaches the maximal limit for solubilization of the curcumin, and sedimentation will occur as there will be no micelles to effectively solubilize the remaining curcumin (Torres et al., 2011). Tween 80 and oleic acid had comparable capabilities of solubilizing curcumin which is attributed to the similar reported range of N_{agg} from 20 to 100 (Glenn et al., 2005; Arai et al., 2016; Penttilä et al., 2019). The N_{agg} of individual bile salts is reportedly very low (3 – 26), and so should have provided sufficient micelles to the system to solubilize curcumin (Madenci and Egelhaaf, 2010). However, the reduction in efficacy of solubilizing curcumin may be due to the large aggregation evident in Table 4-1 as well as the variability of bile acids present.

Table 4-3 Average hydrodynamic diameter measured before and after addition of curcumin into micellar solutions at varying pH (pH 7 and 2), and at NaCl concentrations of 0.0 M, 0.1 M and 1.0 M.

Samples	Hydrodynamic diameter (nm)											
	Before addition of curcumin						After addition of curcumin					
	pH 7			pH 2			pH 7			pH 2		
		0.1M NaCl	1.0M NaCl		0.1M NaCl	1.0M NaCl		0.1M NaCl	1.0M NaCl		0.1M NaCl	1.0M NaCl
Tween 80	11.6 ± 0.5	11.7 ± 0.6	12.1 ± 0.6	11.4 ± 0.4	11.5 ± 0.1	13.2 ± 0.6	15.4 ± 0.1	17.3 ± 0.4	17.5 ± 0.9	17.2 ± 0.6	17.2 ± 0.1	20.4 ± 0.7
Oleic acid	98.1 ± 2.5	232.4 ± 12.4	105.5 ± 0.5	166.6 ± 2.6	190.1 ± 0.1	107.4 ± 41.3	175.2 ± 4.6	211.3 ± 67.4	191.7 ± 1.7	337.5 ± 50.7	199.5 ± 6.4	189.3 ± 0.8

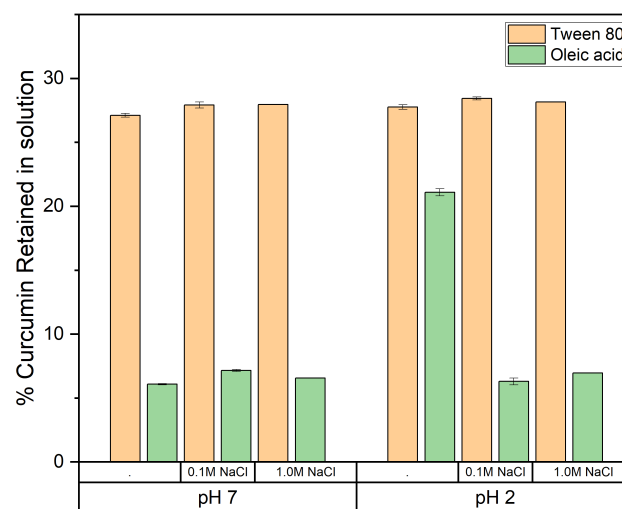


Figure 4-3 Concentration of curcumin remaining in solution at varying conditions of pH and salt concentrations in micellar solutions of Tween 80 and Oleic acid after 1 h.

The particle size of the Tween 80 micelles remained relatively stable under all conditions tested (see Table 4-3), indicating that the micelles were neither affected by pH nor salt concentrations and therefore more stable than those of oleic acid. The micelle sizes increased on addition of curcumin and the increases might be expected to be similar if similar amounts of solubilization occurred. The concentrations of curcumin apparently retained in solution by the micelles were also consistent under all conditions (see Figure 4-3), further demonstrating the stability of the micelles with solubilized curcumin. Tween 80 is a non-ionic surfactant and therefore the effect of salt and pH on Tween 80 micelles are generally reported as negligible (Qazi et al., 2020).

Conversely, with oleic acid, there was a large variability in the results obtained with regard to pH and salt conditions. With the addition of curcumin to oleic acid, the micelles clearly increased in size, but there was no identifiable trend. Moreover, the resulting micelles were very polydisperse under all conditions. The concentration of curcumin retained in solution also remained consistently lower than that with Tween 80, with only the pH 2 condition having a spike at the lowest salt concentration.

In acidic pH, the particle size of oleic acid nearly doubled. Oleic acid is an anionic surfactant that on dissolution in water will generate a negatively charged ionic head and positively charged counter ion (Saha et al., 2023). It is well-documented that the aggregation of oleic acid is highly affected by pH and the degree of protonation of the head group determines whether either micelles, vesicles, or oily droplet phases are formed (Edwards et al., 1995; Kaibara et al., 1997; Nakano et al., 2002; Salentinig et al., 2010; Janke et al., 2014). At pH above 9.85 (above the apparent pK_a value of the oleic acid carboxyl group), the fatty acid is deprotonated, and the solution exists as a suspension of spherical micelles at the CMC. As the pH decreases to a value close to the apparent pK_a (8.0-8.5), there is a balance of protonated and deprotonated micelles and spontaneous formation of highly polydisperse vesicles that may be multilamellar. Additionally, oleic acid would exist in its protonated form with a further decrease in pH values, resulting in the transition into nonlamellar liquid-crystalline structures and subsequently formation of oil droplets due to the reduced hydrophilic nature of the head group. According to Salentinig et al. (2010), it was reported that the particle size of oleic acid

also changes with the pH, with micelles being approximately 120 nm in diameter and decreasing to 60 nm for vesicles. These transitions of oleic acid with pH are known to be reversible. At pH 2, oleic acid would be totally protonated causing the formation of oil droplets, resulting in a large increase in particle size. It is possible that with such structures most of the curcumin would be trapped in the oil droplets, rather than existing in its insoluble crystalline form within the sediment.

Oleic acid aggregation is also known to be sensitive to salt concentration as electrostatic repulsion between headgroups of oleic acid influences the phase stability. Although at low salt concentration (0.1 M), there was a slight increase in size, at high salt concentrations (1.0 M), micelle sizes decreased slightly – but this was not consistent. At pH 7, which is close to the apparent pK_a of 8.0-8.5, the oleic acid should exist in both its protonated and deprotonated form, with its protonated form slightly dominating (Edwards et al., 1995; Salentinig et al., 2010). At low salt concentrations, the electrostatic repulsive interaction between the head group and Na^+ is large, which may result in an increase in area of lipid headgroup (Aota-Nakano et al., 1999). Consequently, this repulsion can lead to the formation of larger micelles or vesicles, or even increased CMC concentrations. Conversely, the addition of salt at higher concentration introduces more Na^+ and Cl^- ions that could potentially screen the electrostatic charges (Hoque et al., 2021). The screening effect would reduce the repulsion between partially ionized head groups that would allow a tighter packing, forming smaller micelles.

According to Kaibara et al. (1997), at low pH (pH 2) the carboxyl group of oleic acid is in its completely protonated form. This would have led to the increased hydrophobic interaction and formation of oil droplets, hence the effect of addition of salt at low concentration is less pronounced. At higher salt concentration, the ions may still screen any residual charges. However, due to the low ionization state, the size was not observed to decrease significantly. Moreover, any further increase in higher salt concentrations would have not been feasible as an aggregation of vesicles would occur, which is likely caused by a “salting out” effect of NaCl (Maurer and Nguyen, 2016).

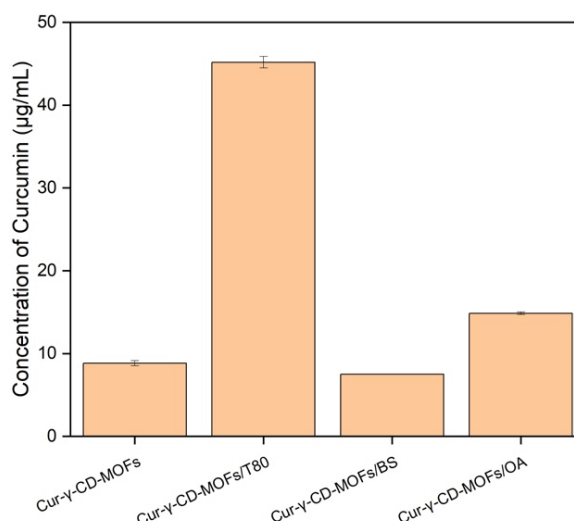


Figure 4-4 Concentration of curcumin remaining in solution after dissolution of Cur-γ-CD-MOFs in distilled water ± solutions containing micelles of Tween 80 (T80), bovine bile salts (BS), and oleic acid (OA) at 100x their CMC.

Generally, curcumin is known to be poorly soluble in water, with a typical solubility limit of 0.6 µg/mL (Górnicka et al., 2023). When γ-CD-MOFs with encapsulated curcumin are placed in contact with water the γ-CD-MOFs disintegrate and so the trapped curcumin within its cavity should be “released”. In the absence of micelles, the released curcumin should therefore rapidly crystallize. The presence of micelles showed a positive influence in the uptake of curcumin, so it was inferred that if the γ-CD-MOF system was augmented with the same surfactant micelles, at least some of the “released” curcumin would be taken up by these micelles and thereby improve its potential bioaccessibility. Based on encapsulation efficiency calculations, 10 mg of Cur-γ-CD-MOFs dispersed in water was predicted to give a maximal 64 µg/mL of curcumin released in the aqueous phase. The results show (Figure 4-4) that on their own Cur-γ-CD-MOFs resulted in 14.08% (9.01 µg/mL) of this maximal value of curcumin dispersed in the aqueous ‘solution’. With Tween 80 micelles in combination with Cur-γ-CD-MOFs there was an increase in the apparent solubility of curcumin to 71% (45.89 µg/mL) of this maximal value. This was followed by oleic acid micelles, which increased the apparent solubility by 9% of this maximal value (to 14.78 µg/mL). Conversely, bile salts were observed to have a slightly negative effect in solubilizing curcumin, giving a reduction of the apparent solubility of curcumin to 7.49 µg/mL.

Based on results collected in Figure 4-2a, Tween 80 and oleic acid exhibited similar capabilities of taking up curcumin within the micelles formed. However, Figure 4-3 shows that the curcumin retained in solution by Tween 80 was at least double that of oleic acid, which also corresponded to results collected in Figure 4-4. With the reported N_{agg} of Tween 80 and oleic acid being within the same range, the lowered curcumin retention may be characterized as being due to the different hydrophile-lipophile (HLB) values of the surfactants. The HLB value is a nondimensional numerical value allocated to surfactant molecules that characterizes their balance between hydrophilic and lipophilic portions (Schmidts et al., 2012). The value can be used, with caution, to indicate the likely suitability of the surfactant for certain formulations and/or stabilization of a system. The HLB values reported for Tween 80 and oleic acid are approximately 15 and 1, respectively (Hait and Moulik, 2001; Barkat et al., 2011). Tween 80 is thus much more hydrophilic in nature, dispersible in water, and its high HLB value indicates that the molecule is better able to stabilize oil-in-water (O/W) emulsions (Hong et al., 2018; Wang et al., 2023). In contrast, oleic acid is lipophilic in nature, largely immiscible with water and in fact may precipitate when added to water. This leads to inferior lowering of the surface tension and its better suitability for stabilizing W/O emulsions (Yu et al., 2015). Since the Cur- γ -CD-MOFs were dispersed in water, Tween 80 would therefore have an advantage over oleic acid in solubilizing curcumin in water.

Although the bile salts showed a slight capability of solubilizing curcumin in water, it was observed that when used in combination with Cur- γ -CD-MOFs, there was a *reduction* in apparent solubility of curcumin in water. It is probable that in the presence of both bile salts and γ -CD-MOFs, the curcumin ends up in several different states: partly solubilized in micelles, partly remaining encapsulated within γ -CD-MOFs and/or partly molecularly dissolved. However, studies have shown that when bile salts interact with drug-cyclodextrin-complexes, bile salts may displace or compete with the molecules within the cyclodextrin cavity, which may have led to the adverse effect of reduced apparent solubility of target compounds (Cuoco et al., 2023). It was also postulated that the dynamic exchange between bile salts and drug complexes would actually boost the free drug concentration to exceed

supersaturation which would induce a higher permeation or bioavailability. However, this was not demonstrated in a study conducted by Eriksen et al. (2022) due to the variation of bile salts concentration in rats and humans, and it was concluded that the competitive displacement was affected by several factors.

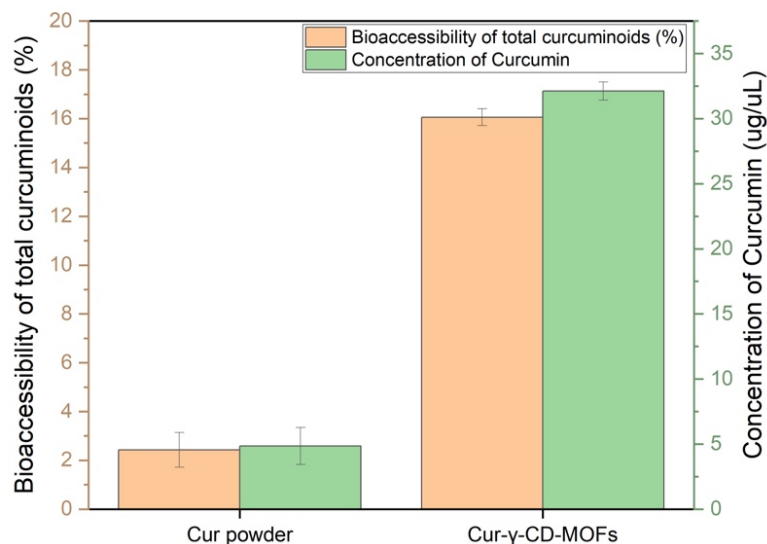


Figure 4-5 Bioaccessibility and concentration of total curcuminoids of curcumin (Cur powder) and curcumin encapsulated in γ -CD-MOFs (Cur- γ -CD-MOFs) after *in vitro* gastrointestinal digestion.

Commercially available curcumin is composed of bisdemethoxycurcumin, demethoxycurcumin and curcumin, all classed as curcuminoids. Based on Figure 4-5, it appears that curcumin, in the presence of bile salts, has an increased bioaccessibility resulting from its increased apparent solubility as compared to the reported solubility of 0.6 $\mu\text{g/mL}$ in water. The bioaccessibility of curcumin + bile salts is approximately 2%, measuring at 5 $\mu\text{g/mL}$. On the other hand, when encapsulated in γ -CD-MOFs the apparent solubility of curcumin is significantly improved and sustained throughout intestinal digestion conditions, thereby improving its bioaccessibility (16%). A comparison of the results shown in Figure 4-4 indicates that Cur- γ -CD-MOFs dissolved in pure water resulted in a retention of curcumin concentration of nearly 10 $\mu\text{g/mL}$, whereas with the addition of bile salts during *in vitro* digestion, the apparent solubility increased nearly 3.2x to approximately 32 $\mu\text{g/mL}$ and is also approximately 6x more bioaccessible than curcumin + bile salts. Moreover, the displacement of curcumin from the γ -CD-MOFs by bile

salts was not observed. Due to the susceptibility of the γ -CD-MOFs to amylase activity from the pancreatin, it is suggested that the collapse of γ -CD-MOFs may counteract the negative displacement effect (Pan et al., 2022). Thus, it has been proven that in the presence of the appropriate type of micelles, the bioaccessibility of a bioactive compound could be further enhanced when used in conjunction with γ -CD-MOFs.

With reports of bioaccessibility of curcumin exceeding 50% when delivered in emulsions, solid lipid particles and oleogels, a 15% bioaccessibility in Cur- γ -CD-MOFs may not seem comparable (Verma et al., 2021; Gonçalves et al., 2024; Ma et al., 2024). This could be influenced by the method of quantifying bioaccessibility and sample preparation involved. Compounds which are insoluble in water generally require an extraction procedure for further quantification and the extraction efficiency plays a role in the subsequent quantification of curcumin within the micellar phase. In some instances, the quantification of curcumin could be either spectrophotometrically or with the use of HPLC. Although spectrophotometric method has proven to be simple, but the precision is low. Non-curcumin colour interfering pigments would cause an overestimation, and the detection method does not provide relative composition of individual curcuminoids (Kotra et al., 2019). HPLC could effectively separate and quantify curcuminoids mixture, but the method is still limited by various factors in the optimization of method (e.g. flow rate, run time, gradient elution, etc.).

The stability of encapsulated material, photochemically and thermally, is known to be improved by γ -CD-MOFs (Chen et al., 2021). Due to its unique host-guest structure, the degradation of curcumin within the cavities and pores of γ -CD-MOFs were significantly delayed and instead protected. With formulations involving the use of lipids or proteins, at certain physiological conditions, the dispersibility may be impacted due to coalescence, aggregation, or separation which would in turn limit the action of lipase and pancreatin in simulated intestinal conditions (Dong et al., 2022). Moreover, as these interfaces degrade due to enzymatic reactions, curcumin could be degraded due to exposure to free radicals (Araiza-Calahorra et al., 2018). Furthermore, the biocompatibility of γ -CD-MOFs could potentially favour the

absorption of curcumin and thereby influencing its bioavailability which would be further explored in the next study.

4.5 Conclusions

This study found that the micelles of the surfactants Tween 80, Tween 60, oleic acid and bovine bile salts showed a positive retention of curcumin, an otherwise insoluble crystalline material, within micellar solutions. All the micelles had a maximum limit by which they could solubilize curcumin – at a molar concentration of micelles:curcumin = 5:1. This was thought largely due to the N_{agg} of the micelles and the amount of surfactant molecules available to form micelles at a fixed concentration. Depending on the nature of the surfactants, pH and salt concentrations can significantly influence the ability of the surfactant to form micelles and the stability of the resulting encapsulating structures. Dispersed in combination with γ -CD-MOFs, Tween 80 and oleic acid markedly improved the apparent solubility of curcumin when compared to using Cur- γ -CD-MOFs alone. Although bovine bile salts are known to displace curcumin and compete for the cavity spaces of the γ -CD-MOFs, this negative effect was not seen in *in vitro* intestinal digestion. This may be due to the susceptibility of γ -CD-MOFs to amylase.

This study has therefore shown the potential for γ -CD-MOFs for sustained release, particularly if they are supplemented by the presence of an appropriate surfactant micelle system. In future studies, an emulsion system in combination with Cur- γ -CD-MOFs + micelles could be investigated for further improvement of the bioaccessibility and bioavailability of curcumin.

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Chapter 5

Bioaccessibility and Absorption of Curcumin: A Look into γ -Cyclodextrin Metal-Organic Frameworks

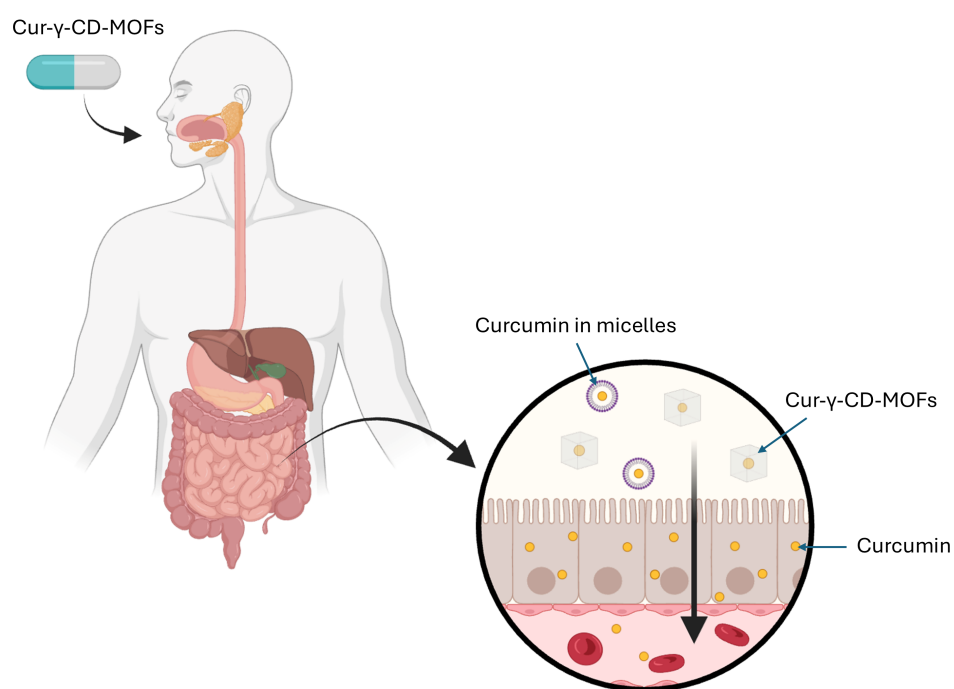


Figure 5-1 Graphical abstract of delivery Cur- γ -CD-MOFs in capsule form and its absorption.

5.1 Abstract

Curcumin, derived from *Curcuma longa*, has long been recognized for its potential pharmacological effects. However, its bioaccessibility and bioavailability remain low. To address this limitation, we evaluated the efficiency of delivery via curcumin-encapsulated γ -cyclodextrin metal-organic frameworks (Cur- γ -CD-MOFs), in comparison to coarse emulsions formulated with sodium caseinate and Tween 80, using simulated *in vitro* gastrointestinal digestion. Our findings indicate that the bioaccessibility of curcumin was highest when encapsulated in γ -CD-MOFs, delivered as a capsule to avoid exposure to gastric conditions, achieving a sixfold increase compared to native (non-encapsulated) curcumin. In contrast, incorporating Cur- γ -CD-MOFs into emulsions did not improve curcumin solubilization and bioaccessibility due to the structural instability of γ -CD-MOFs under acidic gastric conditions. Moreover, the prolonged solubilization of curcumin via capsule-delivered γ -CD-MOFs throughout *in vitro* gastrointestinal digestion enhanced its transport and absorption across the mouse intestinal epithelial tissue. These results highlighted the potential of γ -CD-MOFs as an effective delivery system for improving bioaccessibility and absorption of bioactive compounds.

5.2 Introduction

Curcumin, derived from the root of *Curcuma longa* (turmeric), is a mixture of three principal compounds – curcumin, demethoxycurcumin and bisdemethoxycurcumin – collectively referred to as curcuminoids (Li et al., 2019; Agrawal and Jaiswal, 2022). Due to their physiologically active functionality as a natural antioxidant contributing to the prevention of multiple diseases, curcuminoids have garnered a lot of attention throughout the past decade (Zeng et al., 2017). Curcumin's bioavailability is limited due to poor absorption, significant liver metabolism, and excretion via the gall bladder. It is also pH-sensitive and becomes increasingly more unstable in basic pH (> pH 7). Therefore, to enhance its therapeutic effects and practical applications, various encapsulation techniques – such as liposomes, nanoparticles, emulsions, gels, cyclodextrin inclusion complexes – have been developed,

demonstrating improved stability and controlled delivery of curcumin (Górnicka et al., 2023; Zhao et al., 2024).

γ -Cyclodextrin metal-organic frameworks (γ -CD-MOFs) are porous, versatile, crystalline materials composed of γ -cyclodextrin (γ -CD) units linked by metal ions, such as potassium or other alkaline metals. The γ -CD-MOFs preserve the γ -CD hydrophobic central cavity and outer hydrophilic surface, and so provide excellent biocompatibility and enhances the solubility and stability of various less polar bioactive compounds encapsulated within them (Han et al., 2018). Following a detailed characterization study by Oh et al. (2023) confirming their structural integrity, curcumin was thought to be encapsulated not only within the hydrophobic cavities (via van der Waals forces and hydrophobic interactions), but also between γ -CD pairs in the crystallites, via hydrogen bonding between hydroxyl groups. Further analysis also showed that micelles could take up curcumin prematurely released from cavities of the γ -CD-MOFs as they dissociate in an aqueous environment (Oh et al., 2025). Therefore, supplementing the γ -CD-MOFs encapsulation system within an appropriate surfactant-rich system, such as an oil-in-water (O/W) emulsion, may further boost the fraction of curcumin that would be potentially bioavailable.

Emulsions are widely used for delivering bioactives, potentially protecting hydrophobic compounds from degradation and allowing their controlled release, though the emulsion stability and release will depend on the chosen emulsifier. Sodium caseinate (NaCas) is a natural composite mixture of milk proteins that generally imparts excellent stabilization of O/W emulsions via electrostatic and steric repulsion at the O-W interface (Dickinson, 1999; Du et al., 2018). Tween 80, a synthetic nonionic surfactant, is also an efficient emulsifier (Kaur and Mehta, 2017). These two very different types of emulsifiers, digestible and non-digestible, were therefore chosen for comparison in the first direct assessment of curcumin bioaccessibility via Cur- γ -CD-MOFs, with and without O/W emulsions, when subjected to simulated gastrointestinal conditions. In addition, this is the first study to evaluate encapsulated curcumin absorption in an *ex vivo* intestinal transport model, in this case via an Ussing chamber. The findings provide valuable insights into

the potential of γ -CD-MOFs to enhance curcumin bioaccessibility and absorption.

5.3 Materials and methods

5.3.1 Materials

Curcumin (from *Curcuma longa* (turmeric), powder, >65% purity), γ -cyclodextrin (γ -CD, >90% purity), sodium caseinate (NaCas), D-glucose, mannitol, Nile Red (technical grade) and dimethyl sulfoxide (DMSO, 99.9%) were purchased from Sigma-Aldrich Company Ltd (Dorset, UK). Tween 80 was purchased from Cambridge Bioscience (Cambridge, UK). Commercial sunflower oil (Flora Food Group, Amsterdam, Netherlands) was purchased from a supermarket. Potassium hydroxide (KOH, pellets, ACS reagent, >85% purity), hydrochloric acid (HCl, analytical reagent grade, S.G. 1.18, ~37%), and sodium hydroxide (NaOH, analytical reagent grade, pellets) were purchased from Thermo Fisher Scientific (Loughborough, UK). Pepsin (from porcine gastric mucosa, lyophilized powder, 500 U/mg), pancreatin (from porcine pancreas, 8 USP) and bovine bile (dried, unfractionated) were purchased from Sigma-Aldrich (Dorset, UK). Salts to prepare digestive buffers, i.e., potassium chloride (KCl), potassium dihydrogen sulfate (KH_2PO_4), sodium bicarbonate (NaHCO_3), sodium chloride (NaCl), magnesium chloride hexahydrate ($\text{MgCl}_2(\text{H}_2\text{O})_6$), ammonium carbonate ($(\text{NH}_4)_2\text{CO}_3$), and calcium chloride dihydrate ($\text{CaCl}_2(\text{H}_2\text{O})_2$) were purchased from Sigma-Aldrich. A total starch assay kit (K-TSTA-100A) was purchased from Megazyme Ltd (Bray, Ireland). HPLC grade methanol (>99.9%), acetonitrile (>99.9%), ethyl acetate (>99.7%), curcumin, demethoxycurcumin, and bisdemethoxycurcumin were purchased from Sigma Aldrich. Formic acid (for mass spectrometry) was purchased from Honeywell (Seelze, Germany). All solutions were prepared with pure deionized water (dH_2O) (with a resistivity of not less than 18.4 M Ω cm at 25°C) (Merck Millipore, Darmstadt, Germany).

5.3.2 Encapsulation of curcumin

5.3.2.1 Preparation of Cur- γ -CD-MOFs

The preparation γ -CD-MOFs and their encapsulation of curcumin were conducted following established methods and full details are given in previous

publications (Smaldone et al., 2010; Oh et al., 2023). Briefly, the process involved dissolving γ -CD and KOH in deionized water, filtering and allowing methanol diffusion into the sample for 7 days. The crystals were then dried under vacuum at 30°C overnight (Genevac EZ-2 Plus Evaporating System, Marshall Scientific, New Hampshire, USA).

Curcumin was encapsulated into the CD-MOFs using an impregnation method, in which a 10:1 molar ratio of γ -CD-MOFs to curcumin was simultaneously dispersed and dissolved in methanol. Subsequently, the mixture was centrifuged, washed with methanol to remove the non-encapsulated fraction of curcumin, and then dried overnight under vacuum, yielding Cur- γ -CD-MOFs.

5.3.2.2 Preparation of coarse emulsions

Curcumin was dissolved in sunflower oil to a final concentration of 2 mg/mL under magnetic stirring for 30 min. NaCas was dissolved in water 3% w/w and oil-in-water emulsions (80:20 w/w) were prepared by homogenizing the oil and NaCas solution via an Ultra Turrax T25 homogenizer (IKA-Werke GmbH & Co., Staufen, Germany) at 21,000 rpm for 1 min. Emulsions where Cur- γ -CD-MOFs were dispersed in the oil phase were prepared in the same way. In the remainder of this work these are referred to as MOF/Cas emulsions, whereas the former are referred to as Cur/Cas emulsions. Emulsions were also prepared in exactly the same way but using 3% w/w Tween 80 as the emulsifier, referred to below as Cur/T80 and MOF/T80 emulsions.

5.3.3 *In vitro* gastrointestinal digestion (static model)

A static *in vitro* digestion model was conducted according to the INFOGEST digestion model published by Brodkorb et al. (2019).

Oral phase: exactly 5 mL of emulsion was mixed with 4 mL of SSF at pH 7, 25 μ L of 0.3 M CaCl₂, and 0.975 μ L of dH₂O in a 50 mL Falcon tube. The digestion tube was incubated in a shaking incubator (Incu-Shake MAXI, SciQuip Ltd, Shropshire, UK) operated at 37°C, 115 rpm. The salivary amylase was omitted because of the expected short residence time (< 1 min).

Gastric phase: The subsequent 10 mL of oral sample was mixed with 8 mL of SGF at pH 3, 0.5 mL of pepsin (2000 U/mL) and 5 μ L of CaCl_2 (0.3 M). The pH was adjusted to pH 3 using 1 M HCl solution. The solution was then incubated for 2 h.

Intestinal phase: A similar volume of SIF electrolyte was added to the above gastric 'chyme'. This was followed by the addition of 40 μ L of CaCl_2 (0.3 M), and 3 mL of bile (10 mM) and 5 mL of pancreatin solution (100 U/mL trypsin activity) that were made up in SIF electrolyte solution. Once the pH was adjusted to 7 with a 1 M NaOH solution, the sample was further incubated for 2 h.

An important but slight variation from the protocol was adapted for samples containing non-encapsulated curcumin and Cur- γ -CD-MOFs. It was assumed that these samples would be delivered in a capsule form that would ensure they remain intact until reaching the intestinal phase. Therefore, enzymes relating to the oral phase (salivary amylase) and gastric phase (pepsin) were omitted in the 'digestion' of these vehicles. The volumes of curcumin of each of the samples were adjusted to ensure that all had the same starting concentration of a curcumin, $C_{\text{initial}} = 2 \text{ mg/mL}$.

5.3.4 Droplet size measurements

Static light scattering (SLS) was used to measure the particle size distribution (PSD) of the emulsions via a Malvern Mastersizer 3000 (Malvern Instruments, Worcestershire, UK) assuming a refractive index and absorption of the dispersed phase of 1.47 and 0.01, respectively. The mean particle size of the emulsions was reported as volume mean diameter (d_{43}) since this is more sensitive to droplet aggregation.

5.3.5 ζ -potential measurements

The ζ -potentials of aqueous dispersions of the non-emulsion and emulsion samples were determined via a Zetasizer (Nano ZS series, Malvern Instruments, Worcestershire, UK) at 25°C with the use of a folded capillary cell (Model DTS 1070, Malvern Instruments, Worcestershire, UK). Samples before and after *in vitro* digestion were collected at appropriate time intervals

and were either dispersed in phosphate buffer (pH 7.0) or SGF buffer (pH 3.0) or SIF buffer (pH 7.0).

5.3.6 *In vitro* release and bioaccessibility of CUR

The amount of curcumin released from the curcumin-loaded delivery systems in comparison to native (non-encapsulated) curcumin was measured during *in vitro* gastrointestinal digestion. The sampling was set at every 1 h of gastric conditions and/or at 20 min intervals during intestinal conditions. The bioaccessibility of curcumin was determined for all digesta at the end of the 2 h of intestinal phase. Each sample was centrifuged at $20,000 \times g$ for 10 min at 4°C. Any curcumin that was 'solubilized' and therefore potentially bioaccessible was assumed to be present within the middle layer of supernatant fraction, i.e., not present as a sediment of large particles at the bottom of the tube, nor trapped in any oil layer at the top. We assume that such material is held in some sort of complex mixed bile salt micelles and for brevity we therefore refer to the concentration of curcumin in this layer as $C_{micelle}$. Curcumin in this middle layer was therefore extracted with 500 μ L ethyl acetate, three times. The ethyl acetate was evaporated off under vacuum and the precipitated curcumin was re-dissolved in acetonitrile. The concentration of curcumin in these acetonitrile solutions was analyzed using ultra high-performance liquid chromatography (UHPLC) analysis, using an Agilent 1260 Infinity II LC system coupled with DAD detector. The measurement wavelength was 425 nm and the separation column was an Ascentis Express C18 (150 mm $\times$ 4.6 mm, 2.7 μ m) fitted with a Phenomenex guard column. The mobile phase A was 0.1% aqueous formic acid, the mobile phase B was 0.1% formic acid in acetonitrile. A flow rate of 1 mL/min and a maximum pressure limit of 400 bar was used for elution. The gradient elution program was: 0 min, 82% of A; 10.8-16.8 min, 68% of A; 16.8-19.2 min, 40% of A; 19.2-21.7 min, 0% of A; 21.7-23.8min, 82% of A. The injection volume was 10 μ L and the column temperature was maintained at 35°C. External curcuminoid standards bisdemethoxycurcumin, demethoxycurcumin and curcumin were used to quantify the elution peaks via a calibration curve in acetonitrile ranging in curcuminoid concentrations from 0.1 μ M to 20.0 μ M. The resultant % Bioaccessibility of curcuminoids was thus calculated as:

$$\text{Bioaccessibility (\%)} = C_{\text{micelle}} / C_{\text{initial}} \times 100 \quad (4)$$

5.3.7 Glucose release

The glucose released during the *in vitro* gastrointestinal digestion was quantified by using a total starch assay kit (AOAC Method 76-13.01), with slight modification, to assess the breakdown of γ -CD-MOFs. Digesta samples were collected at 1 h intervals in the gastric phase and at 20 min intervals in the intestinal phase.

After centrifuging the digesta at 10,000 rpm for 15 min, 50 μ L of supernatant, in duplicate, was pipetted into a test tube and mixed well with 5 mL of sodium acetate buffer. After storage at 50°C in a water bath for 10 min, 50 μ L of amyloglucosidase was added to one sample tube and 50 μ L of buffer was added to the other (control tube). The tubes were then incubated for a further 30 min at 50°C. Once cooled, 1 mL of each solution was centrifuge at 13,000 rpm for 5 min. This was followed by the transfer of 50 μ L of supernatant to 2 fresh test tubes and the addition of 1.5 mL GOPOD (Glucose Oxidase/Peroxidase) reagent. After further incubation at 50°C for 20 min, the absorbance at 510 nm was measured and compared against that for a series of standard glucose solutions against the reagent blank using Tecan Spark 10M plate reader (Zürich, Switzerland).

5.3.8 *Ex vivo* intestinal transport and absorption of curcumin using mouse jejunum

The *ex vivo* intestinal transport study protocol used was previously reported by Niaz and Mackie (Niaz and Mackie, 2024), which utilized an Ussing chamber and mouse jejunum.

Intestinal tissue was obtained from 6-week-old C57BL6 mice that had been freshly anesthetized, provided by the Central Biomedical Services, University of Leeds, UK. The protocol was approved by the University of Leeds Animal Welfare Ethical Review Board and was conducted under the appropriate license (PEL number: XDE639D76) in accordance with the UK Home Office Animals Scientific Procedures Act (ASPA) 1986.

Following extraction, the intestinal tissue was rinsed twice with ice-cold Krebs-Ringer bicarbonate (KRB) solution and immediately immersed in cold D-glucose solution (10mM, pH 7.4) before being placed on ice for no longer than 30 min prior to use. A 2 cm of intestinal segment was excised from the jejunum, and the serosal layer was removed. The tissue was then secured onto a cassette and positioned between two chambers, ensuring the mucosal side faced the apical chamber while the serosal side was oriented towards the basolateral chamber.

To each chamber, 1 mL of the respective solution was added: Ringer's solution with glucose for the apical chamber and Ringer's solution with mannitol for the basolateral chamber. Transepithelial electrical resistance (TEER) values were continuously monitored throughout the experiment, with any intestinal tissue displaying a TEER measurement below $20 \Omega \text{ cm}^{-2}$ considered not viable and excluded from further analysis.

The absorption study was carried out on the native curcumin dispersions, Cur- γ -CD-MOFs samples and the Cur/Cas and MOF/Cas and emulsions. Prior to injection into the apical side of the Ussing chamber, samples were subjected to gastrointestinal digestion (as described in section 5.3.3) and 0.5 mL of digesta collected at the endpoint of digestion were added to the chamber. At 60 and 120 min, 500 μL of sample was collected from each chamber and replaced with equal volumes of corresponding buffer. The jejunum tissue was also collected at the end of the experiment. Curcuminoids were extracted from the samples and quantified as described in section 5.3.6.

5.3.9 Microstructural characterization

5.3.9.1 Optical microscopy

The microstructure of samples was imaged using a light microscope (Nikon, SMZ-2T, Tokyo, Japan) equipped with a digital camera (Leica MC120 HD).

5.3.9.2 Confocal scanning laser microscopy (CLSM)

Confocal laser scanning microscopy (CLSM) was carried out using a Zeiss LSM880 upright Airyscan microscope (Cambridge, UK) with a 10x/0.30 and 20x/0.8 objective lens. After washing the jejunum tissue with PBS (phosphate-buffered saline) from the absorption studies with Cur- γ -CD-MOFs samples,

the tissue was fixed on a microscope slide using 4% PFA solution for 3 h. Nile Red (1 $\mu\text{g/mL}$ in DMSO) was used to stain the tissue, using an excitation wavelength of 561 nm and emission wavelength of 665 nm. For curcumin identification its natural fluorescence was used, via excitation and emission wavelengths of 488 nm and 544 nm, respectively. Images were processed using the image analysis software Fiji (GNU General Public License, Schindelin et al., 2012).

5.3.10 Statistical analysis

Statistical analysis was carried out using OriginPro 2024b (OriginLab, Northampton, USA) and statistical significance was taken at $p < 0.05$. Data were collected in triplicate. Results in tables are expressed as means $\pm$ standard deviations, the latter represented in figures by the error bars.

5.4 Results and discussion

5.4.1 Particle sizes of emulsions stabilized by sodium caseinate and Tween 80

The volume mean diameter (d_{43}) and droplet size distributions of the four coarse emulsions, supplemented by corresponding microscopy images, are summarized in Table 5-1 and depicted in Figure 5-2. The initial droplet size range (approx. 25 μm) was within the typical range of macroemulsions (0.5 – 100 μm) (Ma and Chatterton, 2021). When the pH was reduced to 3 in the gastric phase, the droplet size of emulsions stabilized by NaCas increased significantly and remained high throughout the 2 h duration of gastric conditions. Upon transitioning to intestinal the phase (pH 7), the droplet size of the NaCas-stabilized emulsions decreased significantly, reducing to approximately half of their size observed in the gastric phase. In contrast, emulsions formulated with Tween 80 exhibited no significant change in droplet size during the gastric phase, but their size gradually increased during the intestinal phase.

Table 5-1 Mean droplet size of initial emulsions and as emulsions undergo *in vitro* gastrointestinal digestion.

Samples	d_{43} (μm)				
	Initial	Gastric phase		Intestinal phase	
	0 min	60 min	120 min	180 min	240 min
Cur/Cas	26.1 $\pm$ 1.6 ^a	221.0 $\pm$ 100.3 ^a	131.3 $\pm$ 44.5 ^a	37.9 $\pm$ 2.9 ^a	125.2 $\pm$ 96.0 ^a
MOF/Cas	26.0 $\pm$ 1.3 ^a	217.7 $\pm$ 46.4 ^a	228.2 $\pm$ 34.7 ^a	79.0 $\pm$ 47.8 ^b	55.8 $\pm$ 6.4 ^b
Cur/T80	23.8 $\pm$ 0.1 ^b	21.6 $\pm$ 0.2 ^b	24.5 $\pm$ 1.6 ^b	39.8 $\pm$ 23.0 ^a	46.4 $\pm$ 8.0 ^c
MOF/T80	23.7 $\pm$ 1.8 ^b	20.9 $\pm$ 1.6 ^b	25.9 $\pm$ 1.1 ^c	33.6 $\pm$ 2.69 ^a	42.5 $\pm$ 5.8 ^c

Values expressed as mean $\pm$ standard deviation. ^{a-c} Different lower-case letters indicate a statistically significant difference down the column in different GI phases ($p < 0.05$).

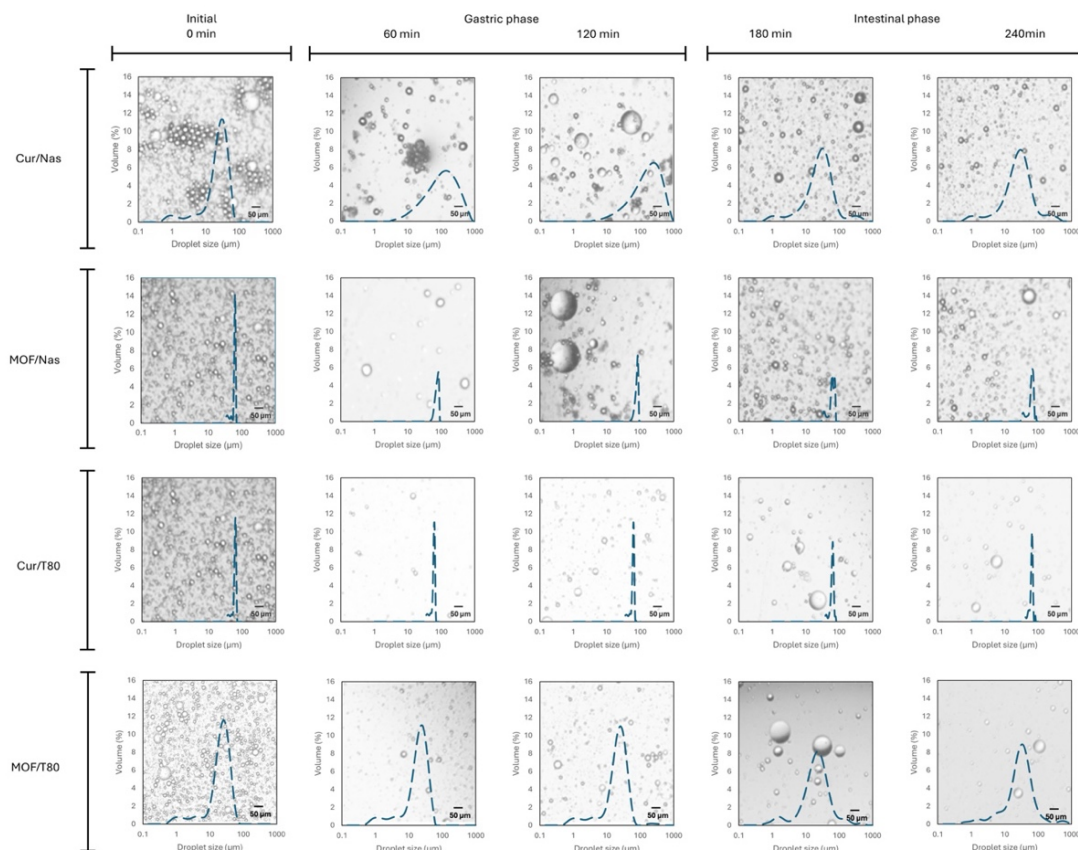


Figure 5-2 Microscopy images with superimposed droplet size distributions of initial emulsions and after their exposure to 60 and 120 min of gastric phase, followed by intestinal conditions, at 180 and 240 min, of simulated digestion.

The significant increase in d_{43} with NaCas during simulated gastric conditions can be largely attributed to the destabilization of the emulsion system due to the presence of pepsin. Pepsin cleaves the interfacial NaCas, breaking down the protein layer, contributing to droplet coalescence (Malaki Nik et al., 2011; Kenmogne-Domguia et al., 2012). The proteolysis of the interfacial film has weakened capabilities of steric stabilization and electrostatic repulsion because any remaining adsorbed protein fragments will be shorter and/or may no longer carry enough charge. Precipitation of the NaCas can occur as the pH approaches its isoelectric point (pI , ~ 4.6) (Van Hekken and Strange, 1993; Sato et al., 2015; Ma and Chatterton, 2021; Ha et al., 2023). Even though the NaCas starts to become (positively) charged again at pH 3, the net charge on the adsorbed protein is still lower than at pH 7, leading to diminished electrostatic repulsion between droplets, promoting droplet flocculation and coalescence (Srinivasan et al., 1996). The relatively high ionic strength –

primarily contributed by the level (47.2 mM) of NaCl but also due to the presence of other salts in the gastric phase – exacerbates emulsion destabilization via electrostatic screening effects (Dickinson and Davies, 1999; Liao et al., 2022). Additionally, the presence of the divalent cations, Mg^{2+} and Ca^{2+} can further destabilize the emulsions via ionic bridging (Dickinson, 2019; Li et al., 2020).

The reduction of droplet size of NaCas-stabilized emulsions in the intestinal conditions can be attributed to the restoration of NaCas solubility at neutral pH, as well as re-established surface charge and hence higher electrostatic repulsion (Xi et al., 2020; Ma and Chatterton, 2021), even though the ionic strength remains high. However, the addition of pancreatin, containing a mixture of lipolytic and proteolytic enzymes in the intestinal phase also influences the droplet size. The lipases hydrolyse triglycerides in the oil droplets to monoglycerides and free fatty acids (FFAs) that act as natural low molecular weight emulsifiers, helping to stabilize smaller droplet sizes (Armand et al., 1999; Malaki Nik et al., 2011; Song et al., 2021). As digestion progresses, proteolytic breakdown of NaCas may also further reduce its emulsion stabilizing capacity as adsorbed chains become even shorter and/or less charged (Sabouri et al., 2017; Böttger et al., 2019). It has also been suggested that other lipid digestion products, such as micelles and vesicles formed from bile salts, FFAs, and monoglycerides, may aggregate with the remaining oil droplets and give the appearance of larger overall ‘droplet’ (particle) sizes (Sarkar et al., 2019; Zhang et al., 2023).

The Tween 80-stabilized emulsions were much more stable to the raised acidity and ionic strength of gastric conditions due the non-ionic nature of Tween 80 (Qazi et al., 2020). Tween 80 provides purely steric repulsion, its hydrophilic ‘head’ extending into the aqueous phase and its hydrophobic tail anchored in the oil phase (Roldan-Cruz et al., 2016), whilst it is also not susceptible to enzymatic breakdown. Nevertheless, a slight increase in droplet size was observed as the intestinal phase progressed. This may have been due to droplet aggregation with lipolysis products, as mentioned above, and/or displacement of Tween 80 from the interface by these products, causing partial coalescence and larger droplet size (Vinarov et al., 2012).

5.4.2 ζ -potentials of emulsions stabilized by sodium caseinate and Tween 80

The ζ -potential measurements corroborated the emulsion stability changes indicated by the particle size measurements described above. A higher absolute ζ -potential value is generally an indication of stronger charge (negative or positive) on the particle surface and therefore a greater degree of electrostatic repulsion between the particles, thereby reducing the likelihood of aggregation (Cano-Sarmiento et al., 2018). As expected, emulsions formulated with NaCas exhibited a shift in ζ -potential from negative (-20 to -30 mV) to positive (15 – 20 mV) under gastric conditions, followed by a return to a lower magnitude negative value ($\sim$ -10 mV) during the intestinal phase (Figure 5-3), considering the pI of the adsorbed protein. Although Tween 80 is a non-ionic surfactant, emulsions formulated with Tween 80 showed a moderately negative ζ -potential ($\sim$ -25 mV) during the gastric phase, that decreased to $\sim$ -10 mV in the intestinal phase.

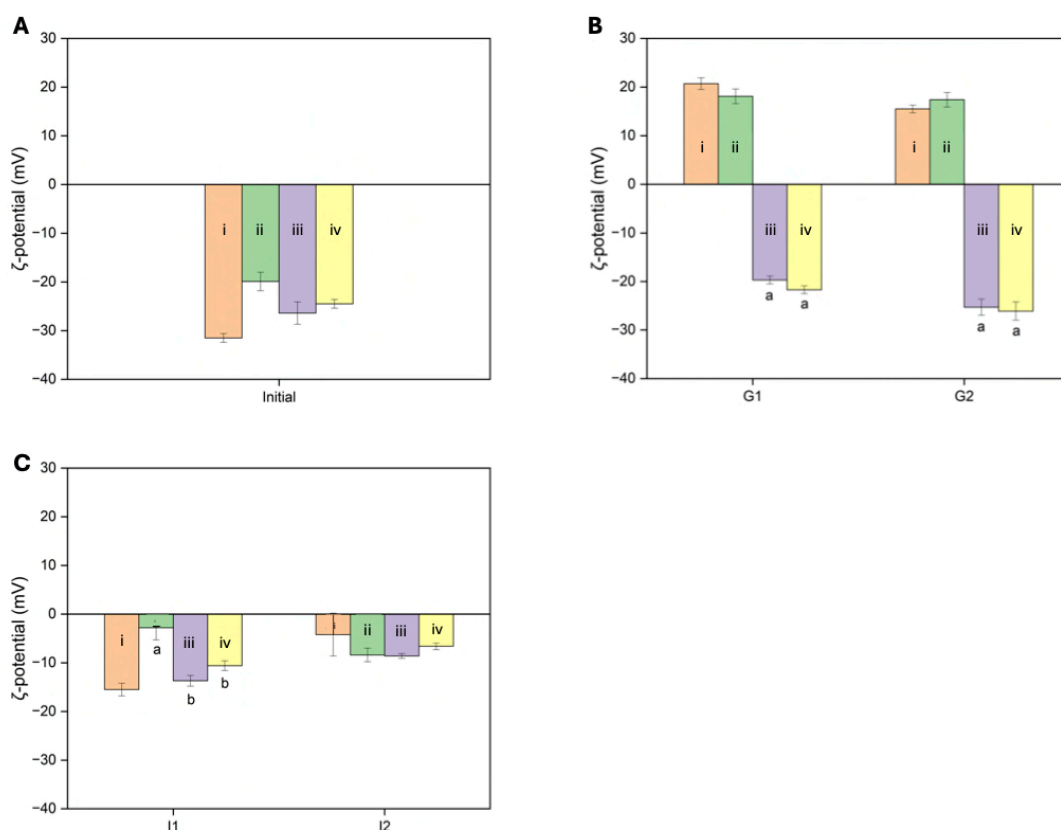


Figure 5-3 Measured ζ -potential of emulsions (A) pre-digestion (initial) and at hourly intervals during simulated gastrointestinal digestion during (B) gastric phase and (C) intestinal phase. Columns i (orange) represents sodium caseinate-stabilized emulsion incorporating curcumin, ii (green) represents sodium caseinate-stabilized emulsion incorporating Cur- γ -CD-MOFs, iii (purple) represents Tween 80-stabilized emulsion incorporating curcumin, and iv (yellow) represents Tween 80-stabilized emulsion incorporating Cur- γ -CD-MOFs. Columns with different letters represent significant difference within each group of 4 samples at $p < 0.05$.

The initial negative charge with Tween 80 might be due to co-adsorption of surfactant impurities or the preferential adsorption of hydroxyl ions from water (Witayaudom and Klinkesorn, 2017; Zhao et al., 2023) or other anions, whilst the moderate changes during digestion were probably due to adsorption of components released during the digestion. The slight lowering of the ζ -potential during the intestinal phase probably contributed to the increased droplet aggregation and/or coalescence observed. Thus, the changes in ζ -potential and therefore electrostatic repulsion coincided with observed droplet coalescence and flocculation, as described in section 5.4.1.

5.4.3 Controlled release and bioaccessibility

The bioaccessibility of curcuminoids from emulsion systems was assessed in comparison to native curcumin and Cur- γ -CD-MOFs and the results are summarized in Figure 5-4. According to Górnicka et al. (2023), the solubility of curcumin in water is poor – approximately 0.6 $\mu\text{g/mL}$. With the presence of bile salts within the simulated intestinal fluid the apparent solubility of curcuminoids increased to nearly 60 $\mu\text{g/mL}$, increasing the bioaccessibility to 3.6%. In the NaCas-stabilized emulsions the bioaccessibility of curcuminoids tripled, to 11.9 % compared to this 3.6 % ‘baseline’ level, and increased 5x to 19.2 % in the Tween 80-stabilized emulsions. Amongst all the samples, the curcuminoids “released” from the Cur- γ -CD-MOFs demonstrated the highest bioaccessibility, at 21.3%. However, incorporating the Cur- γ -CD-MOFs *into* the emulsions (MOF/Cas and MOF/T80) adversely affected curcumin delivery, with less than 5% of curcuminoids remaining bioaccessible – see Figure 5-4.

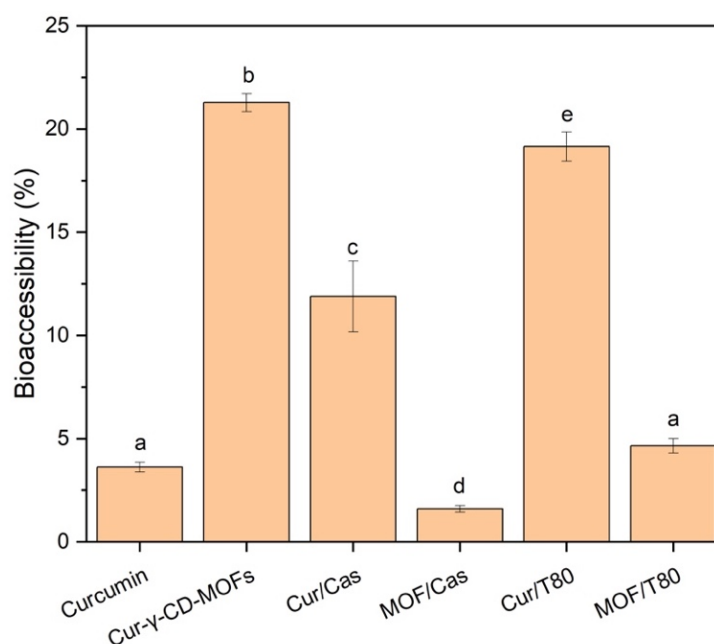


Figure 5-4 Bioaccessibility of total curcuminoids after *in vitro* gastrointestinal digestion of: curcumin, Cur- γ -CD-MOFs and sodium caseinate-stabilized emulsions incorporating curcumin and Cur- γ -CD-MOFs; Tween 80-stabilized emulsions incorporating curcumin and Cur- γ -CD-MOFs. Columns with different letters represent significant difference within each group of 5 samples at $p < 0.05$.

Based on Figure 5-5a, it can be said that sustained release – characterized by a gradual and prolonged release of curcuminoids over time – was achieved with Cur- γ -CD-MOFs alone and both curcumin emulsion samples (Cur/Cas and Cur T80). However, this trend was not observed in emulsions containing Cur- γ -CD-MOFs (MOF/Cas and MOF/T80). Moreover, the % release of curcuminoids for MOF/Cas was slightly lower and for MOF/T80 only slightly higher than native non-encapsulated curcumin.

To further investigate the mechanism of release of curcuminoids from systems containing Cur- γ -CD-MOFs, glucose levels in the digesta were quantified, since the amylases present will digest the γ -CD to linear or branched oligosaccharide and so degrade the γ -CD-MOFs (Hartlieb et al., 2017; Pan et al., 2022; Chen et al., 2023). The results (Figure 5-5b) indicated that nearly all γ -CD-MOF was degraded in emulsion samples, whereas only approximately half the γ -CD-MOF appeared to be degraded in the non-emulsion samples. The reasons for this are not clear, but it is possible that γ -CD-MOFs interact with the surface of the emulsion droplets, and this somehow facilitates access of the enzymes degrading the system.

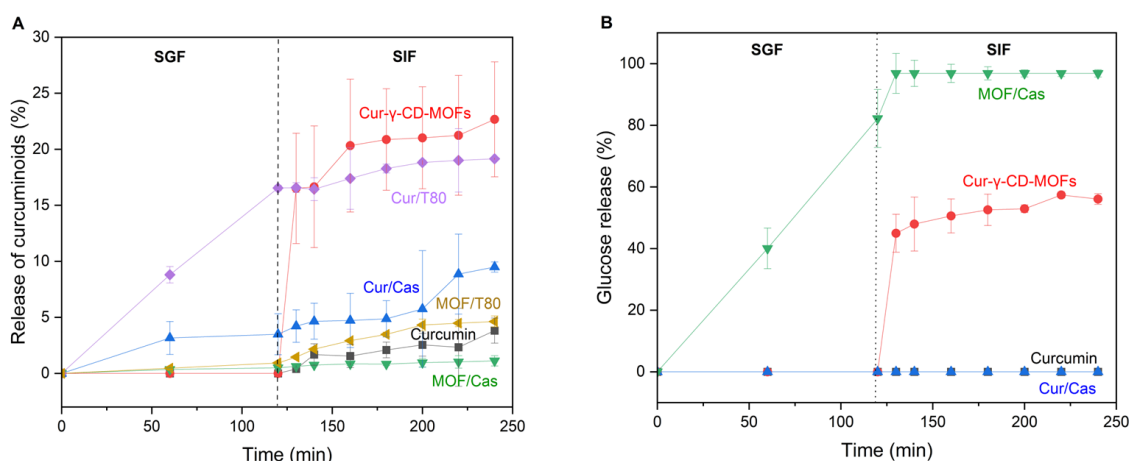


Figure 5-5 (A) *In vitro* controlled release of curcuminoids from native curcumin, Cur- γ -CD-MOFs and emulsion systems and (B) Glucose released during *in vitro* gastrointestinal digestions. The symbols ● represents Cur- γ -CD-MOFs, ◆ represents Tween 80-stabilized emulsion incorporating curcumin, ▲ represents sodium caseinate-stabilized emulsion incorporating curcumin, ◀ represents Tween 80-stabilized emulsion incorporating Cur- γ -CD-MOFs, ■ represents curcumin and ▼ represents sodium caseinate-stabilized emulsion incorporating Cur- γ -CD-MOFs.

Tween 80 is more effective in reducing the interfacial tension than NaCas, due to its lower molecular weight and ability to pack more tightly together at interfaces (Barkat et al., 2011; Liu et al., 2020). This facilitates smaller droplet sizes and therefore better dispersion of curcuminoids dissolved in the oil, enhancing their bioaccessibility because smaller droplets mean a higher interfacial area and thus more efficient lipolysis (Infantes-Garcia et al., 2020). In addition, the non-ionic nature of Tween 80 makes it relatively stable to changes in pH and salt concentration, unlike protein-based emulsifiers such as sodium caseinate. This stability across gastrointestinal conditions helps emulsion integrity and allows more consistent micellization and therefore solubilization of curcumin, ultimately enhancing bioaccessibility.

Post-synthesis, the permanent porosity of γ -CD-MOFs enables encapsulation of bioactive compounds like curcumin, which can contribute to their improved solubility and bioaccessibility (Pan et al., 2022; Rodríguez-Martínez et al., 2023; Si et al., 2024). Most studies suggest that curcumin is encapsulated within the hydrophobic cavity of the γ -CD or held between γ -CD pairs via hydrogen bonding (Chen et al., 2021; Zhou et al., 2021; Oh et al., 2023). In many cases, the relatively weak metal-ligand coordination bonds are susceptible to rapid degradation in aqueous environments. According to Feng et al. (2020), water molecules displace the metal-linker coordination bonds of MOFs, initiating hydrolysis that disrupts the structure. The stability of MOFs is dependent on the charge density of the metal ions, strength of coordinating bonds, and ligand configuration (Ding et al., 2019). Low-valency metal ions tend to form MOFs with weaker coordinate bonds since it generally requires fewer linkers to balance the charge. Conversely, to balance the charges with higher valency metal ions more linkers are required, which can lead to higher co-ordination number of metal clusters and enhance the MOF stability. Moreover, it has been reported that MOFs with larger cavities and longer linkers are less stable due to a more open structure and lower density. This exposes the MOF to easier attack from the water molecules (Feng et al., 2020). For this reason α -CD and β -CD are more water-resistant than γ -CDs due to their smaller inner cavities (Dummert et al., 2022; Topuz and Uyar, 2024).

Degraded γ -CD-MOFs are thought to rapidly release the trapped curcumin from within the γ -CD cavities which then, in the absence of any other components, transforms to its insoluble crystalline form or be solubilized to some extent within the mixed 'micelles' formed by the bile salts and any associated lipids or lipid degradation products formed during digestion (Oh et al., 2025). The micelle-solubilized curcumin is presumed to be far more bioaccessible. It is likely that the curcumin held tightly between the hydrophilic γ -CD pairs also contributed to the improved apparent solubility of curcumin, leading to its improved bioaccessibility. Thus, these two release locations may together be responsible for the sustained release observed in the case of Cur- γ -CD-MOFs alone. Because γ -CD is also susceptible to amylolytic hydrolysis, there should be a further slight increase in apparently solubilized curcuminoids during intestinal digestion, as observed here and in agreement with the observations of Lv et al. (2022). This further indicated that the pores of the γ -CD-MOFs could be slowly hydrolysed, releasing curcumin gradually over time. It is interesting, however, that even at the end of the intestinal digestion, at least 40% of the expected glucose content of the Cur- γ -CD-MOFs had still not been released. Thus, the Cur- γ -CD-MOFs are more resistant to degradation than expected for γ -CD-MOFs and a gradient of concentration from the γ -CD-MOFs to mixed micelles may still be governing the rate of release of curcuminoids.

Interestingly, confocal microscopy revealed that in the emulsion systems the Cur- γ -CD-MOFs were mainly situated at the oil-water interface (Figure C-1). This was surprising since the exterior of γ -CD-MOFs is expected to be hydrophilic and so they should preferentially stay within the aqueous phase. This adsorption to the interface somehow seems to facilitate either the dissolution of the Cur- γ -CD-MOFs and/or the release of curcumin molecules from them, that results in less bioaccessible (i.e., less micelle-solubilized) curcumin and therefore presumably more large crystals that sediment out.

Cur- γ -CD-MOFs are likely to be quite unstable to gastric conditions (pH 3), depending on the composition and coordination of the metal centers. At low pH, H^+ ions could potentially compete with K^+ ions for binding sites on the cyclodextrin (ligand) in the MOF structure, leading to the structural instability and degradation of the MOF (Manousi et al., 2019). This is largely due to the

coordination between monovalent K^+ and cyclodextrin being relatively weak compared to that of higher valency metal species, which could form stronger metal-ligand bonds that may be more resistant to proton displacement in acidic environments. In addition, hydroxyl groups within cyclodextrin can be protonated at low pH (Ding et al., 2019; Feng et al., 2020). This can further alter the γ -CD structure and be detrimental to the stability of MOF. Thus, γ -CD was quantifiable as glucose even in the absence of amylase due to the disassociation of MOFs. Any re-stabilized oil droplets formed at the end of the intestinal phase probably cannot efficiently solubilize any curcumin released from the Cur- γ -CD-MOFs, whilst the curcuminoids themselves may also begin to degrade at the more alkaline pH of the intestinal digesta. Overall then, incorporating Cur- γ -CD-MOFs into oil-in-water emulsions does not seem to be particularly advantageous in terms of improving delivery of active curcuminoids. However, the proposed initial delivery is a capsule form that would protect the Cur- γ -CD-MOFs during the gastric phase.

Since the Tween 80-stabilized emulsions containing Cur- γ -CD-MOFs did not perform any better than NaCas-stabilized emulsions, the degradation of the Cur- γ -CD-MOFs within them was assumed to be similar and thus there does not seem to be any particular advantage of using a synthetic, non-digestible low molecular weight emulsifier.

5.4.4 *Ex vivo* transport via intestinal mucosa

Post-digestion, the bioaccessibility data discussed above suggest that the increasing levels of curcuminoids released and available for intestinal absorption should be in the following order: MOF/Cas < Curcumin < Cur/Cas < Cur- γ -CD-MOFs. To be bioaccessible, compounds must be transported from the apical side of intestinal epithelia cells to the basolateral side to enter systemic circulation (Zeng et al., 2017). Therefore, absorption experiments were performed to assess the transport and absorption of curcuminoids via intestinal epithelial cells (from mice, as described in the Materials and Methods). The results obtained (Figure 5-6), of the concentration of curcuminoids on the apical and basolateral sides of the tissue and within the tissue itself, indicate an almost identical sequence in terms of increasing uptake: Curcumin < MOF/Cas < Cur/Cas < Cur- γ -CD-MOFs.

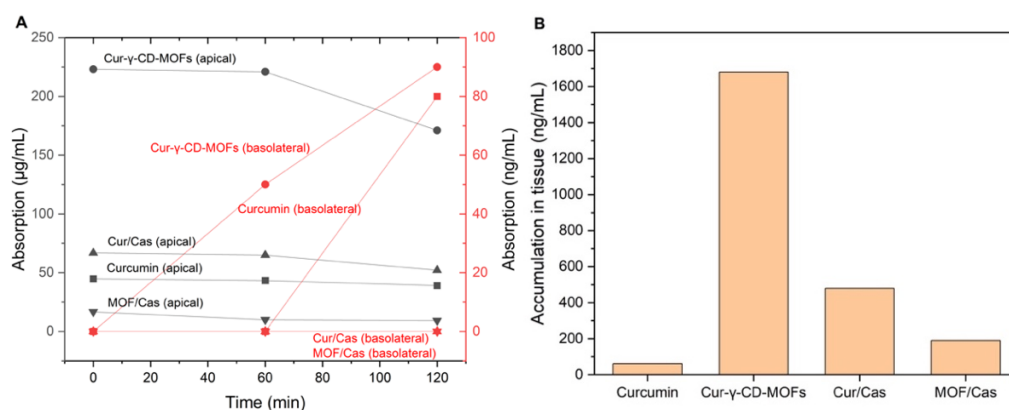


Figure 5-6 (A) Absorption of curcuminoids across mouse jejunum in an Ussing chamber for 4 different delivery systems with black lines representing the apical side and red lines representing basolateral side. The symbols ● represents Cur-γ-CD-MOFs, ▲ represents sodium caseinate-stabilized emulsion incorporating curcumin, ■ represents curcumin and ▼ represents sodium caseinate-stabilized emulsion incorporating Cur-γ-CD-MOFs. (B) The concentration of curcuminoids accumulated within the jejunum tissue

According to the literature, curcuminoids are best absorbed in the upper part of small intestine, and least favored in ileum, with no significant difference in the intestinal regions between the duodenum and jejunum (Wang et al., 2017). For this reason, the jejunum section of the mouse intestine was selected for use in the Ussing chamber. Pure curcumin is naturally poorly soluble in water and exhibits low permeability across intestinal barriers. The exact absorption mechanism of solubilized curcumin remains unclear. Caco-2 cell monolayers have been widely used to determine the permeation rate and mechanism of curcumin uptake. Studies suggest that curcumin absorption occurs via both passive (diffusive) and carrier-mediated active transport (Yu and Huang, 2011; Xue et al., 2017; Zeng et al., 2017; Wang et al., 2017; Li et al., 2023b). Curcumin is thought to be a substrate of P-glycoprotein (P-gp) and so curcumin transport may be affected by P-gp efflux, which pumps drugs out of cells, thus reducing absorption (Xue et al., 2017; Wang et al., 2017). Curcumin has also been proposed to function as an inhibitor of the efflux transporter P-gp and Multidrug Resistance Protein (MRP), potentially improving the absorption and bioavailability (Wortelboer et al., 2003; Hsieh et al., 2014; Li et al., 2023a). However, these studies are conducted using various types and

concentration of co-administered substrates and source of cells, making it challenging to compare.

Bioaccessibility has been reported as being slightly improved by incorporation into bile salt mixed micelles but absorption is predicted to remain poor, consistent with the observed results (Ye et al., 2024). It has been suggested that an oil droplet dominant formulation may be more advantageous (Zheng and McClements, 2020). When incorporated into an oil-in-water emulsion, higher absorption might be related to lipolysis and the formation of a higher concentration and richer range of composition of mixed micelles, better able to accommodate curcuminoid molecules.

The bioavailability of bioactive compounds forming inclusion complexes with γ -CD-MOFs has been described as enhanced due to their improved apparent solubility (He et al., 2020; Li et al., 2023b). Encapsulation within CD-MOFs was described as a multi-phase system in which free curcumin, free cyclodextrins, drug entrapped in CD complexes, and dispersed drug within solution exists in equilibrium (Li et al., 2018). Topuz and Uyar (2024) noted that hydrophilic γ -CDs may not easily traverse the gastrointestinal mucosa, and their presence does not directly influence the permeability of incorporated compound through the mucosa. This was in agreement of a study conducted by Nasr et al. (2023) where unlike α - and β -CD, γ -CD induces only a weak membrane permeability, because its larger cavity does not effectively extract phospholipids from membranes. Conversely, the curcumin-CD complexes act as a delivery reservoir, maintaining a constant free curcumin concentration for diffusion across the intestinal membrane. Although the MOF/Cas emulsion yielded lower bioaccessibility than pure curcumin (Figure 5-4), the solubilization effect of γ -CD-MOFs likely contributed to higher absorption (Figure 5-6B). Prolonged release of curcuminoids at the absorption site may enhance absorption via a combined effect of bioaccumulation by passive diffusion and active transport across the membrane in a concentration-dependent manner. It should also be noted that the starting concentration on the apical side for all samples is directly related to the bioaccessible fraction of curcuminoids as the endpoint of *in vitro* gastrointestinal digestion and differs due to the variation in delivery systems. These differences do not directly influence the absorption of curcuminoids across the intestinal tissue.

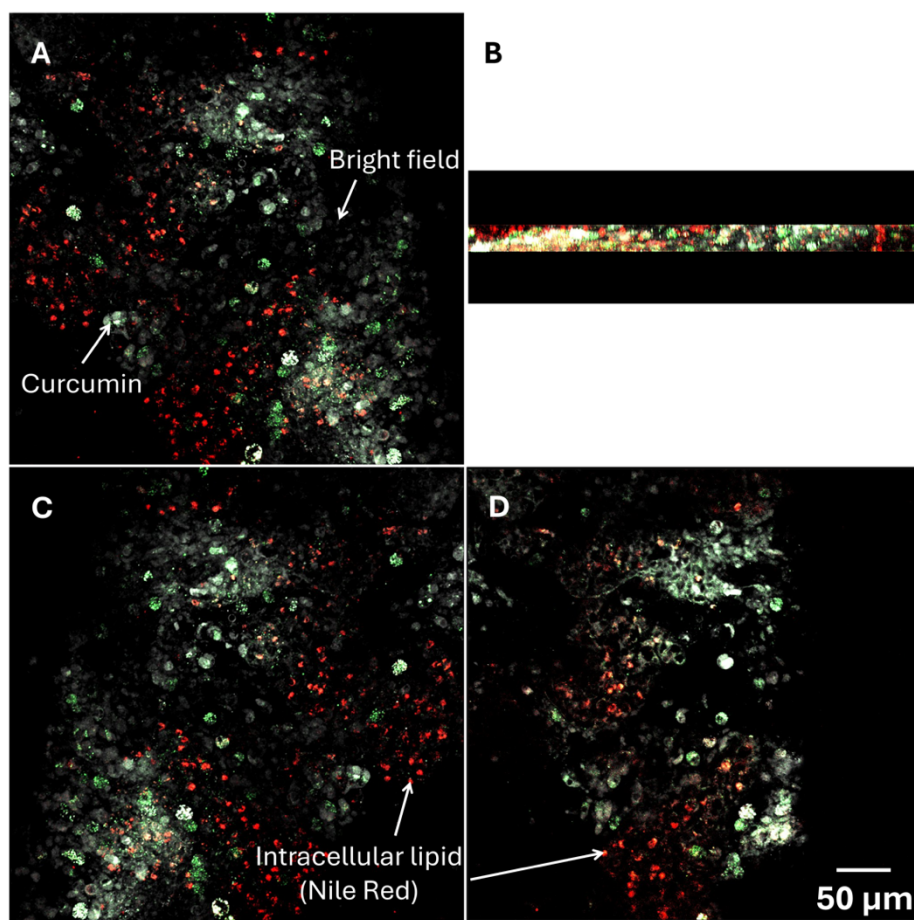


Figure 5-7 Confocal micrographs illustrating the absorption of curcuminoids from cur- γ -CD-MOFs across mouse jejunum from (A) apical side to (C) basolateral side. (B) = cross-section on z-plane and (D) two-dimensional microscopy image of the absorption in the tissue. The red represents the intracellular lipid stained by Nile Red, the green represents the natural fluorescent curcumin, and grey scale represents the bright field.

By the end of the experiment, in the majority of observations, the curcuminoids were found in the tissue (see Figure 5-7). These results suggested that curcuminoids directly released from γ -CD-MOFs or transported via the intermediary of mixed micelles could accumulate in the intestinal epithelial layer. This accumulation may retard further absorption from the gut and increase the residence time of curcuminoids within the mucus, thereby prolonging their bioactive effects (Niaz and Mackie, 2024). The accumulation was more prominent in Cur- γ -CD-MOFs as the average particle size of the bioaccessible fraction within the supernatant layer was below 200 nm (Table C-1), and within the reported range of pore size of gastrointestinal mucus of

100 – 200 nm (Wang et al., 2023). However, this may not be the case for some coarse emulsions since the droplet size appeared to exceed this range. A reduction in particle sizes to < 200 nm may therefore promote diffusion across intestinal epithelial cells via transcellular uptake.

5.5 Conclusions

The application of highly porous γ -CD-MOFs in biological and medical applications (i.e., drug release) requires excellent chemical stability, including strong resistance to hydrolysis or structural collapse in physiological environments. Although the stability of γ -CD-MOFs may be less than desirable, the method of delivery plays a critical role in determining their rate of degradation and therefore enhancement of both the bioaccessibility and transepithelial absorption of curcumin encapsulated within them. If delivered in capsule form, Cur- γ -CD-MOFs would be protected from the acidic gastric environment that could otherwise degrade γ -CD-MOFs and destabilize any emulsions into which they have been incorporated. This would allow γ -CD-MOFs to increase the apparent solubility of curcumin in a sustained manner, thus resulting in a higher absorption rate. Prolonged solubilization of curcumin could potentially persist into the colon, where fermentation occurs. Consequently, the fate of γ -CD-MOFs and unabsorbed curcumin in an *ex vivo* fermentation model presents an opportunity for future research.

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Chapter 6

General Discussion

6.1 Introduction

Curcumin, a naturally occurring polyphenolic compound derived from the rhizomes of *Curcuma longa* (turmeric), has been extensively studied for its diverse pharmacological properties. This includes anti-inflammatory, antioxidant, anticancer effects, contributing to its therapeutic versatility (Sabet et al., 2021; Ahmad et al., 2024; Nunes et al., 2024; Han et al., 2024). Despite its promising bioactivity, curcumin's clinical application is severely limited by its poor bioavailability, primarily due to its low aqueous solubility, rapid metabolic degradation, and instability under physiological conditions (Dei Cas and Ghidoni, 2019; Zhao et al., 2024). These challenges have prompted researchers to explore innovative encapsulation and delivery systems, such as emulsions, nanoparticles, liposomes, and gels, to enhance curcumin's solubility, stability and bioavailability. Among these, γ -cyclodextrin metal-organic frameworks (γ -CD-MOFs) have emerged as a particularly promising strategy due to their unique structural properties and biocompatibility.

In this thesis, I have investigated and demonstrated that γ -CD-MOFs can serve as an effective delivery system for curcumin, with the potential to improve the delivery of other poorly soluble bioactive compounds. By integrating these findings, this chapter aims to provide a comprehensive understanding of the functional properties of γ -CD-MOFs and their potential applications in drug delivery, while identifying future research directions to optimize their performance.

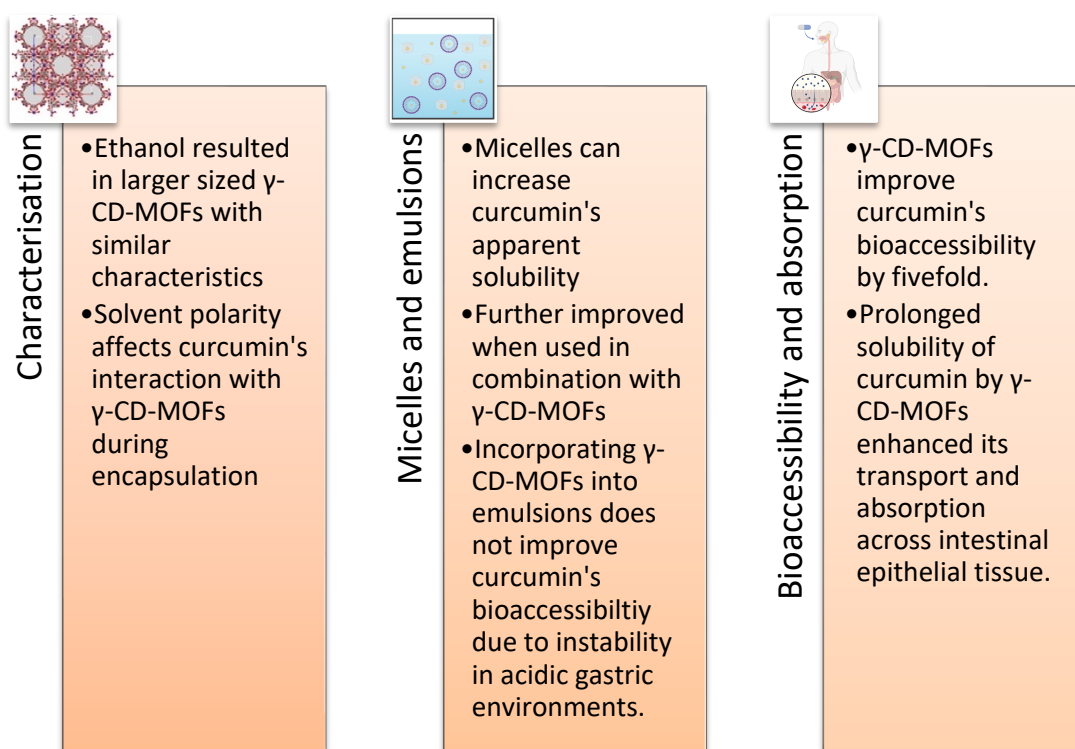


Figure 6-1 Summarized results based on the three studies conducted on γ -CD-MOFs in improving the bioaccessibility of curcumin.

6.2 Summary of results

The first assessment of the current evidence was a literature review conducted in **Chapter 2: An Overview of Cyclodextrin Metal-Organic Frameworks (CD-MOFs)**. The review highlighted the potential of CD-MOFs, particularly γ -CD-MOFs, as delivery systems for poorly soluble bioactive compounds like curcumin. The following knowledge gaps were identified and targeted to ensure the novelty of this thesis: (1) the impact of synthesis conditions (e.g., solvent choice) on γ -CD-MOFs properties and the encapsulation efficiency, (2) the synergistic effects of γ -CD-MOFs with micelles and emulsion systems, and (3) the bioaccessibility and bioavailability of curcumin in digestion and absorption models.

The first series of experiments focused on the synthesis and characterization of γ -CD-MOFs (**Chapter 3**). It was suggested that solvents with different polarities could impact the morphology of MOFs (e.g., crystal structure and

crystal lattice) thereby impacting their performance (Suresh et al., 2022; Song et al., 2023). Two solvents, ethanol and the more conventionally used methanol, were compared for their impact on γ -CD-MOFs properties. Key findings showed that while both solvents yielded γ -CD-MOFs with similar encapsulation efficiency (EE) and loading capacity (LC) for curcumin, ethanol resulted in larger crystal sizes due to its lower polarity, which slowed nucleation and growth rates. This suggests that the choice of synthesis solvent primarily influences the physical properties of γ -CD-MOFs rather than their functional performance, with the porous structure of γ -CD-MOFs being critical for encapsulation of bioactive compounds.

This was followed by an investigation on the choice of encapsulation solvent, similarly methanol and ethanol, on the EE and LC. Results suggest that solvent polarity plays a role in curcumin's interaction with γ -CD-MOFs. Hydrophobic compounds preferentially react with less polar solvent, leading to a weaker interaction with the hydrophobic cavity of γ -CD-MOFs and a lowered EE and LC. Therefore, this interaction should be considered when selecting the encapsulation solvent. The EE and LC can be manipulated and optimized for future investigations.

Preliminary results show that γ -CD-MOFs can improve the apparent solubility of curcumin. However, the limited stability of γ -CD-MOFs in aqueous environments is a critical factor in their performance as delivery systems. As γ -CD-MOFs are susceptible to disintegration in water, encapsulated curcumin could be prematurely released. Interestingly, Cur- γ -CD-MOFs exhibited lower water solubility and slower dissolution compared to γ -CD-MOFs alone, a striking result that currently lacks a clear explanation. This observation suggests that curcumin may not only be encapsulated within the cavities of γ -CD-MOFs but could also be located between γ -CD-MOF crystallites or γ -CD pairs, on the outer surfaces of the crystals, or in other structural arrangements. This distribution could help explain some of the later findings, especially how γ -CD-MOFs perform when combined with other delivery systems.

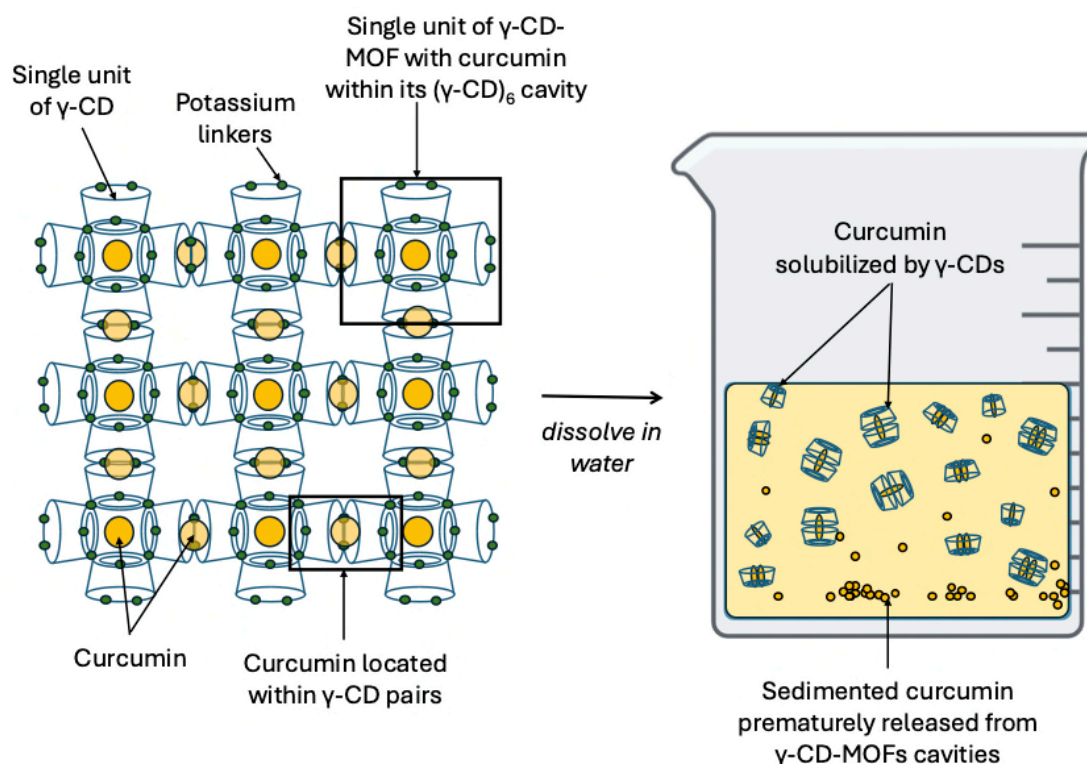


Figure 6-2 Encapsulation of curcumin within cavities of γ-CD-MOFs and γ-CD pairs in the extended network of Cur-γ-MOFs and the consequence when dissolved in water.

It was next essential to understand whether the performance of γ-CD-MOFs could be further enhanced by combining them with surfactant micelles (**Chapter 4**). Micelles, such as Tween 80, were found to improve the apparent solubility of curcumin released from γ-CD-MOFs, creating a boosted effect that enhanced curcumin's bioaccessibility. The presence of bovine bile salts added during simulated *in vitro* gastrointestinal digestion also slightly enhanced the bioaccessibility of curcumin in combination with γ-CD-MOFs compared to just γ-CD-MOFs.

As micelles were found to enhance the apparent solubility of curcumin, the effects of incorporating Cur-γ-CD-MOFs into an emulsion system was explored (**Chapter 5**). It was hypothesized that emulsions could supplement mixed micelles to the system post-digestion and further solubilize curcumin. However, the instability of γ-CD-MOFs in acidic gastric conditions limit their effectiveness in emulsion systems, highlighting the need for protective delivery methods such as capsules.

Finally, the bioaccessibility and absorption of curcumin delivered via γ -CD-MOFs were evaluated using simulated gastrointestinal digestion and an *ex vivo* intestinal transport model. Key findings showed that γ -CD-MOFs significantly improved curcumin's bioaccessibility, particularly when delivered in capsule form, which protected the MOFs from degradation in acidic gastric conditions. In the intestinal phase, a sustained release of curcumin was observed and the concentration solubilized by γ -CD-MOFs and bile salts outperformed coarse emulsions stabilized by sodium caseinate and Tween 80. The *ex vivo* model confirmed that γ -CD-MOFs enhanced curcumin's absorption across a mouse jejunum due to the prolonged solubilization provided by γ -CD-MOFs.

In summary, this thesis has demonstrated the potential of γ -CD-MOFs as effective delivery systems for curcumin, with the ability to enhance its solubility, bioaccessibility and absorption. By optimizing synthesis conditions, encapsulation methods, and delivery systems, γ -CD-MOFs can be tailored to meet the challenges of delivering poorly soluble bioactive compounds, paving the way for their application in drug delivery and functional foods.

6.3 Limitations

Although this thesis has provided valuable insights into the synthesis, characterization, and application of γ -CD-MOFs for the encapsulation and delivery of curcumin, several limitations should be acknowledged. These limitations highlight areas for future research and methodological improvements.

The EE and LC of curcumin in γ -CD-MOFs were found to be influenced by the choice of solvent and encapsulation method. As methanol generally provided higher EE and LC compared to ethanol, methanol was ultimately chosen as the encapsulation solvent to maximize the EE and LC. However, the associated toxicity with the use of methanol could limit their practical application and is considered unsafe for consumption (Tephly, 1991; Nekoukar et al., 2021). As methanol is nonpolar in nature, it may interact with γ -CD-MOFs and exist within the tight junctions within the crystals. Although the encapsulation process involved an overnight vacuum drying that should

have ensured the complete evaporation of methanol, there may still be concerns with the use of methanol in food products that could potentially be consumed. At present, there is no literature that focused on the interaction of solvent with bioactive compounds and the effects in their inclusion in the cavity of γ -CD-MOFs during encapsulation. It is recommended that studies should carefully consider such interactions and negate toxicity effects with preliminary tests focusing on optimizing the EE and LC when forming the inclusion complexes.

The concentration of curcumin encapsulated, EE, and LC was estimated based on the unencapsulated fraction of curcumin within the supernatant post-encapsulation (Moussa et al., 2016; Chen et al., 2021). The estimated EE was then used to determine the quantity of Cur- γ -CD-MOFs used for subsequent analysis (e.g. release, solubilization, and bioaccessibility). This could lead to the over- or underestimation of encapsulated fraction and could be ineffective for delivering therapeutic doses of curcumin. Moreover, this estimation does not take into account the possibility of curcumin degradation (e.g. light, temperature, pH, oxygen, etc.) that may occur during the encapsulation process (Wang et al., 1997; Zheng et al., 2017; Appendino et al., 2022). Due to the nature of the interaction of curcumin and γ -CD-MOFs, the true concentration of encapsulated curcumin could not be determined easily. Dissolution of Cur- γ -CD-MOFs in water would not completely “release” the curcumin as the compound is still held within the γ -CD pairs and Cur- γ -CD-MOFs do not dissociate in non-polar solvents. There is therefore a need to develop a method to fully degrade the MOFs and extract curcumin in a stable state for more accurate quantification of encapsulated curcumin.

The next limitation of this work is the reliance on *in vitro* models to simulate gastrointestinal digestion and absorption. While *in vitro* models, such as the INFOGEST protocol, provide a controlled and reproducible environment, they do not fully replicate the complexity of human physiological conditions (Brodkorb et al., 2019; Wang et al., 2023; Faubel et al., 2023). For example, continuous enzyme addition, control of pH changes, and peristaltic movements. The predetermined sequential digestion stages of static models occur under fixed conditions and is more like a batch process. Realistically, the simple, cost-effective static model is advantageous for preliminary studies,

and is often suited for evaluating endpoints resulting from food digestion such as the bioaccessibility of nutrients (Egger et al., 2017; Rojas-Bonzi et al., 2020). To better study the release, absorption, and metabolism of nutrients or drugs, the dynamic models could provide more details on their bioavailability. The choice between the two very much depends on the research goals, complexity of the study, and available resources.

The Ussing chamber allows analysis of drug absorption properties in a more physiologically relevant way compared to purely *in vitro* models involving the use of cell culture. This is due to the use of intact intestinal tissue with complex cellular interactions and allows for study of regional differences in absorption along the intestine. However, as there is limited availability of healthy human tissue, animal tissue is often used (Westerhout et al., 2015; Streekstra et al., 2022). Therefore, species-specific variation is expected and may not be completely representative to intestinal absorption in humans. Although the mice used were kept on a controlled diet, there may still be variation in tissue due to age, weight or health status (Xu et al., 2021). For this reason, replicates were not recommended and may yield different results. Moreover, the short viable time of harvested intestinal tissue is a major disadvantage of the system which limits the use for longer-term studies (Ghiselli et al., 2021).

6.4 Future directions and recommendations

6.4.1 Development of more stable MOF structures

The present study utilized γ -CD-MOFs synthesized using potassium ions as linkers. However, the structural stability of these MOFs in aqueous and acidic environments remains a challenge for their application in drug delivery. Future research should focus on developing more stable MOF structures by exploring alternative metal ions, cross-linking strategies, or surface modifications.

The diversity of metal components ranging from monovalent, divalent, trivalent to tetravalent cations could be explored to develop a more stable MOF structure. Metal ions of higher valency are said to enhance MOF structure through the formation of stronger coordination bonds with the cyclodextrin ligands (Feng et al., 2020). At present, zinc and iron have been proposed as metal ligands in synthesis of MOFs, but its applications may be limited due to

toxicity risks associated with the use of these metal ions such as leaching and accumulation in the body (Safdar Ali et al., 2021; Zhong et al., 2023; Xing et al., 2023). The stability and inertness may be the key concern and should be thoroughly tested for digestion, absorption, and potential toxicity to meet regulatory standards. Calcium salts and compounds are often used in food products as supplements or fortifications and is generally considered safe and beneficial for human health. Calcium-based CD-MOFs has already been synthesized and showed considerable potential for molecular separations, water purifications and electronic and magnetic applications (Xian et al., 2021; Sukatis et al., 2022; Shi et al., 2024). In combination with cyclodextrin, it may be possible to synthesize a biocompatible MOFs that offers a better aqueous stability and promising applications in food, health, and pharmaceuticals.

6.4.2 Exploration of colonic fermentation model

While the small intestine is the primary site of nutrient absorption, the colon is home to a variety of microbes that can metabolize compounds that are not digested in the upper gastrointestinal tract (Ośko et al., 2024). Given that curcumin was estimated to not have been completely released post-digestion, it is possible that curcumin or intact Cur- γ -CD-MOFs could be delivered to the colon. Future research should explore the use of colonic fermentation models to evaluate the fate of γ -CD-MOFs and their encapsulated compounds in the colon. This could involve the use of *in vitro* colonic fermentation models, such as the batch fermentation models, TIM-2 system or MiGut (Veintimilla-Gozalbo et al., 2021; Davis Birch et al., 2023).

Curcumin has been shown to have prebiotic-like effect, promoting the growth of beneficial bacteria, such as *Lactobacillus* and *Bifidobacterium*, while inhibiting the growth of pathogenic bacteria, thus re-establishing gut microbiota balance (Neyrinck et al., 2021; Enayati et al., 2023; Zhu and He, 2024). The encapsulation of curcumin in γ -CD-MOFs could enhance its stability and bioaccessibility in the colon, potentially amplifying its prebiotic effects. The impact of γ -CD-MOFs on microbial diversity, metabolic activity, and the production of short-chain fatty acids (SCFAs) which contributes to host health could also be studied.

The metabolism and biotransformation of bioactive compounds by gut microbiota can result in the production of bioactive metabolites with enhanced therapeutic properties. Curcumin metabolism in the GI tract is said to be complicated and yield a range of metabolites such tetrahydrocurcumin (THC), dihydroferulic acid (DFA), 1-(40hydroxy-3-methoxyphenyl)-2-propanol, hexahydrocurcumin (HHC), and octahydrocurcumin (OHC) (Tan et al., 2015; Li et al., 2017). The microbial metabolites, particularly THC and HHC, have been suggested to have increased bioactivity compared to the parent compound (Zam, 2018; Huang et al., 2018; Hundshammer et al., 2021). Hence, the bioactive metabolites produced through colonic fermentation could be identified and quantified and their bioactivity could be further assessed.

6.4.3 *In vivo* studies and pharmacokinetic evaluation

In vivo studies could be crucial for fully understand how well γ -CD-MOFs can deliver curcumin and other active compounds. While *in vitro* studies have shown that γ -CD-MOFs can improve curcumin's solubility and bioaccessibility, *in vivo* research is needed to confirm whether these improvements actually increase bioavailability (Bohn et al., 2018). Pharmacokinetic studies in living organisms will provide important data on how curcumin is absorbed, distributed, metabolized, and excreted when delivered through γ -CD-MOFs (Fischer and Chan, 2007; Faa-Berthon et al., 2021; Teix -Roig et al., 2023). Key measurements like the maximum plasma concentration (C_{max}), time to reach C_{max} (T_{max}), area under the plasma concentration-time (AUC), and half-life ($t_{1/2}$) will help explain how the system performs in the body. Safety testing, including toxicity studies, tissue analysis, and immune response checks, is also needed to ensure γ -CD-MOFs do not cause harmful effects on organs (Simon-Yarza et al., 2018).

The route of administration can also impact how well γ -CD-MOFs work, so *in vivo* studies should look at how different methods, such as oral, intravenous, or pulmonary delivery, affect pharmacokinetics, tissue distribution, and effectiveness. (Benet, 1978; Stielow et al., 2023; Kim and De Jesus, 2025) For example, intravenous delivery might be better for quick action, while pulmonary delivery could be suited for targeting lung conditions. Long-term stability and how γ -CD-MOFs break down in the body are also important to

ensure they do not accumulate and cause harm. Overall, *in vivo* studies are key to confirming the safety and effectiveness of γ -CD-MOFs and their potential use in delivering curcumin and other drugs.

6.5 Implications of current findings

In conclusion, the future of γ -CD-MOFs as a sustained delivery system holds great promise and this thesis has contributed to the understanding the use of γ -CD-MOFs in enhancing the bioaccessibility and absorption of poorly soluble bioactive compounds like curcumin. It was demonstrated that γ -CD-MOFs can improve the apparent solubility and sustained release of curcumin, particularly when delivered in capsule form to avoid degradation in acidic gastric conditions. The prolonged solubilization of curcumin encapsulated within γ -CD-MOFs not only led to a higher bioaccessibility but also improved the absorption across intestinal epithelial tissues. However, incorporating γ -CD-MOFs into emulsion systems did not yield similar benefits, likely due to structural instability under gastric conditions. These insights highlighted the importance of delivery methods in optimizing the functionality of γ -CD-MOFs, suggesting that capsule-based delivery may be more advantageous. Future research should explore long-term stability and potential applications of γ -CD-MOFs in various physiological environments to further validate their efficacy as delivery vehicles.

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Appendix A
Supporting information for Chapter 3

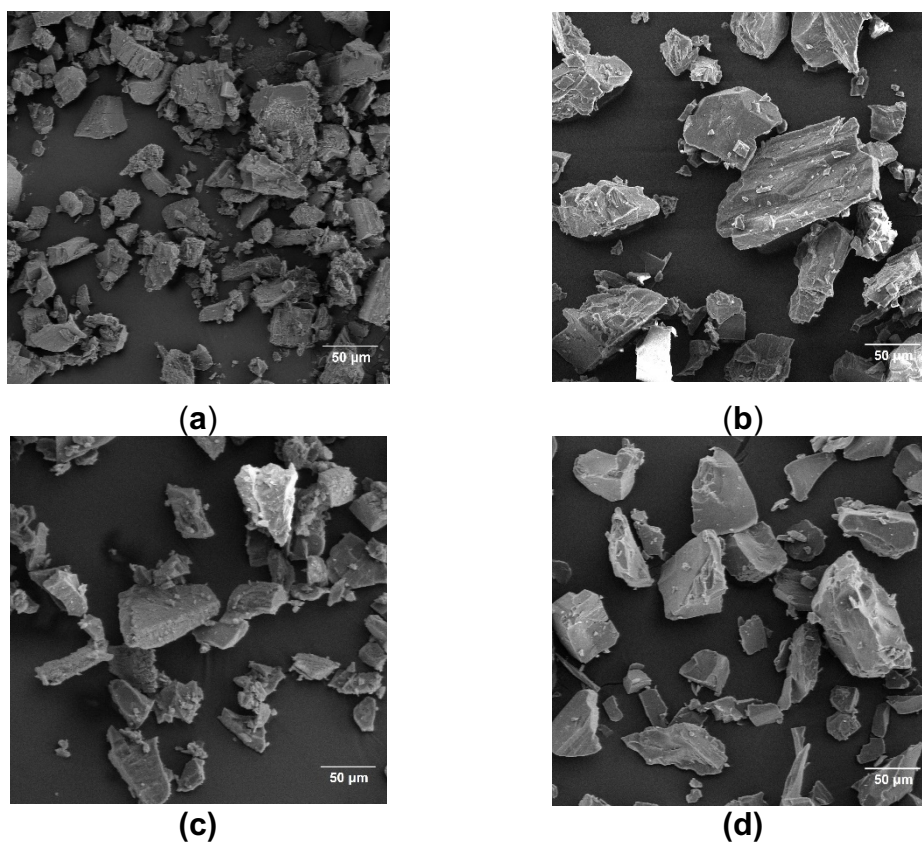


Figure A-1 SEM images of: (a) Cur- γ -CD-MOFs (methanol-synthesized crystals, encapsulated via methanol), (b) Cur- γ -CD-MOFs (methanol-synthesized crystals, encapsulated via ethanol), (c) Cur- γ -CD-MOFs (ethanol-synthesized crystals, encapsulated via methanol), and (d) Cur- γ -CD-MOFs (ethanol-synthesized crystals, encapsulated via ethanol).

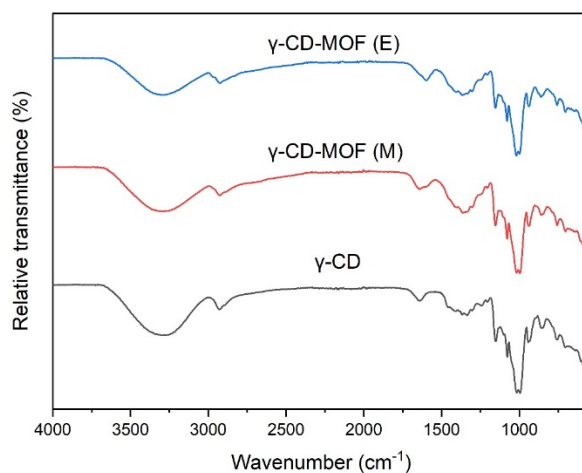


Figure A-2 FT-IR patterns of γ -CD, γ -CD-MOFs (methanol-synthesized), and γ -CD-MOFs (ethanol synthesized).

The FT-IR spectra of γ -CD, methanol-synthesised γ -CD-MOFs, and ethanol-synthesised γ -CD-MOFs shown in Figure A-2 have similar characteristic peaks. The strong, broad peak at 3000 – 3500 cm⁻¹ indicated -OH stretching vibration; peak at approximately 1637 cm⁻¹ accounted for a hydrate water vibration whereas 1360 cm⁻¹ corresponded to -OH plane bending vibration. Thus, indicating that the change in synthesis solvent does not affect the intermolecular interaction in the γ -CD-MOFs significantly.

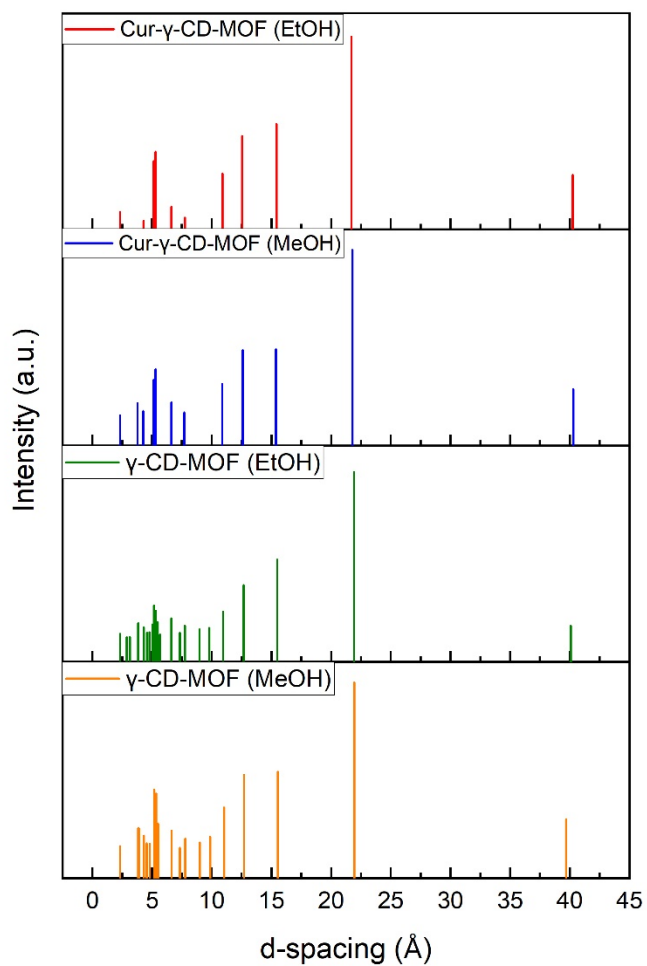
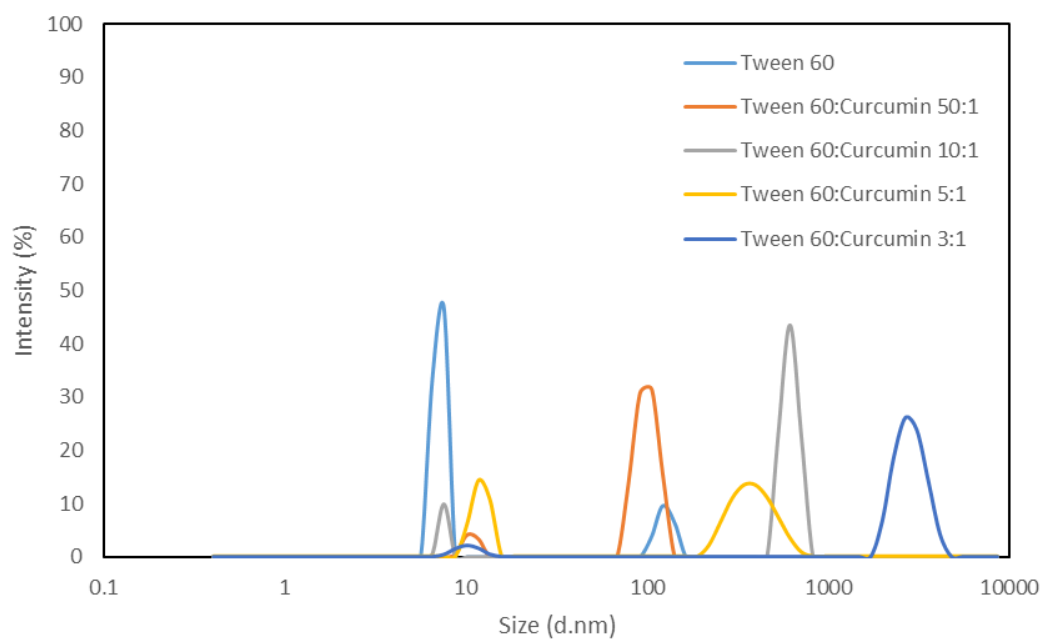


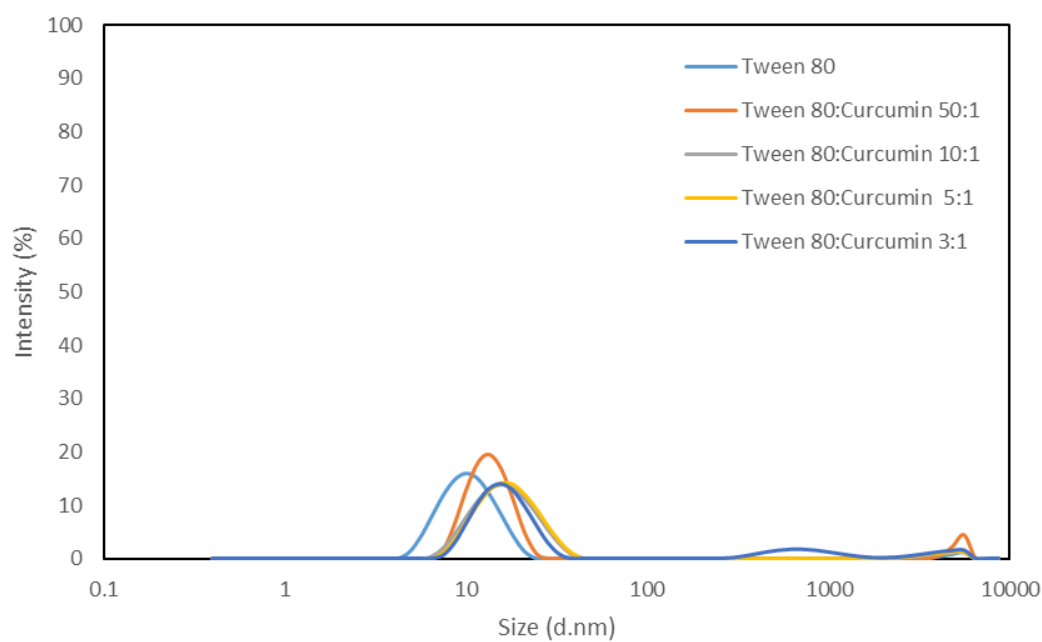
Figure A-3 Diffraction peaks expressed in d-spacing of free γ -CD-MOFs and Cur- γ -CD-MOFs.

Appendix B

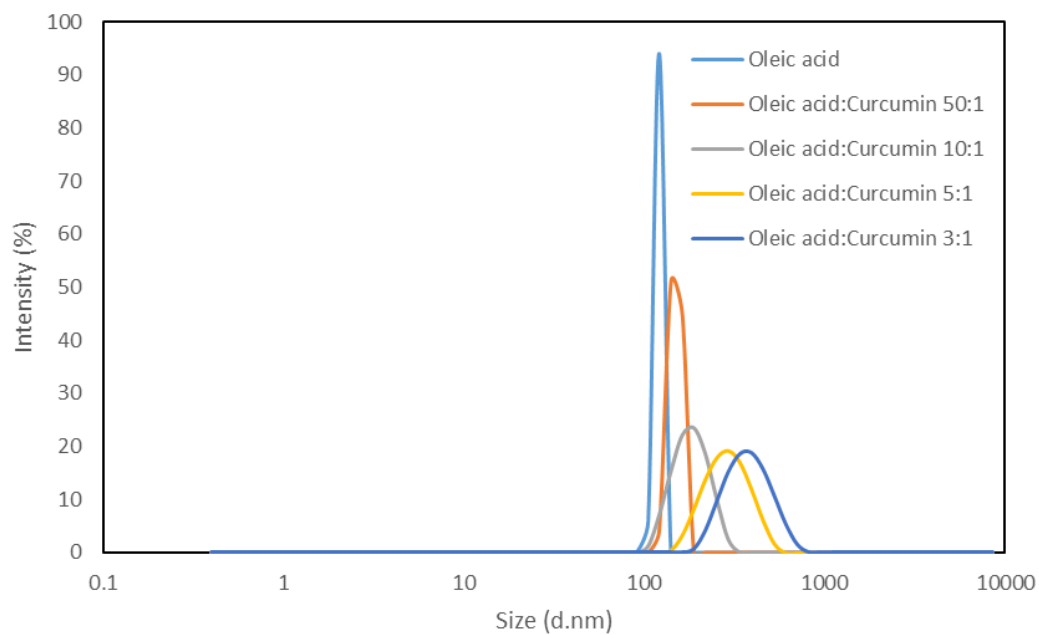
Supporting information for Chapter 4



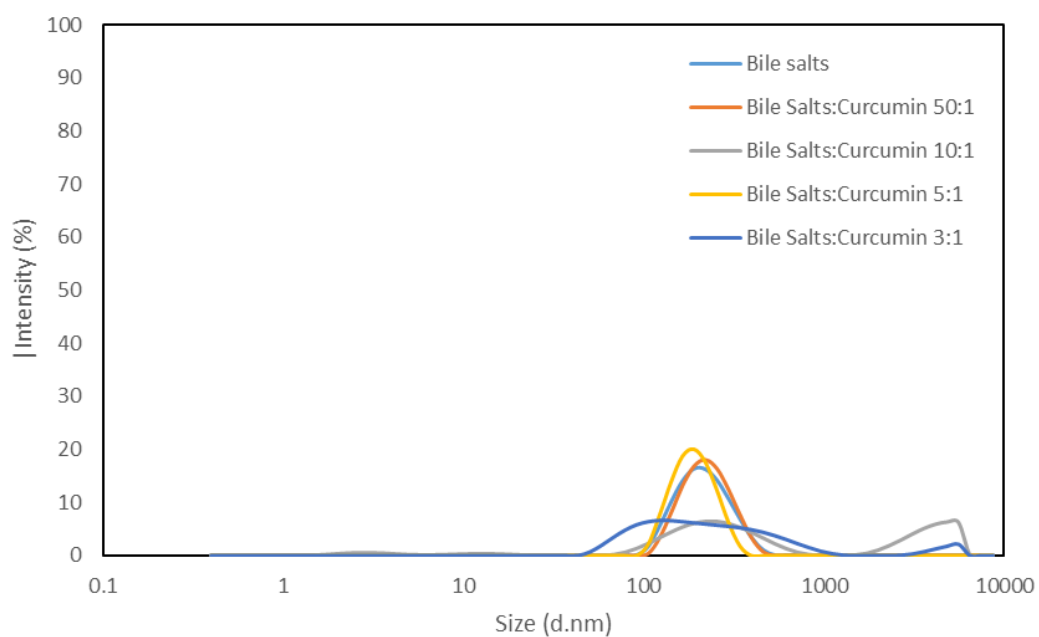
(a)



(b)

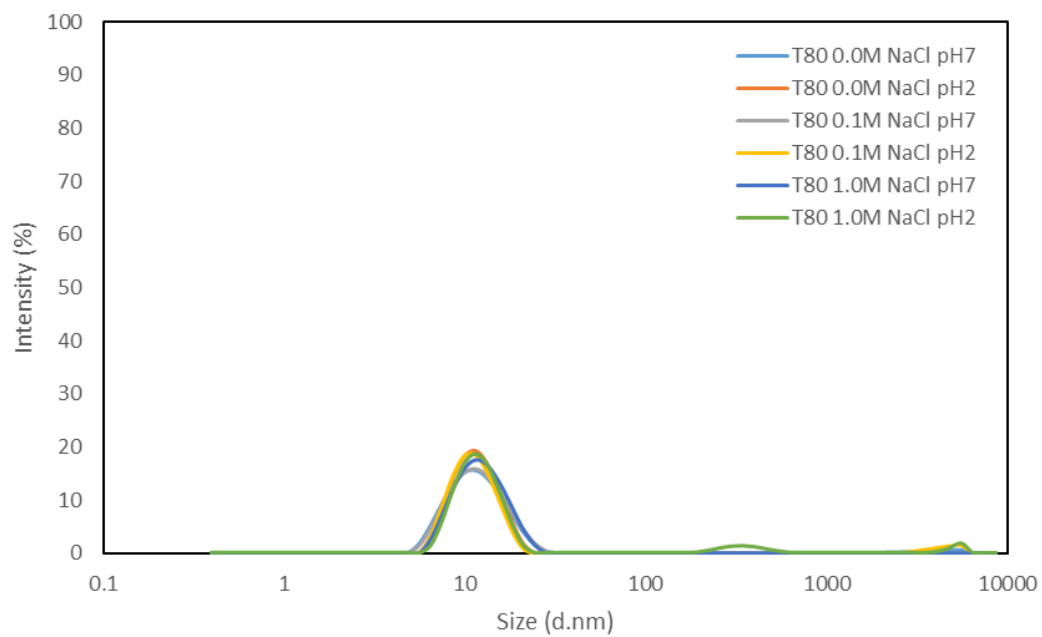


(c)

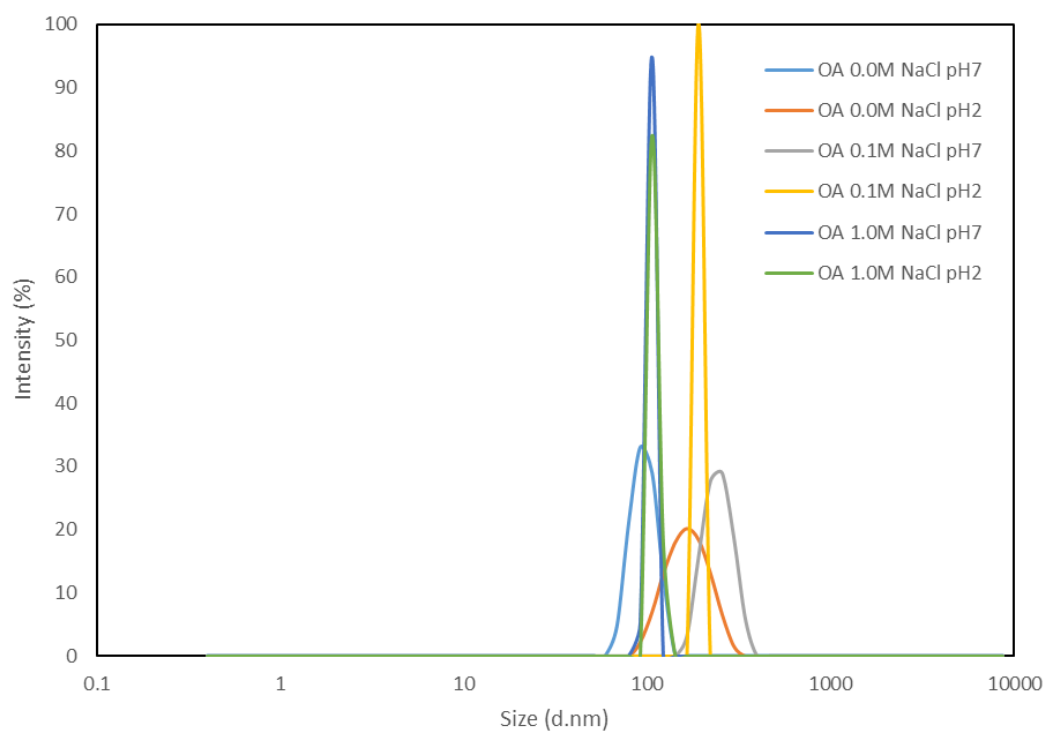


(d)

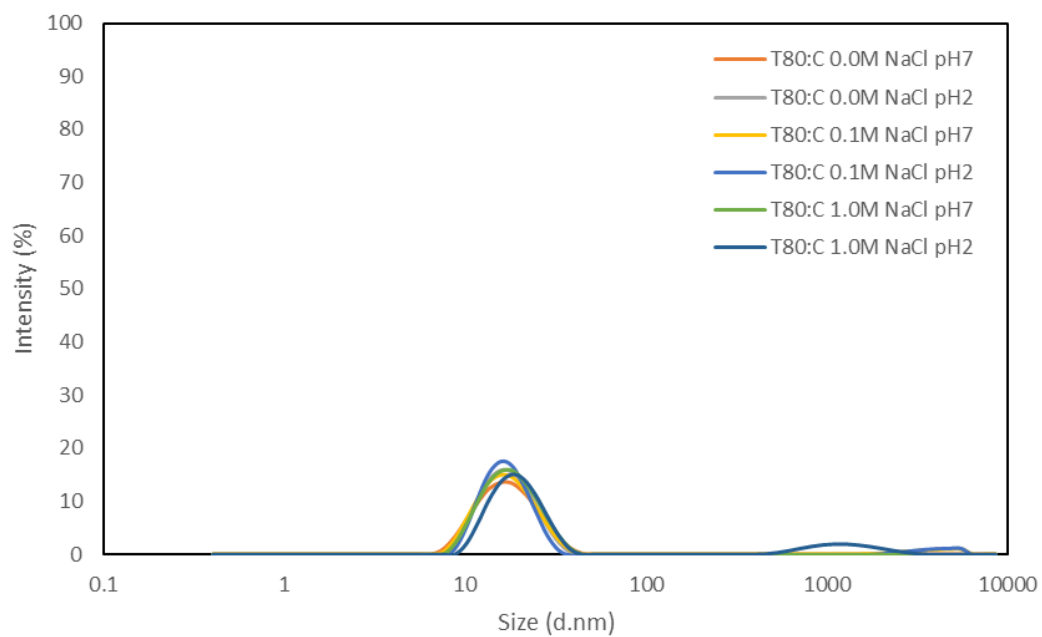
Figure B-1 Size distribution curve of micelles and micelles with increasing molar concentration of curcumin: (a) Tween 60, (b) Tween 80, (c) Oleic acid, and (d) Bovine bile salts.



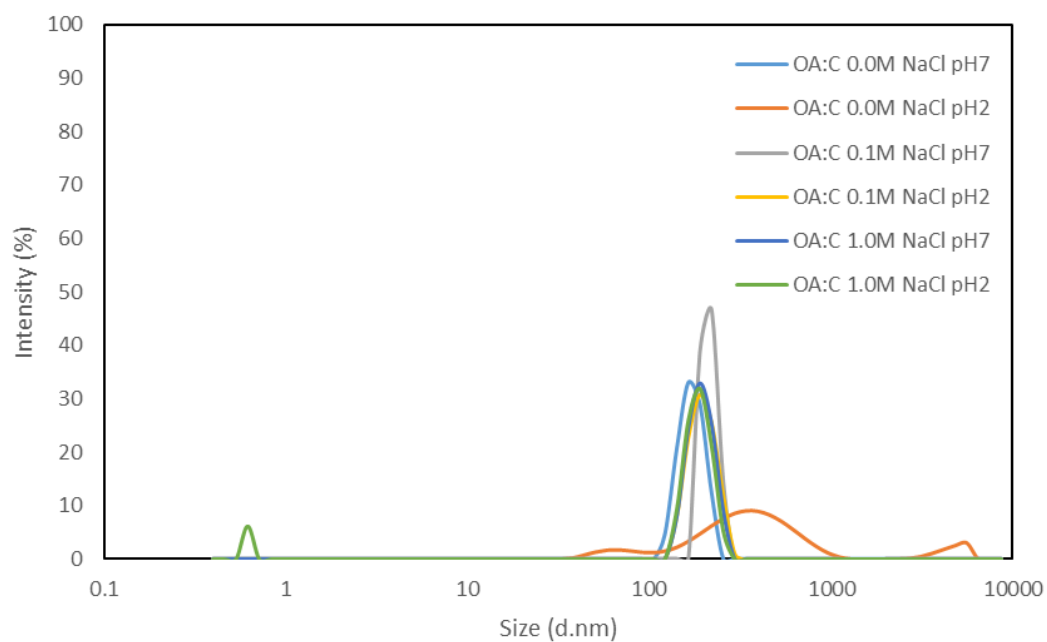
(a)



(b)



(c)



(d)

Figure B-2 Size distribution curve of micelles at varying pH (pH 7 and pH 2) and at NaCl concentrations of 0.0 M, 0.1 M of (a) Tween 80 and (b) Oleic acid, and with curcumin under the same conditions of (c) Tween 80 and (d) Oleic acid.

Appendix C
Supporting information for Chapter 5

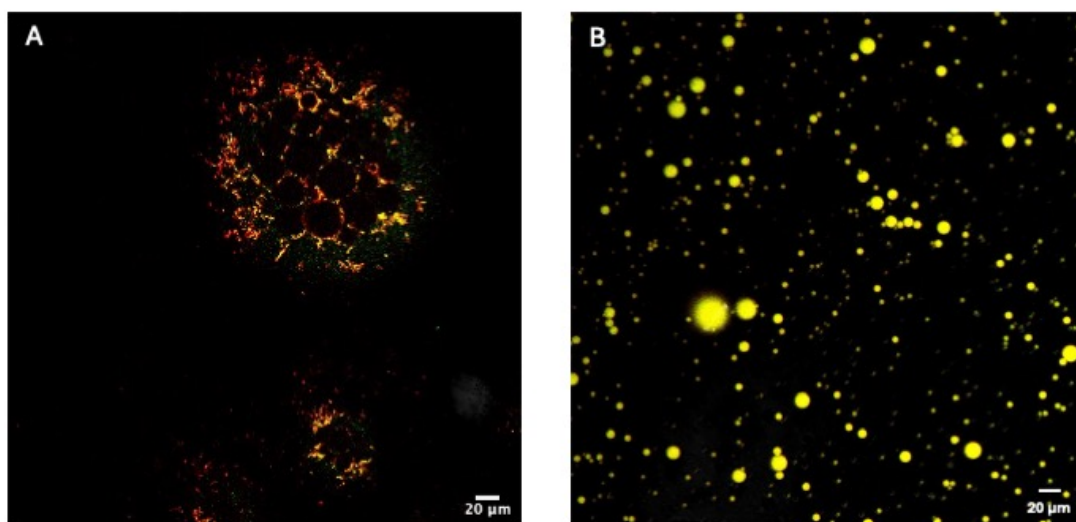


Figure C-1 Two-dimensional confocal microscopy images of (A) freshly prepared Cur-γ-CD-MOFs emulsion stabilized by sodium caseinate and (B) curcumin taken up by droplets post-digestion of the Cur-γ-CD-MOFs emulsion.

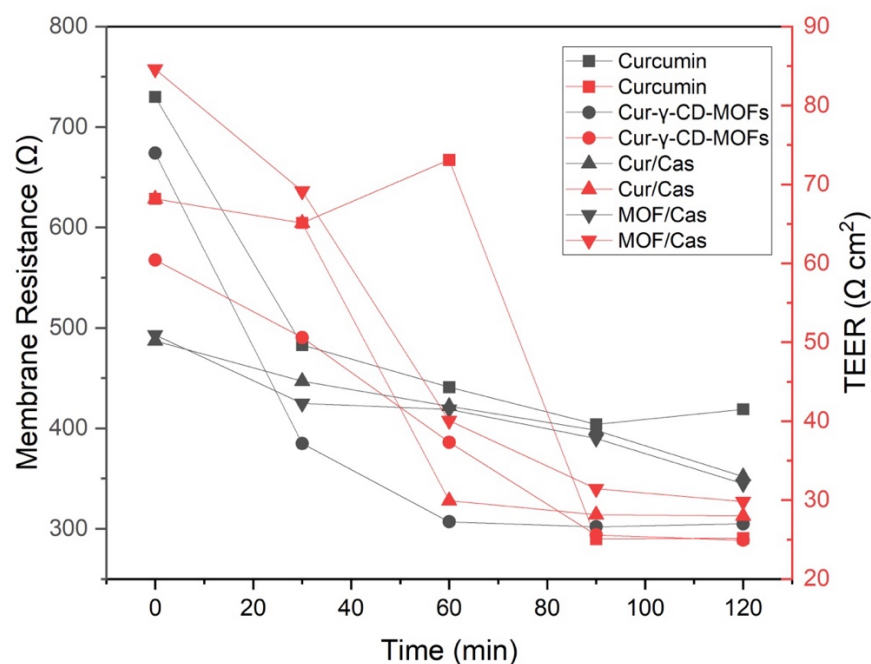


Figure C-2 Tissue viability of mouse jejunum tissue used in Ussing chamber studies to assess the absorption of curcuminoids in the four delivery systems. Black lines represent the membrane resistance; red lines represent transepithelial electrical resistance (TEER).

Table C-1 The average particle size of the bioaccessible fraction within the supernatant layer of four delivery systems post-digestion.

Samples	Hydrodynamic meter (nm)
Curcumin	217.8 ± 3.6
Cur-γ-CD-MOFs	171.7 ± 17.7
Cur/Cas	259.4 ± 164.3
MOF/Cas	189.7 ± 37.9