

Investigation of Nonionic Surfactant–Cyclodextrin Hydrogels for Hydrophobic Drug Delivery

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DIVISION OF FOOD AND PHARMA/CHEMICAL ENGINEERING | DEPARTMENT OF
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Investigation of Nonionic Surfactant– Cyclodextrin Hydrogels for Hydrophobic Drug Delivery

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by

Jingdan Hou

Process and Life Science engineering

June 2026

Supervisor: Stefan Ulvenlund

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A Flowable “Jelly”: A Soft Therapeutic Material That Behaves More Like the Human Body

Although the human body contains 206 bones, it is by no means a rigid system. Approximately 60% of the human body consists of water, and the water content of many soft tissues is even higher. From a materials science perspective, the human body can therefore be regarded as a large, soft, naturally occurring hydrogel. This raises an important question: when diseases, injuries, or surgical procedures leave cavities and wounds within the body, what kind of material is most suitable for supporting tissue repair? The answer may not lie in rigid materials, but rather in a soft, water-rich, and even flowable gel.

In my research, I attempted to develop a novel hydrogel material capable of behaving both like a liquid and a solid. Ideally, it should be injectable like a liquid, allowing it to flow into deep wounds and irregular cavities, while subsequently recovering a jelly-like state after injection and remaining localized at the target site to support tissue repair. To achieve this goal, the capacity of six different nonionic surfactants to form gels together with cyclodextrin was investigated. Nonionic surfactants can be thought of as tiny molecular bridges between water and oil, two substances that normally do not mix. One end of the molecule prefers water, while the other prefers oil, allowing it to bring the two together. Because these molecules do not carry an electrical charge in water, they are known as *nonionic* surfactants. Four of these (alkyl glycosides) were initially expected to form stable gels; however, the experimental results were unsatisfactory. After high-speed centrifugation, these samples underwent phase separation and failed to maintain stable structures. In contrast, systems composed of Tween (polysorbate) 20 or Tween (polysorbate) 80 together with α -cyclodextrin exhibited markedly different and more promising behavior. Under specific compositional ratios, these systems behaved like elastic gels under static conditions, but temporarily flowed like liquids when subjected to stress, such as during injection through a syringe.

One of the most remarkable observations appeared during the later stages of the experiments. Immediately after preparation, the samples resembled ordinary liquids and flowed freely. However, after standing for a period of time, the material underwent a striking transition. When the glass vial was inverted, the sample remained firmly attached to the bottom without flowing downward, as though it had transformed into an elastic gel. This “solid-like at rest, liquid-like under stress” behavior directly corresponds to its potential biomedical applications. The material could first be injected into irregular cavities in a liquid-like state and subsequently reform into a soft gel capable of filling wounds and remaining localized at the treatment site.

More importantly, this hydrogel system may also serve as a carrier for poorly water-soluble drugs. In this way, therapeutic agents would no longer need to circulate extensively throughout the entire body before reaching the target tissue; instead, they could act in close proximity to the wound site. Such localized delivery may not only improve therapeutic efficacy but also

reduce unwanted effects on healthy tissues elsewhere in the body.

Although considerable work remains before these materials can be translated into clinical practice, such studies already suggest a promising new direction for biomedical materials. Future therapeutic materials may no longer function merely as passive fillers, but instead behave more like living tissues themselves—soft, adaptive, flowable, and actively involved in the body's own healing processes.

Abstract

Supramolecular hydrogels have attracted increasing attention in drug delivery, tissue engineering, and injectable biomaterials because of their high water content, good biocompatibility, and dynamic network structures. Cyclodextrin-based supramolecular systems can form network structures through host–guest interactions. These systems usually require mild cross-linking conditions and allow flexible control of material properties, which gives them potential for biomedical applications. However, studies on the gelation behavior of different nonionic surfactant–cyclodextrin systems and their structure–property relationships are still limited.

In this study, six nonionic surfactants were selected, including the polysorbates 20 and polysorbate 80, and the alkyl glycosides α -dodecyl maltoside, β -dodecyl maltoside, decyl glucoside, and lauryl glucoside. These surfactants were mixed with α -cyclodextrin and β -cyclodextrin to investigate their gelation behavior and possible assembly mechanisms. The study first screened the phase behavior of the systems by changing the surfactant type, cyclodextrin type, water content, and molar ratio. The study further carried out rheological measurements, nuclear magnetic resonance analysis, injectability evaluation, and in vitro curcumin release experiments.

The results showed that different surfactants formed different assembly structures with cyclodextrins. Most alkyl glycoside systems showed phase separation or precipitation after centrifugation and failed to form stable hydrogels. The nuclear magnetic resonance results indicated that the strong host–guest interactions between α -cyclodextrin and dodecyl maltoside favored the formation of dense aggregates rather than continuous three-dimensional networks.

In contrast, the systems formed by Polysorbate 20 or Polysorbate 80 with α -cyclodextrin showed better stability. The systems formed stable hydrogels at higher α -cyclodextrin/Polysorbate 80 molar ratios and lower water contents. Rheological measurements showed that these hydrogels had clear viscoelastic behavior. The storage modulus remained higher than the loss modulus. The systems also showed obvious shear-thinning and thixotropic recovery behavior. These results indicated that the hydrogels possessed dynamic and reversible network structures together with good injectability.

The study also prepared curcumin-loaded systems using Polysorbate 80/ α -cyclodextrin hydrogels as carriers. The results showed that the hydrogels could encapsulate curcumin–cyclodextrin inclusion complexes and provide sustained drug release. Different curcumin–cyclodextrin inclusion ratios influenced the release behavior. These findings suggested that the supramolecular structures and host–guest interactions inside the systems affected the drug diffusion process.

Acknowledgement

As my two years of Master's studies at Lund University are drawing to a fulfilling close, I find myself reflecting on what has been my very first experience studying abroad. Throughout these two years, I encountered countless challenges and opportunities, but more importantly, I was fortunate enough to meet many remarkable mentors and friends whose support and companionship profoundly enriched both my academic journey and personal growth.

First and foremost, I would like to express my sincere gratitude to my supervisor and examiner, Stefan Ulvenlund and Marie Wahlgren, for their unwavering support throughout my master's thesis project. At the very beginning of the project, Marie patiently listened to my interest in conducting research on hydrogels and kindly introduced Stefan as a co-supervisor for the project. Despite her busy schedule, Marie consistently offered generous guidance, encouragement, and insightful advice throughout the course of my work.

I am equally grateful to Stefan, who devoted an extraordinary amount of time and effort to this project. From defining the project scope and designing experiments to troubleshooting challenges during the research process and holding weekly meetings, his dedication and commitment were evident at every stage. Beyond the project itself, both of my supervisors offered me tremendous encouragement and confidence whenever we discussed my future academic and professional aspirations. Their recognition of my academic potential has meant more to me than words can fully express. I am deeply thankful for all of their support, patience, and trust.

I would also like to thank Johan Evenäs for his guidance and assistance with NMR measurements and data analysis, which greatly improved my understanding of how to approach and interpret complex datasets.

My deepest gratitude goes to my family, especially my mother and grandmother. I am profoundly thankful for their open-mindedness, their unwavering emotional and financial support for my academic pursuits, and for always giving me the freedom to make my own choices. Because of them, I have been able to devote myself wholeheartedly to the scientific research I truly love, without the burden of financial worries. Their love and support have given me a sense of security that allowed me to pursue my dreams with confidence and peace of mind.

I would also like to thank my dearest friends, Fan Yawen and Li Ying. During my years living and studying abroad, they were always the first people I wanted to share my joys with and the first ones I turned to in times of sadness and uncertainty. Even though we were separated by half the world, I never stopped feeling their presence by my side.

To my friends and classmates in Lund — Dong Ziyi, Zhu Ruiqian, Xu Chengqi, and Xue Ruilin — thank you for making me feel loved and accompanied far away from home. Your friendship has given me strength, comfort, and confidence throughout this journey.

Lastly, I would like to leave a few words for my future self: no matter what lies ahead, whether in times of success or adversity, never lose sight of your original aspirations. Stay confident, stay resilient. Having left home and traveled thousands of miles, you must keep striving, and strive even harder.

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1 Background

Hydrogels have attracted increasing attention in the biomedical and pharmaceutical fields owing to their high water content, tunable properties, and excellent biocompatibility. Among them, injectable hydrogels, which can be administered through minimally invasive approaches, are considered ideal materials for localized sustained drug delivery. For example, in postoperative intervention for solid tumors, hydrogel formulations loaded with anticancer and anti-inflammatory agents can be injected into the postoperative cavity. Such systems not only provide structural support to the residual cavity but, more importantly, facilitate the elimination of residual cancer cells, thereby reducing the risks of recurrence and metastasis while promoting wound healing and tissue recovery.

Among various hydrogel systems, supramolecular hydrogels constructed through non-covalent interactions have received considerable research attention because of their reversible and dynamic mechanical properties, which make them highly adaptable to diverse application scenarios. The supramolecular system investigated in this study focuses on nonionic surfactants and cyclodextrins. Nonionic surfactants are widely utilized in pharmaceutical applications due to their low toxicity, favorable biocompatibility, and excellent solubilization capacity, and they have been extensively applied in drug solubilization, stabilization, controlled-release delivery, as well as in the construction of thermosensitive hydrogels and nanocarrier systems.

Cyclodextrins, characterized by their hydrophobic inner cavity and hydrophilic outer surface, can improve the solubility, stability, and bioavailability of poorly water-soluble drugs through inclusion complex formation. Consequently, they have been broadly employed in drug solubilization, controlled-release, and targeted delivery systems.

Although cyclodextrin-based supramolecular systems have demonstrated great potential in drug delivery applications, the gelation behavior and structure–property relationships between different nonionic surfactants and α -/ β -cyclodextrins remain insufficiently understood. In addition, comprehensive evaluations of the mechanical properties, drug release behavior, and injectability of such supramolecular hydrogels are still limited.

Therefore, in this study, six nonionic surfactants were selected to construct supramolecular hydrogel systems with α -/ β -cyclodextrins. Their gelation behavior and possible mechanisms were systematically investigated, while their rheological properties, drug release characteristics, and injectability were further evaluated to explore their potential feasibility as injectable drug delivery carriers.

2 Aim of the thesis

The aim of this thesis is to investigate the gelation behavior and properties of supramolecular hydrogels formed by six nonionic surfactants in combination with α - and β -cyclodextrins. The surfactants were selected from the two classes *polysorbates* and *alkylglycosides*. The study focused on understanding how the molecular structure of the surfactants and the type of cyclodextrin influence hydrogel formation, as well as identifying the possible mechanisms responsible for gelation or the absence of gelation in different systems.

In addition, the rheological properties of selected hydrogel formulations were evaluated in order to assess their mechanical strength and viscoelastic behavior. A specific hydrogel formulation was further investigated for its potential biomedical application through in vitro curcumin release studies and injectability testing. The overall objective was therefore not only to explore the structure–property relationship governing hydrogel formation, but also to evaluate the feasibility of these supramolecular hydrogels as potential injectable drug delivery systems.

3 Theoretical background

3.1 Cyclodextrin Structure and Host–guest Inclusion Mechanism

3.1.1 Chemical Structure and Characteristics of Cyclodextrins

Cyclodextrins are a class of cyclic oligosaccharides composed of multiple glucopyranose units linked through α -(1,4)-glycosidic bonds. Depending on the number and type of monomeric units, cyclodextrins can be categorized into several types, among which α -, β -, and γ -cyclodextrins, consisting of six, seven, and eight glucopyranose units, respectively, are the most common.

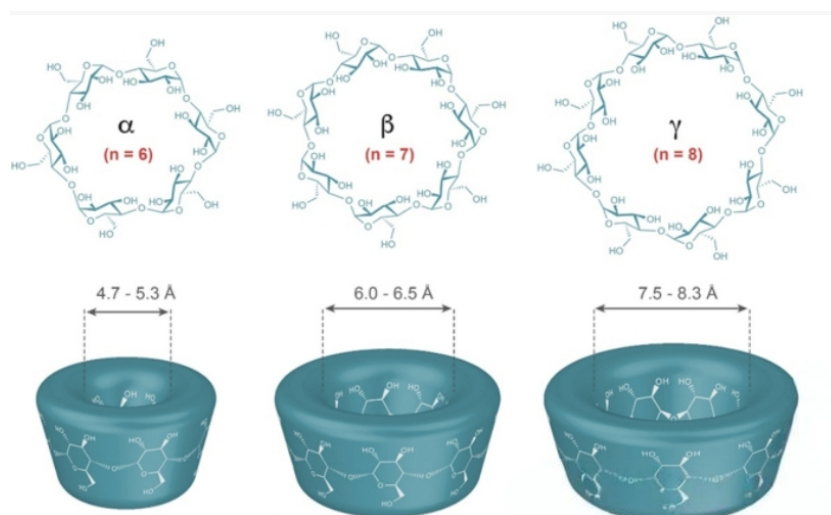


Figure 3.1.1.1. α -, β - and γ -Cyclodextrin structures and cavity sizes.

X-ray crystallographic studies have demonstrated that cyclodextrins exhibit a truncated cone-like structure. The secondary hydroxyl groups at the C2 and C3 positions are distributed on the wider rim, whereas the primary hydroxyl groups at the C6 position are located on the narrower rim. Consequently, the external surface of cyclodextrins is hydrophilic, while the internal cavity formed by ether oxygen atoms and hydrogen atoms is relatively hydrophobic. Owing to this unique amphiphilic architecture, cyclodextrins can form host–guest inclusion complexes with a wide range of hydrophobic molecules ¹.

The present study involves α - and β -cyclodextrins. Although these two molecules differ by only one glucopyranose unit, they exhibit substantial differences in molecular structure and physicochemical properties. α -cyclodextrin has a molecular weight of 972 g/mol, a cavity diameter of 4.7–5.3 Å, and a water solubility of 14.5% (w/v) at 25°C. β -cyclodextrin has a molecular weight of 1135 g/mol, a cavity diameter of 6.0–6.5 Å, and a water solubility of 1.85% (w/v) at 25°C ². These pronounced differences strongly influence the formation of

cyclodextrin–hydrophobic molecule/structure inclusion complexes because the size of the hydrophobic cavity determines the degree of compatibility with guest molecules or structures.

3.1.2 Thermodynamics and Driving Forces of Host–guest Inclusion

A wide variety of guest molecules can form inclusion complexes with cyclodextrins, including vitamin D₂, naphthol, steroids, hydrocarbons, and even gaseous molecules such as ethylene and carbon dioxide ³. It has also been reported that cyclodextrins are capable of forming supramolecular assemblies with crown ethers and metal cations ⁴. Owing to the relatively large size of crown ether molecules, the participation of multiple cyclodextrin units is required for complex formation. These findings further demonstrate that the size compatibility between the hydrophobic cavity of cyclodextrins and the guest molecule is a key factor governing the formation of inclusion complexes.

The formation of host–guest inclusion complexes involves the encapsulation and retention of hydrophobic molecules or structures within the hydrophobic cavity of cyclodextrins. This process does not involve the formation or cleavage of covalent bonds and is primarily driven by hydrophobic interactions⁵. From a thermodynamic perspective, water molecules initially confined within the cyclodextrin cavity are replaced by hydrophobic guest molecules or structures, resulting in an increase in system entropy (ΔS) and a decrease in Gibbs free energy (ΔG), thereby producing a more stable low-energy state ⁶.

$$\Delta G = \Delta H - T\Delta S \quad (1)$$

Additional noncovalent interactions involved in inclusion complex formation include hydrogen bonding and van der Waals interactions. Because these interactions are noncovalent in nature, the formation of inclusion complexes is dynamic and reversible.

3.1.3 Dynamic and Nonstoichiometric Supramolecular Assembly

Although idealized stoichiometric models such as 1:1, 1:2, and 2:1 are commonly used to describe cyclodextrin inclusion complexes⁷, the actual supramolecular aggregates formed in aqueous systems often deviate substantially from these theoretical structures ^{8,9}. In practical systems, the formation of cyclodextrin-based aggregates depends not only on simple host–guest inclusion but also on multiple intermolecular forces, aggregation behavior, and dynamic self-assembly processes ¹⁰.

In aqueous environments, guest molecules may simultaneously interact with multiple cyclodextrin molecules, while neighboring inclusion complexes can further associate through hydrogen bonding, hydrophobic interactions, van der Waals forces, and cooperative stacking effects ^{10,11}. Under specific experimental conditions, nonstoichiometric supramolecular assemblies are therefore more likely to form, particularly in concentrated systems or in systems containing amphiphilic molecules capable of aggregation ^{9,12}.

Furthermore, the actual composition ratio within precipitated or aggregated phases does not necessarily correspond to the initial feed ratio. During phase separation and self-assembly, selective binding and component enrichment of either cyclodextrins or guest molecules may occur. These phenomena arise from differences in molecular packing efficiency, hydration behavior, cooperative molecular effects, and the thermodynamic stability of the final supramolecular aggregates ⁸.

Overall, the formation of cyclodextrin-based supramolecular assemblies should not be simply regarded as the generation of standard stoichiometric inclusion complexes with fixed structures and compositions. Instead, these systems are more appropriately defined as dynamic multicomponent assembly systems in which inclusion interactions coexist with molecular aggregation and cooperative supramolecular self-assembly ^{10,11}.

3.2 Nonionic Surfactants and Their Interactions with Cyclodextrins

Surfactants are amphiphilic molecules containing both hydrophilic and hydrophobic groups that can orient at interfaces between two phases, thereby reducing interfacial tension. Owing to this property, surfactants are widely applied in detergency, emulsification, dispersion, wetting, solubilization, and foam stabilization. In aqueous solution, when the concentration exceeds the critical micelle concentration (CMC), surfactant molecules further self-assemble into structures such as micelles and vesicles, which play important roles in drug delivery and material fabrication ¹³.

Based on the charge characteristics of their hydrophilic groups, surfactants can be classified into anionic, cationic, zwitterionic, and nonionic surfactants. Nonionic surfactants are characterized by uncharged hydrophilic moieties, commonly including polyoxyethylene chains and glycosyl groups. Compared with ionic surfactants, nonionic surfactants generally exhibit lower toxicity, superior biocompatibility, and reduced sensitivity to pH and ionic strength variations. Consequently, they have found extensive application in the fields of food science, cosmetics, biomedicine, and functional materials ¹⁴.

In pharmaceutical formulations, nonionic surfactants are commonly used as emulsifiers, solubilizers, wetting agents, and stabilizers in oral, injectable, and topical formulations. Polyoxyethylene surfactants, for example, can improve the stability and release efficiency of hydrophobic drugs in aqueous media through micellar encapsulation ²¹. Furthermore, certain nonionic surfactants can enhance membrane permeability and drug delivery efficiency, rendering them highly valuable in nanocarriers, liposomes, and hydrogel-based delivery systems.

Six nonionic surfactants belonging to two major categories were investigated in this study.

3.2.1 Polyoxyethylene-Based Surfactants

In this study, the group of polyoxyethylene-based surfactants was represented by polysorbate 20 and polysorbate 80. Polyoxyethylene-based nonionic surfactants employ polyoxyethylene chains as hydrophilic groups and exhibit excellent water solubility, biocompatibility, and micelle-forming ability. Polysorbate 20 and polysorbate 80 are polyoxyethylene derivatives of sorbitan fatty acid esters. polysorbate 20 contains a lauric acid hydrophobic chain, whereas polysorbate 80 contains an oleic acid hydrophobic chain ¹⁵. Because these molecules contain both hydrophobic fatty chains and hydrophilic polyoxyethylene segments, they can form stable micelles in aqueous solution and further generate supramolecular aggregates through hydrophobic interactions, self-assembly behavior, and host–guest inclusion interactions with cyclodextrins. In particular, the polyoxyethylene segments can undergo threading interactions with cyclodextrin to construct polyrotaxane structures, thereby promoting the formation of three-dimensional hydrogel networks ¹⁶. Owing to their low toxicity, high stability, and excellent solubilization capability, these surfactants are widely used in drug delivery, biomaterials, and supramolecular hydrogel research.

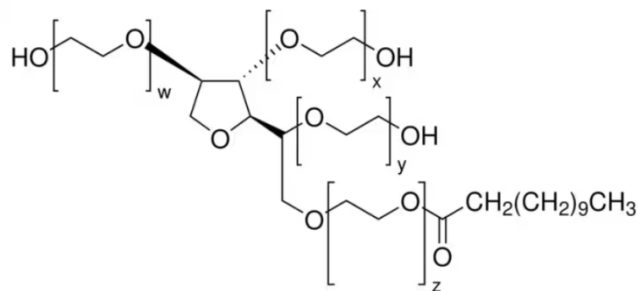


Figure 3.2.1.1. Chemical structure of polysorbate 20.

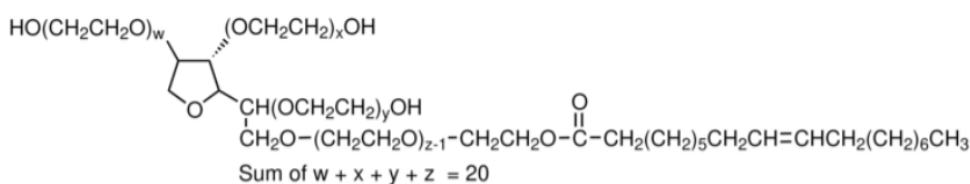


Figure 3.2.1.2. Chemical structure of polysorbate 80.

3.2.2 Alkyl Glycoside Surfactants

The group of alkyl glycosides was represented by α -dodecyl maltoside (α -DDM), β -dodecyl maltoside (β -DDM), decyl glucoside (DG), and lauryl glucoside (LG). Alkyl glycoside nonionic surfactants are environmentally friendly surfactants formed by glycosidic linkage between natural sugar moieties and fatty alcohols. They exhibit excellent biodegradability, low irritation, and environmental compatibility. Variations in alkyl chain length and sugar structure influence their critical micelle concentration, micelle size, and self-assembly behavior.

The main difference between α -DDM and β -DDM is the spatial arrangement of the dodecyl chain at the anomeric carbon in the glycosidic bond. In α -DDM, the alkyl chain is in an axial position, while in β -DDM, the alkyl chain is in an equatorial position. This small structural difference affects the micelle structure, self-assembly behavior, and membrane protein stabilization ability of the two surfactants ¹⁷.

DG and LG are commercially used alkyl glycoside nonionic surfactants. They differ from DDM in that they are polydisperse materials, rather than well-defined chemical compounds. Both compounds use glucose as the hydrophilic head group, but they have different alkyl chain lengths. DG contains a polydisperse alkyl chains with an average length of 10. Similarly, LG contains alkyl chains with an average length of 12. The difference in chain length affects their surface activity, self-assembly behavior, and micelle properties ¹⁸. LG has longer alkyl chains, so it usually shows a lower critical micelle concentration (CMC) and stronger hydrophobic interactions. As a result, LG forms more stable micelles. DG has shorter alkyl chains, so it has higher water solubility and a higher CMC. DG also tends to form smaller and more dynamic micelles ¹⁸. Both LG and DG show good biocompatibility, low toxicity, and good biodegradability. They are therefore widely used in cosmetics, drug delivery systems, and membrane protein studies ¹⁹. DG has milder detergent properties, so it can better maintain the

native structure of some membrane proteins. LG has stronger hydrophobic interactions, so it is more effective in solubilizing lipids and other hydrophobic compounds ^{19,20}.

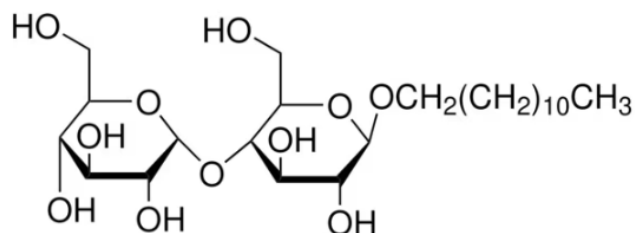


Figure 3.2.2.1. Chemical structures of α -dodecyl maltoside.

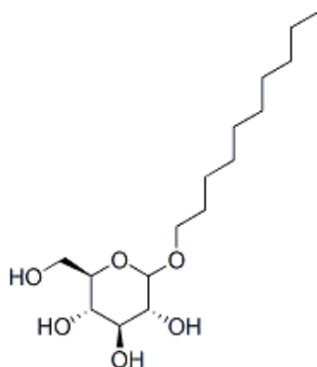


Figure 3.2.2.2. Chemical structures of decyl glucoside

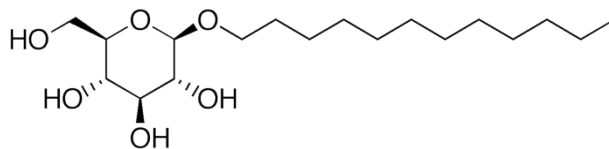


Figure 3.2.2.3. Chemical structures of lauryl glucoside

3.2.3 Supramolecular Interactions Between Cyclodextrins and Nonionic Surfactants

Because nonionic surfactants contain hydrophobic tail groups, previous studies have demonstrated that these hydrophobic structures can form host–guest inclusion complexes with cyclodextrins ^{22–28}. In water-containing ternary systems, these complexes may further self-assemble through hydrogen bonding and hydrophobic interactions into supramolecular network structures, thereby generating hydrogels with unique physicochemical properties ²⁹. Unlike chemically covalently crosslinked hydrogels, these physically crosslinked supramolecular hydrogels possess dynamic and reversible characteristics, endowing them with tunable mechanical properties.

3.3 Mechanism of Supramolecular Hydrogel Formation

3.3.1 Host–guest Assembly and Polypseudorotaxane Formation

The formation of supramolecular hydrogels in cyclodextrin (CD)/nonionic surfactant systems mainly comes from host–guest interactions and self-assembly processes. Cyclodextrins have a

hydrophilic outer surface and a relatively hydrophobic inner cavity. This special structure allows CDs to include hydrophobic guest molecules, such as alkyl chains or polypropylene oxide (PPO) segments from nonionic surfactant^{9,30}. Hydrophobic interactions mainly drive this inclusion process. At the same time, water molecules inside the CD cavity are released, which further supports complex formation⁸.

After the inclusion complexes form, these complexes do not stay isolated. The host–guest complexes can further assemble into larger structures through intermolecular interactions. Hydrogen bonding, hydrophobic interactions, and van der Waals forces help stabilize these assemblies³¹. These interactions gradually build ordered supramolecular structures and provide the basis for hydrogel network formation.

In CD–surfactant systems, the host–guest inclusion complexes may further organize into necklace-like supramolecular arrangements³². The CD molecules act like ‘beads’, while the polymer chain acts like the ‘string’. The threaded CDs can further stack together through hydrogen bonding between neighboring CD molecules³³. This process promotes the formation of larger supramolecular aggregates and helps connect nearby polymer chains.

The threading behavior strongly depends on the compatibility between CDs and polymers. Flexible hydrophobic chains usually show better inclusion behavior because they can more easily fit into the CD cavity³⁴. The size match between the guest chain and the CD cavity also affects the stability of the inclusion complex².

3.3.2 Network Formation and Physical Crosslinking

As the supramolecular assemblies continue to interact with each other, a three-dimensional network gradually forms throughout the system. Unlike chemically crosslinked hydrogels, supramolecular hydrogels do not rely on permanent covalent bonds. The network mainly forms through reversible physical interactions³⁵. In cyclodextrin/nonionic surfactant systems, host–guest inclusion interactions play the main role in network formation. Hydrogen bonding, hydrophobic interactions, and van der Waals forces also help stabilize the structure^{31,36}.

The formation of this interconnected network limits the large-scale movement of molecules in the system. At the same time, the network can trap a large amount of water inside the structure³⁷. This high water content gives hydrogels their soft and flexible properties. The water molecules remain inside the network because the supramolecular structure creates many small spaces that can hold water through physical interactions.

The crosslinking points in supramolecular hydrogels are dynamic rather than permanent. Noncovalent interactions can continuously break and reform under external stimulation or environmental changes³⁸. Because of this reversible behavior, the hydrogel network can respond to changes such as shear force, temperature, or concentration. This dynamic property is also closely related to behaviors such as injectability and self-healing ability³⁹.

3.3.3 Balance Between Gelation and Phase Separation

Although cyclodextrin-surfactant interactions can promote supramolecular assembly, not all assemblies can form stable hydrogels. Different structures may appear in the system, such as gels, precipitates, or phase-separated mixtures. The final state depends on the balance between intermolecular interactions and molecular movement.

Stable hydrogel formation requires an extended three-dimensional network throughout the whole system. The supramolecular assemblies must connect with each other while still keeping enough flexibility and mobility. Under these conditions, the network can continuously rearrange and retain large amounts of water inside the structure.

If the intermolecular interactions become too strong, the system may form dense local aggregates instead of an extended network³⁶. These compact structures can gradually form crystalline domains and lose their dynamic behavior. As a result, the assemblies may separate from the solution and form precipitates³³.

On the other hand, if the intermolecular interactions are too weak, the assemblies cannot connect into a continuous network⁴⁰. In this case, the system remains fluid and stable gelation does not occur. Hydrogel formation only happens when the interaction strength and molecular mobility remain in a suitable balance. The supramolecular interactions must be strong enough to stabilize the network but still dynamic enough to allow structural rearrangement.

Different assembly states can also explain the experimental differences observed in transparent gels, precipitated systems, and phase-separated samples⁴¹.

3.3.4 Effect of Water Content and Nonstoichiometric Assembly

Water content has an important effect on supramolecular assembly and hydrogel formation. In cyclodextrin-based systems, water is not only a solvent. Water also affects hydrophobic interactions, hydrogen bonding, molecular movement, and the assembly process^{8,42}.

When the water content is low, the intermolecular interactions usually become stronger, while molecular movement becomes more limited. Under these conditions, the system may form dense aggregates or precipitates instead of a uniform hydrogel network⁴³. In contrast, when the water content is too high, the distance between molecules increases and intermolecular interactions become weaker. As a result, the assemblies may not connect into a continuous three-dimensional network⁴¹. A suitable water content is therefore necessary because it helps maintain a balance between intermolecular interactions and molecular mobility.

CD-surfactant systems also show dynamic and nonstoichiometric assembly behavior. Unlike traditional molecular complexes with fixed compositions, supramolecular assemblies in these systems do not always have a constant host–guest ratio⁴⁴. Different local structures can exist at the same time. Some surfactant chains may only be partially threaded into the CD cavity, while other regions may contain different numbers of CD molecules around the guest chain³³.

The host–guest interactions in these systems are also reversible. Inclusion complexes can continuously form and dissociate in solution³⁵. Because of this dynamic behavior, the hydrogel network remains structurally heterogeneous and can continuously rearrange over time. Different formulation conditions, such as concentration and water content, can therefore strongly affect the final gel structure and stability.

3.4 Rheological Properties of Supramolecular Hydrogels

3.4.1 Viscoelastic Properties

Supramolecular hydrogels are typical viscoelastic materials. These materials show both solid-like and liquid-like behavior⁴⁵. Unlike chemically crosslinked hydrogels, supramolecular hydrogels do not contain permanent covalent bonds. Their three-dimensional network mainly

forms through dynamic and reversible noncovalent interactions, such as hydrogen bonding, host–guest interactions, hydrophobic interactions, and van der Waals forces ^{31,35}. These reversible interactions allow the hydrogel network to respond to external stress and deformation.

Researchers usually study the viscoelastic properties of hydrogels through oscillatory rheological measurements ⁴⁶. Two important parameters are the storage modulus (G') and the loss modulus (G''). The storage modulus reflects the elastic behavior of the material and describes the ability of the hydrogel to store deformation energy. The loss modulus reflects the viscous behavior and represents energy dissipation during deformation ⁴⁷.

In stable hydrogel networks, the storage modulus is usually higher than the loss modulus:

$$G' > G'' \quad (2)$$

This means that the elastic behavior dominates the system and that a stable three-dimensional network has formed ³⁷. In contrast, when G'' becomes higher than G' , the system behaves more like a viscous liquid and usually cannot maintain a stable gel structure.

3.4.2 Linear Viscoelastic Region and Frequency Sweep

The linear viscoelastic region (LVER) is an important parameter in rheological analysis ⁴⁶. In this region, both the storage modulus (G') and the loss modulus (G'') remain almost constant as the strain increases. This result means that the internal hydrogel network stays stable and is not damaged by deformation ⁴⁵. Researchers usually determine the LVER before other rheological measurements because later tests are normally performed within this stable range.

Frequency sweep analysis is commonly used to study the structural stability of supramolecular hydrogels at different frequencies and hence timescales ⁴⁷. During this test, researchers monitor the changes in G' and G'' with angular frequency:

$$G', G'' \text{ vs. } \omega \quad (3)$$

For stable hydrogels, G' usually remains higher than G'' over the whole tested frequency range ³⁷. At the same time, both moduli often show only small changes with frequency. This behavior suggests that the hydrogel contains a stable and connected three-dimensional network structure. Supramolecular hydrogels usually show some frequency-dependent changes because their networks are formed through dynamic noncovalent interactions ³⁵. The reversible crosslinking points can continuously dissociate and reform during deformation. Because of this behavior, supramolecular hydrogels are more dynamic and responsive than permanently crosslinked covalent hydrogels ³¹.

3.4.3 Shear-Thinning and Thixotropic Recovery Behavior

Another important rheological property of supramolecular hydrogels is shear-thinning behavior. In shear-thinning systems, the viscosity decreases when the shear rate increases ⁴⁶. When external shear stress is applied, some reversible supramolecular interactions temporarily break apart. This process partially destroys the hydrogel network and increases the fluidity of the system ³⁵. After the shear force is removed, the noncovalent interactions can gradually reform. The hydrogel network can then recover its original structure. This property is very important for injectable hydrogels because the material can flow through a syringe needle during injection and then recover its gel structure after administration ³⁹.

Many supramolecular hydrogels also show thixotropic recovery behavior. Thixotropy means that the material recovers its structure and mechanical properties after shear stress is removed, but that this recovery is relatively slow ⁴⁸. Researchers usually study this behavior through alternating high-strain and low-strain rheological tests. During these tests, the hydrogel network breaks under high strain and reforms under low strain.

Researchers often use the recovery of the storage modulus (G') to evaluate the self-recovery ability of hydrogels ³⁸. A rapid increase of G' after large deformation suggests that the hydrogel network can quickly rebuild its structure. Researchers also use the recovery ratio to measure the extent of structural recovery.

These dynamic rheological properties make supramolecular hydrogels useful in many biomedical fields. Researchers have studied these materials for applications such as injectable drug delivery, tissue engineering, and minimally invasive therapy systems ^{31,37}.

3.5 Injectable Supramolecular Hydrogels and Injectability Evaluation

3.5.1 Characteristics of Injectable Hydrogels

Injectable hydrogels must be easily extruded from a syringe in a flowable form and subsequently undergo in situ transformation into a gel state upon reaching the target site. These hydrogels can encapsulate bioactive molecules or even cells and possess significant advantages including targeted delivery and minimal invasiveness, making them particularly important in tissue engineering and drug delivery applications ⁴⁹.

Compared with conventional chemically crosslinked hydrogels, injectable hydrogels are more commonly formed through physical crosslinking mechanisms, including thermosensitive, pH-sensitive, ionic, and host–guest interactions, as well as chemical crosslinking methods such as enzymatic and photo-induced reactions. The high structural flexibility associated with these systems provides tunable rheological properties, including shear-thinning behavior.

Injectable hydrogels intended for biomedical applications are generally considered to require the following characteristics ⁴⁹:

1. The precursor solution should flow smoothly under moderate pressure and rapidly solidify upon reaching the target site.
2. The hydrogel should rapidly establish sufficient mechanical strength after injection.
3. The gel should maintain structural integrity and mechanical stability throughout the desired treatment period.
4. Both the hydrogel and its precursor materials should exhibit excellent biocompatibility and minimal cytotoxicity.
5. The hydrogel should possess controllable biodegradability.
6. The polymeric structure should be amenable to functional modification for tunable biological performance.

3.5.2 Parameters for Injectability Assessment

The earliest operational standards and testing methods for injectability were proposed by Ritschel and Suzuki for evaluating the injection force required for suspensions and oily formulations using specific syringe–needle combinations ⁵⁰.

Parameters commonly used to evaluate injectability include ^{51,52}:

Plunger-break loose force (PBF): the force required to initiate plunger movement.

Maximum force ($F_{\max}$): the peak force measured throughout the entire injection process.

Dynamic glide force (DGF): the average force required to maintain continuous plunger movement during the plateau phase.

Total work of injection (J): the cumulative energy consumed throughout the injection process, obtained by integrating the area under the distance-force curve.

These parameters derived from the distance-force curve not only reflect the mechanical injection behavior of formulations but are also closely associated with controllability, safety, and therapeutic outcomes during clinical administration. Previous studies have shown that injection force profiles of similar injectable products may exhibit batch-to-batch variability and insufficient uniformity, which correspond to clinical sensations such as force fluctuation, plunger jerking, or blockage perceived by physicians during administration ⁵³.

3.5.3 Clinical Significance of Injectability

From a clinical perspective, unstable injection force can reduce the precision of filler administration. The injector may feel uneven extrusion or sudden release of force during plunger movement. This problem is more important for highly viscous hydrogel fillers or fillers that need accurate control of injection depth and dosage. Variations in the force profile may lead to dosing errors, inaccurate filler placement, and uneven local distribution of the material. In some cases, unstable extrusion may cause part of the filler to be placed too close to the skin surface. The Tyndall effect is an optical light-scattering phenomenon that clinical studies have linked to skin discoloration due superficial placement of filler materials. Superficial overfilling may also cause subcutaneous nodules and irregular local contours ^{54,55}.

Therefore, parameters such as plunger-break loose force (PBF), maximum force ($F_{\max}$), and dynamic glide force (DGF) are not only indicators of injection initiation resistance, peak operational load, and continuous injection stability but also partially reflect the difficulty of controlling the injection process. High or highly fluctuating PBF and $F_{\max}$ values may increase the risk of injection instability, whereas stable DGF values are closely associated with smooth and controllable administration. Meanwhile, the total work of injection comprehensively reflects the cumulative operational energy required throughout the injection process and is particularly important for evaluating the clinical acceptability of highly viscous or large-volume formulations.

Overall, it is insufficient to merely considering peak injection force. Rather, the uniformity of injectability performance, the smoothness of the force profile, and operational consistency may exert more direct influences on clinical safety, injection accuracy, and therapeutic efficacy. Consequently, injectability-related parameters have gradually become important indicators for evaluating the clinical usability of injectable formulations and delivery devices, while also

providing valuable guidance for formulation development, process optimization, and quality control.

3.6 Curcumin Delivery and Release

3.6.1 Physicochemical Characteristics of Curcumin

Curcumin ((E,E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is the principal polyphenolic pigment extracted from the rhizome of *Curcuma longa* and is accompanied by several closely related compounds, including demethoxycurcumin and bisdemethoxycurcumin ⁵⁶.

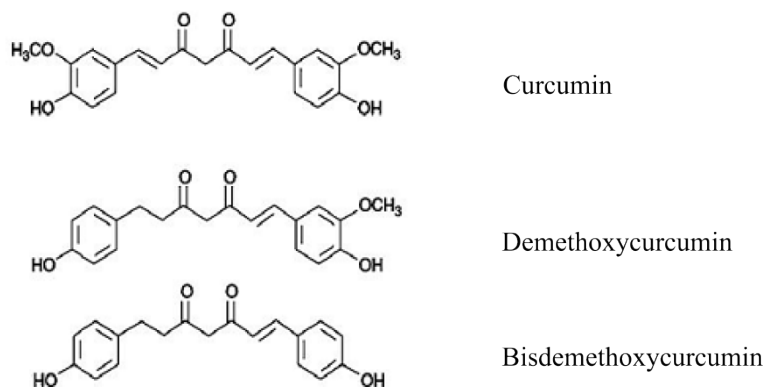


Figure 3.6.1.1. Chemical structures of curcumin and derivatives.

Studies have demonstrated that curcumin possesses broad and significant biological activities, including antioxidative ^{57,58}, anti-inflammatory ⁵⁹, antitumor ⁶⁰, antiviral ⁶¹, and antibacterial ⁶² effects. Owing to its low intrinsic toxicity, strong hydrophobicity, and excellent anticancer and anti-inflammatory properties, curcumin is widely used as a model hydrophobic drug in drug delivery research, particularly for investigating drug release behavior and biological activity in hydrophobic drug delivery systems.

3.6.2 Curcumin Delivery Strategies

Despite its high therapeutic potential, curcumin suffers from poor gastrointestinal stability, low membrane permeability, extensive hepatic metabolism, and rapid systemic clearance, resulting in low oral bioavailability. Animal and clinical studies have indicated that oral absorption efficiency of curcumin in humans is substantially lower than that in rodents, and even substantially increased doses often fail to achieve plasma concentrations comparable to those observed in rats ⁶³. To maximize the pharmacological efficacy of curcumin and improve its bioavailability, numerous novel drug delivery systems have been developed based on the pathological characteristics of different diseases ⁶⁴. In anticancer applications, nanotechnology-based delivery systems—including nanoparticles ⁶⁵, dendrimers ⁶⁶, liposomes ⁶⁷, exosomes ⁶⁸, micelles, and microsphere/microbubble carriers—have been extensively investigated to enhance the solubility, bioavailability, and stability of curcumin. In addition, cyclodextrins have been introduced into formulation design to address challenges associated with hydrophobic drug delivery. Due to their hydrophilic exterior and hydrophobic cavity, cyclodextrins can encapsulate hydrophobic drug molecules through host–guest interactions, thereby improving drug solubility, stability, and release behavior ⁶⁹.

3.6.3 Curcumin-Loaded Cyclodextrin Hydrogels

Based on the above research background, the present study employed injectable hydrogels as a drug delivery platform and curcumin as the anticancer active agent for local treatment of postoperative cavities in solid tumors. The system was designed to achieve sustained local eradication of residual cancer cells following surgery, thereby reducing the risks of tumor metastasis and recurrence. Simultaneously, the shear-thinning property of injectable hydrogels enables smooth injection under external force and rapid recovery of the gel state after administration into the postoperative cavity, thereby providing structural support to the cavity wall. Regarding drug release, the release process is primarily governed by diffusion through the three-dimensional hydrogel network, exhibiting a typical diffusion-controlled release profile that enhances local drug retention time and concentration at the lesion site.

In this study, cyclodextrin-curcumin inclusion complexes were further combined with polysorbate 80 to construct curcumin-loaded hydrogel systems. In vitro release studies were conducted to investigate the drug release behavior and release kinetics of hydrogels prepared using cyclodextrin-curcumin complexes with different inclusion ratios, with the aim of elucidating the relationship between system composition and drug release performance.

4 Material and Methods

4.1 Material

Key characteristics of the materials used in the study are compiled in Table 4.1.1.

Table 4.1.1. Specifications of material used in hydrogel preparation and assay.

Material	Supplier	Batch Number	Purity Level
α -Cyclodextrin (α -CD)	Wacker Chemie AG	BCCH9973	$\geq 99.0\%$
β -Cyclodextrin (α -CD)	Wacker Chemie AG	BCCN4975	$\geq 98.0\%$
α -Dodecyl Maltoside (α -DDM)	GLYCON Biochemicals GmbH	D99020	$> 99\%$
β -Dodecyl Maltoside (β -DDM)	GLYCON Biochemicals GmbH	D97002	$> 99.0\%$
Decyl Glucoside (DG) 50% solution	BASF.	-	Commercial C10 G1.1
Lauryl Glucoside (LG) 50% solution	BASF.	-	Commercial C12.5 G1.1
Polysorbate 20	Sigma-Aldrich	SLBZ5658	$\geq 97.0\%$

Polysorbate 80	CRODA Inc	0002125315	Super refined
Curcumin	Sigma-Aldrich	081M1611V	$\geq 65\%$
Dimethylsulfoxide-d6 (DMSO-d ₆)	ARMAR AG	3058	99.8%
Di-sodium hydrogenphosphate dodecahydrate	MERCK	87479	99%
Potassium dihydrogen phosphate	VWR CHEMICALS	16C244139	99.8%
Potassium Chloride	MERCK	87374	99.5%
Sodium Chloride	VWR CHEMICALS	22J044107	99.9%

DG and LG are commercial-grade alkyl glycosides consisting of mixtures of homologues with varying alkyl chain lengths and degrees of polymerization. According to the supplier's specifications, molecular weight calculations were based on the average compositions DG (C10G1.1) and LG (C12.5G1.1), where C_n denotes the average alkyl chain length and G_x denotes the average number of glucose units per molecule.

4.2 Methods

4.2.1 Preparation of Cyclodextrin-Nonionic Surfactant-Water System

Material Pretreatment

To remove water from the materials, selected samples were subjected to drying treatment.

α -CD and β -CD were dried overnight in an oven at 120 °C.

DG and LG were delivered as 50% solutions. These were predried in an oven at 60 °C until a nearly solid state was obtained, followed by further drying in a vacuum oven at 60 °C. The dried sample was ground into a white powder using a mortar and pestle, and the powder was evenly spread in the vacuum oven for continuous drying and stored under dry conditions until use.

Preparation Methods

In the preliminary stage of formulation development, four factors were investigated: surfactant type (six types), cyclodextrin type (two types), water content (two levels), and the molar ratio of surfactant to cyclodextrin (three ratios). By combining all factor levels, a total of 60 samples were initially prepared. Each formulation was prepared as a single 2 g sample.

It should be noted that, for α -DDM and β -DDM, only formulations containing α -CD were investigated, while formulations containing β -CD were excluded.

Table 4.2.1.1. Experimental factors and levels for ternary system design.

Factor	Levels
Nonionic surfactant	α -DDM, β -DDM, DG, LG, polysorbate 20, polysorbate 80
Cyclodextrin	α -CD, β -CD
Water content	90%, 80%
Molar ratio (CD:surfactant)	1:1, 1:2, 2:1

Furthermore, based on the comparative analysis of the phase behavior and physicochemical characteristics of the preliminary samples, three additional formulations composed of α -cyclodextrin and polysorbate 80 at a molar ratio of 2:1 were prepared, with water contents of 70%, 65%, and 60%, respectively.

To facilitate centrifugation and observation of phase characteristics, centrifuge tubes made from glass without external scratches or abrasions were used during sample preparation. Prior to use, the tubes were thoroughly cleaned, rinsed with ethanol, dried in an oven at 120 °C to prevent residual water stains, and subsequently cooled in a desiccator. These procedures were performed to ensure the accuracy of the component ratios within the system and to minimize deviations in phase behavior caused by the introduction of extraneous moisture.

During the preparation process, surfactants were added to centrifuge tubes in either powder or viscous liquid form. To facilitate handling and improve operational convenience, cyclodextrin solutions were used instead of cyclodextrin powder during formulation. This approach minimized the influence of electrostatic interactions associated with the powder and promoted the formation of a more homogeneous system. The mixtures were prepared in conical flasks under magnetic stirring at 50 °C, with the flasks sealed using Parafilm to prevent water evaporation. Stock solution containing cyclodextrin at mass fractions of 20% and 30% were prepared, and the additional water content introduced by the cyclodextrin solutions was calculated accordingly.

The surfactant was weighed into a centrifuge tube, followed by the addition of the calculated amount of extra water. The mixture was vortexed to obtain a relatively homogeneous surfactant–water system. After the surfactant had visibly dissolved and dispersed uniformly, the cyclodextrin solution was added gradually with continuous vortex mixing until a macroscopically homogeneous system was formed. The resulting samples were then centrifuged at 3500 rpm (RCF=2100 $\times$ g) for 15 min at room temperature (25 °C).

4.2.2 Rheological Tests

Hydrogel samples that showed no phase separation after centrifugation and exhibited non-flowing behavior upon inversion during phase behavior observations were subjected to amplitude sweep measurements to determine the linear viscoelastic region (LVER). After having established the LVER, the samples were analyzed by strain and frequency sweep tests to identify the crossover point between the storage modulus (G') and loss modulus (G''). For each sample, both amplitude sweep and frequency sweep measurements were performed in duplicate to ensure reproducibility of the results.

Flow curve measurements were carried out by varying the shear rate over a defined range to evaluate the steady-state shear behavior of the samples. Thixotropy tests were conducted using a stepwise shear protocol to assess the time-dependent structural recovery behavior of the materials. All measurements were performed at room temperature (25 °C), and the resulting data were plotted and analyzed using Origin.

Based on the sample formulation with optimal storage modulus performance (2 α -CD: 1 polysorbate80), a curcumin delivery carrier was obtained by adjusting the water content (reducing it from 80% to 70%). This curcumin-free hydrogel with a water content of 70% was referred to as a pure hydrogel. Curcumin-loaded hydrogels prepared using two different curcumin-to-cyclodextrin inclusion ratios were further characterized by amplitude scanning measurements and LVER determination, strain and frequency scanning analysis and cross-evaluation, as well as flow curves and thixotropic measurements.

All rheological experiments were performed on a Kinexus rheometer (Malvern Panalytical, Malvern, UK).

4.2.3 NMR

This analysis was conducted only for six samples consisting of α -cyclodextrin and α -DDM/ β -DDM systems with three different molar ratios at a water content of 90%.

Sample Preparation

Following centrifugation, the six samples exhibited distinct supernatant and precipitate phases. The supernatant and precipitate phases were separated, and their weights prior to drying were recorded. The samples were then dried overnight in an oven at 120 °C, after which the dried weights were measured and recorded again. Approximately 10 mg of the dried precipitate was collected and dissolved in 0.75 mL of DMSO- d_6 until complete dissolution was achieved. Subsequently, approximately 0.6 mL of the resulting solution was transferred into an NMR tube for analysis.

NMR experiments were performed on a Bruker Avance Neo 500 MHz spectrometer kindly made accessible to the project by RG Discovery AB (Lund, Sweden).

Result Analysis

The obtained spectra were analyzed using MestReNova.

4.2.4 Stoichiometry determination and mass balance analysis

The stoichiometries of the precipitated α -CD/ α -DDM α -CD/ β -DDM complexes were determined by NMR. Precipitates obtained from samples prepared at initial α -CD/DDM molar ratios of 1:1, 1:2, and 2:1 were isolated by centrifugation, dried, and analyzed by NMR. Stoichiometric ratios were obtained from integration of characteristic proton resonances assigned to α -CD and surfactant species.

Mass balance calculations were subsequently performed to estimate the concentrations of α -CD and surfactant remaining in the supernatant phase after precipitation. Initially, the masses of α -CD, surfactant, and water added to each sample were recorded together with the theoretical molar ratio and total dry mass of the system. After centrifugation, the supernatant and

precipitate phases were separated and weighed individually. For both phases, the total mass, dry mass, and dry mass percentage were determined after drying.

The difference between the initial dry mass and the combined dry masses of the separated phases was used to evaluate mass balance closure and estimate the amount of retained water associated with the precipitate phase. The amount of supernatant trapped within the precipitate was estimated from the retained water content, and the dissolved solids associated with this trapped supernatant were corrected for in subsequent calculations. The dry masses assigned to the precipitate and supernatant phases were then used to calculate the mass ratios between α -CD and surfactant under different assumed stoichiometries for the precipitate (1:1, 1:2, and 2:1 α -CD:DDM). Based on the NMR results, the 2:1 stoichiometry was identified as the limiting precipitate composition and was subsequently used for interpretation of the final supernatant concentrations. An anhydrous precipitate composition was assumed throughout the calculations. For each assumed stoichiometry, the amounts of α -CD and surfactant remaining in the supernatant were calculated from the difference between the initially added quantities and the quantities assigned to the precipitated phase. These values were subsequently converted to concentrations (mg/mL and mM) using the measured supernatant volumes.

4.2.5 In Vitro Release Assay

Based on the results from the rheological testing, the hydrogel composed of α -cyclodextrin and polysorbate 80 at a molar ratio of 2:1 and a water content of 70% was used as the drug delivery matrix. Curcumin- α -cyclodextrin inclusion complexes with two different molar ratios were prepared and subsequently incorporated into the hydrogel system to obtain curcumin-loaded hydrogels with a final water content of 70%.

It should be noted that curcumin is highly sensitive to light and is prone to hydrolysis in aqueous environments, leading to degradation. Therefore, all experimental procedures were conducted under light-protected conditions, and the samples were characterized as soon as possible after preparation.

The absorbance of different samples in the in vitro release assay was measured using a Varian Cary 50 Bio UV–Vis spectrophotometer.

Preparation of curcumin-loaded hydrogel:

- **Curcumin- α -CD complex**

Curcumin and cyclodextrin powders were accurately weighed at molar ratios of 1:1 and 1:3. The powders were placed in a mortar and mixed thoroughly. During grinding, absolute ethanol was added dropwise until a uniform paste formed. The paste was then kept under light-protected conditions for 1 h. After the reaction, the samples were transferred to a vacuum oven (Gallenkamp, UK) and dried at 25 °C under reduced pressure (indicated pressure: 800 mbar) for 3 days. The dried products were then ground into fine powders using a mortar.

- **Hydrogel formation**

The required amount of inclusion complex powder and water was calculated based on the designed loading ratio. The inclusion complex powder, polysorbate 80, and water were mixed thoroughly by vortexing. A spatula was used during mixing to prevent powder aggregation. The mixtures were then centrifuged under the same conditions described above.

Experimental procedure for release studies :

- Construction of curcumin standard curve

A total of 20 mg of curcumin was weighed and dissolved completely in 10 mL of absolute ethanol. Then, 370 μ L of the solution was transferred into a mixture of 4.63 mL of absolute ethanol and 95 mL of pure PBS to prepare a stock solution with a curcumin concentration of 7.4 mg/L. The stock solution was then serially diluted two-fold five times using 5% ethanol–PBS buffer, giving a total of six curcumin solutions with different concentrations.

A full-wavelength scan was carried out using the 7.4 mg/mL stock solution. The solution showed a strong absorption peak at 430 nm, where the absorbance reached its maximum value. Therefore, all later absorbance measurements were performed at 430 nm. The absorbance of each solution was measured five times sequentially, and the average value was used for analysis. A calibration curve was plotted with concentration as the x-axis and absorbance as the y-axis. Linear regression fitting was then carried out in Excel to obtain the regression equation and the coefficient of determination (R^2).

- Sample design

The release studies were carried out in 15mL sample tubes made of glass. 5 mL of gel was added to each tube, and 5 mL of buffer solution then carefully added on top of the gel. Seven sampling time points were set at 1, 2, 4, 8, 12, 24, and 48 h. Each time point corresponded to a single, designated sample tube.

Before each measurement, blank correction was carried out using unloaded hydrogels containing 5 mL of buffer solution as the control group. After sampling and before analysis, the solutions were filtered through a hydrophilic syringe filter membrane (Millipore, USA). The absorbance of each sample was measured three times, and the average value was used for analysis.

To examine whether the gel level changed during the course of the study, control samples were also prepared for both molar ratios. These samples contained curcumin-loaded hydrogels without the addition of PBS solution. Photographs were taken at each time point for comparison.

4.2.6 Injectability Test

Injectability tests were performed on an XT2i instrument from Stable Micro Systems, using 3 mL disposable syringes from Hence-Ject. The instrumental setup is described in detail in Fig. 4.2.6.1. The injectability of a pure hydrogel composed of 2:1 α -CD:TW80 with a water content of 70%, as well as two curcumin-loaded hydrogels with the same composition but containing inclusion complexes at different ratios, was evaluated and quantified.

The hydrogels were prepared using the same method as described above. The samples were then transferred into the 3 mL disposable syringes for testing. The sample volume in each syringe was kept as consistent as possible (2.8–3.0 mL). During transfer, the formation of bubbles and air gaps was avoided as much as possible. The filled syringes were left to stand overnight to allow the systems to reach equilibrium before testing.

Before measurement, the texture analyzer was calibrated for both force and distance.

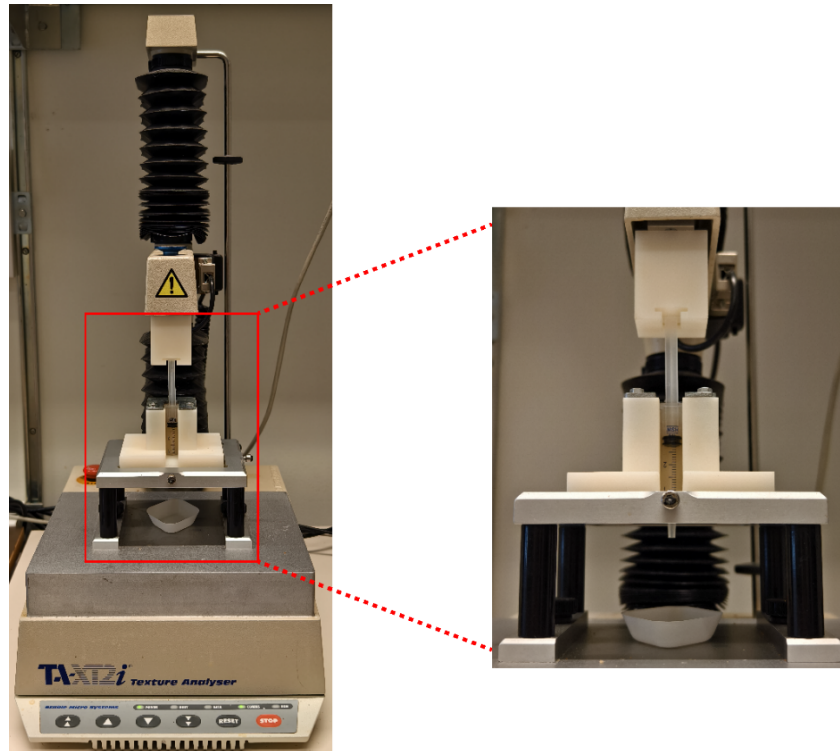


Figure 4.2.6.1. Analog system of injection carried out by a software-controlled texture analyzer in compression mode. As shown in Figure 4.2.6.1, the syringe was fixed in a custom holder. A probe with a surface area similar to that of the syringe plunger was used to push the sample out of the syringe. The syringe was tested without a needle attached. During each test cycle, the probe of the texture analyzer moved downward by 5 mm at a constant speed of 0.5 mm/s and then stopped. This process pushed the material out of the syringe. At the same time, the instrument recorded the Distance-Force curve. The test cycles continued until all of the material in the syringe had been extruded.

Injectability was evaluated by extracting and calculating the maximum force ($F_{\square_{ax}}$) and dynamic glide force (DGF) from the distance-force curves. $F_{\square_{ax}}$ was defined as the peak force recorded before complete extrusion of the material, while DGF represented the steady force required to maintain continuous extrusion of the formulation through the syringe. Raw data were visualized using Origin, and the post-peak plateau region between 0.5 and 4.5 mm was manually selected for calculation of the average force value to determine the DGF.

Previous studies have suggested that total injection work provides a more comprehensive and accurate representation of extrusion behavior than a single distance-force curves parameter alone. This approach is particularly suitable for injection experiments that generate large volumes of raw data, as the texture analyzer records more than 10,000 data points in a single test, far exceeding the information represented by isolated parameters such as $F_{\square_{ax}}$ and DGF. In addition, variations in syringe and needle specifications may also influence the measured force values. Therefore, total injection work was further evaluated in this study. After removal of negative values from the raw dataset, the area under the complete 5 mm distance-force curves was integrated using Origin to calculate the injection work for different samples across individual injection cycles.

During testing, the force profiles obtained from the first injection cycle of all four samples differed markedly from those of the subsequent injection cycles. Compared with later cycles of the same sample, the first cycle exhibited an abnormal increase in force and unstable glide behavior after approximately 1.4 mm displacement. Therefore, the first injection cycle for each sample was treated as a distinct case and analyzed separately from the remaining injection cycles.

In addition, for some samples, the final injection cycle showed a sharp increase in force before reaching the full 5 mm displacement due to insufficient remaining material, which caused the plunger to contact the bottom of the syringe barrel. These cycles were included in the analysis of F_{ax} only and were excluded from DGF analysis.

5 Results

5.1 Systems containing α -DDM and α -CD

5.1.1 Phase Behavior Observation

The phase behavior and physical appearance of α -CD/ α -DDM systems with different water contents and molar ratios before and after centrifugation are shown in *Figure 5.1.1.1*.

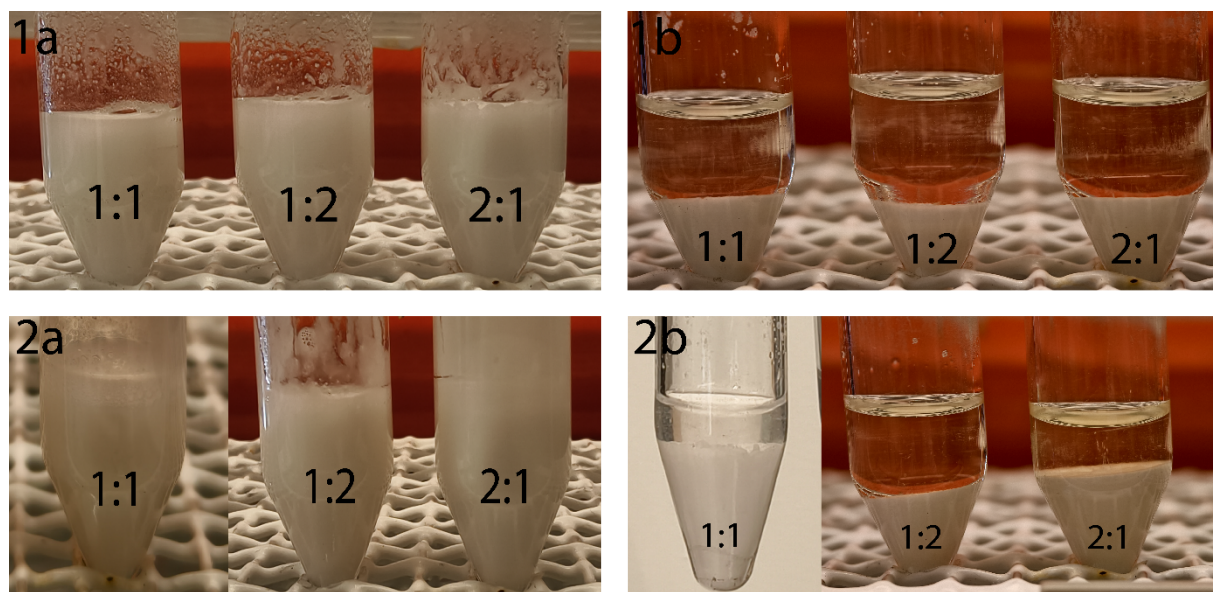


Figure 5.1.1.1. Phase characteristics of α -CD/ α -DDM systems with different water contents and molar ratios. (1a) 90% water content before centrifugation and (1b) after centrifugation. (2a) 80% water content before centrifugation and (2b) after centrifugation.

At all three molar ratios, samples with 90% water content appeared as milky, low-viscous suspensions before centrifugation, exhibiting obvious particle adhesion to the tube wall. Upon inversion, the suspensions flowed freely, indicating good fluidity. After centrifugation, a distinct two-phase separation was observed, with a denser solid separating from a clear aqueous solution. The amount of precipitate was similar among the three molar ratios.

Compared with the corresponding samples containing 90% water, the 1:1 and 1:2 samples with 80% water content exhibited moderately increased viscosity, while still retaining the ability to flow upon inversion. In contrast, the 2:1 sample with 80% water content exhibited a substantially more viscous appearance and showed no observable flow behavior upon inversion. Nevertheless, after centrifugation, phase separation was observed.

It should be noted that the 1:1 sample in Figure 2b appears to contain more visible precipitate than the other samples. However, this observation may be influenced by differences in the geometry of the glass sample vials, particularly the shape of the conical bottom section. Therefore, repeating the experiment using identical vials and standardized imaging conditions would facilitate a more accurate visual comparison of the precipitation behavior.

5.1.2 NMR

Samples were prepared at the specified molar ratios. After vortex mixing and centrifugation, no gel formation was observed. Instead, the system underwent phase separation into a supernatant and a solid precipitate. To determine the composition of the lower precipitated phase, α -cyclodextrin/ α -DDM mixtures with molar ratios of 1:1, 1:2, and 2:1 were centrifuged (350 rpm, 15 min) to separate the supernatant and precipitate. The precipitated fractions were collected, dried, dissolved in DMSO- d_6 , and analyzed by 1H NMR spectroscopy.

The spectra obtained for the three samples are shown below. In all spectra, resonances in the range of 3.2–5.6 ppm were assigned to the glucose-ring protons of α -cyclodextrin, whereas signals between 0.8 and 1.6 ppm originated from the alkyl-chain protons of DDM. Integration of the selected characteristic peaks hence gives the molar ratio of cyclodextrin to DDM in the precipitated fraction.

1:1 α -CD : α -DDM

Integration of the spectrum in *Figure 5.1.2.1* gave a CD:DDM molar ratio of approximately 1.6:1, suggesting enrichment of α -cyclodextrin in the precipitate relative to the initial feed composition.

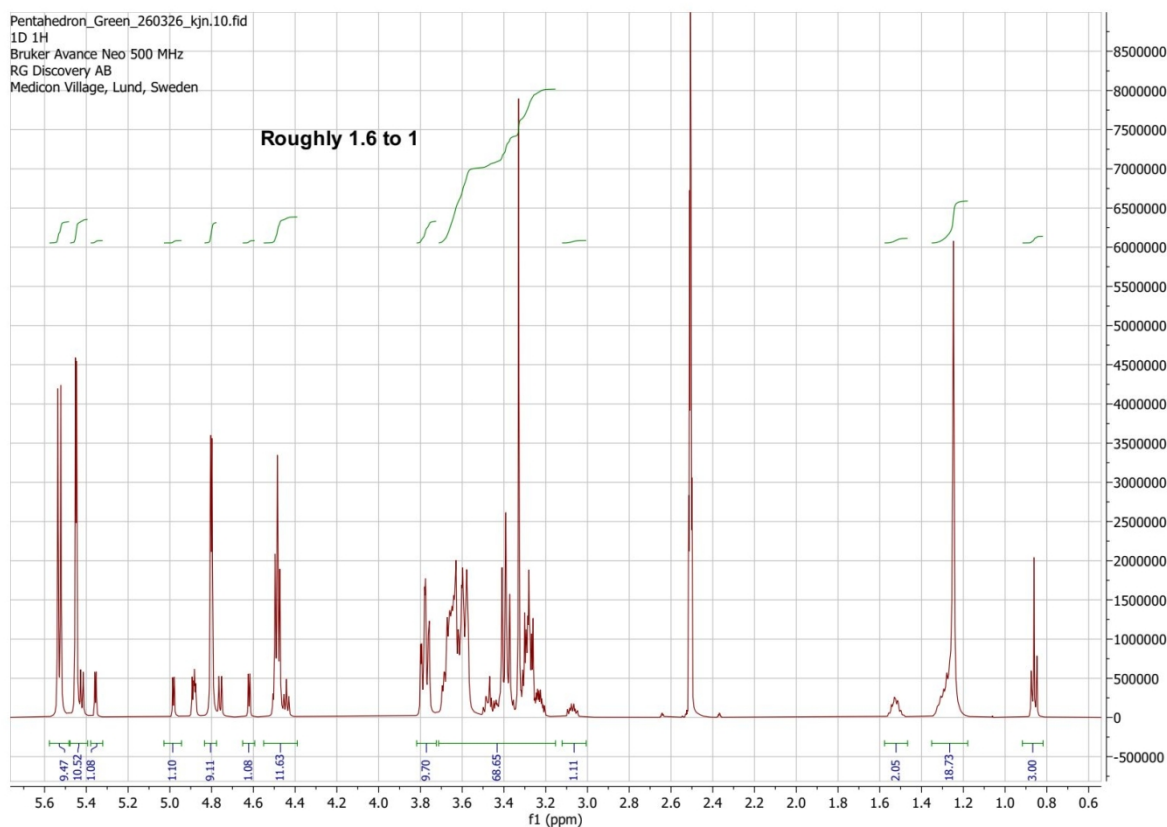


Figure 5.1.2.1. ^1H NMR spectrum of the precipitated α -CD/ α -DDM complex obtained from the initial 1:1 formulation.

1:2 α -CD : α -DDM

Integration analysis of the selected characteristic peaks in Figure 5.1.2.2 indicated that the molar ratio of α -cyclodextrin to α -DDM in the precipitated fraction was approximately 1.3:1. Compared with the previously analyzed sample exhibiting a molar ratio of 1.6:1, the lower relative α -cyclodextrin content observed in this precipitate suggests that a greater proportion of α -DDM participated in the formation of the precipitated assemblies. These findings indicate that the composition of the solid phase is highly sensitive to the initial formulation conditions and that the supramolecular assemblies formed after phase separation do not necessarily retain the original feed ratio. The substantial difference between the initial composition and the final precipitated fraction further suggests selective incorporation of α -DDM during precipitation and supports the formation of non-stoichiometric host–guest assemblies rather than well-defined 1:1 inclusion complexes.

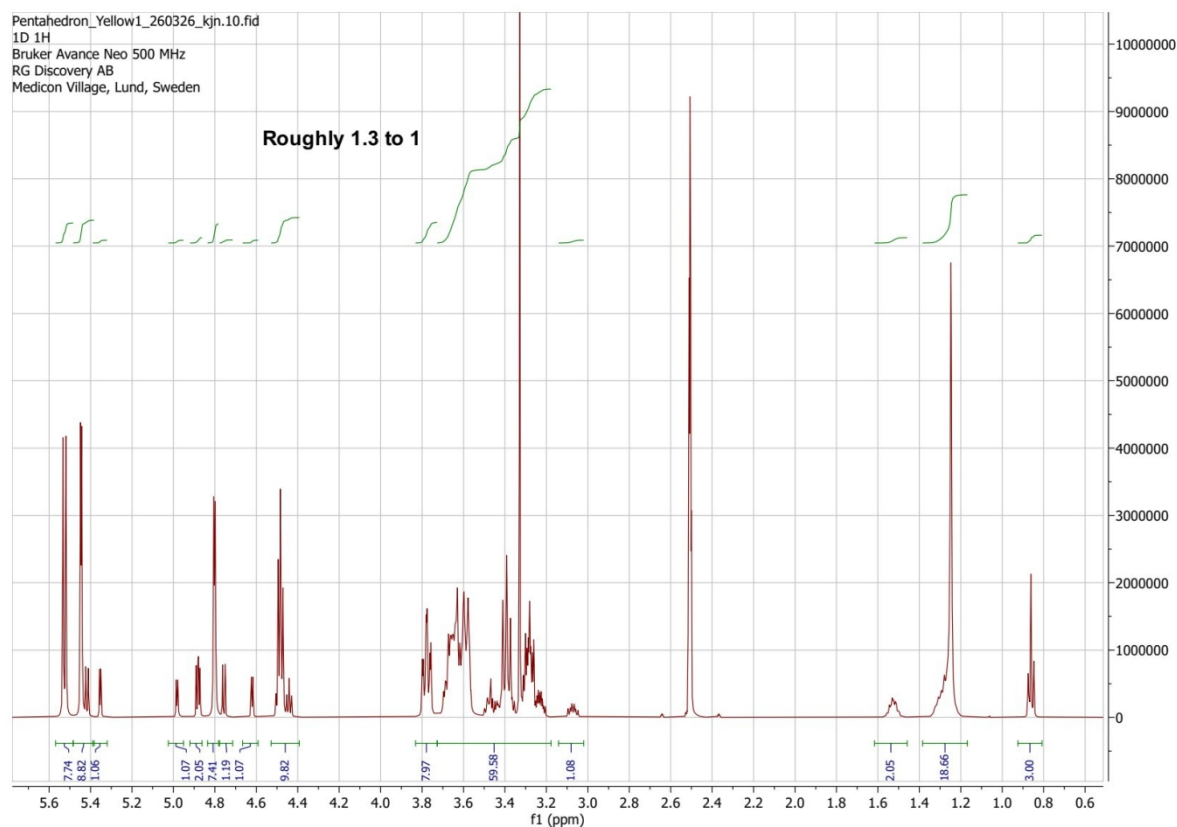


Figure 5.1.2.2. ^1H NMR spectrum of the precipitated α -CD/ α -DDM complex obtained from the initial 1:2 formulation.

2:1 α -CD : α -DDM

Integration of the spectrum in Figure 5.1.2.3 shows that the molar ratio of α -cyclodextrin to α -DDM in the precipitated fraction was approximately 2.0:1. Among all phase-separated samples, this formulation exhibited the highest relative α -cyclodextrin content in the precipitate. The composition agrees with the stoichiometry expected for α -CD inclusion complexes with medium-chain surfactants, such as C12G2.

In general, the deviation between the actual composition of the precipitated fraction and the theoretical composition expected for ideal, stoichiometrically well-defined inclusion complex indicates that the assembly process results in the formation of complexes with both 1:1 and 2:1 stoichiometry. It may also indicate that the process is not governed solely by host–guest inclusion interactions. Additional factors, including intermolecular hydrogen bonding, aggregation behavior, and selective phase partitioning under high-water-content conditions, are also likely involved. Furthermore, the absence of gel formation suggests that the resulting supramolecular assemblies were unable to establish a continuous three-dimensional network structure and instead formed cyclodextrin-rich precipitated aggregates.

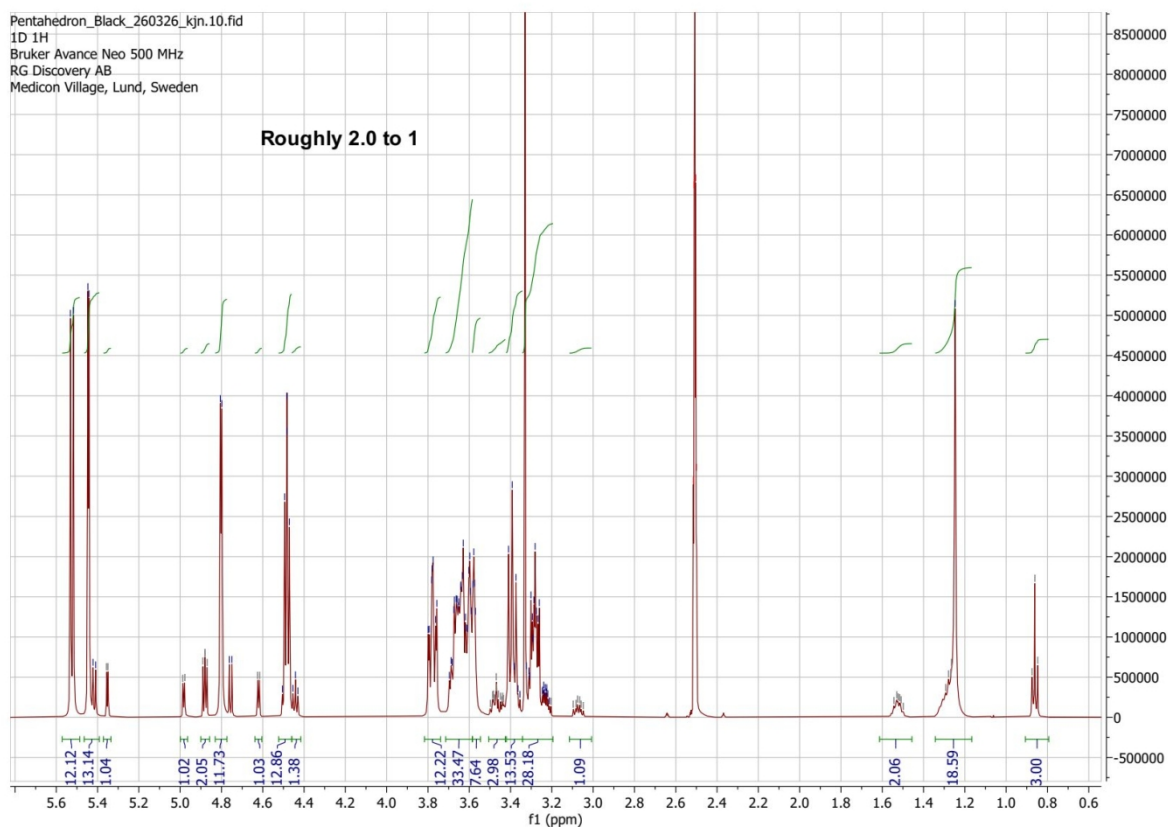


Figure 5.1.2.3. ^1H NMR spectrum of the precipitated α -CD/ α -DDM complex obtained from the initial 2:1 formulation.

5.1.3 Stoichiometry and mass balance analysis of α -CD/ α -DDM precipitates

Table 5.1.3.1. Stoichiometries of precipitated complexes from α -CD/ α -DDM and α -CD/ β -DDM determined by NMR.

System	Theoretical molar ratio	Stoichiometry observed in precipitate
α -CD/ α -DDM	1:1	1.6:1
	1:2	1.3:1
	2:1	2.0:1
α -CD/ β -DDM	1:1	1.7:1
	1:2	1.5:1
	2:1	2.0:1

The stoichiometries of the precipitated complexes determined by NMR are summarized in Table 5.1.3.1.

For the α -CD/ α -DDM system, the observed stoichiometries of the precipitated complexes suggest a tendency towards a limiting 2:1 stoichiometry (α -CD : α -DDM), although deviations were observed at lower initial α -CD ratios.

As shown in *Table 5.1.3.1*, when the theoretical molar ratios were 1:1 and 1:2, the precipitated complexes exhibited observed stoichiometries of 1.6:1 and 1.3:1, respectively, whereas a 2:1 stoichiometry was obtained when the feed ratio was 2:1.

Based on the experimentally observed limiting stoichiometry determined by NMR, mass balance calculations were performed assuming an anhydrous precipitated complex with a 2:1 stoichiometry (α -CD : α -DDM). For samples prepared using an initial 2:1 molar ratio, the calculated supernatant concentrations were 10.1 mM α -CD and 5.0 mM α -DDM. Detailed calculation procedures and results are presented in the Appendix.

It is worth noting that the measured α -CD concentration in the supernatant is substantially lower than the reported aqueous solubility of α -CD in water (132 mM).

5.2 Systems containing β -DDM and α -CD

5.2.1 Phase Behavior Observation

The phase behavior and physical appearance of α -CD/ β -DDM systems with different water contents and molar ratios before and after centrifugation are shown in *Figure 5.2.1.1*.

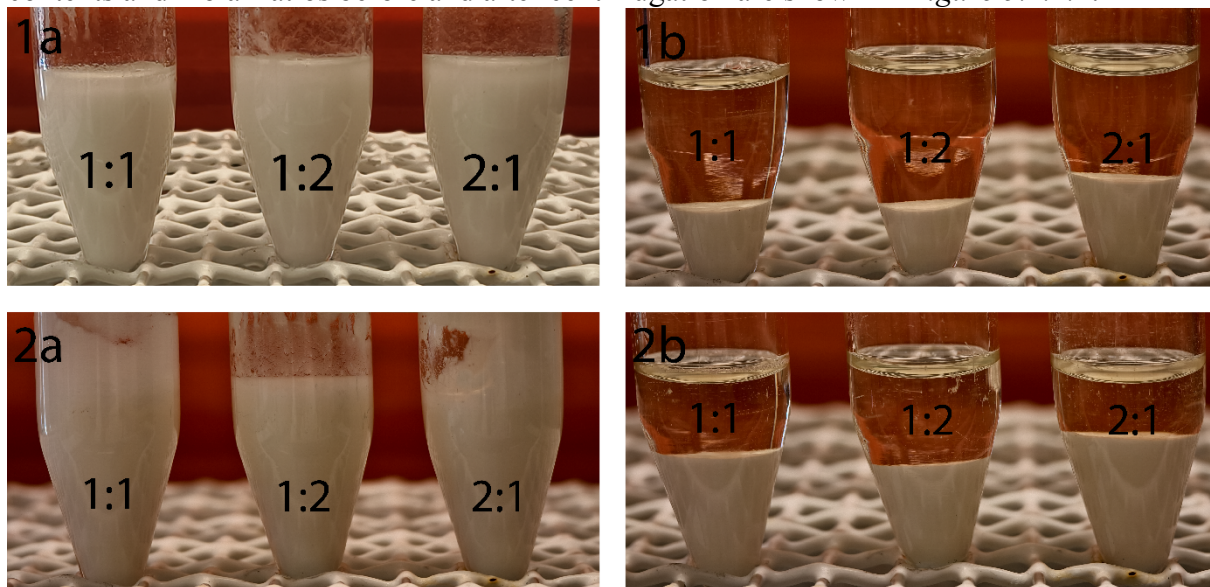


Figure 5.2.1.1. Phase characteristics of α -CD/ β -DDM systems with different water contents and molar ratios. (1a) 90% water content before centrifugation and (1b) after centrifugation. (2a) 80% water content before centrifugation and (2b) after centrifugation.

Samples at 3 different molar ratios with 90% water content appeared as low-viscous suspensions prior to centrifugation, exhibiting obvious particle adhesion to the tube wall. Upon inversion, the liquids flowed freely. After centrifugation, the 1:1 and 1:2 samples showed similar amounts of precipitate. The 2:1 sample produced slightly more precipitate and a relatively smaller volume of supernatant compared with the 1:2 sample. Based on visual observation, the amount of precipitate increased in the order of 1:2 < 1:1 < 2:1.

Compared with the corresponding samples containing 90% water, the 1:1 and 1:2 samples with 80% water content were slightly more viscous, but still flowed upon inversion. In contrast, the 2:1 sample with 80% water content was markedly more viscous and did not flow when inverted. After centrifugation, a distinct two-phase separation was observed, with a solid-like phase in the bottom, overlaid by a clear supernatant. Compared with the samples containing 90% water, the amount of precipitate increased noticeably, accompanied by a reduction in the volume of supernatant. The relative amount of precipitate remained in the order of $1:2 < 1:1 < 2:1$.

5.2.2 NMR

Samples were prepared at the specified molar ratios. After vortex mixing and centrifugation, no gel formation was observed. Instead, the system underwent phase separation into a supernatant and a solid precipitate. To determine the composition of the lower precipitated phase, α -cyclodextrin/ β -DDM mixtures with molar ratios of 1:1, 1:2, and 2:1 were centrifuged (350 rpm, 15 min) to separate the supernatant and precipitate. The precipitated fractions were collected, dried, dissolved in DMSO- d_6 , and analyzed by 1H NMR spectroscopy. The spectra obtained for the three samples are shown below:

1:1 α -CD : β -DDM

Spectra were assigned, analysed and integrated as previously described. According to *Figure 5.2.2.1*, the molar ratio of α -cyclodextrin to β -DDM in the precipitated fraction was approximately 1.7:1, suggesting enrichment of α -cyclodextrin within the solid phase relative to the initial feed composition.

Similar to the α -cyclodextrin/ α -DDM systems described above, the discrepancy between the initial formulation ratio and the composition of the precipitated fraction indicates that the supramolecular assemblies formed during phase separation were non-stoichiometric in nature.

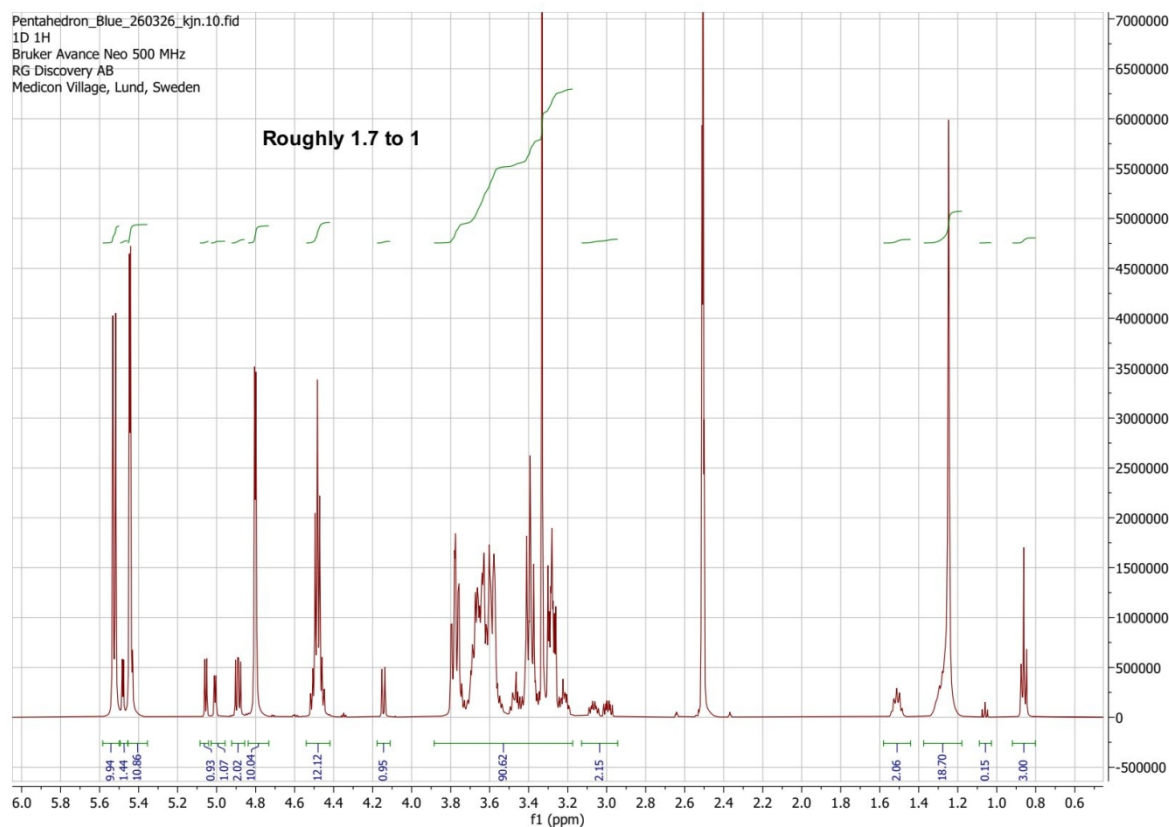


Figure 5.2.2.1. ^1H NMR spectrum of the precipitated α -CD/ β -DDM complex obtained from the initial 1:1 formulation.

1:2 α -CD : β -DDM

Integration analysis of the spectrum in Figure 5.2.2.2 indicated that the molar ratio of α -cyclodextrin to β -DDM in the precipitated fraction was approximately 1.5:1.

Compared with the α -cyclodextrin/ β -DDM system initially prepared at a 1:1 molar ratio, the precipitated fraction obtained from the 1:2 formulation exhibited a lower relative α -cyclodextrin content, indicating that a greater proportion of β -DDM participated in the formation of the solid assemblies. Nevertheless, the composition of the precipitated phase remained enriched in α -cyclodextrin relative to the initial feed ratio, suggesting selective incorporation behavior during phase separation.

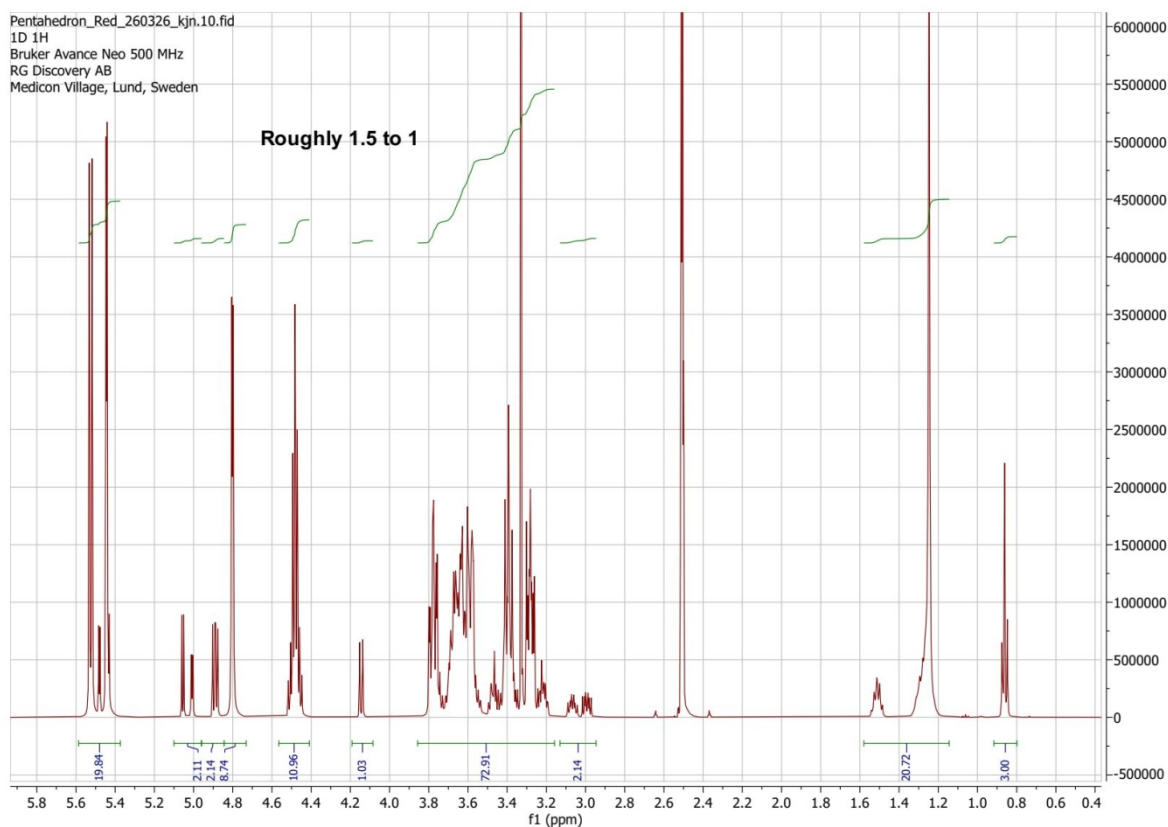


Figure 5.2.2.2. ^1H NMR spectrum of the precipitated α -CD/ β -DDM complex obtained from the initial 1:2 formulation.

2:1 α -CD : β -DDM

For the spectrum in Figure 5.2.2.3 the molar ratio of α -cyclodextrin to β -DDM in the precipitated fraction was approximately 2.0:1.

Just as for the corresponding system containing α -DDM, this formulation exhibited the highest relative α -cyclodextrin content within the precipitated phase, indicating that excess α -cyclodextrin preferentially participated in solid-phase assembly during precipitation.

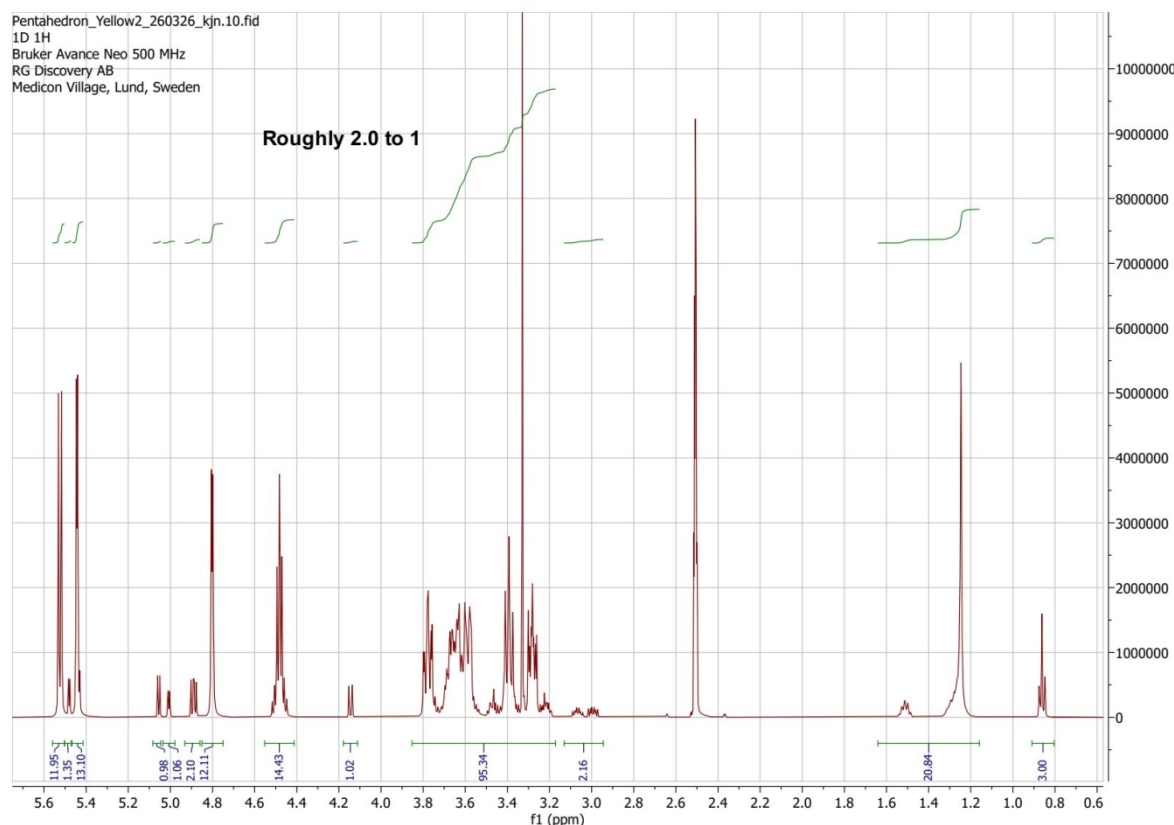


Figure 5.2.2.3. ^1H NMR spectrum of the precipitated α -CD/ β -DDM complex obtained from the initial 2:1 formulation.

5.2.3 Stoichiometry and mass balance analysis of α -CD/ β -DDM precipitates

The stoichiometries of the precipitated complexes determined by NMR are summarized and compared with the data for the β -DDM systems in *Table 5.1.3.1*.

For the α -CD/ β -DDM system, the observed stoichiometries of the precipitated complexes suggest a tendency towards a 2:1 (α -CD : β -DDM) composition, although variations were observed depending on the initial mixing ratio.

As shown in *Table 5.1.3.1*, when the theoretical molar ratios were 1:1 and 1:2, the precipitated complexes exhibited observed stoichiometries of 1.5:1 and 1.7:1, respectively, whereas a 2:1 stoichiometry was obtained when the theoretical ratio was 2:1.

Mass balance calculations were also performed for the α -CD/ β -DDM system assuming an anhydrous precipitated complex with a 2:1 stoichiometry (α -CD : β -DDM), consistent with the limiting stoichiometry observed by NMR. For samples prepared using an initial 2:1 molar ratio, the calculated supernatant concentrations were 19.5 mM α -CD and 9.7 mM β -DDM. Detailed calculation procedures and results are presented in the Appendix.

As in the α -DDM system, the measured α -CD concentration in the supernatant was also substantially lower than the reported aqueous solubility of α -CD in water (132 mM).

It is also noteworthy that higher supernatant concentrations were obtained for the α -CD/ β -DDM system than for the corresponding α -CD/ α -DDM system. This clearly suggest that the relatively

minor structural difference between α -DDM and β -DDM has a large impact on the properties of the complexed formed with α -CD.

5.3 Decylglucoside (DG)

5.3.1 Phase Behavior Observation

The phase behavior and physical appearance of α -CD/DG and β -CD/DG systems with different water contents and molar ratios before and after centrifugation are shown in *Figure 5.3.1.1* and *Figure 5.3.1.2*.

Systems Comprising α -Cyclodextrin

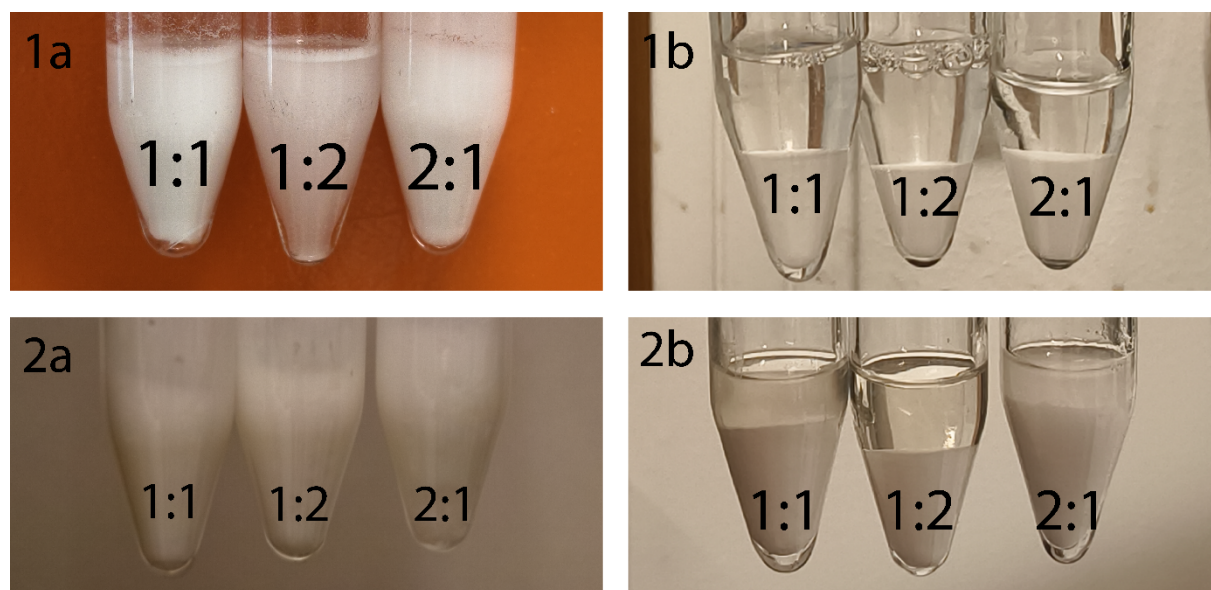


Figure 5.3.1.1. Phase characteristics of α -CD/DG systems with different water contents and molar ratios. (1a) 90% water content before centrifugation and (1b) after centrifugation. (2a) 80% water content before centrifugation and (2b) after centrifugation.

Before centrifugation, the samples containing 90% water appeared as milky white suspensions with no obvious adhesion to the tube wall. After centrifugation, clear phase separation was observed, and noticeable differences were found in the volume of the supernatant among the samples.

The samples containing 80% water also appeared as milky white suspensions prior to centrifugation, but were more viscous and exhibited obvious adhesion to the tube wall. Following centrifugation, pronounced phase separation was observed, accompanied by a marked decrease in the supernatant volume and an increase in the precipitated phase compared with the samples containing 90% water.

Systems Comprising β -Cyclodextrin

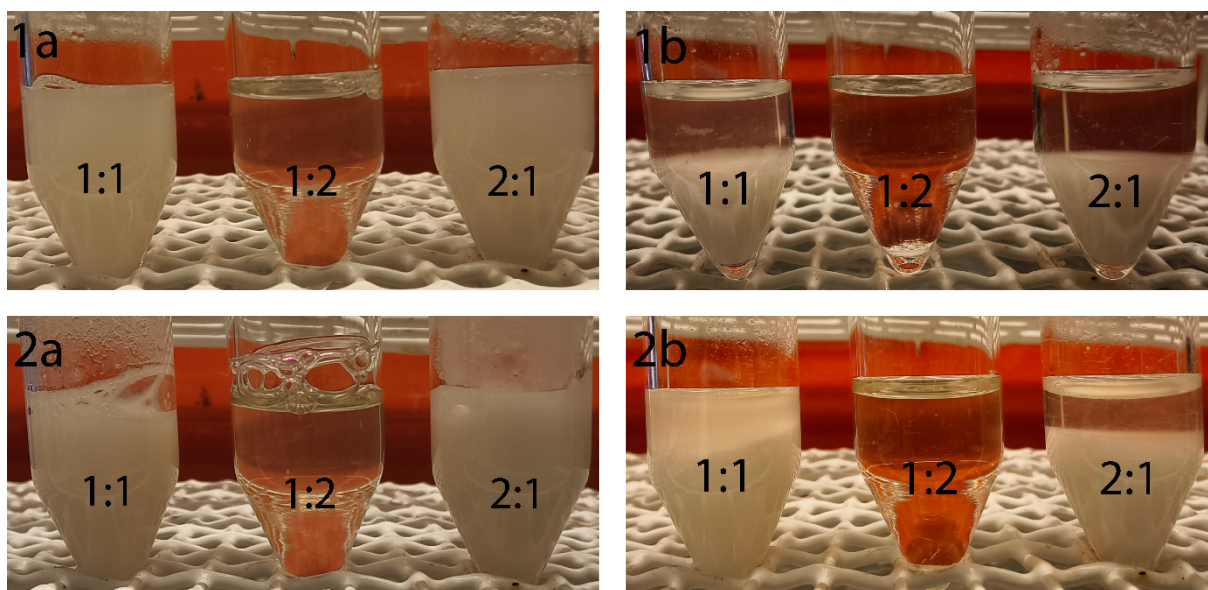


Figure 5.3.1.2. Phase characteristics of β -CD/DG systems with different water contents and molar ratios. (1a) 90% water content before centrifugation and (1b) after centrifugation. (2a) 80% water content before centrifugation and (2b) after centrifugation.

Among the samples containing 90% water, the 1:1 and 2:1 samples appeared as white suspensions and showed no obvious adhesion to the tube wall after vortex. In contrast, the 1:2 sample was a clear and transparent solution that flowed upon inversion. After centrifugation, no noticeable change was observed in the 1:2 sample. However, obvious phase separation occurred in the 1:1 and 2:1 samples, with flocculent material present at the phase interface.

For the samples containing 80% water, the 1:1 and 2:1 samples appeared as slightly viscous white suspensions. After vortexing, particle adhesion to the tube wall was observed in the 1:1 sample and was more pronounced in the 2:1 sample; neither sample flowed upon inversion. The 1:2 sample remained a clear and transparent solution and flowed upon inversion. After centrifugation, no obvious change was observed in the 1:2 sample. In contrast, the 1:1 and 2:1 samples exhibited clear phase separation with flocculent material at the interface. Compared with the corresponding samples containing 90% water, the precipitated phase increased noticeably. The upper phase of the 1:1 sample appeared turbid, which was likely caused by disturbance during handling.

After centrifugation and subsequent vortex, none of the samples returned to their original state. Samples that previously showed no observable flow behavior upon inversion became flowable after re-vortex. In addition, some samples exhibited obvious white precipitates at the bottom of the tube that could not be fully redispersed by vortex.

5.4 Laurylglucoside (LG)

5.4.1 Phase Behavior Observation

The phase behavior and physical appearance of α -CD/LG and β -CD/LG systems with different water contents and molar ratios before and after centrifugation are shown in Figure 5.4.1.1 and Figure 5.4.1.2.

Systems Comprising α -Cyclodextrin

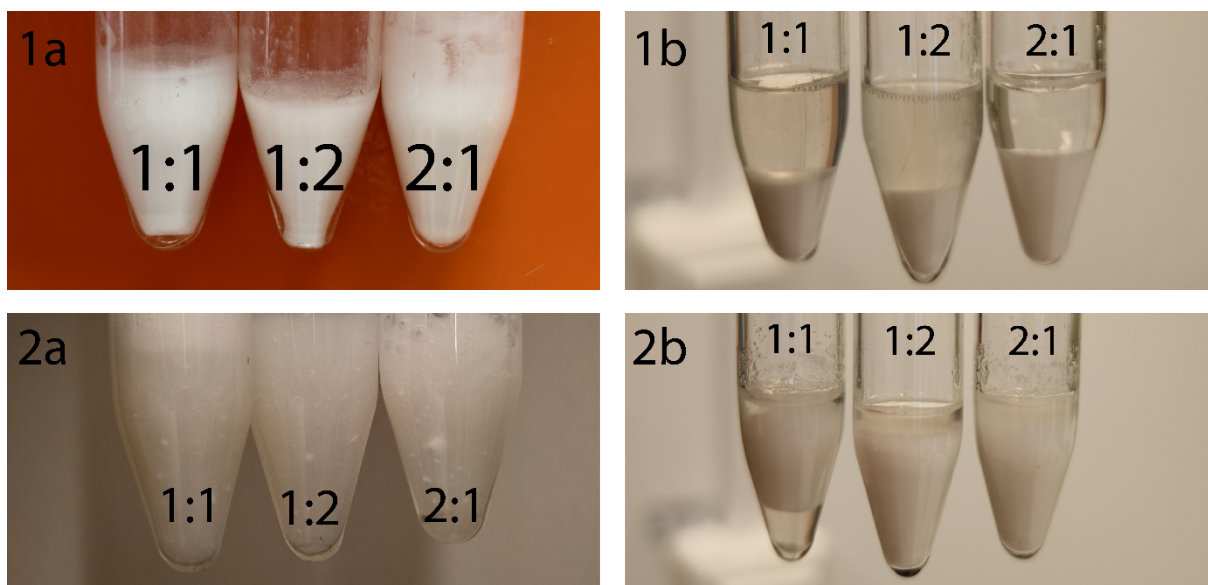


Figure 5.4.1.1. Phase characteristics of α -CD/LG systems with different water contents and molar ratios. (1a) 90% water content before centrifugation and (1b) after centrifugation. (2a) 80% water content before centrifugation and (2b) after centrifugation.

Among the samples containing 90% water, only the 2:1 sample exhibited relatively obvious adhesion to the tube wall, while all three molar ratios appeared as milky white low-viscous suspensions. Clear phase separation was observed after centrifugation.

The samples containing 80% water contained insoluble particles and exhibited phase separation after centrifugation.

5.4.1.1 Systems Comprising β -Cyclodextrin

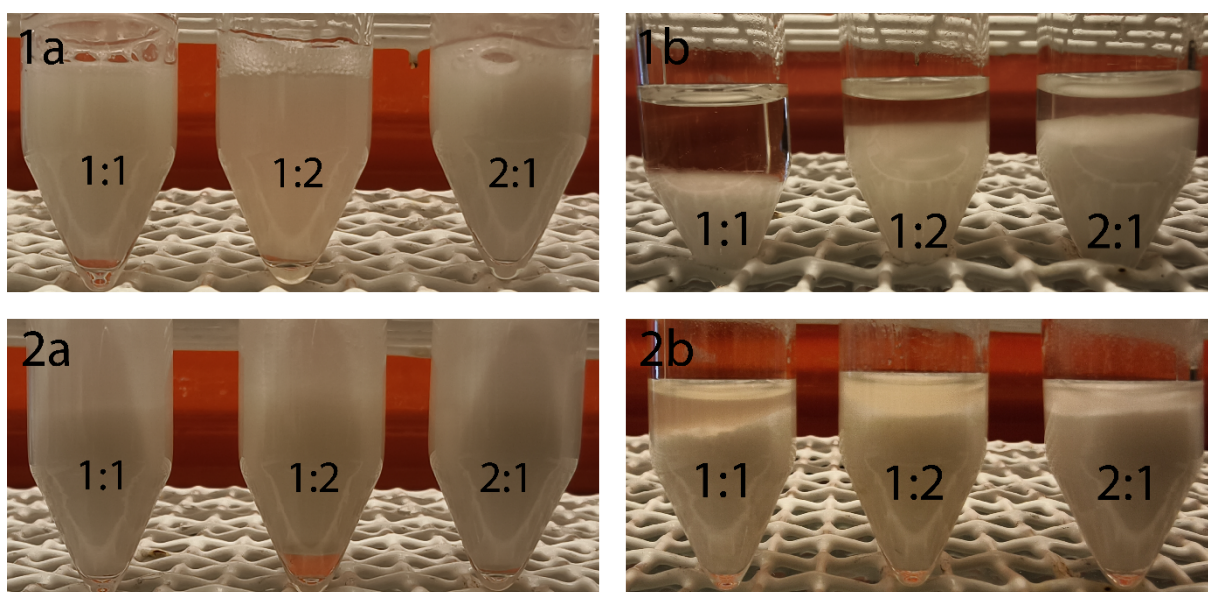


Figure 5.4.1.2. Phase characteristics of β -CD/LG systems with different water contents and molar ratios. (1a) 90% water content before centrifugation and (1b) after centrifugation. (2a) 80% water content before centrifugation and (2b) after centrifugation.

Before centrifugation, the samples containing 90% water appeared as milky white liquids, and only the 2:1 sample failed to display observable flow behavior upon inversion. After centrifugation, phase separation was observed in all samples, with white flocculent material present at the phase interface.

The samples containing 80% water appeared as viscous milky white suspensions prior to centrifugation. Obvious adhesion to the tube wall was observed after vortex, and none of the samples flowed upon inversion. In the 1:2 sample, highly viscous LG solution remained at the bottom of the tube and could not be fully dispersed by vortex. After centrifugation, phase separation occurred in all three samples, and the upper phase appeared turbid, which was likely caused by disturbance during handling.

After centrifugation and subsequent vortex, none of the samples returned to their original state. Samples that previously showed no observable flow behavior upon inversion became flowable after re-vortex. In addition, some samples exhibited obvious white precipitates at the bottom of the tube that could not be fully redispersed by vortex.

5.5 Polysorbate 20

5.5.1 Phase Behavior Observation

The phase behavior and physical appearance of α -CD/polysorbate 20 and β -CD/polysorbate 20 systems with different water contents and molar ratios before and after centrifugation are shown in *Figure 5.5.1.1* and *Figure 5.5.1.2*.

Systems Comprising α -Cyclodextrin

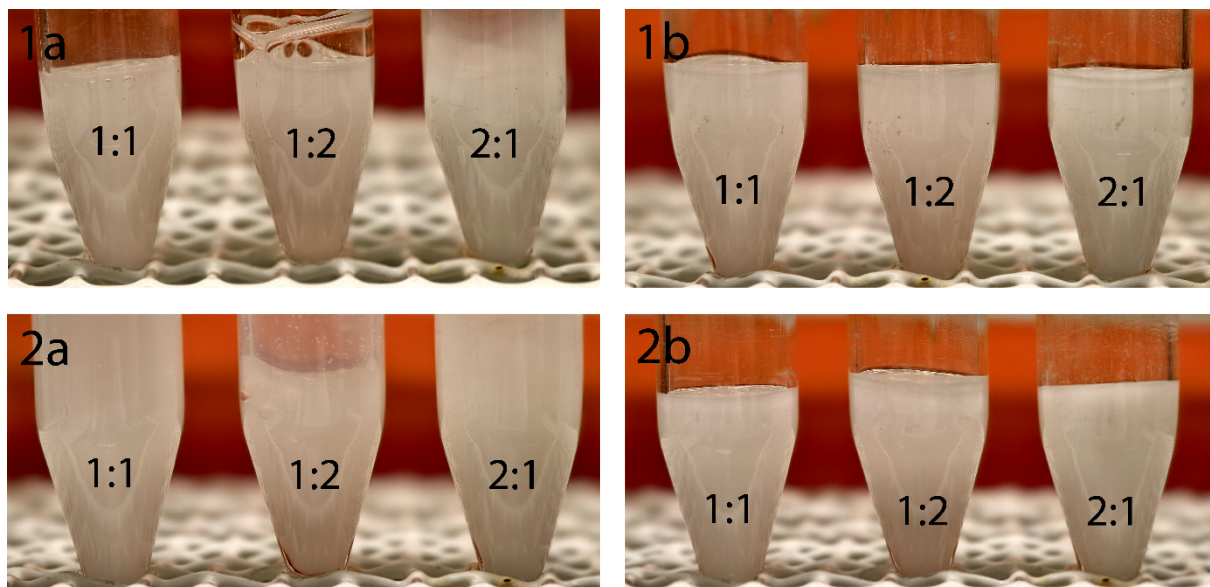


Figure 5.5.1.1. Phase characteristics of α -CD/polysorbate 20 systems with different water contents and molar ratios. (1a) 90% water content before centrifugation and (1b) after centrifugation. (2a) 80% water content before centrifugation and (2b) after centrifugation.

The samples containing 90% water appeared as white milky suspensions. The 1:1 and 1:2 samples exhibited a more water-like consistency and showed no obvious adhesion to the tube wall after vortex, whereas the 2:1 sample displayed noticeable wall adhesion and did not flow upon inversion. Following centrifugation, no phase separation was observed in any of the samples. Upon inversion, the 1:1 and 1:2 samples remained flowable, while only a very small amount of the 2:1 sample flowed downward.

The samples containing 80% water appeared as slightly more viscous white milky suspensions with obvious wall adhesion, and none of the samples flowed upon inversion. After centrifugation, none of the samples flowed when inverted; however, movement of the liquid surface was observed in the 1:2 sample, indicating a tendency to flow downward.

Systems Comprising β -Cyclodextrin

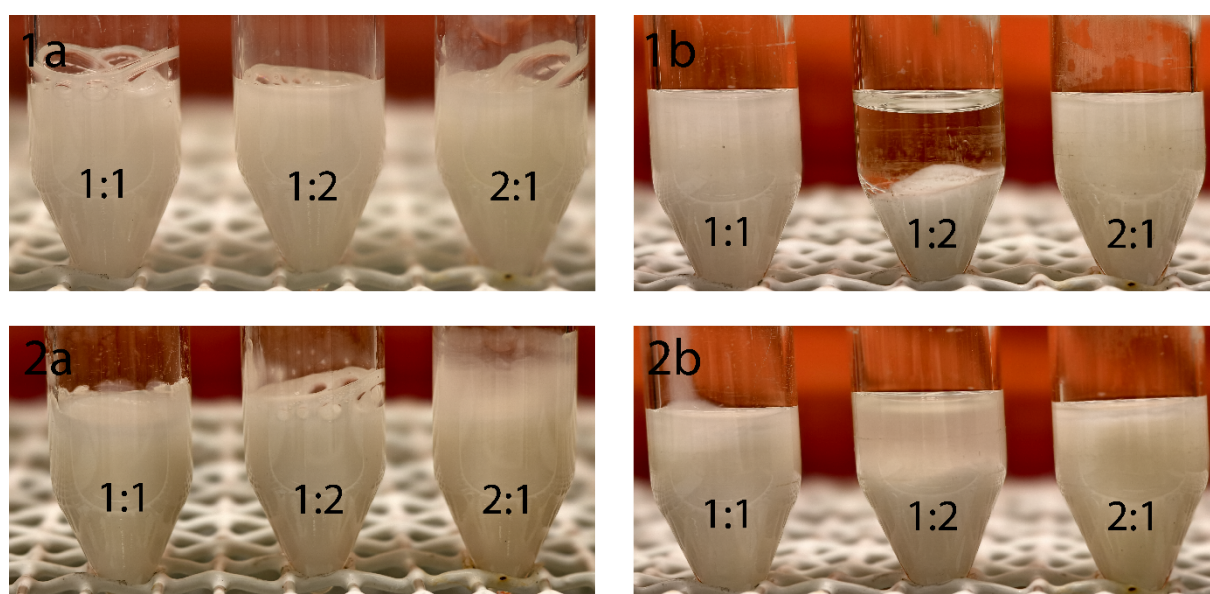


Figure 5.5.1.2. Phase characteristics of β -CD/polysorbate 20 systems with different water contents and molar ratios. (1a) 90% water content before centrifugation and (1b) after centrifugation. (2a) 80% water content before centrifugation and (2b) after centrifugation.

All samples containing 90% water appeared as white liquids, and only the 2:1 sample exhibited slight adhesion to the tube wall after vortex. Following centrifugation, phase separation only existed in the 1:2 sample. Upon inversion, the 1:1 and 2:1 samples remained flowable.

Among the samples containing 80% water, the 2:1 sample showed obvious adhesion to the tube wall and did not flow upon inversion. After centrifugation, phase separation was observed only in the 1:2 sample. The 1:1 sample remained flowable upon inversion, whereas the 2:1 sample showed no observable flow behavior.

5.5.2 Rheological test

Rheological characterisation was performed on the sample that showed no phase separation after centrifugation, i.e. 1:1 α -CD:polysorbate 20 with 80% of water.

80%water content α CD-polysorbate 20 hydrogels

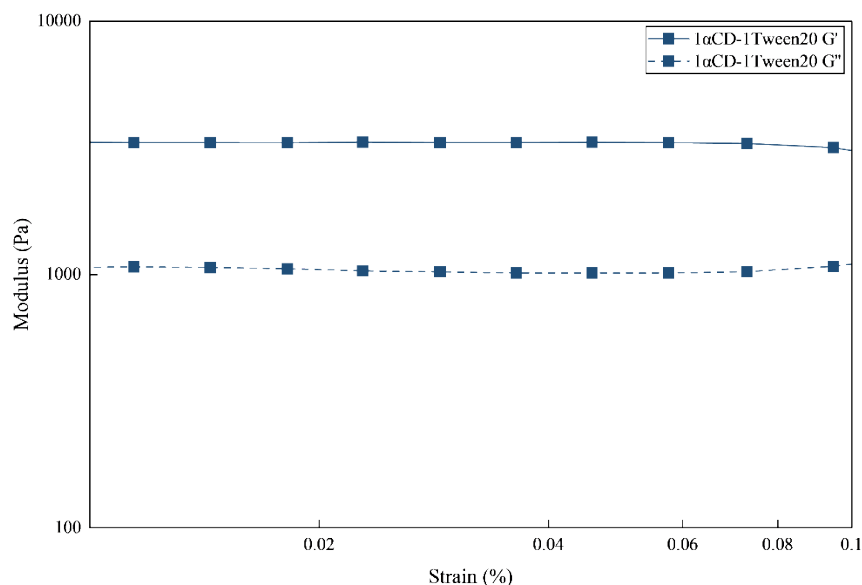


Figure 5.5.2.1. Viscoelastic properties of 1 α CD-1polysorbate 20 hydrogel evaluated by oscillatory amplitude sweep measurements.

The oscillatory rheological results demonstrated typical supramolecular gel characteristics. During the amplitude sweep test, the storage modulus (G') remained consistently higher than the loss modulus (G''), indicating that the systems were dominated by elastic behavior.

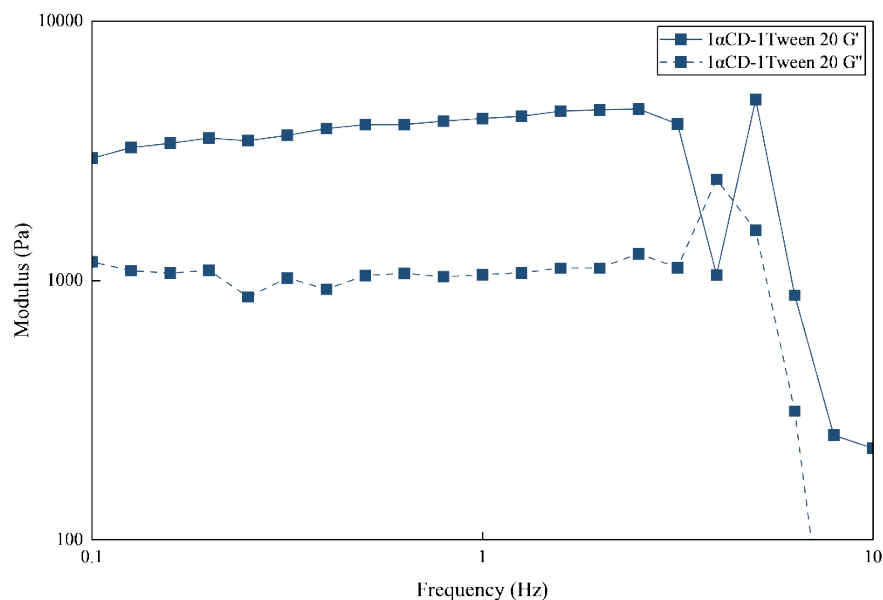


Figure 5.5.2.2. Frequency-dependent viscoelastic properties of 1 α CD-1polysorbate 20 hydrogel obtained from oscillatory frequency sweep measurements.

The frequency sweep results further demonstrated that G' remained consistently higher than G'' throughout the entire tested frequency range, further confirming the presence of stable gel

structures in the systems. In addition, the modulus values exhibited a certain degree of frequency dependence with increasing frequency.

5.6 Polysorbate 80

5.6.1 Phase Behavior Observation

The phase behavior and physical appearance of α -CD/polysorbate 80 and β -CD/ polysorbate 80 systems with different water contents and molar ratios before and after centrifugation are shown in *Figure 5.6.1.1* and *Figure 5.6.1.2*.

Systems Comprising α -Cyclodextrin

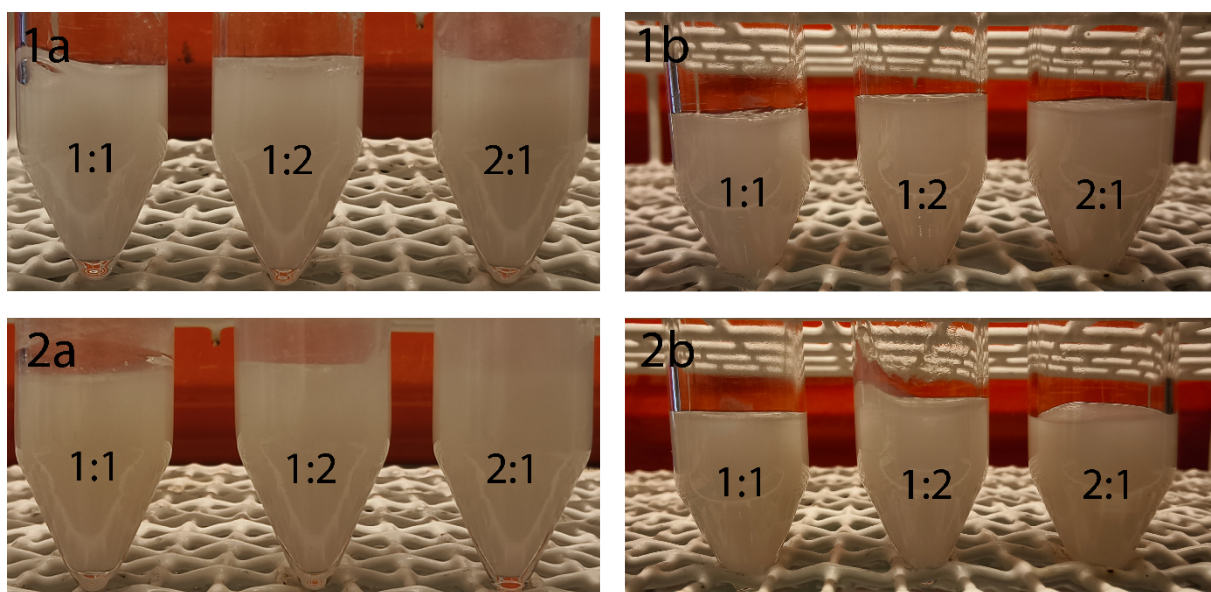


Figure 5.6.1.1. Phase characteristics of α -CD/polysorbate 80 systems with different water contents and molar ratios. (1a) 90% water content before centrifugation and (1b) after centrifugation. (2a) 80% water content before centrifugation and (2b) after centrifugation.

Before centrifugation, the samples containing 90% water appeared as white viscous liquids, and only the 2:1 sample showed no observable flow behavior upon inversion. After centrifugation, no obvious phase separation was observed in the 1:1 or 1:2 samples, although the 1:2 sample exhibited slightly more noticeable fluidity upon gentle agitation. The 2:1 sample also remained monophasic; however, a thin aqueous layer was observed at the interface, which appeared turbid and was presumed to consist of a mixture of flocculent material and water.

Before centrifugation, the samples containing 80% water appeared as white viscous liquids, and the 1:2 and 2:1 samples showed no observable flow behavior upon inversion. Following centrifugation, all three samples remained monophasic. A thin aqueous layer containing slight flocculent material was observed at the interface of the 1:1 sample.

Systems Comprising β -Cyclodextrin

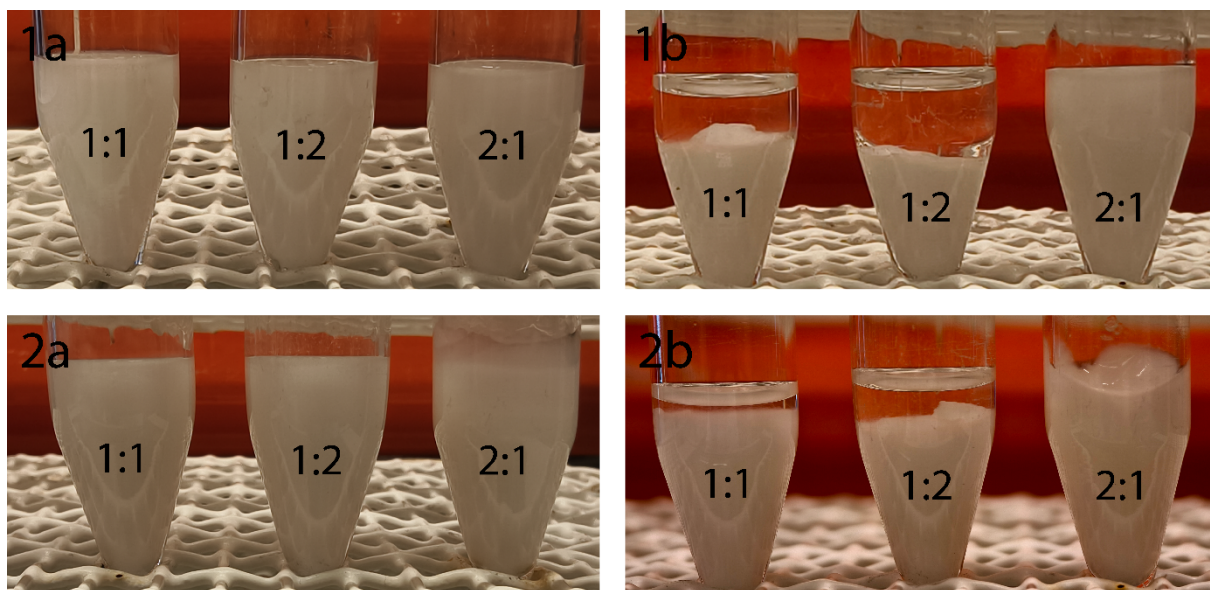


Figure 5.6.1.2. Phase characteristics of β -CD/polysorbate 80 systems with different water contents and molar ratios. (1a) 90% water content before centrifugation and (1b) after centrifugation. (2a) 80% water content before centrifugation and (2b) after centrifugation.

Before centrifugation, all samples containing 90% water showed as white viscous liquids and were able to flow upon inversion. After centrifugation, the 1:1 sample showed obvious phase separation, with white flocculent material observed at the phase interface. The 1:2 sample also exhibited phase separation, although the interface was clear and no flocculent material was observed; the precipitated phase displayed a soft gel-like appearance. In contrast, no phase separation was observed in the 2:1 sample after centrifugation.

Before centrifugation, the 1:1 and 1:2 samples containing 80% water appeared as white viscous liquids and were able to flow upon inversion, whereas the 2:1 sample showed no observable flow behavior upon inversion. After centrifugation, the 1:1 sample exhibited phase separation, with a thin supernatant water layer and the presence of flocculent material. The 1:2 sample showed more pronounced phase separation without observable flocculent material. In contrast, no phase separation in the 2:1 sample, and no thin water layer existed at the interface.

5.6.2 Rheological test

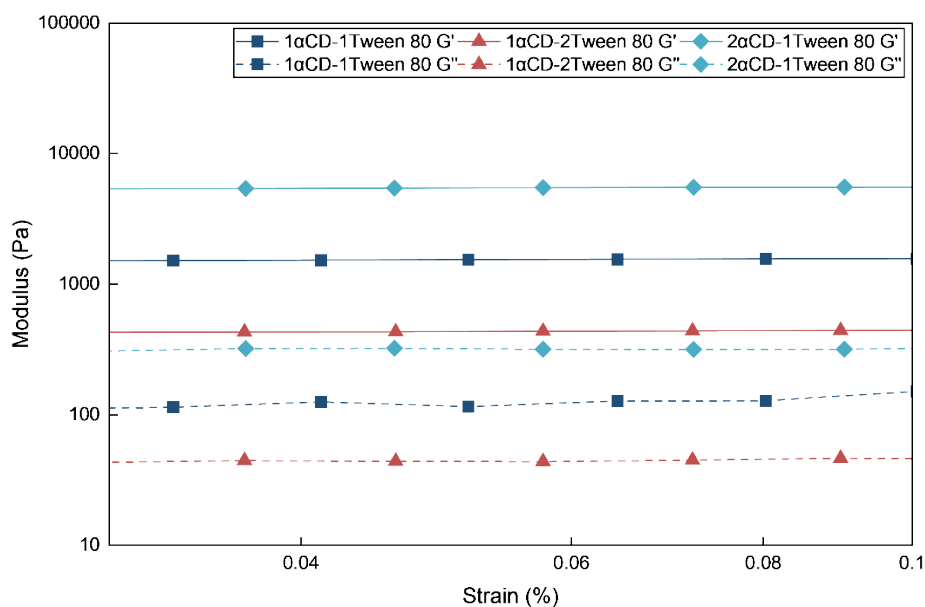


Figure 5.6.2.1. Viscoelastic properties of 1αCD-1polysorbate 80 hydrogel, 1αCD-2polysorbate 80 hydrogel, 2αCD-1polysorbate 80 hydrogel evaluated by oscillatory amplitude sweep measurements.

At 80% water content, αCD-polysorbate 80 hydrogels with different molar ratios all exhibited typical gel characteristics, with the storage modulus (G') remaining higher than the loss modulus (G''). However, significant differences in modulus values were observed among the different systems. Specifically, the 2αCD-1polysorbate 80 hydrogel exhibited the highest G' value and maintained a relatively stable variation trend during both amplitude sweep and frequency sweep tests, followed by the 1αCD-1polysorbate 80 hydrogel. In contrast, the 1αCD-2polysorbate 80 hydrogel showed the lowest G' value and exhibited more pronounced frequency dependence.

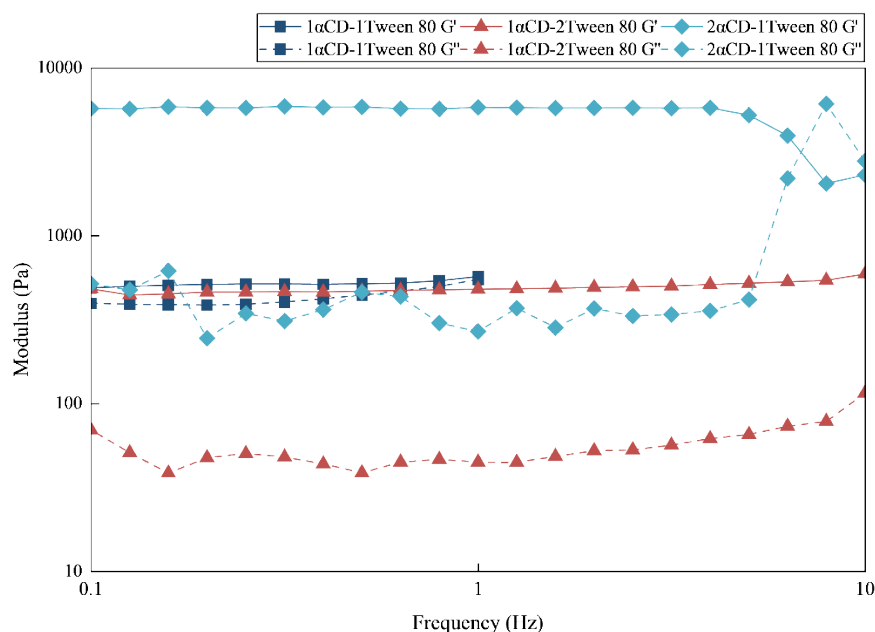


Figure 5.6.2.2. Frequency-dependent viscoelastic properties of 1 α CD-1polysorbate 80 hydrogel, 1 α CD-2polysorbate 80 hydrogel, 2 α CD-1polysorbate 80 hydrogel obtained from oscillatory frequency sweep measurements.

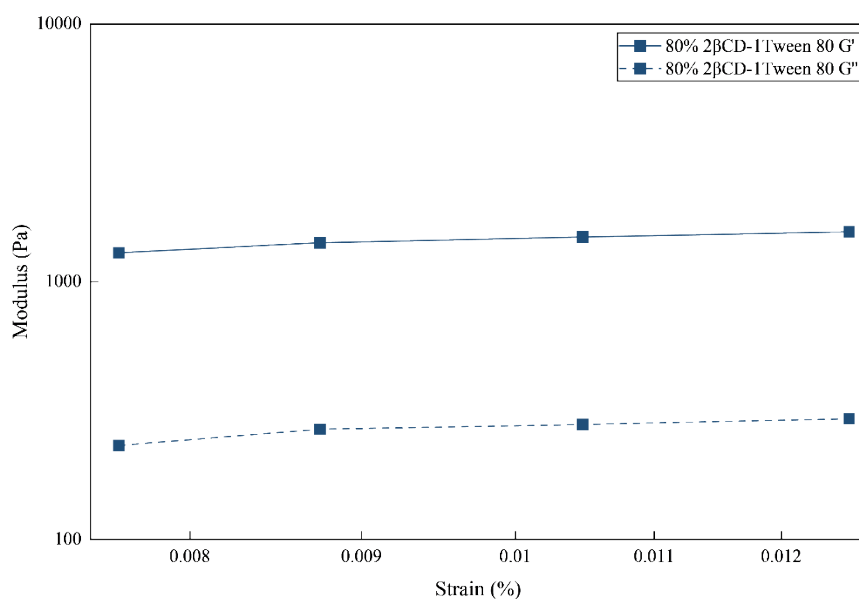


Figure 5.6.2.3. Small-strain viscoelastic response of 2 β CD-1polysorbate 80 hydrogel showing storage modulus (G') and loss modulus (G'') within the linear viscoelastic region.

In addition, when the cyclodextrin type was varied under the same 2:1 molar ratio condition, the G' value of the 2 β CD-1polysorbate 80 hydrogel was significantly lower than that of the 2 α CD-1polysorbate 80 hydrogel. Moreover, the α -CD-based system exhibited more stable modulus variation during the frequency sweep test, whereas the β -CD based system showed more pronounced fluctuations.

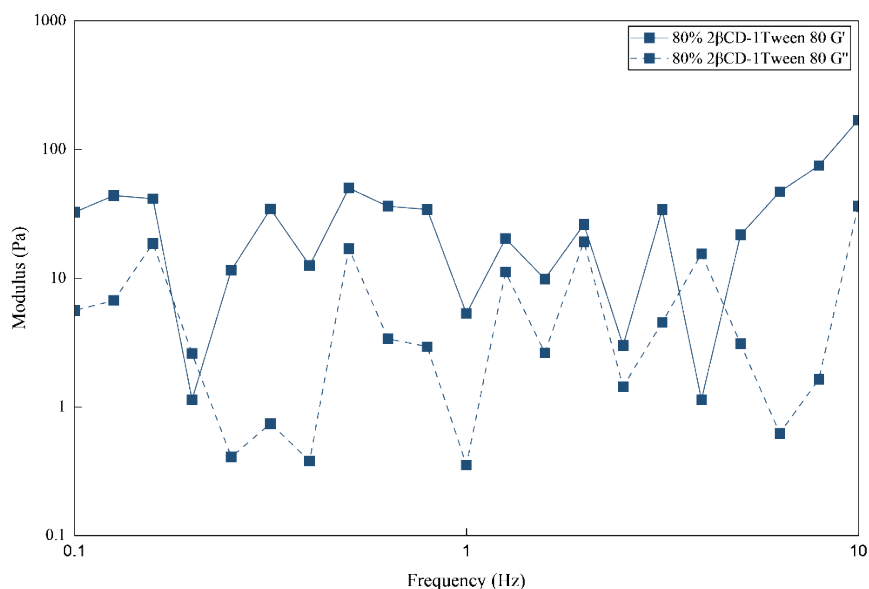


Figure 5.6.2.4. Frequency-dependent viscoelastic behavior of 2 β CD-polysorbate 80 hydrogel obtained from oscillatory frequency sweep measurements.

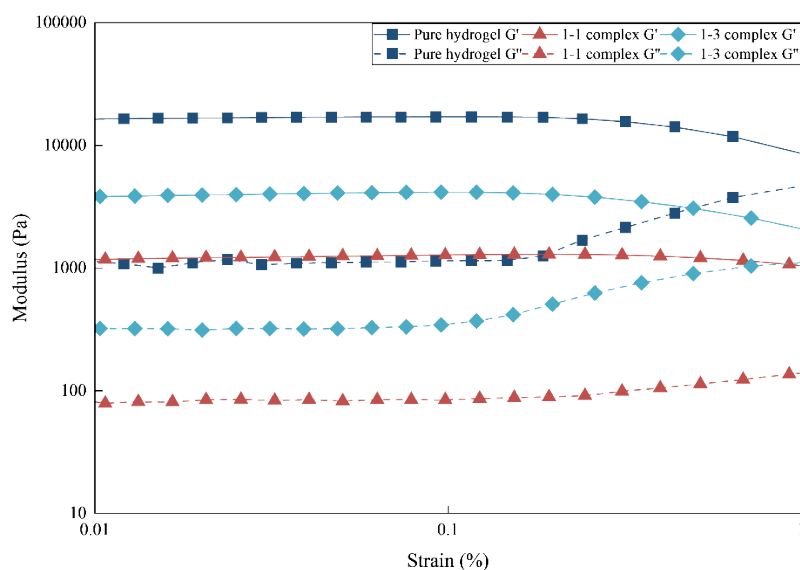


Figure 5.6.2.5. Viscoelastic properties of Pure hydrogel, 1-1 complex hydrogel, and 1-3 complex hydrogel evaluated by oscillatory amplitude sweep measurements.

Rheological characterization was also used to compare the behavior of CD-polysorbate gels (henceforth referred to as ‘pure hydrogel’) with those obtained after inclusion of curcumin (‘complex hydrogel’). At 70% water content and an α -CD:polysorbate 80 ratio of 2:1, the pure hydrogel, 1-1 complex hydrogel, and 1-3 complex hydrogel all exhibited typical gel behavior, with the storage modulus (G') remaining higher than the loss modulus (G''). Among them, the pure hydrogel showed the highest G' value, indicating the strongest elastic network structure,

followed by the 1-3 complex hydrogel, while the 1-1 complex hydrogel exhibited the lowest G' value.

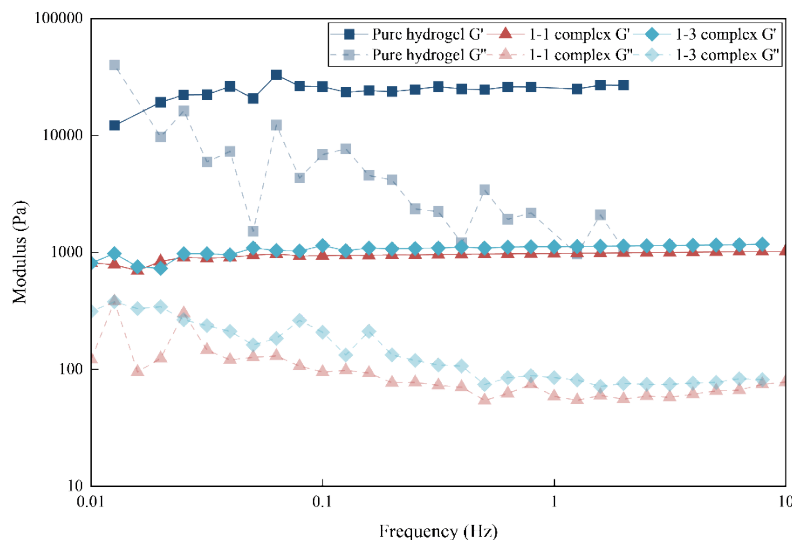


Figure 5.6.2.6. Frequency-dependent viscoelastic properties of Pure hydrogel, 1-1 complex hydrogel, and 1-3 complex hydrogel obtained from oscillatory frequency sweep measurements.

The frequency sweep results showed that all three systems maintained storage modulus (G') values higher than loss modulus (G'') throughout the tested frequency range, indicating the presence of relatively stable gel structures. Among them, the pure hydrogel exhibited the highest overall modulus values with minimal variation, whereas the drug-loaded systems, particularly the 1-1 complex hydrogel, showed more pronounced frequency dependence.

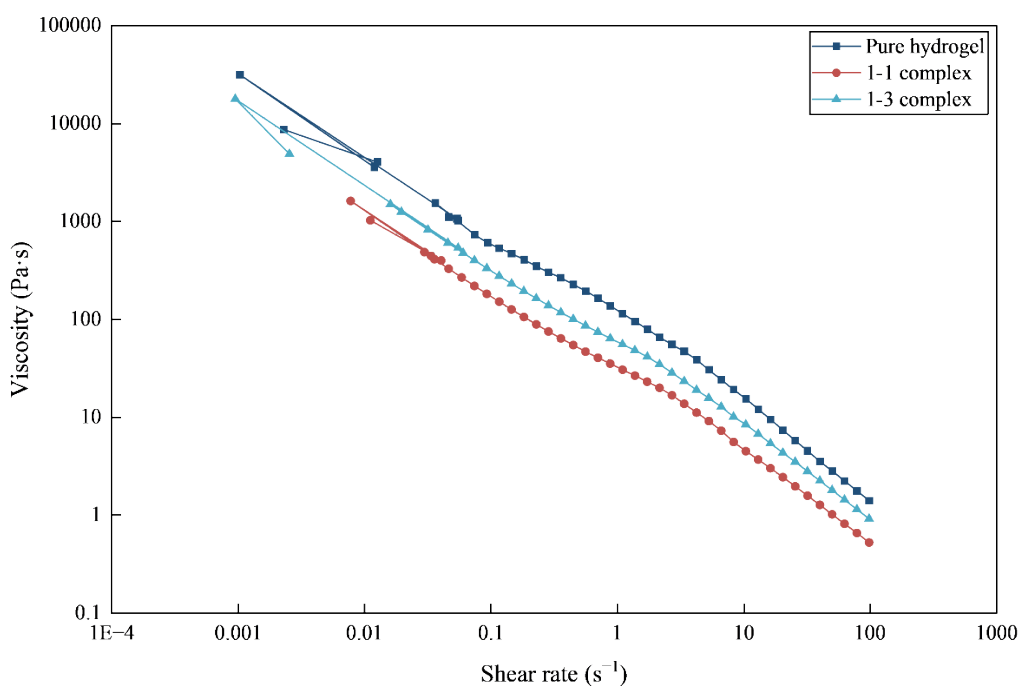


Figure 5.6.2.7. Viscosity as a function of shear rate for Pure hydrogel, 1-1 complex hydrogel, and 1-3 complex hydrogel.

The shear viscosity test demonstrated that all three systems exhibited pronounced shear-thinning behavior, with viscosity decreasing as the shear rate increased. In addition, the pure hydrogel exhibited the highest viscosity throughout the entire shear range, followed by the 1-3 complex hydrogel, while the 1-1 complex hydrogel showed the lowest viscosity.

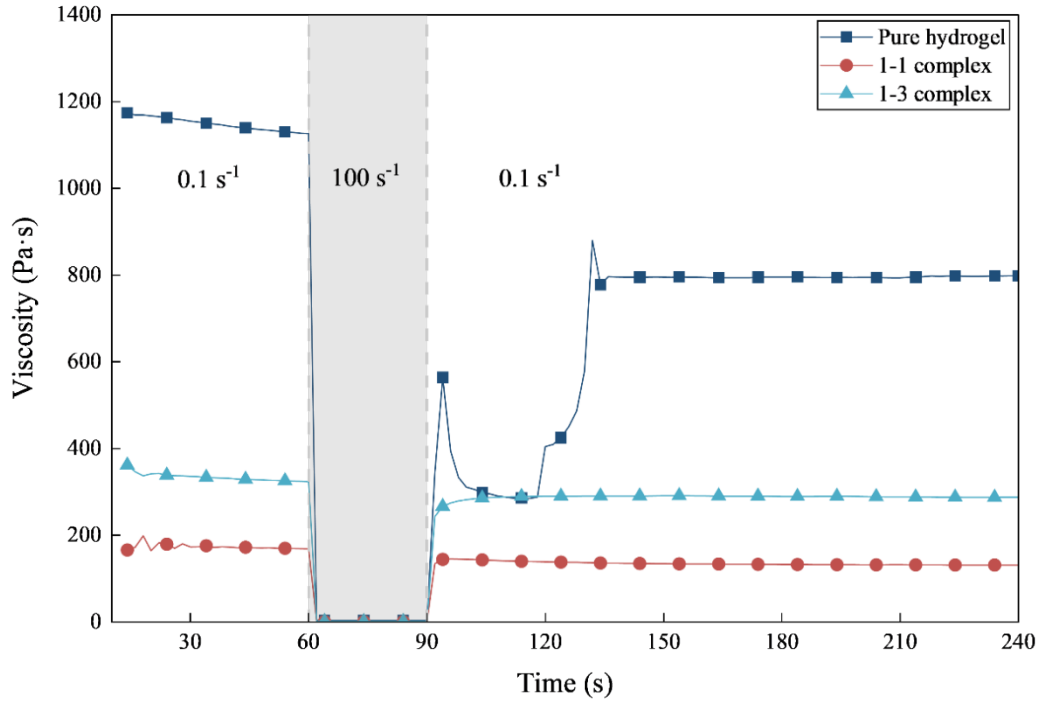


Figure 5.6.2.8. Three-step thixotropic recovery curves of Pure hydrogel, 1-1 complex hydrogel, and 1-3 complex hydrogel under alternating shear rates (0.1 s^{-1} , 100 s^{-1} , 0.1 s^{-1}).

The recover ratio was calculated by the standard equation:

$$\text{Recovery ratio} = \frac{\eta_3}{\eta_1} \times 100\% \quad (4)$$

The recovery ratio of Pure hydrogel, 1-1 complex hydrogel, and 1-3 complex hydrogel from thixotropy test were calculated and the results are presented in Figure 5.6.2.9.:

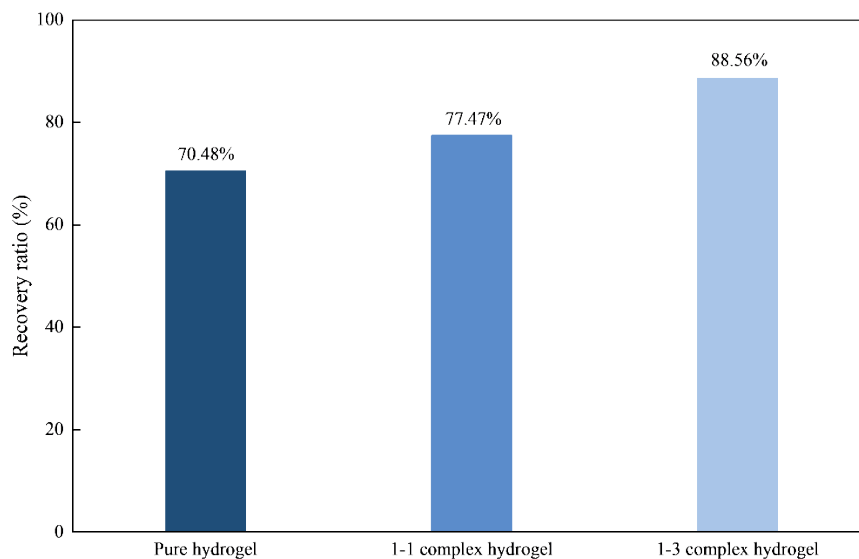


Figure 5.6.2.9. Recovery ratio of hydrogels calculated as η_3/η_1 after three-step thixotropy testing.

The results of the three-step thixotropic recovery test showed that the viscosities of all three hydrogels decreased rapidly under high shear conditions, while partial viscosity recovery was observed after returning to low shear conditions, indicating that the systems possessed a certain degree of structural recovery capability. Among them, the 1-3 complex hydrogel exhibited the highest recovery ratio, followed by the pure hydrogel, whereas the 1-1 complex hydrogel showed the lowest recovery ratio.

5.6.3 In Vitro Release Assay

The results obtained using the test method described in Section 4.2.5 are shown below:

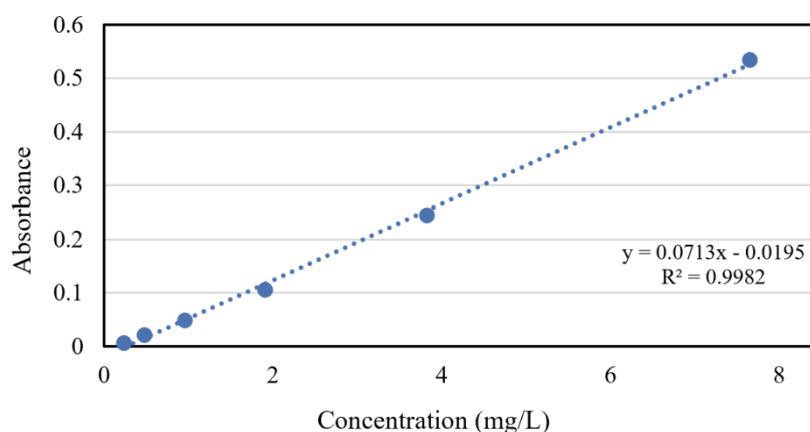


Figure 5.6.3.1. Calibration curve of curcumin determined by UV–Vis spectrophotometry at 430 nm.

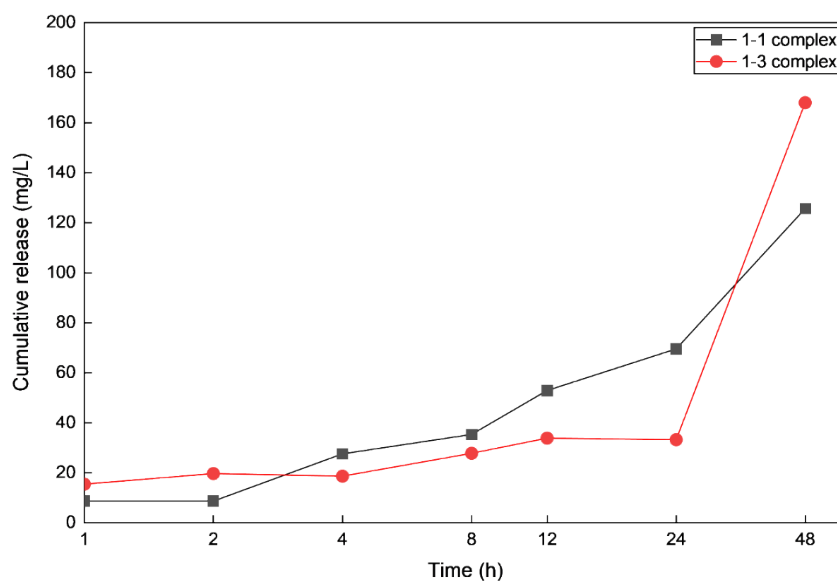


Figure 5.6.3.2. In vitro release profiles of curcumin from 1-1 and 1-3 complexes hydrogel at different time points over 48 h.

The release concentration profiles of curcumin from the 1:1 and 1:3 complex hydrogels over 48 h are shown in *Figure 5.6.3.2*. For the 1:1 complex hydrogel, the release concentration remained relatively stable during the initial 1–2 h period, followed by a gradual increase from 4 to 24 h and a more pronounced increase at 48 h. In the 1:3 complex hydrogel group, slightly higher release concentrations were observed during the early stage (1–12 h) compared with the 1:1 complex hydrogel. The release concentration remained nearly unchanged between 12 and 24 h, followed by a sharp increase at 48 h. Overall, both hydrogels exhibited very low release concentrations throughout the experimental period.

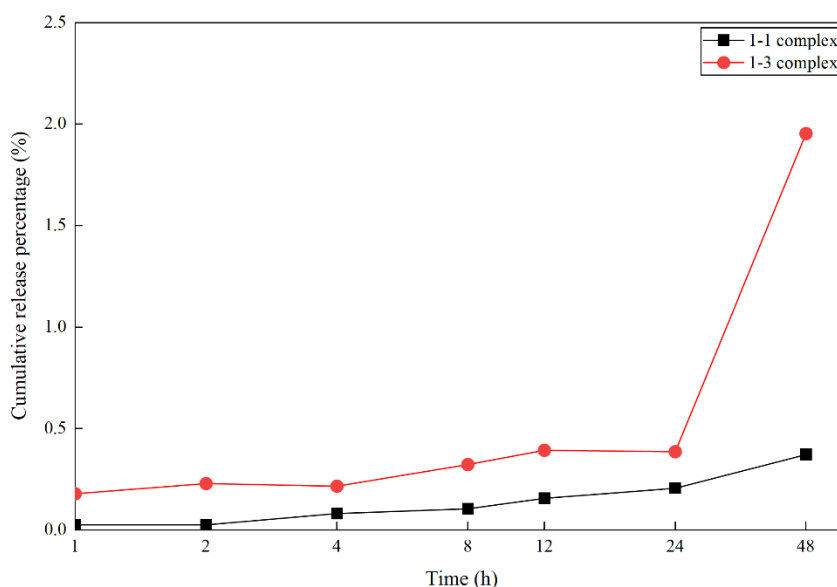


Figure 5.6.3.3. In vitro release percentage profiles of curcumin from 1-1 and 1-3 complexes hydrogel at different time points over 48 h.

Figure 5.6.3.3 presents the cumulative release percentages of curcumin from the 1:1 and 1:3 complex hydrogels. The cumulative release percentage was calculated as the ratio of the cumulative released amount at each time point to the total drug loading amount, and the detailed calculation process is provided in the Appendix. Compared with the 1:1 complex hydrogel, the 1:3 complex hydrogel exhibited consistently higher cumulative release percentages throughout the 48 h period. For the 1:1 complex hydrogel, the cumulative release percentage gradually increased from 0.0255% at 1 h to 0.3717% at 48 h. In contrast, the cumulative release percentage of the 1:3 complex hydrogel increased from 0.1794% at 1 h to 1.9530% at 48 h. Although the cumulative release percentages of both hydrogel systems gradually increased over time, the overall release levels remained low and did not exceed 2% after 48 h.

5.6.4 Injectability test

After manually selecting the $F_{\max}$ values from the distance–force curves obtained for the four samples, the results of the first injection cycle were summarized in the bar chart shown below. The empty syringe group showed a much higher $F_{\max}$ than the other three groups. In contrast, the hydrogels containing the complexes showed lower $F_{\max}$ values than the pure hydrogel.

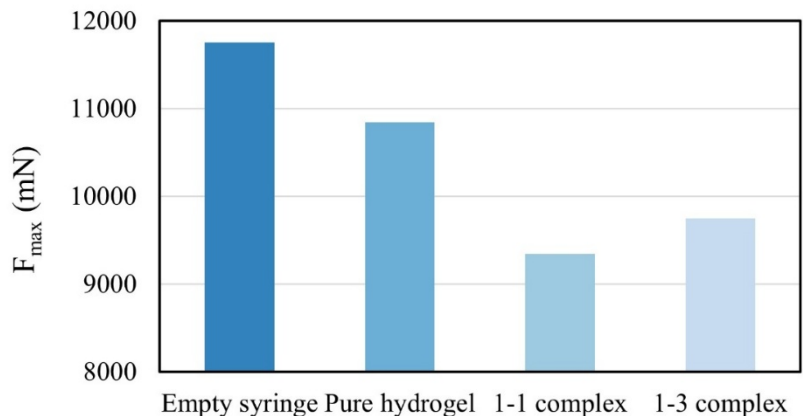


Figure 5.6.4.1. Initial extrusion peak force ($F_{\max}$) during the first injection cycle for Empty syringe, Pure hydrogel, 1–1 complex hydrogel, and 1–3 complex hydrogel samples.

The $F_{\max}$ values of the injection cycles after the first cycle are shown in the line chart below. All samples showed noticeable fluctuations in $F_{\max}$, especially the 1:1 complex hydrogel. However, the overall trend could still be observed. The $F_{\max}$ values generally followed the order: 1:3 complex hydrogel > Pure hydrogel > 1:1 complex hydrogel > Empty syringe.

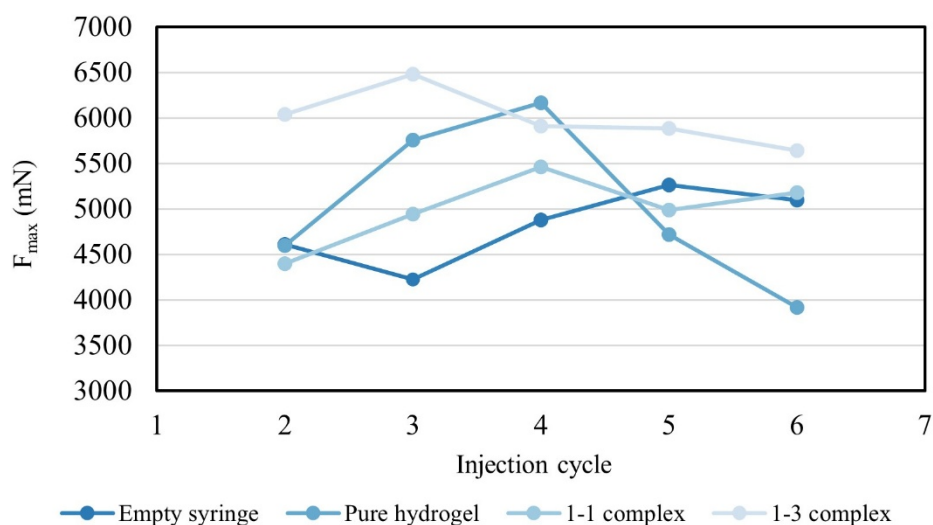


Figure 5.6.4.2. Evolution of maximum extrusion force ($F_{\max}$) during continuous injection cycles for Empty syringe, Pure hydrogel, 1–1 complex hydrogel, and 1–3 complex hydrogel samples.

Individual replicate measurements of DGF are shown as colored dots, while black markers and error bars represent the mean $\pm$ standard deviation (SD) for each group. The Empty syringe group contained six replicates ($n = 6$), whereas the Pure hydrogel, 1–1 complex, and 1–3 complex groups each contained eight replicates ($n = 8$).

The Empty syringe group exhibited relatively small error bars, indicating good reproducibility among replicates. In terms of mean values, the Pure hydrogel group showed the highest DGF, whereas the 1–1 complex group exhibited the lowest DGF.

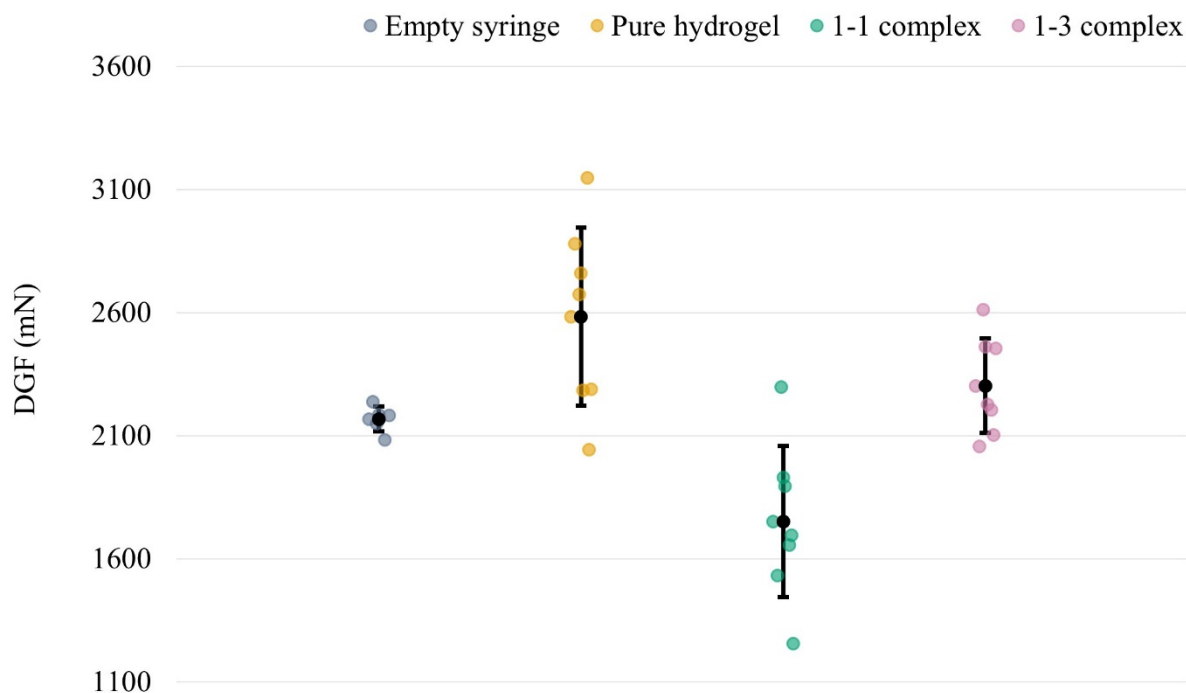


Figure 5.6.4.3. Distribution of Dynamic Glide Force (DGF) Values for Empty syringe, Pure hydrogel, 1–1 complex hydrogel, and 1–3 complex hydrogel samples.

The total injection work consumed during the first injection cycle for the four samples is compared below. All groups containing injectable formulations exhibited markedly higher injection work than the Empty syringe group. Among them, the 1-1 complex group showed the highest total injection work, whereas the 1-3 complex group exhibited the lowest value.

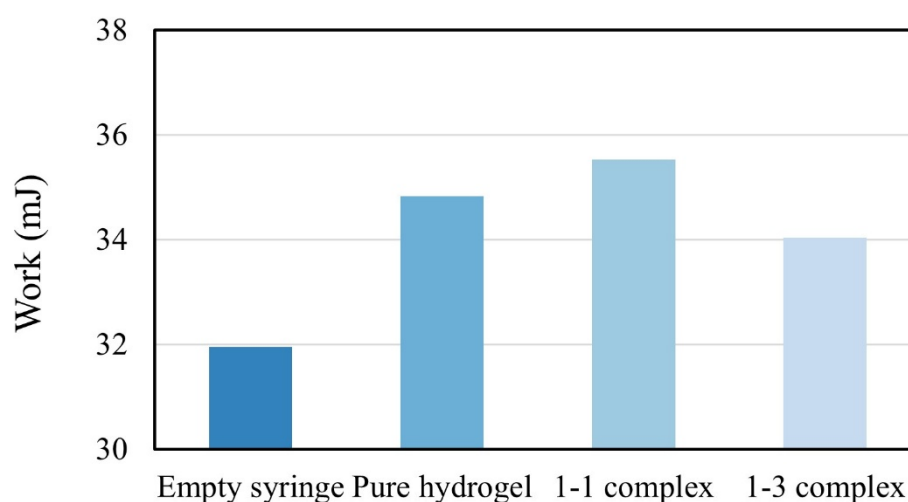


Figure 5.6.4.4. Total work done to extrude 1st injection cycle for Empty syringe, Pure hydrogel, 1–1 complex hydrogel, and 1–3 complex hydrogel samples.

For injection cycles 2–5, all sample groups except the Empty syringe group exhibited noticeable fluctuations in injection work. Among them, the Pure hydrogel group showed consistently higher overall injection work consumption.

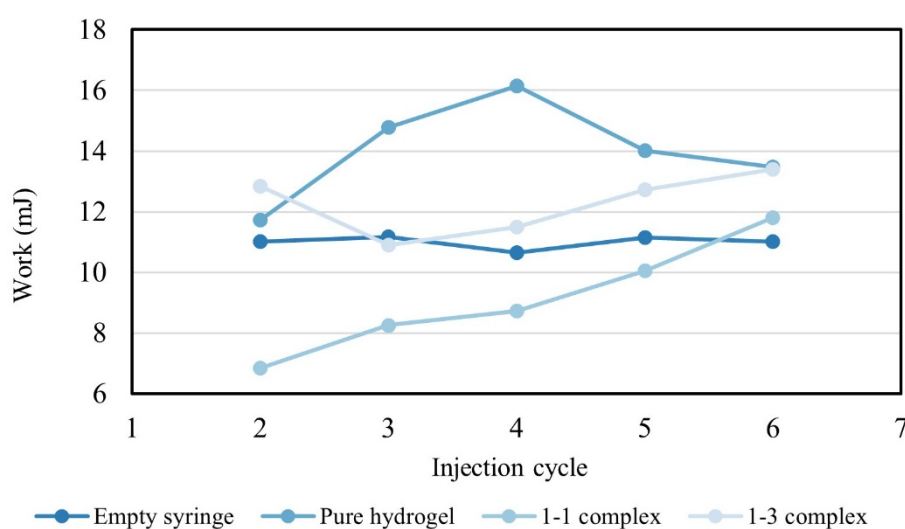


Figure 5.6.4.5. Evolution of work done during continuous injection cycles for Empty syringe, Pure hydrogel, 1–1 complex hydrogel, and 1–3 complex hydrogel samples.

6 Discussion

6.1 Relationship Between Surfactant Structure and Hydrogel Formation

6.1.1 Systems Exhibiting Phase Separation and Precipitation

In the α -CD/ α -DDM and α -CD/ β -DDM systems, all samples exhibited distinct phase separation after centrifugation, accompanied by the formation of cyclodextrin-rich precipitates. Although some formulations displayed relatively high viscosity or temporary non-flow behavior at low water contents, their structures were unable to remain stable under centrifugal conditions. These results suggest that the systems preferentially form localized aggregates or microcrystalline precipitates rather than a genuinely percolated supramolecular hydrogel network.

Previous studies have demonstrated that surfactants in aqueous solution inherently exhibit a strong tendency toward self-assembly. Once the concentration exceeds the critical micelle concentration (CMC), surfactant molecules preferentially form thermodynamically more stable micellar or vesicular structures through hydrophobic interactions, thereby minimizing the free energy of the system⁷⁰. In CD/DDM systems, surfactant self-aggregation and cyclodextrin inclusion complexation are essentially two competing processes. Thermodynamic studies have shown that only when a sufficient concentration of CD is present can the capture of surfactant monomers by CD effectively suppress micellization and promote the formation of inclusion complexes⁷⁰. Therefore, in the α -CD/DDM system, under relatively low molar ratios such as 1:1 or 1:2, DDM molecules are more likely to maintain their micellar state rather than being completely converted into stable α -CD inclusion complexes. As a result, free DDM, micellar DDM, and inclusion-complexed DDM coexist within the system, which disrupts the formation of ordered supramolecular assemblies and further reduces the likelihood of constructing a continuous hydrogel network.

Furthermore, the failure of the α -CD/DDM system to exhibit macroscopic gelation may also be related to the microscopic assembly mode of the α -CD/DDM inclusion complexes. Previous studies have shown that α -CD exhibits strong inclusion capability toward long hydrophobic guest chains. Owing to its relatively small cavity size, α -CD tends to form structures in which multiple α -CD molecules cooperatively encapsulate a single hydrophobic chain, rather than forming a simple 1:1 inclusion complex. Early thermodynamic studies proposed that, in the α -CD/DDM system, the 2:1 (α -CD : DDM) complex represents an important, and possibly dominant, binding configuration, in which two α -CD molecules collectively encapsulate the alkyl chain of DDM⁷¹. The NMR results obtained in this study also suggest the formation of α -CD/DDM inclusion complexes with a stoichiometry approaching 2:1 in the precipitated phase. Subsequent studies on α -CD/surfactant and α -CD/polymer inclusion complexes further revealed that α -CD molecules tend to stack continuously along the hydrophobic chain direction and assemble into channel-type crystalline structures stabilized by intermolecular hydrogen bonding⁷². Within such structures, the hydrophobic chains are extensively shielded by α -CD molecules, leading to a significant reduction in the hydrophobic interactions that would otherwise contribute to chain entanglement and dynamic network formation. Meanwhile, the inclusion complexes preferentially undergo parallel alignment and localized ordered packing,

rather than forming a three-dimensional crosslinked network throughout the system. Consequently, the system is more likely to generate localized columnar aggregates or microcrystalline domains instead of a supramolecular hydrogel possessing a continuous spatial network. This may represent an important reason why the α -CD/DDM system failed to exhibit macroscopic gelation even at a 2:1 feed ratio.

In the present study, neither DG (decyl glucoside) nor LG (lauryl glucoside) formed stable hydrogels in the presence of either α -CD or β -CD. Direct structural characterization of DG/LG–cyclodextrin inclusion complexes remains relatively limited in the current literature. Nevertheless, previous studies have demonstrated that α -CD tends to undergo host–guest inclusion interactions with linear alkyl chains⁷³, which may further assemble into threaded structures or channel-type aggregates⁷⁴.

However, the formation of stable supramolecular hydrogels depends not only on host–guest inclusion itself, but also on the subsequent establishment of a percolated network with long-range structural organization. The absence of macroscopic gelation in the DG and LG systems suggests that their interactions with α -CD or β -CD may be insufficient to drive the subsequent construction of a continuous supramolecular network.

This phenomenon may be related to the structural characteristics of alkyl polyglucoside molecules. Compared with conventional linear surfactants, the glucoside headgroups of DG and LG possess strong hydration capability and a certain degree of steric hindrance, which may restrict the effective threading and ordered stacking of α -CD along the hydrophobic alkyl chains, thereby hindering the formation of channel-type aggregates.

In addition, commercial alkyl polyglucosides are typically not single-component structures but instead exhibit a certain distribution of alkyl chain lengths and relatively high system polydispersity. They may also contain small amounts of unreacted fatty alcohols, oligosaccharides, by-products, and inorganic salts. Such impurities may further disrupt the ordered arrangement of supramolecular assemblies and weaken the ability of the system to form a continuous network structure.

During the experiments, the DG and LG systems also exhibited pronounced phase separation and irreversible aggregation behavior. Particularly under low-water-content conditions, although the viscosity of the systems increased significantly, large amounts of precipitates were still formed after centrifugation, and some samples could not recover their initial homogeneous state even after repeated vortex mixing. These observations indicate that the aggregates formed within the systems lacked sufficient dynamic reversibility.

Considering the relatively low purity of commercial-grade DG and LG, hydrophobic impurities or long-chain by-products present in the systems may have preferentially accumulated in the precipitated phase during centrifugation, leading to the formation of dense cake-like aggregates. Owing to the strong hydrophobic associations and localized adhesive interactions within these aggregates, they remained difficult to redisperse even after prolonged agitation, thereby causing partially irreversible sedimentation behavior in the systems.

As discussed previously, the formation of supramolecular hydrogels depends not only on the presence of host–guest interactions, but also on network percolation induced by cooperative intermolecular interactions. When supramolecular interactions preferentially promote localized

aggregation rather than long-range connectivity, the system generally exhibits precipitation or phase-separated aggregates instead of forming stable hydrogels ³³.

It is noteworthy that some DG and LG samples exhibited relatively high viscosity or even temporary non-flow behavior prior to centrifugation, yet regained fluidity after re-vortexing. This suggests that the systems may have formed only weak and discontinuous transient aggregates rather than stable network structures possessing sufficient crosslink density. Similar phenomena have also been reported in other cyclodextrin–surfactant systems, in which the systems may exist in an intermediate state between precipitation and gelation ⁷⁵. Their final phase behavior is therefore jointly governed by multiple factors, including molecular compatibility, concentration, and solvent ⁷⁶.

6.1.2 Formation of Stable Hydrogels in Polysorbate-Based Systems

In comparison to the α -DDM, β -DDM, DG, and LG systems, the polysorbate 20 and polysorbate 80 systems exhibited distinctly different behaviors. In particular, in the presence of α -cyclodextrin (α -CD), both polysorbate-based systems were capable of forming homogeneous gel systems without obvious phase separation, which further supports conclusions reported in previous studies.

Previous studies have demonstrated that increasing the concentration of α -cyclodextrin (α -CD) gradually enhances both the crosslinking density and the degree of polyrotaxane aggregation in polysorbate 80/ α -CD hydrogels. As a result, the systems exhibit faster gelation rates, higher mechanical strength, denser network structures, and more stable gel states. At the same time, the corresponding drug release rate and hydrogel degradation rate are reduced accordingly ⁷⁷.

Notably, α -CD/polysorbate 80 hydrogels exhibited superior mechanical properties compared with α -CD/polysorbate 20 hydrogels. The key factor underlying this difference lies in the distinct hydrophobic chain structures of polysorbate 20 and polysorbate 80. Polysorbate 20 mainly contains saturated laurate chains, whereas polysorbate 80 possesses longer oleate chains containing a *cis* double bond. The longer hydrophobic chains not only provide a larger threading region for α -CD inclusion, but also enhance intermolecular hydrophobic associations, thereby favoring the formation of supramolecular crosslinking networks with higher density ^{77,78}.

In addition, the stronger chain entanglement effect among the longer alkyl chains further improves the overall stability and mechanical strength of the hydrogel network.

On the other hand, the *cis* double bond present in polysorbate 80 molecules may also play an important role in the gelation process. As discussed previously, systems containing linear saturated chains, such as DDM, tend to form inclusion complexes that undergo regular packing and further develop into crystalline aggregates, ultimately leading to precipitation or localized phase separation. Based on existing studies, it can be inferred that the double bond in polysorbate 80 introduces chain bending and conformational irregularity, thereby hindering the close packing and crystallization tendency of the inclusion complexes ⁷⁴.

Consequently, the unsaturated structure of polysorbate 80 is more favorable for the formation of flexible and continuous supramolecular networks rather than rigid precipitated particles.

Furthermore, in both the polysorbate 20 and polysorbate 80 systems, α -CD and β -CD exhibited markedly different gelation behaviors. This difference is most likely related to the compatibility between the cavity size of the cyclodextrins and the hydrophobic chains of the polysorbate

molecules. Previous studies have shown that the relatively small cavity of α -CD is more suitable for accommodating hydrophobic chain segments, whereas when the cavity size increases to that of β -CD or γ -CD, the binding stability between the cyclodextrin and the alkyl chain decreases significantly, making the formation of stable gel systems more difficult ⁷⁷.

As a result, α -CD is more likely to form stable threaded structures that can further assemble into supramolecular crosslinked networks resembling “molecular necklaces.” In contrast, cyclodextrins with larger cavities exhibit poorer geometric compatibility with the hydrophobic chains of polysorbate molecules, leading to reduced threading efficiency and weaker intermolecular interactions. Consequently, such systems are more prone to localized aggregation or phase separation and are therefore less capable of forming stable hydrogels.

6.2 Stoichiometry and solubility behavior of α -CD/DDM supramolecular complexes

The NMR results show that both the α -CD/ α -DDM and α -CD/ β -DDM systems tend to form precipitated complexes with a limiting 2:1 stoichiometry (α -CD:surfactant). The measured stoichiometries changed slightly at different initial molar ratios. However, the precipitates moved closer to a 2:1 composition in all cases, especially when the initial molar ratio was 2:1. Previous studies have reported that α -CD commonly forms 2:1 inclusion complexes with surfactants containing medium-length alkyl chains ². Previous work has also shown that increasing the cyclodextrin concentration favours the formation of 2:1 host–guest complexes ⁷⁹. The behavior observed in this work is therefore consistent with the expected limiting stoichiometry for this type of system.

The samples prepared at initial molar ratios of 1:1 and 1:2 did not fully reach the limiting 2:1 stoichiometry. The lower amount of α -CD in these systems may reduce the formation of the preferred 2:1 complexes under the experimental conditions. The equilibrium between dissolved and precipitated species in solution may also affect the final composition of the precipitates.

Both the α -CD/ α -DDM and α -CD/ β -DDM systems showed the same limiting 2:1 stoichiometry. However, the calculated supernatant concentrations were clearly different. The α -CD/ β -DDM system showed higher residual concentrations of both α -CD and surfactant in the supernatant than the α -CD/ α -DDM system. The concentrations differed by approximately a factor of two between the two systems. These results show that the α -CD/ α -DDM and α -CD/ β -DDM complexes have different solubility behavior, even though they exhibit the same limiting stoichiometry. Small changes in surfactant configuration therefore appear to strongly affect the solubility of the resulting supramolecular complexes.

The calculated α -CD concentrations in the supernatants were also much lower than the reported aqueous solubility of α -CD (132 mM) ⁸⁰. In the presence of surfactant, the α -CD concentration decreased to about 10–20 mM, corresponding to a reduction of more than one order of magnitude.

6.3 Influence of Water Content and Molar Ratio on Network Formation

In numerous literature reports, water content is commonly characterized by equilibrium water content or swelling ratio and is regarded as a critical factor governing the mechanical properties and gelation behavior of hydrogels. Since hydrogels are intrinsically water-swollen network

structures, variations in their hydration state can directly influence key performance parameters, including modulus, toughness, and viscoelastic response.

Under conditions of high water content, the effective concentration of cyclodextrin–surfactant inclusion complexes becomes significantly diluted, resulting in a reduced density of supramolecular crosslinking points within the system. At the same time, the average intermolecular distance between adjacent inclusion complexes increases, which further limits the aggregation of these complexes into larger supramolecular assemblies and ordered packing structures. Although localized host–guest interactions may still occur, these relatively isolated supramolecular interactions are insufficient to generate a continuous percolated network structure and therefore cannot effectively support stable macroscopic gelation^{81–83}.

These observations indicate that water content not only influences the density of supramolecular interactions and the degree of network connectivity, but also ultimately determines the overall structural integrity and mechanical performance of the hydrogel system.

However, excessively low water content may also adversely affect the formation of supramolecular networks. In the present study, this was mainly manifested by the pronounced mixing inhomogeneity observed in low-water-content samples (e.g., water content $\leq 65\%$), as well as the significantly prolonged time required for the systems to reach a homogeneous and stable state. Previous studies on low-water-content gel systems have shown that high-concentration conditions can substantially increase system viscosity, thereby restricting molecular diffusion and hindering sufficient homogeneous mixing among the components. Under such conditions, rapid local aggregation may occur, leading to the formation of highly concentrated supramolecular domains rather than uniformly distributed continuous network structures.

Furthermore, the restricted molecular mobility may also impede further dynamic rearrangement within the system, making it more difficult to form thermodynamically stable and homogeneous supramolecular assemblies^{83,84}. Therefore, although reducing water content may strengthen intermolecular interactions and promote supramolecular aggregation, excessively high system concentrations may instead reduce the homogeneity of the network structure and impair the processability of the system.

It should be noted that the currently available literature mainly focuses on covalently crosslinked or polymer-crosslinked hydrogel systems, whereas the physically crosslinked non-covalent supramolecular hydrogels investigated in the present study may exhibit somewhat different behavior. Consequently, the above discussion regarding network formation under low-water-content conditions remains largely based on reasonable interpretations derived from existing theories and experimental observations. Systematic rheological and injectability studies on low-water-content systems have not yet been conducted in this work, and the underlying mechanisms therefore require further experimental validation in future studies.

On the other hand, the pronounced dependence of gelation behavior on the cyclodextrin/surfactant molar ratio further highlights the importance of stoichiometric balance in supramolecular assembly. Previous studies have shown that surfactants in aqueous solution intrinsically tend to form thermodynamically stable micelles or vesicles, while cyclodextrin inclusion complexation competes with this self-assembly process. When the cyclodextrin concentration is insufficient, surfactant molecules preferentially remain in their aggregated states. Only when the cyclodextrin concentration becomes sufficiently high can surfactant

molecules be effectively “extracted” from these aggregates to form stable host–guest inclusion complexes, thereby shifting the system from a micellization equilibrium toward a supramolecular complexation equilibrium^{70,85,86}.

Therefore, the differences in gelation behavior observed at different cyclodextrin/surfactant molar ratios essentially reflect the delicate thermodynamic balance between self-aggregation and host–guest complexation, while appropriate stoichiometric matching represents a key factor in constructing stable supramolecular networks. Among the three investigated ratios, the 2:1 molar ratio provided a relatively sufficient amount of cyclodextrin to enhance the probability of host–guest inclusion formation while still maintaining effective supramolecular organization. Such a balanced composition favors the formation of supramolecular crosslinking points with an appropriate density, thereby promoting the establishment of a stable and continuous network structure^{34,87}.

In contrast, when the cyclodextrin content was insufficient, as in the 1:2 system, the number of effective inclusion complexes that could be formed was limited, resulting in an inadequate degree of network connectivity.

6.4 Rheological Properties and Network Stability of Selected Hydrogels

6.4.1 α -CD/polysorbate 20 Hydrogel at a 1:1 Molar Ratio and 80% Water Content

For the α -CD–polysorbate 20 hydrogel with 80% water content, oscillatory rheological measurements demonstrated typical characteristics of a supramolecular gel. In the amplitude sweep tests, the storage modulus (G') remained consistently higher than the loss modulus (G''), indicating that the system was dominated by elastic behavior and suggesting the formation of a stable three-dimensional network structure within the hydrogel. Moreover, both moduli remained nearly constant within the low-strain region, reflecting good structural stability of the gel network. As the strain increased, G' gradually decreased, indicating that the applied external force progressively disrupted the non-covalent interactions within the system, thereby demonstrating the dynamic and reversible nature of the network.

The frequency sweep results further showed that G' remained higher than G'' throughout the entire tested frequency range, further confirming the existence of a stable gel structure. In addition, the moduli exhibited a certain degree of frequency dependence, suggesting that the network was primarily maintained by reversible host–guest interactions and hydrophobic associations rather than permanent covalent crosslinking. Such dynamic characteristics are advantageous for reversible structural rearrangement under external stress, thereby providing the basis for the injectability and self-recovery behavior of the hydrogel.

6.4.2 α -CD/polysorbate 80 Hydrogels with Three Different Molar Ratios and β -CD/polysorbate 80 Hydrogel at a 1:1 Molar Ratio and 80% Water Content

The rheological results of the three α -CD hydrogels with different molar ratios at 80% water content demonstrated that the cyclodextrin-to-surfactant molar ratio plays a crucial role in the formation of supramolecular networks. Among the tested formulations, the 2:1 α -CD : polysorbate 80 hydrogel exhibited the highest storage modulus and the most stable rheological behavior, indicating that a higher α -CD content is favorable for the formation of a denser host–guest crosslinked network. This may be attributed to the greater number of cyclodextrin molecules providing additional inclusion sites, thereby enhancing network connectivity and

improving gel stability. In contrast, when the proportion of polysorbate 80 was excessively high, the insufficient amount of cyclodextrin limited the formation of a fully interconnected three-dimensional network, resulting in lower moduli and weaker structural stability.

On the other hand, the differences observed between α -CD and β -CD further highlight the importance of cavity-size matching in hydrogel formation. The smaller cavity of α -CD exhibits better compatibility with the alkyl chains of polysorbate 80, enabling the formation of more stable host-guest inclusion structures and thereby promoting the construction of supramolecular networks. In contrast, the larger cavity of β -CD may weaken the inclusion interactions, leading to reduced network stability. Consequently, compared with β -CD, α -CD is more favorable for the formation of supramolecular hydrogels with higher elasticity and structural stability.

6.4.3 Pure α -CD/polysorbate 80 Hydrogel and Two Curcumin-Loaded Hydrogels at a 2:1 Molar Ratio and 70% Water Content

The above results demonstrate that the introduction of inclusion complexes significantly influences the network structure and dynamic behavior of supramolecular hydrogels. The pure hydrogel (i.e. the system without curcumin) exhibited the highest storage modulus and viscosity, indicating that, in the absence of curcumin, α -CD and polysorbate 80 were able to form a more complete and densely crosslinked supramolecular network.

In contrast, the inclusion-complex-loaded systems comprising curcumin exhibited reduced moduli, suggesting that the presence of the complexes partially interfered with the host-guest interactions between cyclodextrin and the surfactant, thereby decreasing the degree of network crosslinking. Among these systems, the 1:1 complex hydrogel showed the lowest G' and viscosity, indicating that it induced the greatest disruption to the original network structure.

However, although the mechanical strength of the 1:3 complex hydrogel was lower than that of the pure hydrogel, it exhibited the highest recovery ratio, indicating superior dynamic reversibility. This suggests that an appropriate proportion of inclusion complexes may enhance the reconfigurability of non-covalent interactions within the network, enabling the system to recover its structure more rapidly after high-shear disruption. Consequently, although the 1:3 complex hydrogel possessed lower overall rigidity, it demonstrated improved structural recovery capability and dynamic responsiveness.

Overall, the pure hydrogel tended to form a high-strength and stable supramolecular network, whereas the inclusion-complex-loaded systems exhibited more pronounced dynamic characteristics. Among them, the 1:3 complex hydrogel achieved a favorable balance between structural stability and recovery performance, which is of considerable importance for the application of injectable supramolecular hydrogels.

6.5 Curcumin Release and Injectable Performance of the Hydrogel System

6.5.1 In Vitro Release Assay

In this study, the release concentration profiles and cumulative release percentage profiles exhibited different trends. This discrepancy mainly arises from the substantial difference in the theoretical drug-loading capacities of the two inclusion-complex hydrogel systems: the total drug loading of the 1:1 complex hydrogel (169 mg) was significantly higher than that of the 1:3 complex hydrogel (43 mg). Accordingly, the release concentration (mg/L) reflects the absolute amount of curcumin released into a unit volume of release medium, whereas the cumulative

release percentage is normalized to the total drug-loading amount and therefore more accurately represents the intrinsic release efficiency and release behavior of the system. Consequently, when comparing the release performance of different hydrogel systems, analyses based solely on release concentration may be strongly influenced by differences in initial drug loading, while cumulative release percentage is more appropriate as the primary parameter for evaluating and comparing release behaviors among different hydrogel systems. Previous studies have demonstrated that the structural characteristics and mechanical properties of hydrogel networks can significantly influence drug release kinetics⁸⁸. The release of bioactive compounds encapsulated within hydrogels is generally governed by two coupled processes: the restricted diffusion of drug molecules through the hydrogel network and the erosion or structural evolution of the hydrogel itself³⁹. In addition, the dynamic crosslinking interactions present in supramolecular hydrogels may lead to time-dependent variations in network pore size, meaning that drug release behavior is also strongly affected by the strength of intermolecular interactions within the system⁸⁹.

According to the cumulative release results, the 1:3 complex hydrogel consistently exhibited a higher release percentage than the 1:1 complex hydrogel throughout the entire 48 h period, indicating a higher drug release efficiency relative to its own loading capacity. However, the cumulative release percentages of both systems remained relatively low within 48 h, with the overall release not exceeding 2%, suggesting that the both the diffusion of curcumin within the hydrogel network and the erosion of the gel were strongly restricted. Compared with the relatively slow release observed during the initial 1-24 h period, the 1:3 complex hydrogel exhibited a pronounced increase in cumulative release during the 24-48 h interval, rising from 0.39% to 1.95%. This behavior may be attributed to a relatively greater extent of surface erosion in the hydrogel during the later stage of release, which facilitated the diffusion and release of curcumin entrapped within the interior of the hydrogel network.

In the present study, the host–guest inclusion interactions formed between α -CD and the surfactants may have generated a relatively stable and dense three-dimensional supramolecular network, thereby restricting the migration and diffusion of curcumin within the hydrogel matrix to a certain extent. Furthermore, owing to the intrinsic hydrophobicity of curcumin, the molecule is more likely to associate with the hydrophobic domains inside the hydrogel rather than partition into the relatively polar release medium, which further reduces its diffusion and release rate into the external medium.

6.5.2 Injectability test

F_{max}

The $F_{\square_{ax}}$ results obtained from the first injection cycle showed that, compared with the empty syringe group containing only an air column, syringes loaded with hydrogel samples exhibited lower initial peak extrusion forces. This observation suggests that the presence of the hydrogel may reduce the interfacial frictional resistance between the plunger and the syringe barrel, thereby facilitating more uniform pressure transmission and smoother plunger movement. According to the Hagen–Poiseuille law, the flow resistance is directly proportional to the fluid viscosity (μ).

Theoretically, according to the Hagen–Poiseuille law, flow resistance is directly proportional to the dynamic viscosity of the fluid⁹⁰. Since the dynamic viscosity of air is substantially lower

than that of viscous sample systems, the air column experiences considerably lower flow resistance within the syringe and can therefore be expelled more readily ⁹¹.

Quite surprisingly, the present study exhibited the opposite trend. In addition to the potential lubricating effect of the samples, this behavior may also be associated with the compressibility of air and its relatively poor stress transmission capability ^{92,93}.

Compressibility refers to the ability of a material to undergo volume changes under applied pressure. Air is a highly compressible medium, whereas hydrogels, owing to their continuous liquid phase, are generally regarded as approximately incompressible systems. The differences in their mechanical responses to applied stress can directly influence the establishment and transmission efficiency of pressure during the injection process.

During the initial stage of injection, when an air column is present within the system, part of the energy applied by the plunger is first consumed in compressing the air rather than being immediately converted into effective pressure for fluid displacement, thereby generating a pressure lag phenomenon. Consequently, a higher instantaneous force is required to initiate the extrusion process, resulting in a higher F_{ax} value. In contrast, a continuous hydrogel phase can transmit pressure more efficiently and establish a stable flow state more rapidly, thereby reducing initial pressure fluctuations and localized stress concentration, which ultimately leads to a lower F_{ax} ⁹².

For the F_{ax} results obtained during the subsequent continuous injection cycles, the same sample exhibited pronounced fluctuations between different cycles, and no clear monotonic trend was observed. Combined with the intermittent extrusion behavior observed during the experiments, these findings suggest that the hydrogel systems may possess considerable structural heterogeneity under continuous shear conditions ^{94,95}. One important factor contributing to local flow instability may be the introduction of air bubbles during sample preparation, transfer, and syringe loading processes ^{93,96,97}. However, beyond the influence of entrapped air bubbles, supramolecular hydrogels themselves possess dynamic and reversible network structures. Under externally applied shear stress, the hydrogel network may continuously undergo localized disruption, reorganization, and alterations in flow pathways, thereby further amplifying the fluctuations in extrusion force.

Furthermore, considering the shear-thinning behavior observed in the rheological measurements, the viscosity of the system would theoretically be expected to decrease progressively during continuous injection, which should consequently lead to a gradual reduction in F_{ax} . However, no obvious decreasing trend was observed in the present study, suggesting that dynamic structural rearrangement and locally heterogeneous flow behavior may have masked the mechanical reduction effect arising solely from shear thinning.

DGF

It should be noted that, owing to the pronounced shear-responsive nature of supramolecular hydrogels, the data obtained from consecutive injection cycles cannot be strictly regarded as independent replicates, since each extrusion process may theoretically alter the internal network structure of the system. Therefore, the mean values and error bars presented in *Figure 5.6.4.3* are intended primarily to provide a visual representation of the extent of data fluctuation, rather than serving as statistical replicates in the conventional sense.

Compared with the empty syringe group, which contained only an air column and therefore possessed a relatively homogeneous internal structure, all hydrogel sample groups exhibited markedly greater data dispersion. This observation further suggests the presence of substantial structural heterogeneity and localized flow instability within the hydrogel systems. In addition to air bubbles introduced during the experimental process, the continuous localized disruption and reorganization of the supramolecular network under shear conditions may also contribute to the pronounced fluctuations in DGF observed between different injection cycles ⁹⁴.

Furthermore, the average DGF values of the Pure hydrogel and 1:3 complex hydrogel groups were both higher than that of the Empty syringe group, which is consistent with theoretical expectations that the presence of sample materials increases the flow resistance that must be overcome during continuous extrusion. In contrast, the average DGF value of the 1:1 complex hydrogel was lower than that of the Empty syringe group, which may indicate that this system undergoes network relaxation and structural collapse more readily under continuous shear conditions, thereby reducing the sustained extrusion resistance required during the subsequent dynamic sliding stage ⁹⁶. This observation further suggests that different inclusion ratios may exert distinct influences on the stability and shear-responsive behavior of the supramolecular network.

Total work

Previous studies have suggested that, compared with individual parameters such as $F_{\square_{ax}}$ or DGF, the total work provides a more comprehensive representation of the overall flow resistance encountered during the injection process and is therefore considered a more appropriate parameter for evaluating injectable performance ⁵². The results of the present study showed that, during the first injection cycle, the empty syringe group required the lowest total work, indicating that the overall energy consumption associated with expelling only the air column was minimal, which is consistent with theoretical expectations.

In contrast, all three hydrogel-containing systems exhibited higher total work values, further demonstrating that the presence of the samples increased the overall flow resistance that needed to be overcome during the injection process. Among them, the pure hydrogel group showed a higher total work than the empty syringe group, indicating that the presence of a continuous hydrogel phase increased the viscous resistance and energy consumption associated with flow.

Furthermore, the 1:1 complex hydrogel group exhibited the highest total work, which may indicate that the introduction of inclusion complexes comprising curcumin enhanced the supramolecular interactions within the system, thereby increasing the complexity of the network structure and the associated flow resistance. The host–guest interactions formed between α -CD and the surfactants may generate a more compact localized three-dimensional network, such that greater energy is required during injection to disrupt and reorganize the network structure.

However, despite containing a higher proportion of α -CD, the 1:3 complex hydrogel group exhibited a slightly lower total work than the 1:1 complex hydrogel group, with values even approaching those of the pure hydrogel group. This observation suggests that the system may undergo structural relaxation and flow-induced reorganization more effectively under shear conditions, thereby reducing the overall energy consumption required for extrusion.

These findings further indicate that, for supramolecular hydrogels, the dynamic reversibility and structural homogeneity of the network may exert a more significant influence on injectable performance than network strength alone.

In the total work results obtained from consecutive injection cycles, the empty syringe group exhibited relatively small fluctuations and maintained a comparatively stable level throughout the experiments. This indicates that the air-column system possessed a homogeneous internal structure and did not undergo significant structural changes during continuous extrusion, resulting in relatively stable energy consumption.

In contrast, all hydrogel-containing systems exhibited more pronounced fluctuations, indicating that the supramolecular hydrogels underwent complex dynamic structural responses under continuous shear conditions. Among them, the pure hydrogel group showed a marked increase in total work during the initial injection cycles, reaching a maximum at the fourth cycle before gradually decreasing thereafter.

This behavior may be associated with the progressive rearrangement of the internal hydrogel network during shear. In the initial stage, continuous shear may induce localized network compression and alterations in flow pathways, thereby temporarily increasing the extrusion resistance. However, as shear continued, the system gradually exhibited shear-thinning behavior, during which the internal network was progressively disrupted and reorganized, leading to a subsequent reduction in total work.

For the 1:1 complex hydrogel group, the total work exhibited an overall increasing trend with increasing injection cycles, but no clear tendency toward stabilization or equilibrium was observed. At the same time, noticeable fluctuations were present between different cycles. Combined with the discontinuous extrusion behavior observed during the experiments, these fluctuations are more likely to originate primarily from the interference of entrapped air bubbles during injection rather than being solely caused by changes in the internal hydrogel network structure. The presence of air bubbles can alter local flow resistance, thereby affecting the instantaneous extrusion pressure and the corresponding energy consumption.

In contrast, although the 1:3 complex hydrogel group also exhibited certain fluctuations, the overall variation was relatively smaller, and the total work remained within a comparatively stable range, indicating more stable flow behavior during continuous injection. It should be noted that the absolute α -CD content was identical in both the 1:1 and 1:3 systems, with the only difference being the ratio between α -CD and curcumin. Therefore, beyond the influence of entrapped air bubbles, the differences observed between the two groups are more likely attributable to the effects of host-guest assembly ratios on the structure and homogeneity of the supramolecular network, rather than simply resulting from an increase in α -CD content.

Overall, in the injectability experiments, the presence of air bubbles during injection led to intermittent extrusion behavior, resulting in considerable data fluctuation and limited reproducibility. These fluctuations were mainly associated with the compressibility of the entrapped gas, whereas the hydrogel itself can be regarded as an approximately incompressible medium and therefore remains capable of transmitting stress relatively efficiently.

Despite the presence of certain random disturbances, hydrogels with different formulation ratios still exhibited distinct overall flow behaviors during consecutive injection cycles, indicating that total work can, to some extent, reflect the structural stability and flow

characteristics of the systems. Taken together, compared with individual instantaneous force parameters, total work provides a more comprehensive characterization of the overall injectable performance and structural response of supramolecular injectable hydrogels during continuous extrusion.

For the *in vitro* release and injectability studies, the primary objective of this work was to investigate and compare the distinct properties of hydrogels prepared with different curcumin- α -cyclodextrin inclusion ratios, particularly with respect to drug release behavior and injection performance. At present, studies focusing on the influence of API-cyclodextrin inclusion ratios on the injectability of supramolecular hydrogels remain relatively limited. Existing research has mainly concentrated on drug release behavior, inclusion stability, and rheological characterization, whereas systematic investigations into practical injectability during continuous extrusion are comparatively scarce.

Previous studies have examined the effects of varying inclusion ratios on solubility differences⁶⁹, or have indirectly inferred potential injection behavior through rheological measurements^{98,99}. Moreover, current investigations involving different cyclodextrin/API inclusion ratios have primarily focused on drug loading and release kinetics, with relatively limited discussion regarding their influence on total work or flow stability during continuous extrusion processes¹⁰⁰.

Therefore, the findings of the present study contribute to the understanding of the relationship between API-cyclodextrin supramolecular assembly ratios and practical injection performance. The results further suggest that total work may serve as an important reference parameter for evaluating the continuous injection behavior of injectable supramolecular hydrogels.

7 Conclusion

This study systematically investigated supramolecular hydrogel systems constructed from nonionic surfactants and cyclodextrins, with the aim of exploring their potential applications as injectable carriers for hydrophobic drug delivery. Two classes of structurally distinct nonionic surfactants were selected, including polysorbates (polysorbate 20 and polysorbate 80) and alkyl glycosides (α -DDM, β -DDM, DG, and LG), which were combined with α - and β -cyclodextrins to construct supramolecular systems. Their gelation behavior, phase stability, rheological properties, injectability, and curcumin release behavior were systematically evaluated.

The experimental results demonstrated that the molecular structures of both the surfactants and cyclodextrins played decisive roles in supramolecular assembly and hydrogel formation. The alkyl glycoside systems exhibited pronounced phase separation or precipitation after centrifugation and failed to form stable hydrogel structures. NMR analysis of the α -CD/DDM system suggested that the strong host-guest interactions between the hydrophobic chains of DDM and α -CD favored the formation of dense aggregated structures rather than continuous three-dimensional networks. These findings indicate that excessively strong localized interactions may restrict molecular mobility within the system, thereby promoting precipitation instead of stable gel formation.

In contrast, systems constructed from polysorbate 20 or polysorbate 80 and α -CD exhibited markedly different behaviors. Under appropriate compositional conditions, particularly at relatively high α -CD/polysorbate 80 ratios and lower water contents, stable supramolecular

hydrogels could be successfully formed. Rheological measurements demonstrated that these hydrogels possessed typical viscoelastic characteristics, with the storage modulus (G') remaining consistently higher than the loss modulus (G'') over a broad frequency range, indicating the formation of stable three-dimensional supramolecular network structures within the systems.

Further investigations revealed that the polysorbate 80/ α -CD hydrogels exhibited pronounced shear-thinning behavior and thixotropic recovery properties. Under externally applied shear stress, the gels temporarily transformed into flowable states, while the gel structures were able to recover after removal of the shear force. Such dynamically reversible rheological behavior is highly advantageous for injectable hydrogel applications, as it enables smooth syringe extrusion while allowing the material to reform stable gel structures after injection, thereby facilitating local retention and structural support.

In the drug delivery studies, curcumin-loaded supramolecular hydrogels were further constructed to evaluate the feasibility of these systems as carriers for hydrophobic drugs. The results demonstrated that the hydrogels were capable of effectively encapsulating curcumin–cyclodextrin inclusion complexes and achieving sustained drug release. Different curcumin-to-cyclodextrin inclusion ratios influenced the drug release behavior, indicating that the internal supramolecular structures and host–guest interactions of the systems further affected drug diffusion and release kinetics.

Overall, this study demonstrates that supramolecular hydrogels formed through host–guest interactions between nonionic surfactants and cyclodextrins possess promising potential for localized injectable drug delivery applications. Furthermore, the findings highlight that achieving an appropriate balance between intermolecular interaction strength and molecular mobility is crucial for supramolecular hydrogel formation. Excessively strong localized aggregation tends to induce precipitation, whereas moderate and dynamically reversible supramolecular interactions are more favorable for the formation of stable and injectable hydrogel networks.

Although the present work mainly focused on system construction and physicochemical characterization, the findings provide an important foundation for the future development of cyclodextrin–surfactant supramolecular biomaterials. Further investigations are still required to better understand the supramolecular structures, long-term stability, biodegradability, cytocompatibility, and in vivo therapeutic performance of these systems, in order to further evaluate their practical biomedical application potential.

8 Future work

8.1 Further Investigation of Rheological Properties and Gelation Behavior

In future studies, the number of rheological measurements could be increased to improve the reliability and statistical significance of the experimental results. In addition, for injectability evaluation, parameters beyond peak injection force, such as the total work of injection, should be incorporated to provide a more comprehensive analysis of hydrogel flow behavior during the injection process.

Regarding gelation behavior, this study investigated several glycoside-based nonionic surfactants, including α -DDM, β -DDM, LG, and DG; however, none of these systems formed ideal cyclodextrin-based hydrogels or exhibited the expected gelation characteristics. Future work could further explore the reasons underlying the failure of gel formation, such as insufficient host–guest inclusion interactions, the influence of hydrophobic chain structures, or differences in microscopic assembly behavior. Moreover, optimization strategies including adjustment of molar ratios, water content, temperature, or the introduction of synergistic components may help improve system performance and facilitate the development of novel hydrogels with enhanced mechanical properties.

8.2 Further Optimization of the Final Candidate Hydrogel System

In the present study, the α -CD/polysorbate 80 hydrogel with 70% water content and a 2:1 molar ratio was selected as the final candidate system and subjected to injectability and in vitro release studies. However, hydrogels with the same composition but lower water contents were not further investigated because homogeneous mixing during sample preparation was difficult to achieve.

Future studies could employ approaches such as inversion centrifuge mixing tubes or other advanced homogenization methods to improve the mixing uniformity of low-water-content samples. This would enable more systematic investigations of their rheological behavior, mechanical properties, and injectability performance, thereby providing a more comprehensive understanding of α -CD/polysorbate 80 hydrogel systems.

8.3 Optimization of the In Vitro Release System

The current in vitro release experiments also allow room for further optimization. First, the release testing method itself could be improved, for example by employing a shaking-incubator system to provide a more stable and reproducible release environment.

In addition, due to the limited aqueous solubility of curcumin, the present study employed PBS containing 5% ethanol as the release medium to facilitate analytical detection. However, the addition of ethanol may not accurately simulate the in vivo environment and could potentially disrupt the hydrogel structure, thereby influencing release behavior. Therefore, future work should aim to establish release systems that more closely resemble the postoperative tumor microenvironment and physiological body fluids, enabling more realistic simulation of curcumin release behavior in vivo and improving the physiological relevance of the experimental results.

8.4 Further Studies on Injectability Performance

There remains substantial room for improvement regarding injectability evaluation under application-relevant conditions.

First, although efforts were made to minimize the presence of bubbles and air columns during sample transfer into syringes, discontinuous extrusion behavior was still observed during testing, indicating that entrapped air remained within the systems, particularly in the inclusion-complex-loaded hydrogels. Future studies could consider centrifugation-assisted degassing after syringe loading, as well as the use of syringe connectors during sample transfer, in order to minimize the formation of air columns between the sample and plunger and thereby improve the stability and accuracy of injection measurements.

Second, the injectability tests performed in this study were conducted without attached needles, and therefore did not account for the additional resistance introduced during actual injection through a needle. When the sample flows from the syringe barrel into the needle, both the contact materials and the internal flow diameter change, resulting in more complex injection resistance behavior. Consequently, future studies should incorporate clinically relevant needles into the testing setup to better simulate practical injection conditions.

Furthermore, considering the local pressure variations that may occur during postoperative cavity injection, future investigations could also involve appropriate animal models to more comprehensively evaluate the injection behavior and distribution of hydrogels under realistic physiological conditions.

9 References

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10 Appendices

10.1 Cyclodextrin-Nonionic Surfactant-Water Sample Preparation

10.1.1 α -DDM/ α -CD/ H_2O

Table 10.1.1.1. Raw data for the preparation of α -DDM/ α -CD/ H_2O systems.

90% Water Content				
Molar ratio	α -CD (g)	20% α -CD solution (g)	α -DDM (g)	Extra H_2O (g)
1 α -CD	0.131	-	0.069	1.8
1 α -DDM	0.131		0.069	1.8021
1 α -CD	0.098	0.49	0.102	1.408
2 α -DDM	0.09762	0.4881	0.102	1.4253
2 α -CD	0.158	0.79	0.042	1.168
1 α -DDM	0.15796	0.7898	0.042	1.1692
80% Water Content				
Molar ratio	α -CD (g)	20% α -CD solution (g)	α -DDM (g)	Extra H_2O (g)
1 α -CD	0.262	-	0.138	1.6
1 α -DDM	0.2629		0.1378	1.6051
1 α -CD	0.195	0.975	0.205	0.82
2 α -DDM	0.19474	0.9737	0.2054	0.8192
2 α -CD	0.317	1.585	0.083	0.332
1 α -DDM	0.32494	1.6247	0.829	0.3382

Note: The black numbers indicate the theoretical amounts calculated according to the formulation design, whereas the blue numbers indicate the actual amounts weighed and added during sample preparation.

10.1.2 β -DDM/ α -CD/ H_2O

Table 10.1.2.1. Raw data for the preparation of β -DDM/ α -CD/ H_2O systems.

90% Water Content				
Molar ratio	α -CD (g)	20% α -CD solution (g)	β -DDM (g)	Extra H_2O (g)
1 α -CD	0.131	-	0.069	1.8
1 β -DDM	0.1322		0.0689	1.81
1 α -CD	0.098	0.49	0.102	1.408
2 β -DDM	0.09754	0.4877	0.1016	1.4054
2 α -CD	0.158	0.79	0.042	1.168
1 β -DDM	0.16306	0.8153	0.0431	1.1718
80% Water Content				
Molar ratio	α -CD (g)	20% α -CD solution (g)	β -DDM (g)	Extra H_2O (g)
1 α -CD	0.262	-	0.138	1.6
1 β -DDM	0.262		0.138	1.609
1 α -CD	0.195	0.975	0.205	0.82
2 β -DDM	0.19652	0.9826	0.2063	0.8195
2 α -CD	0.317	1.585	0.083	0.332
1 β -DDM	0.31564	1.5782	0.0834	0.3316

Note: The black numbers indicate the theoretical amounts calculated according to the formulation design, whereas the blue numbers indicate the actual amounts weighed and added during sample preparation.

10.1.3 DG/ α -CD/ H_2O

Table 10.1.3.1. Raw data for the preparation of DG/ α -CD/ H_2O systems.

90% Water Content				
Molar ratio	α -CD (g)	20% α -CD solution (g)	DG (g)	Extra H_2O (g)
1 α -CD	0.149	0.745	0.051	1.204
1DG	0.14918	0.7459	0.0512	1.2106

1 α -CD	0.118	0.590	0.082	1.328
2DG	0.1199	0.5995	0.0819	1.3322
2 α -CD	0.171	0.855	0.029	1.116
1DG	0.17774	0.8887	0.0289	1.1141
80% Water Content				
Molar ratio	α -CD (g)	20% α -CD solution (g)	DG (g)	Extra H ₂ O (g)
1 α -CD	0.297	1.485	0.103	0.412
1DG	0.30056	1.5028	0.1028	0.4142
1 α -CD	0.237	1.185	0.163	0.652
2DG	0.23694	1.1847	0.1628	0.653
2 α -CD	0.341	1.705	0.059	0.236
1DG	0.34104	1.7052	0.0593	0.2354

Note: The black numbers indicate the theoretical amounts calculated according to the formulation design, whereas the blue numbers indicate the actual amounts weighed and added during sample preparation.

10.1.4 DG/ β -CD/H₂O

Table 10.1.4.1. Raw data for the preparation of DG/ β -CD/H₂O systems.

90% Water Content				
Molar ratio	β -CD (g)	20% β -CD solution (g)	DG (g)	Extra H ₂ O (g)
1 β -CD	0.154	0.77	0.046	1.184
1DG	0.15722	0.7861	0.0461	1.1855
1 β -CD	0.126	0.63	0.074	1.296
2DG	0.12758	0.6379	0.0741	1.291
2 β -CD	0.174	0.87	0.026	1.104
1DG	0.17608	0.8804	0.0257	1.1063
80% Water Content				

Molar ratio	β -CD (g)	20% β -CD solution (g)	DG (g)	Extra H ₂ O (g)
1 β -CD	0.309	1.545	0.091	0.364
1DG	0.3084	1.5420	0.0906	0.3631
1 β -CD	0.251	1.255	0.149	0.596
2DG	0.25084	1.2542	0.1490	0.5946
2 β -CD	0.348	1.740	0.052	0.208
1DG	0.3538	1.769	0.0517	0.2044

Note: The black numbers indicate the theoretical amounts calculated according to the formulation design, whereas the blue numbers indicate the actual amounts weighed and added during sample preparation.

10.1.5 LG/ α -CD/H₂O

Table 10.1.5.1. Raw data for the preparation of LG/ α -CD/H₂O systems.

90% Water Content				
Molar ratio	α -CD (g)	20% α -CD solution (g)	LG (g)	Extra H ₂ O (g)
1 α -CD	0.145	0.725	0.055	1.220
1 LG	0.1552	0.776	0.0549	1.2295
1 α -CD	0.114	0.570	0.086	1.344
2 LG	0.1145	0.5723	0.0863	1.3434
2 α -CD	0.168	0.840	0.032	1.128
1 LG	0.17046	0.8523	0.032	1.1239
80% Water Content				
Molar ratio	α -CD (g)	20% α -CD solution (g)	LG (g)	Extra H ₂ O (g)
1 α -CD	0.29	1.450	0.110	0.440
1 LG	0.2899	1.4496	0.1098	0.4433
1 α -CD	0.227	1.135	0.173	0.692
2 LG	0.232	1.160	0.1733	0.6940

2 α -CD	0.336	1.680	0.064	0.256
1 LG	0.3399	1.6995	0.064	0.2593

Note: The black numbers indicate the theoretical amounts calculated according to the formulation design, whereas the blue numbers indicate the actual amounts weighed and added during sample preparation.

10.1.6 LG/ β -CD/H₂O

Table 10.1.6.1. Raw data for the preparation of LG/ β -CD/H₂O systems.

90% Water Content				
Molar ratio	β -CD (g)	20% β -CD solution (g)	LG (g)	Extra H ₂ O (g)
1 β -CD	0.145	0.725	0.055	1.22
1LG	0.1469	0.7343	0.0555	1.2368
1 β -CD	0.114	0.570	0.086	1.344
2LG	0.11608	0.5804	0.0863	1.3467
2 β -CD	0.168	0.84	0.032	1.128
1LG	0.1672	0.8360	0.0322	1.1228
80% Water Content				
Molar ratio	β -CD (g)	20% β -CD solution (g)	LG (g)	Extra H ₂ O (g)
1 β -CD	0.29	1.45	0.11	0.44
1LG	0.29024	1.4512	0.113	0.4315
1 β -CD	0.227	1.135	0.173	0.692
2LG	0.22676	1.1338	0.1746	0.7063
2 β -CD	0.336	1.68	0.064	0.256
1LG	0.3357	1.6785	0.0639	0.2591

Note: The black numbers indicate the theoretical amounts calculated according to the formulation design, whereas the blue numbers indicate the actual amounts weighed and added during sample preparation.

10.1.7 polysorbate 20/ α -CD/H₂O

Table 10.1.7.1. Raw data for the preparation of polysorbate 20/ α -CD/H₂O systems.

90% Water Content				
Molar ratio	α -CD (g)	20% α -CD solution (g)	polysorbate 20 (g)	Extra H ₂ O (g)
1 α -CD	0.0886	0.443	0.112	1.446
1polysorbate 20	0.0891	0.4455	0.1141	1.4497
1 α -CD	0.0568	0.284	0.143	1.573
2polysorbate 20	0.05794	0.2897	0.1433	1.580
2 α -CD	0.1226	0.613	0.077	1.310
1polysorbate 20	0.12458	0.6229	0.0771	1.3147
80% Water Content				
Molar ratio	α -CD (g)	20% α -CD solution (g)	polysorbate 20 (g)	Extra H ₂ O (g)
1 α -CD	0.177	0.885	0.223	0.892
1polysorbate 20	0.17848	0.8924	0.2262	0.8948
1 α -CD	0.114	0.57	0.286	1.144
2polysorbate 20	0.11748	0.5874	0.2858	1.1532
2 α -CD	0.245	1.225	0.155	0.62
1polysorbate 20	0.24626	1.2313	0.1563	0.6226

Note: The black numbers indicate the theoretical amounts calculated according to the formulation design, whereas the blue numbers indicate the actual amounts weighed and added during sample preparation.

10.1.8 polysorbate 20/ β -CD/H₂O

Table 10.1.8.1. Raw data for the preparation of polysorbate 20/ β -CD/H₂O systems.

90% Water Content				
Molar ratio	β -CD (g)	20% β -CD solution (g)	polysorbate 20 (g)	Extra H ₂ O (g)

1 β -CD	0.0962	0.481	0.104	1.415
1polysorbate 20	0.0962	0.481	0.1078	1.5558
1 β -CD	0.0632	0.316	0.137	1.547
2polysorbate 20	0.06362	0.3181	0.1373	1.5381
2 β -CD	0.13	0.65	0.07	1.28
1polysorbate 20	0.13106	0.6553	0.0838	1.2926
80% Water Content				
Molar ratio	β -CD (g)	20% β -CD solution (g)	polysorbate 20 (g)	Extra H ₂ O (g)
1 β -CD	0.192	0.96	0.208	0.832
1polysorbate 20	0.19392	0.9696	0.2048	0.8443
1 β -CD	0.126	0.63	0.274	1.096
2polysorbate 20	0.12742	0.6371	0.2746	1.0832
2 β -CD	0.26	1.3	0.14	0.56
1polysorbate 20	0.2625	1.3125	0.1455	0.5698

Note: The black numbers indicate the theoretical amounts calculated according to the formulation design, whereas the blue numbers indicate the actual amounts weighed and added during sample preparation.

10.1.9 polysorbate 80/ α -CD/H₂O

Table 10.1.9.1. Raw data for the preparation of polysorbate 80/ α -CD/H₂O systems.

90% Water Content				
Molar ratio	α -CD (g)	20% α -CD solution (g)	polysorbate 80 (g)	Extra H ₂ O (g)
1 α -CD	0.0852	0.426	0.115	1.459
1polysorbate 80	0.0858	0.429	0.1154	1.4550
1 α -CD	0.0554	0.277	0.145	1.579
2polysorbate 80	0.05902	0.2951	0.1520	1.5730

2 α -CD	0.1194	0.597	0.081	1.322
1polysorbate 80	0.11914	0.5957	0.0825	1.3337
80% Water Content				
Molar ratio	α -CD (g)	20% α -CD solution (g)	polysorbate 80 (g)	Extra H ₂ O (g)
1 α -CD	0.1704	0.852	0.23	0.918
1polysorbate 80	0.17124	0.8562	0.2293	0.9195
1 α -CD	0.1106	0.553	0.289	1.158
2polysorbate 80	0.11062	0.5531	0.2945	1.1730
2 α -CD	0.2388	1.194	0.161	0.645
1polysorbate 80	0.2386	1.193	0.1668	0.6508

Note: The black numbers indicate the theoretical amounts calculated according to the formulation design, whereas the blue numbers indicate the actual amounts weighed and added during sample preparation.

10.1.10

polysorbate 80/ β -CD/ H₂O

p

Table 10.1.10.1. Raw data for the preparation of polysorbate 80/ β -CD/H₂O systems.

90% Water Content				
Molar ratio	β -CD (g)	20% β -CD solution (g)	polysorbate 80 (g)	Extra H ₂ O (g)
1 β -CD	0.0928	0.464	0.107	1.429
1polysorbate 80	0.0928	0.4640	0.1051	1.4262
1 β -CD	0.0632	0.316	0.137	1.547
2polysorbate 80	0.0644	0.322	0.1393	1.5427
2 β -CD	0.1268	0.634	0.073	1.293
1polysorbate 80	0.1284	0.642	0.077	1.3111
80% Water Content				

Molar ratio	β -CD (g)	20% β -CD solution (g)	polysorbate 80 (g)	Extra H ₂ O (g)
1 β -CD 1polysorbate 80	0.1856 0.18596	0.928 0.9298	0.214 0.2137	0.858 0.8586
1 β -CD 2polysorbate 80	0.1264 0.1296	0.632 0.648	0.274 0.2783	1.094 1.1080
2 β -CD 1polysorbate 80	0.2536 0.25292	1.268 1.2646	0.146 0.1448	0.586 0.5958

Note: The black numbers indicate the theoretical amounts calculated according to the formulation design, whereas the blue numbers indicate the actual amounts weighed and added during sample preparation.

10.2 Supplementary Graph from Rheological Test

10.2.1 2 α -CD, 1polysorbate80: 70% Pure hydrogel, 1-1 complex hydrogel, 1-3 complex hydrogel

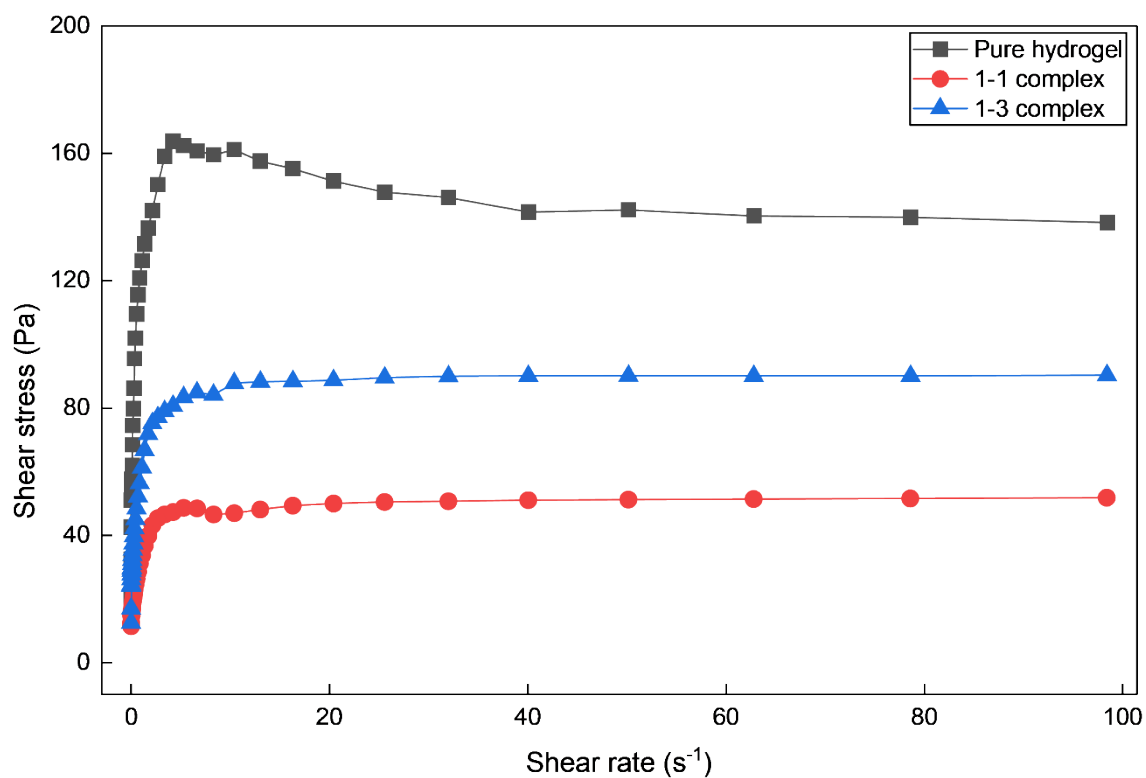


Figure 10.2.1.1. Flow curves (shear stress vs shear rate) of different hydrogels.

10.3 Raw Data and Relative Figures of In Vitro Release Assay

10.3.1 Full-wavelength scanning results and selection of the detection wavelength

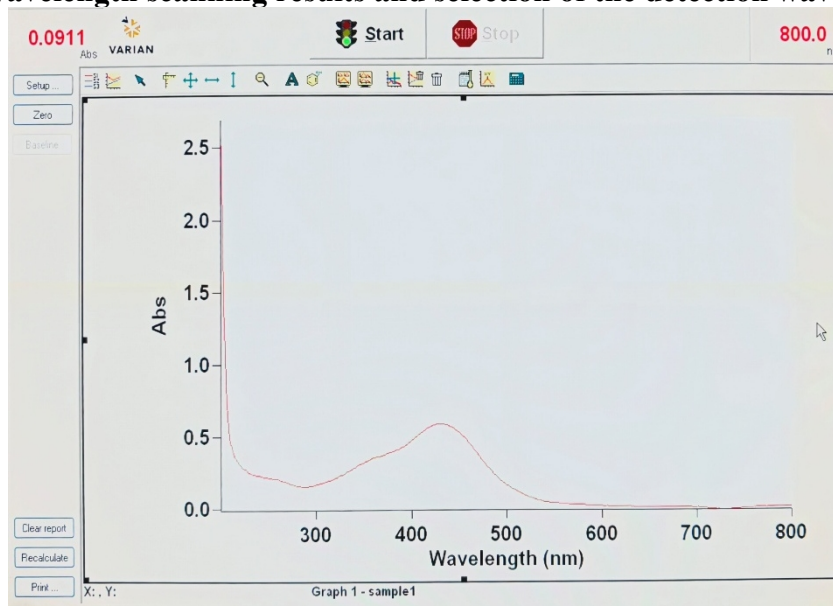


Figure 10.3.1.1. Absorption spectrum obtained from the full-wavelength scan of the 7.4 mg/mL stock solution.

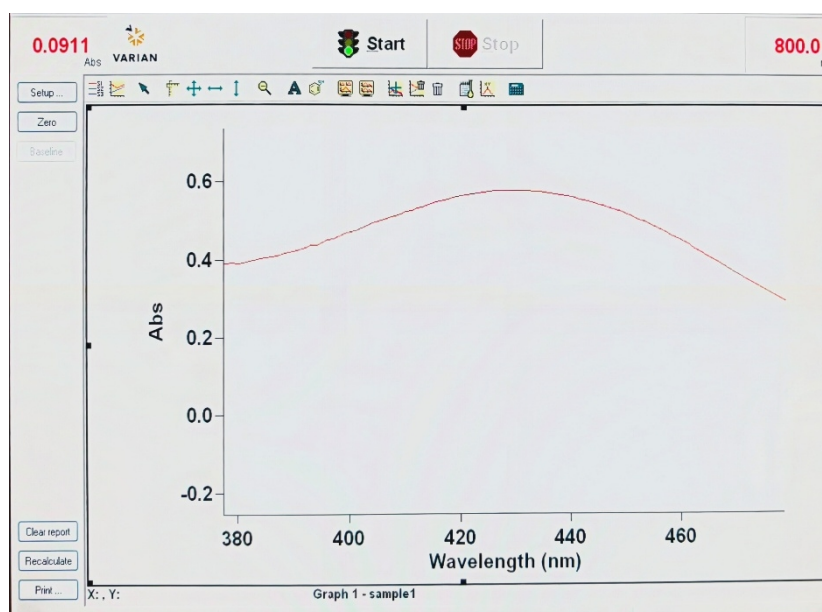


Figure 10.3.1.2. Enlarged view of the absorption peak of the 7.4 mg/mL stock solution in the 400–500 nm range.

10.3.2 Raw and processed data of absorbance detection

1				2							
1h Abs			1h Abs Average	Conc.	2h Abs			2h Abs Average	Conc.		
1-1	0.5979	0.5951	0.5939	0.595633333	8.62739598	0.5867	0.598	0.6033	0.596	8.63253857	
1-3	1.0791	1.0809	1.0805	1.080166667	15.4230949	1.3794	1.3813	1.3931	1.3846	19.6928471	
3				4							
4h Abs			4h Abs Average	Conc.	8h Abs			8h Abs Average	Conc.		
1-1	0.9611	0.9617	0.9623	0.9617	27.5231417	1.2406	1.2396	1.2394	1.239866667	35.3258532	
1-3	1.3011	1.3057	1.3143	1.307033333	18.6049556	0.9702	0.9704	0.9704	0.970333333	27.7653109	
5				6							
12h Abs			12h Abs Average	Conc.	24h Abs			24h Abs Average	Conc.	After correction	
1-1	0.9232	0.9234	0.9238	0.923466667	52.9013558	0.8783	0.8782	0.8788	0.878433333	37.7812062	69.52
1-3	0.7846	0.7846	0.7844	0.784533333	33.8302945	0.8268	0.8271	0.8271	0.827	23.7447405	33.22
7											
48h Abs			48h Abs Average	Conc.							
1-1	0.8762	0.8761	0.8762	0.876166667	125.619448						
1-3	1.1789	1.178	1.1773	1.178066667	167.961664						

Figure 10.3.2.1. Absorbance measured at 430 nm and calculated concentrations for each sample.

The absorbance of each sample was initially measured at 430 nm. Samples with absorbance values higher than 1.5 were diluted appropriately to improve measurement reliability and accuracy. The dilution factor varied depending on the sample. Absorbance values close to 1.0 were considered acceptable for analysis. Detailed dilution information is provided in the table below.

Table 10.3.2.1. Diluted samples used for absorbance measurement.

Dilution factor	Samples
2 times dilution	1-1(2 h), 1-1(8 h), 1-3 (8 h), 1-3(24 h)
3 times dilution	1-3 (12 h), 1-1 (24 h)
4 times dilution	1-1 (24 h)
10 times dilution	1-1 (48 h), 1-3(48 h)

10.3.3 Calculated cumulative release percentage of 1-1 complex hydrogel and 1-3 complex hydrogel during 48 h in vitro release assay

The cumulative percentage of in vitro release was calculated using the following equation:

$$\text{Cumulative Release (\%)} = \frac{M_t}{M_\infty} \times 100\% \quad (5)$$

Table 10.3.3.1. Curcumin release percentage of 1-1 complex hydrogel at different time points.

Time (h)	Concentration (mg/L)	Release amount (mg)	Release percentage (%)
1	8.6274	0.04314	0.0255
2	8.6325	0.04316	0.0255
4	27.5231	0.13762	0.0814

8	35.3259	0.17663	0.1045
12	52.9014	0.26451	0.1565
24	69.52	0.34760	0.2057
48	125.6194	0.62810	0.3717

Table 10.3.3.2. Curcumin release percentage of 1-3 complex hydrogel at different time points.

Time (h)	Concentration (mg/L)	Release amount (mg)	Release percentage (%)
1	15.4231	0.07712	0.1794
2	19.6928	0.09846	0.2290
4	18.6050	0.09303	0.2163
8	27.7653	0.13883	0.3229
12	33.8303	0.16915	0.3934
24	33.22	0.16610	0.3863
48	167.9617	0.83981	1.9530

10.3.4 Surface characteristics of curcumin-loaded hydrogels over time during assay

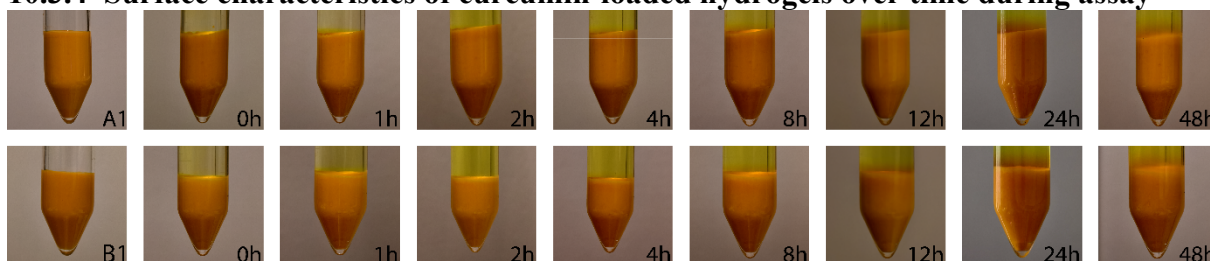


Figure 10.3.4.1. Changes in the hydrogel–buffer interface during the 48 h assay period. A1 represents the 1:1 complex hydrogel, and B1 represents the 1:3 complex hydrogel.



Figure 10.3.4.2. Interfacial changes in the curcumin-loaded hydrogel after prolonged exposure to the release medium (approximately 2 weeks).

No obvious surface erosion was observed during the short-term release study (48 h); however, extended incubation led to medium penetration into the superficial hydrogel layer, resulting in visible interfacial erosion and gap formation. Partial swelling and loosening of the upper hydrogel layer were also observed, indicating progressive matrix degradation over time.

10.4 Visualization of Raw Data in Injectability Test

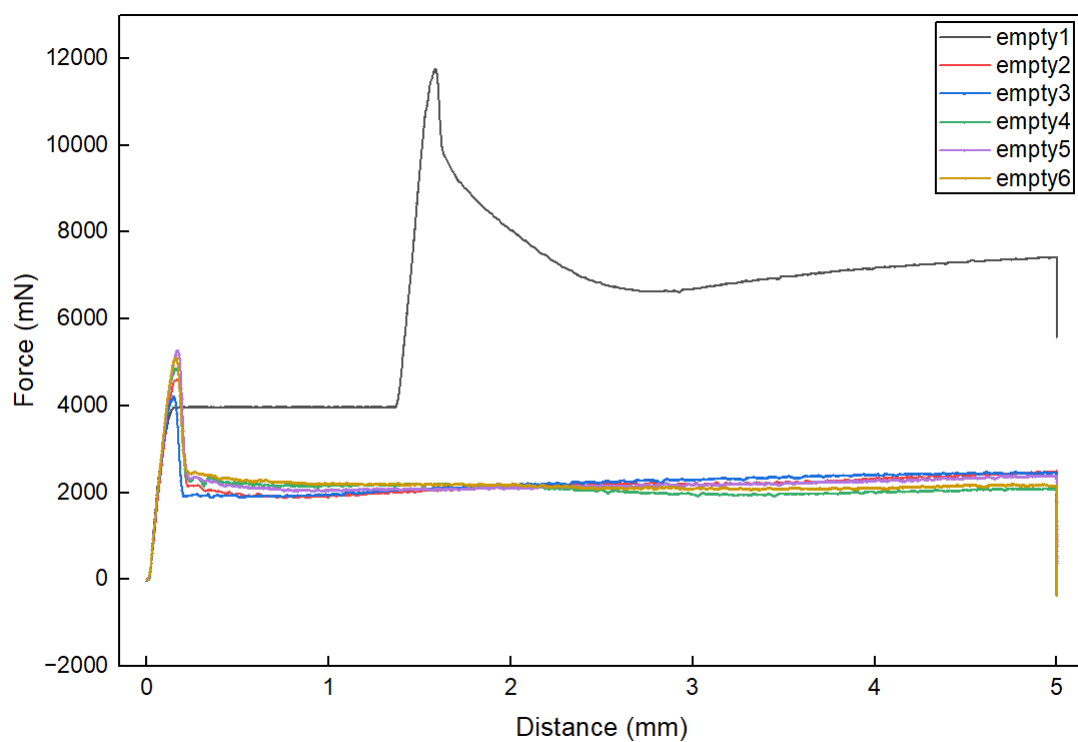


Figure 10.4.1. Visualization of Raw Data in Injectability Test: Force–Distance Curves of empty syringe group.

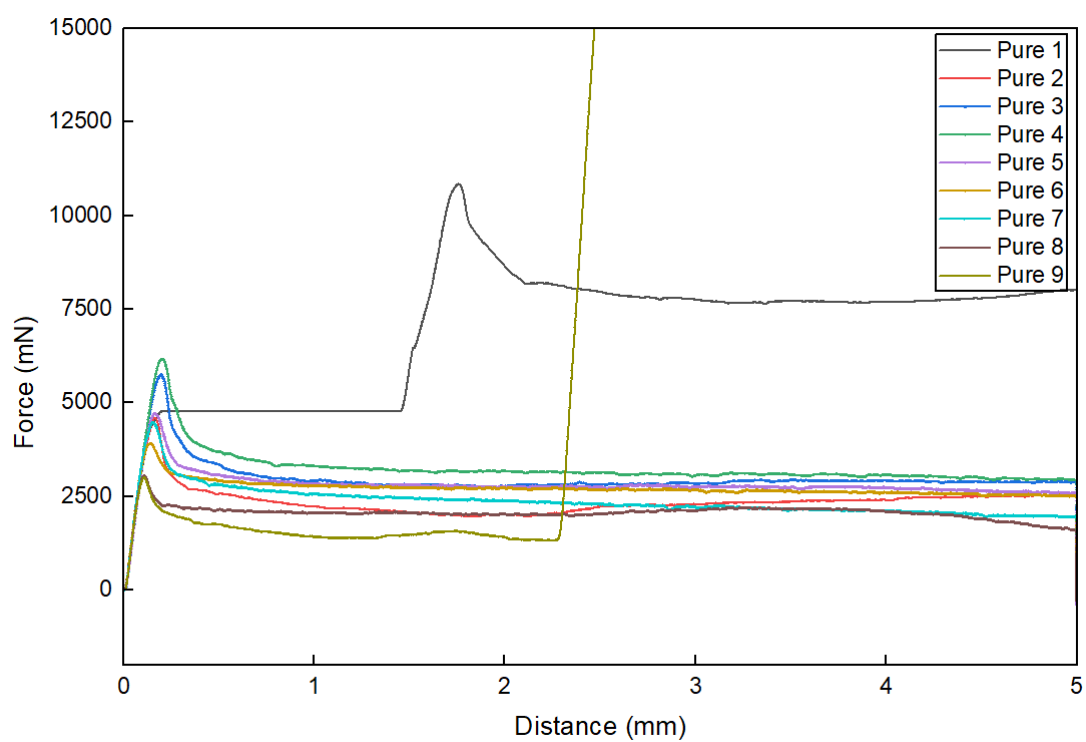


Figure 10.4.2. Visualization of Raw Data in Injectability Test: Force–Distance Curves of pure hydrogel group.

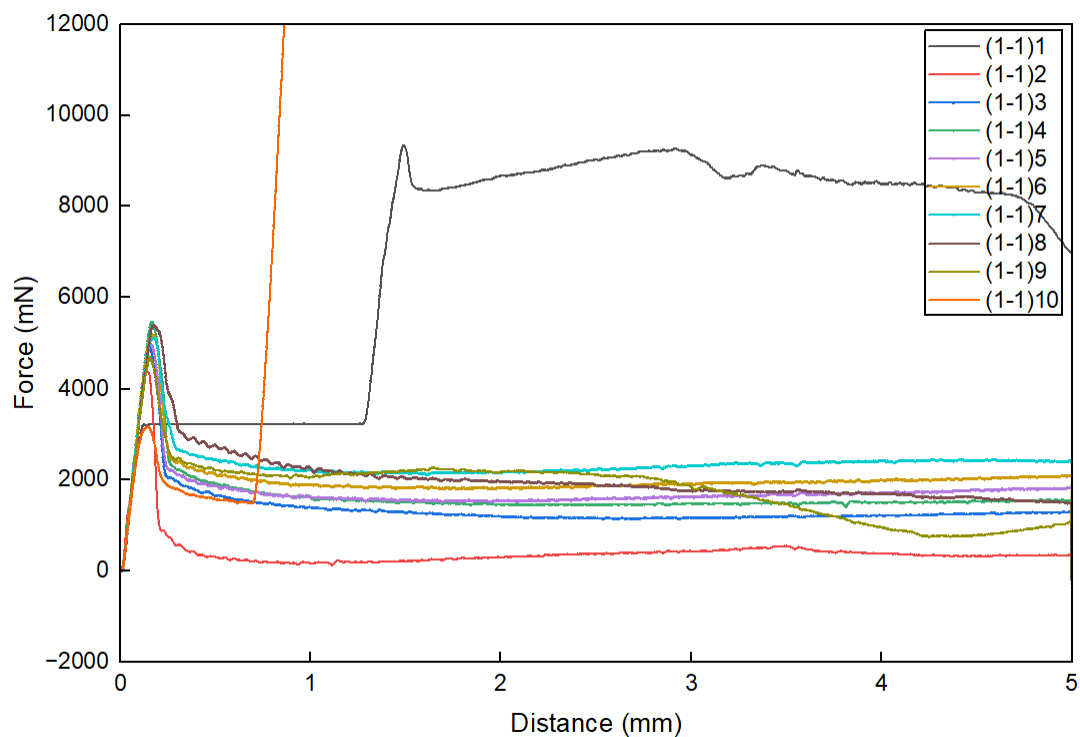


Figure 10.4.3. Visualization of Raw Data in Injectability Test: Force–Distance Curves of 1-1 complex hydrogel group.

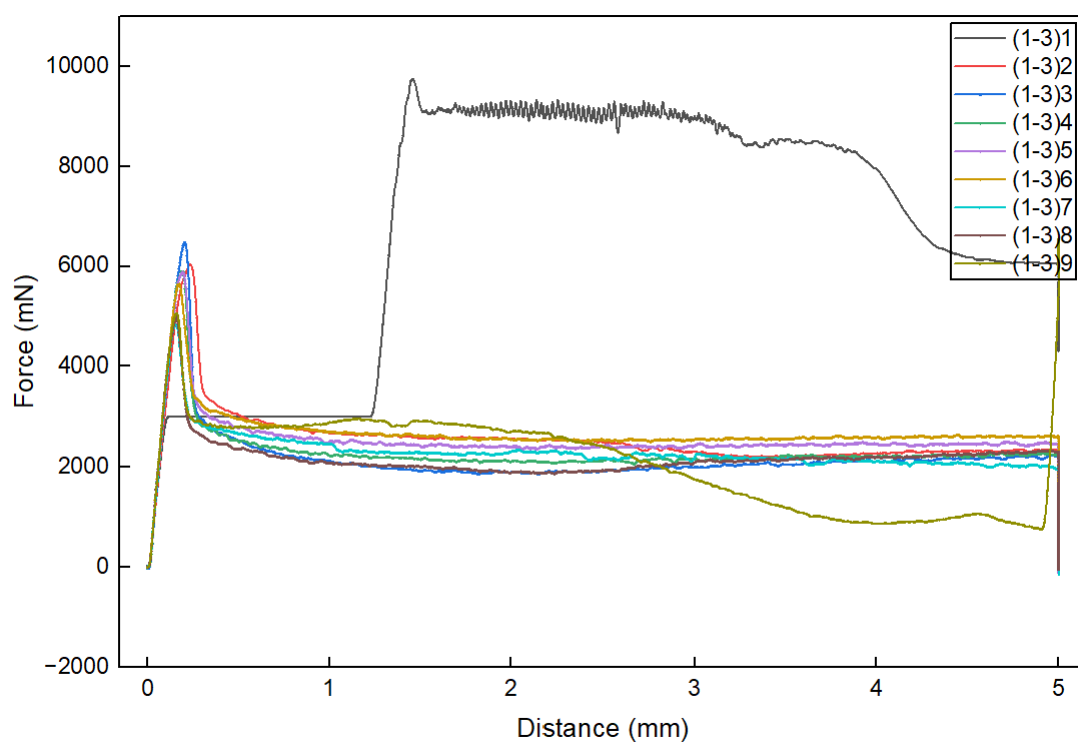


Figure 10.4.4. Visualization of Raw Data in Injectability Test: Force–Distance Curves of 1-3 complex hydrogel group.