

# Inclusion Complexes of Stearyl Glycyrrhetinate with $\gamma$ -Cyclodextrin and Cyclodextrin-Based Metal–Organic Frameworks: Solid-State Characterization and Improved Solubility in Fasted-State Simulated Intestinal Fluid

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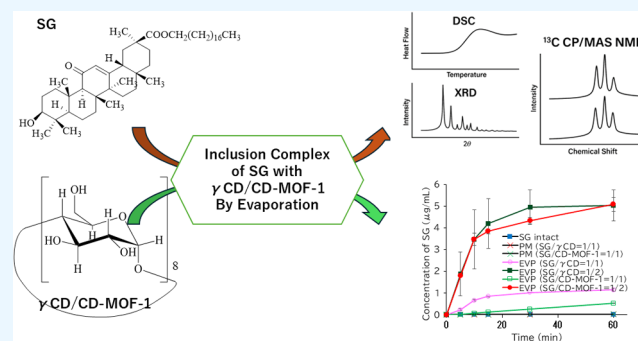
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**ABSTRACT:** In this study, stearyl glycyrrhetinate (SG), a poorly water-soluble derivative of glycyrrhetic acid, was formulated into inclusion complexes with  $\gamma$ -cyclodextrin ( $\gamma$ CD) and cyclodextrin-based metal–organic frameworks (CD-MOF-1) using the evaporation method (EVP) in the solid state. Powder X-ray diffraction and differential scanning calorimetry analyses confirmed the disappearance of SG's crystalline and melting peaks, suggesting complexation and amorphization. Meanwhile, solid-state  $^{13}\text{C}$  CP/MAS NMR and near-infrared spectroscopy revealed chemical shift alternation and peak broadening in C–H and carbon signals. This demonstrates clear molecular interactions and differences in inclusion modes between the SG and the host materials. In solubility tests using fasted-state simulated intestinal fluid, EVP (SG/ $\gamma$ CD = 1/2) and EVP (SG/CD-MOF-1 = 1/2) significantly increased in SG solubility by approximately 194-fold and 193-fold, respectively, compared with intact SG. Both complexes achieved rapid release from the early stages of testing. CD-MOF-1 showed unique dissolution behavior attributed to its rigid three-dimensional framework. These findings suggest that forming inclusion complexes with  $\gamma$ CD or CD-MOF-1 can enhance the aqueous solubility and dissolution performance of SG, offering promising potential for the development of oral SG formulations.



## INTRODUCTION

Glycyrrhizic acid (GL) is a major active component derived from licorice root (*Glycyrrhiza glabra*) and widely used in traditional Kampo medicine, injectable formulations, topical preparations, and oral medications. GL exhibits anti-inflammatory,<sup>1</sup> antitumor,<sup>2</sup> and hepatoprotective properties,<sup>3</sup> and GL-based pharmaceutical formulations have been applied in the treatment of various liver diseases, including drug-induced and alcoholic liver injury.<sup>3</sup>

Stearyl glycyrrhetinate (SG), a lipophilic ester derivative synthesized from stearyl alcohol and 18 $\beta$ -glycyrrhetic acid (GA, obtained by GL hydrolysis),<sup>4</sup> has excellent skin-conditioning effects due to its lipid solubility and is therefore used in dermatological products and cosmetics.<sup>5</sup> However, its poor aqueous solubility<sup>6</sup> has limited pharmaceutical applications and hinders oral absorption.

Cyclodextrins (CDs) are cyclic oligosaccharides composed of D-glucopyranose units linked by  $\alpha$ -1,4-glycosidic bonds. Based on the number of glucose units, they are classified as  $\alpha$ CD,  $\beta$ CD, or  $\gamma$ CD.<sup>7</sup> Their favorable safety profile has enabled their widespread use in the food, cosmetic, and pharmaceutical

industries.<sup>8</sup> CDs form inclusion complexes via their hydrophobic cavities, improving the solubility, stability, and bioavailability of poorly soluble compounds. For example,  $\beta$ CD enhances the solubility and oral bioavailability of curcumin,<sup>9</sup> while  $\gamma$ CD improves the stability of retinol (vitamin A),<sup>10</sup> and quercetin shows improved solubility and preserved antioxidant activity when complexed with CDs.<sup>11</sup> We have previously reported the preparation of SG inclusion complexes with  $\beta$ CD and  $\gamma$ CD using a cogrinding method.<sup>12</sup>

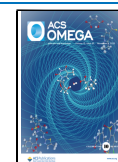
In recent years, metal–organic frameworks (MOFs), known for their high porosity and surface area, have attracted attention as promising drug delivery carriers. Among them,  $\gamma$ -cyclodextrin-based MOF ( $\gamma$ -CD-MOF, also referred to as CD-MOF-1) is synthesized from  $\gamma$ CD, ethanol, and potassium

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hydroxide (KOH).<sup>13</sup> This framework consists of six  $\gamma$ CD units coordinated by potassium ions, forming a three-dimensional microporous structure that exists in  $\alpha$ - and  $\beta$ -hydrated forms.<sup>14</sup> CD-MOF-1 can encapsulate drug molecules within its pores, thereby improving solubility, stability, and membrane permeability.<sup>15</sup> Previous studies have demonstrated its ability to load drugs such as carmofur, 5-fluorouracil, salicylic acid, and ibuprofen, resulting in enhanced bioavailability and controlled release profiles.<sup>15,16</sup>

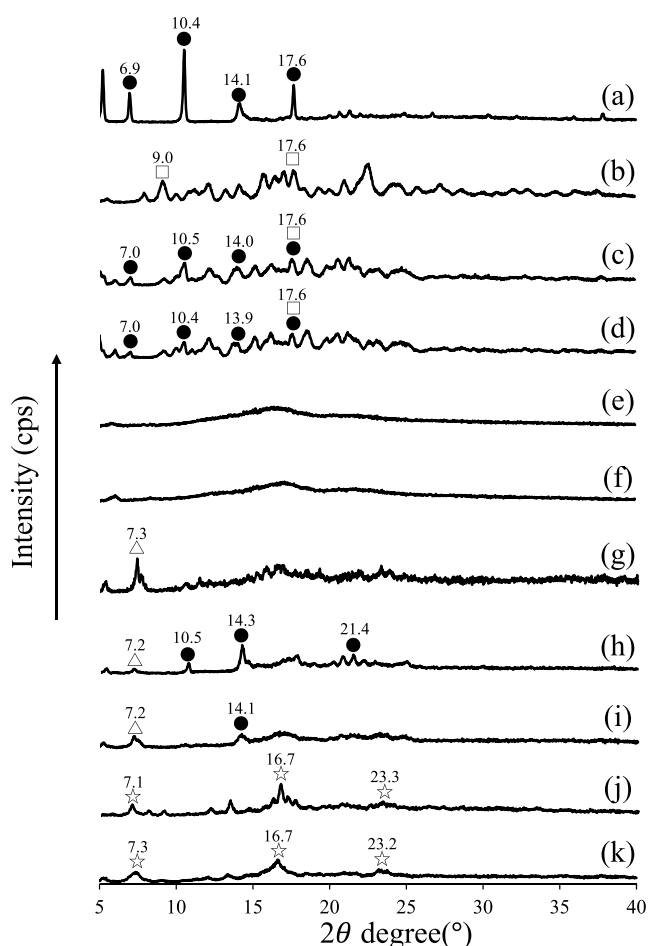
Our research group has also prepared CD-MOF-1 inclusion complexes of coenzyme Q10 (CoQ10)<sup>17</sup> and daidzein,<sup>18</sup> which exhibited improved solubility and antioxidant effects. Building on these findings, we hypothesized that CD-MOF-1 could serve as an effective host for SG, overcoming its poor aqueous solubility and facilitating its use in oral and topical formulations. Previous studies have demonstrated the feasibility of cyclodextrin inclusion complexes with glycyrrhetic acid (GA), improving its solubility and antioxidant activity.<sup>19,20</sup> However, no study has yet addressed the inclusion of stearyl glycyrrhetinate (SG), a highly lipophilic ester derivative of GA, with either the  $\gamma$ CD or CD-MOF systems. Given its poor aqueous solubility and pharmaceutical potential, investigating SG as a model compound provides both scientific novelty and clinical relevance.

Accordingly, the present study aimed to prepare and characterize SG/ $\gamma$ CD and SG/CD-MOF-1 inclusion complexes using a solvent evaporation method to evaluate their physicochemical properties by PXRD, DSC, <sup>13</sup>C CP/MAS NMR, NIR spectroscopy, SEM, and  $T_1$  relaxation measurements and to investigate their solubility and dissolution behavior in fasted-state simulated intestinal fluid (FaSSIF).

## RESULTS AND DISCUSSION

**Powder X-ray Diffraction (PXRD).** PXRD is a widely used technique for evaluating structural changes and crystallinity in CD inclusion complexes. The formation of such complexes typically generates new crystalline phases or amorphous states that differ from the original states of the drug and CD. These changes can be observed in PXRD patterns as the disappearance or shift of diffraction peaks, the appearance of new peaks, or peak broadening.<sup>21</sup>

Among CDs,  $\beta$ CD and  $\gamma$ CD are particularly known for their high crystallinity, exhibiting sharp and characteristic diffraction peaks. The encapsulation of a hydrophobic drug within the hydrophobic cavity of a CD often disrupts the drug's crystalline structure, thereby decreasing crystallinity or the formation of an amorphous state. Consequently, PXRD patterns of the inclusion complexes are distinguishable from those of the individual components or their physical mixtures, indirectly supporting the formation of host–guest complexes.<sup>22</sup> Herein, PXRD analysis was performed to investigate the crystalline states of EVP (SG/ $\gamma$ CD) and EVP (SG/CD-MOF-1) prepared by using the solvent evaporation method (Figure 1). For SG alone, characteristic diffraction peaks were observed at  $2\theta = 6.9^\circ$ ,  $10.4^\circ$ ,  $14.1^\circ$ , and  $17.6^\circ$  (Figure 1a), while  $\gamma$ CD exhibited peaks at  $2\theta = 9.0^\circ$  and  $17.6^\circ$  (Figure 1b). In the physical mixtures (PMs) with SG/ $\gamma$ CD ratios of 1/1, 1/2, and 2/1, diffraction peaks corresponding to SG and  $\gamma$ CD were detected (Figures 1c,d and S1d). In contrast, the PXRD pattern of EVP (SG/ $\gamma$ CD) showed the disappearance of SG- and  $\gamma$ CD-derived peaks, with the emergence of a broad halo pattern typical of amorphous materials (Figures 1e,f and S1e). Such patterns indicate the formation of inclusion complexes

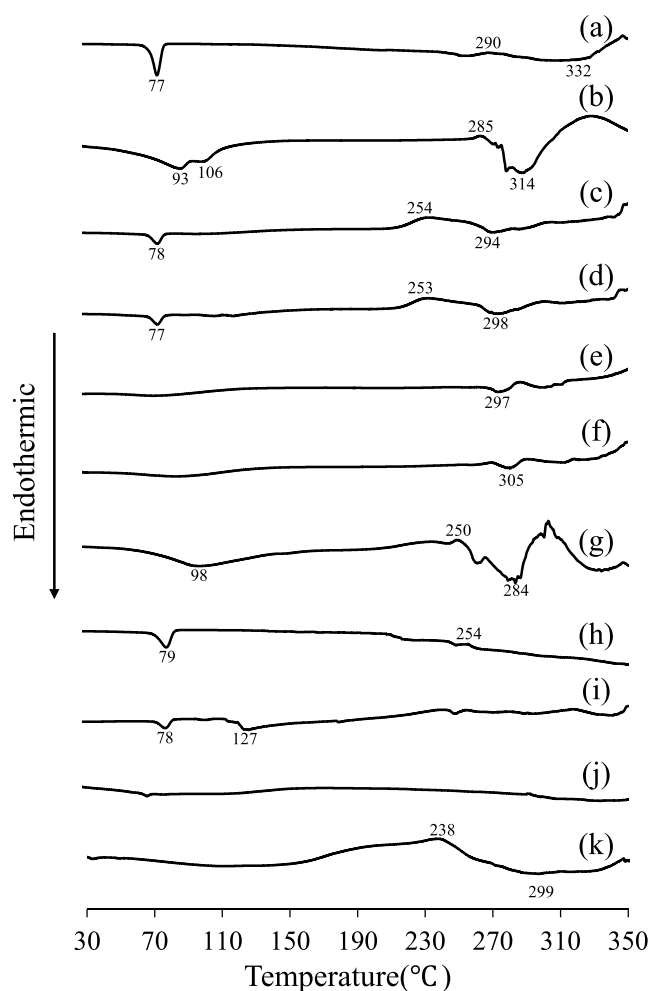


**Figure 1.** PXRD patterns of SG/ $\gamma$ CD and SG/CD-MOF-1 systems: (a) SG intact, (b)  $\gamma$ CD, (c) PM (SG/ $\gamma$ CD = 1/1), (d) PM (SG/ $\gamma$ CD = 1/2), (e) EVP (SG/ $\gamma$ CD = 1/1), (f) EVP (SG/ $\gamma$ CD = 1/2), (g) CD-MOF-1, (h) PM (SG/CD-MOF-1 = 1/1), (i) PM (SG/CD-MOF-1 = 1/2), (j) EVP (SG/CD-MOF-1 = 1/1), and (k) EVP (SG/CD-MOF-1 = 1/2). (●) SG, (□)  $\gamma$ CD, and (Δ) CD-MOF-1.

between CDs and guest molecules.<sup>23</sup> Therefore, EVP (SG/ $\gamma$ CD) may form a host–guest complex.

The PXRD pattern of CD-MOF-1 did not exhibit  $\gamma$ CD-characteristic peaks; instead, it displayed a distinct peak at approximately  $2\theta = 7.3^\circ$  (Figure 1g). EVP (SG/CD-MOF-1) showed new diffraction peaks at  $2\theta = 6.9^\circ$ ,  $16.5^\circ$ , and  $23.1^\circ$ , not observed for CD-MOF-1 alone (Figure S1c). CD-MOF-1 has been reported to exist in  $\alpha$ -type and  $\beta$ -type forms, with structural transformations dependent on the hydration state.<sup>14</sup> Thus, the solvent evaporation process in this study might have induced a phase transition from the  $\alpha$ -type to the  $\beta$ -type. The PXRD pattern of the physical mixture PM (SG/CD-MOF-1) revealed peaks attributable to SG and CD-MOF-1 (Figure 1h,i). In EVP (SG/CD-MOF-1 = 2/1), the SG-derived peak at  $2\theta = 14.1^\circ$  was still observed (Figure S1g), whereas in EVP samples with SG/CD-MOF-1 ratios of 1/1 and 1/2, the SG peaks disappeared, and new ones appeared at  $2\theta = 7.1^\circ$  and  $16.7^\circ$  (Figure 1j,k). Lv et al. reported that neutralized CD-MOF-1 exhibits characteristic peaks at approximately  $2\theta = 5.7^\circ$ ,  $7.0^\circ$ , and  $16.7^\circ$ ,<sup>24,25</sup> consistent with our observations. These results suggest that EVP (SG/CD-MOF-1 = 1/1, 1/2) forms new crystalline phases indicative of host–guest complexation with structural features distinct from those of the parent CD-MOF-1.

**Differential Scanning Calorimetry (DSC).** PXRD analysis suggested the formation of inclusion complexes in EVP (SG/ $\gamma$ CD) and EVP (SG/CD-MOF-1). To verify the formation of such host–guest complexes, DSC, a thermal analysis method for characterizing CD inclusion complexes, was used. Typically, the presence of an endothermic peak corresponding to the melting point of a drug indicates that the drug remains in a crystalline state. In contrast, when the drug is encapsulated within the hydrophobic cavity of a CD, its molecular mobility is significantly restricted, often resulting in the disappearance or significant reduction of the melting peak.<sup>26</sup> In this study, the thermal behavior of SG/ $\gamma$ CD and SG/CD-MOF-1 complexes was investigated by using DSC (Figure 2). The thermogram of SG alone exhibited a sharp



**Figure 2.** DSC curves of SG/ $\gamma$ CD and SG/CD-MOF-1 systems: (a) SG intact, (b)  $\gamma$ CD, (c) PM (SG/ $\gamma$ CD = 1/1), (d) PM (SG/ $\gamma$ CD = 1/2), (e) EVP (SG/ $\gamma$ CD = 1/1), (f) EVP (SG/ $\gamma$ CD = 1/2), (g) CD-MOF-1, (h) PM (SG/CD-MOF-1 = 1/1), (i) PM (SG/CD-MOF-1 = 1/2), (j) EVP (SG/CD-MOF-1 = 1/1), and (k) EVP (SG/CD-MOF-1 = 1/2).

endothermic peak at approximately 77 °C, attributable to its melting point (Figure 2a). In contrast,  $\gamma$ CD and CD-MOF-1 showed an endothermic peak approximately 80 °C due to dehydration and another approximately 270 °C associated with thermal decomposition (Figure 2b,g).

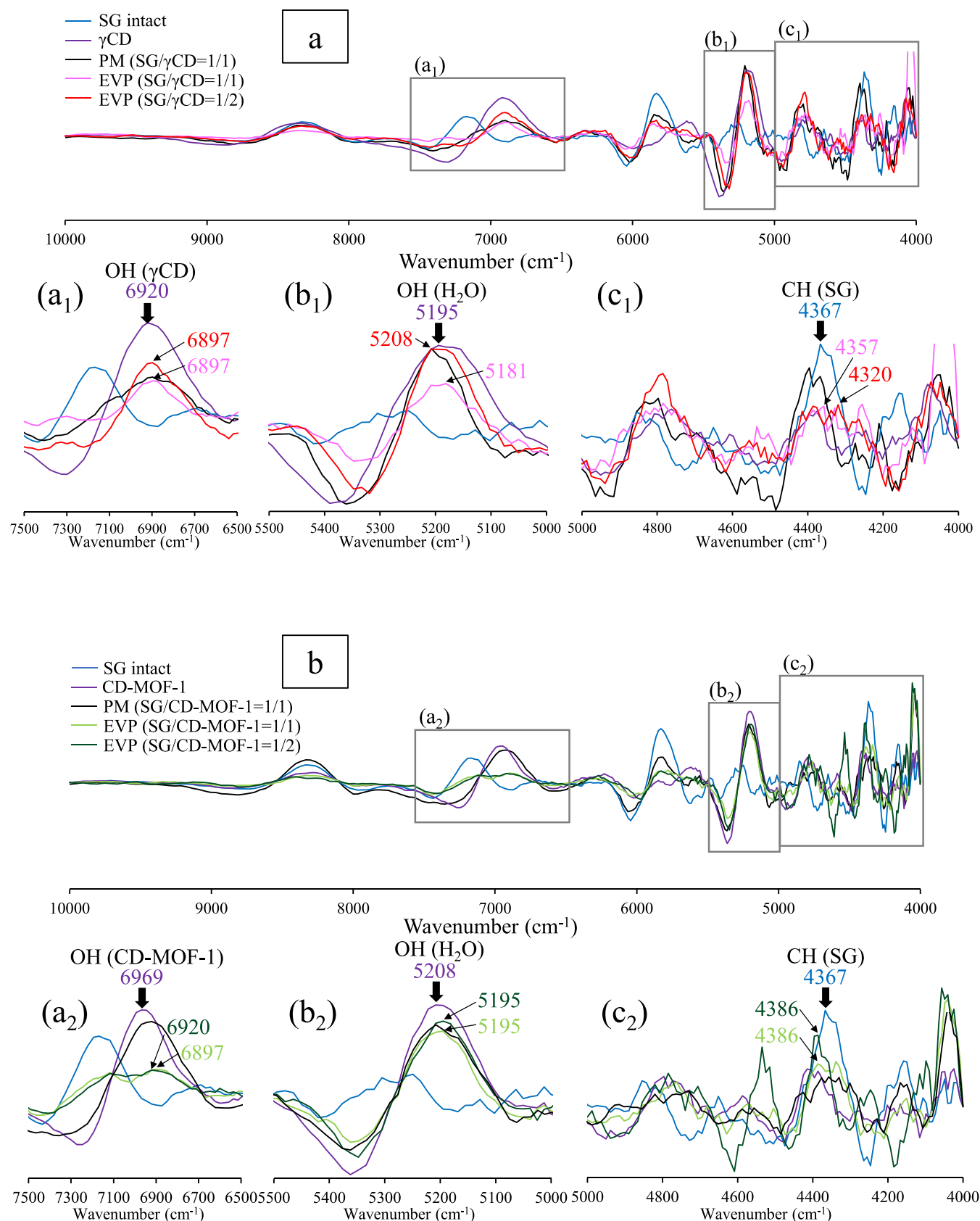
For the physical mixtures PM (SG/ $\gamma$ CD) and PM (SG/CD-MOF-1), the melting peak of SG remained visible at

approximately 77 °C, suggesting the presence of crystalline SG in these mixtures (Figures 2c,d,h,i and S3d,f). Similarly, in EVP samples with an SG-to-host ratio of 2/1, characteristic peaks of SG were observed at approximately 75 °C (Figure S3e,g), indicating partial retention of the crystalline state. However, in EVP (SG/ $\gamma$ CD = 1/1 and 1/2) and EVP (SG/CD-MOF-1 = 1/1 and 1/2), the characteristic melting peak of SG completely disappeared (Figure 2e,f,j,k). This is consistent with previous findings that inclusion complex formation is typically accompanied by the disappearance of the drug's melting peak, the emergence of new thermal events, or shifts and broadening of thermal transitions in DSC thermograms.<sup>27</sup> Taken together, these results suggest that in EVP (SG/ $\gamma$ CD = 1/1 and 1/2) and EVP (SG/CD-MOF-1 = 1/1 and 1/2), SG is successfully included within the cavities of  $\gamma$ CD or CD-MOF-1, resulting in a loss of crystallinity. Therefore, the formation of stable inclusion complexes is strongly supported by these compositions.

**Near-Infrared Absorption Spectroscopy.** PXRD and DSC analyses indicated the formation of inclusion complexes in SG/ $\gamma$ CD and SG/CD-MOF-1 at 1/1 and 1/2 molar ratios. To further verify the presence of intermolecular interactions in these systems, near-infrared (NIR) spectroscopy was performed on SG/ $\gamma$ CD and SG/CD-MOF-1 samples (Figure 3a,b). NIR spectroscopy enables the evaluation of molecular structure and intermolecular interactions through characteristic absorption bands corresponding to vibrational modes of C–H, N–H, and O–H bonds.<sup>28,29</sup>

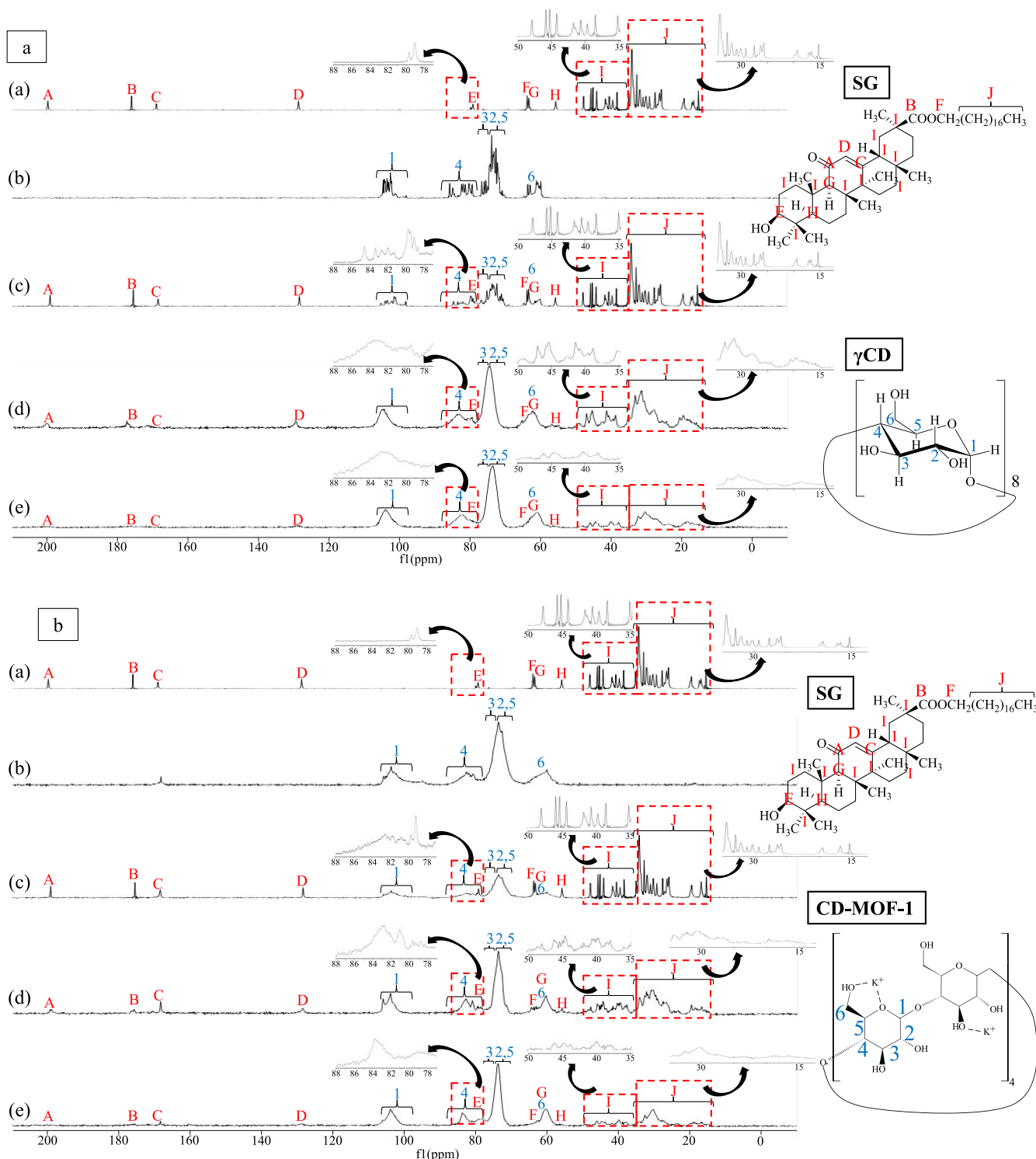
In the SG intact sample, a peak attributed to –CH stretching vibrations was observed at 4367 cm<sup>–1</sup> (Figure 3a:c<sub>1</sub>). In contrast,  $\gamma$ CD showed an –OH stretching band at 6920 cm<sup>–1</sup> and a water-associated band at 5195 cm<sup>–1</sup> (Figure 3a:a<sub>1</sub>,b<sub>1</sub>). For EVP (SG/ $\gamma$ CD = 1/1), the –CH band of SG shifted from 4367 to 4357 cm<sup>–1</sup> and exhibited noticeable broadening (Figure 3a:c<sub>1</sub>). Additionally, the –OH peak of  $\gamma$ CD shifted from 6920 to 6897 cm<sup>–1</sup>, and the water band shifted from 5195 to 5181 cm<sup>–1</sup>, both accompanied by peak broadening (Figure 3a(a<sub>1</sub>,b<sub>1</sub>)). Similarly, in EVP (SG/ $\gamma$ CD = 1/2), the –CH band of SG shifted to 4320 cm<sup>–1</sup>, and both the –OH and water bands shifted to 6897 and 5208 cm<sup>–1</sup>, respectively, with significant broadening (Figure 3a).

In CD-MOF-1, the –OH and water peaks were observed at 6969 and 5208 cm<sup>–1</sup>, respectively (Figure 3b:a<sub>2</sub>,b<sub>2</sub>). In EVP (SG/CD-MOF-1 = 1/1), the –CH band of SG shifted from 4367 to 4386 cm<sup>–1</sup> and showed broadening (Figure 3b:c<sub>2</sub>). The –OH band shifted from 6969 to 6897 cm<sup>–1</sup>, while the water band shifted from 5208 to 5195 cm<sup>–1</sup>, both displaying broadening (Figure 3b:a<sub>2</sub>,b<sub>2</sub>). In EVP (SG/CD-MOF-1 = 1/2), the –CH band remained at 4386 cm<sup>–1</sup>, and the –OH and water bands shifted to 6920 and 5195 cm<sup>–1</sup>, respectively, with notable broadening (Figure 3b). These shifts and broadenings of the –OH and water bands originating from  $\gamma$ CD and CD-MOF-1 suggest that the formation of the inclusion complexes is accompanied by a reduction in free water molecules and consequent changes in the hydrogen bonding network.<sup>30</sup> Furthermore, hydrogen bonding interactions may occur between the –CH groups of SG and the –OH groups of  $\gamma$ CD or CD-MOF-1. These results indicate that SG is successfully encapsulated within the cavities of  $\gamma$ CD and CD-MOF-1, resulting in the formation of inclusion complexes stabilized by intermolecular interactions including hydrogen bonding.



**Measurement of  $^{13}\text{C}$  CP/MAS NMR Spectra.** The results of PXRD, DSC, and NIR measurements suggested that

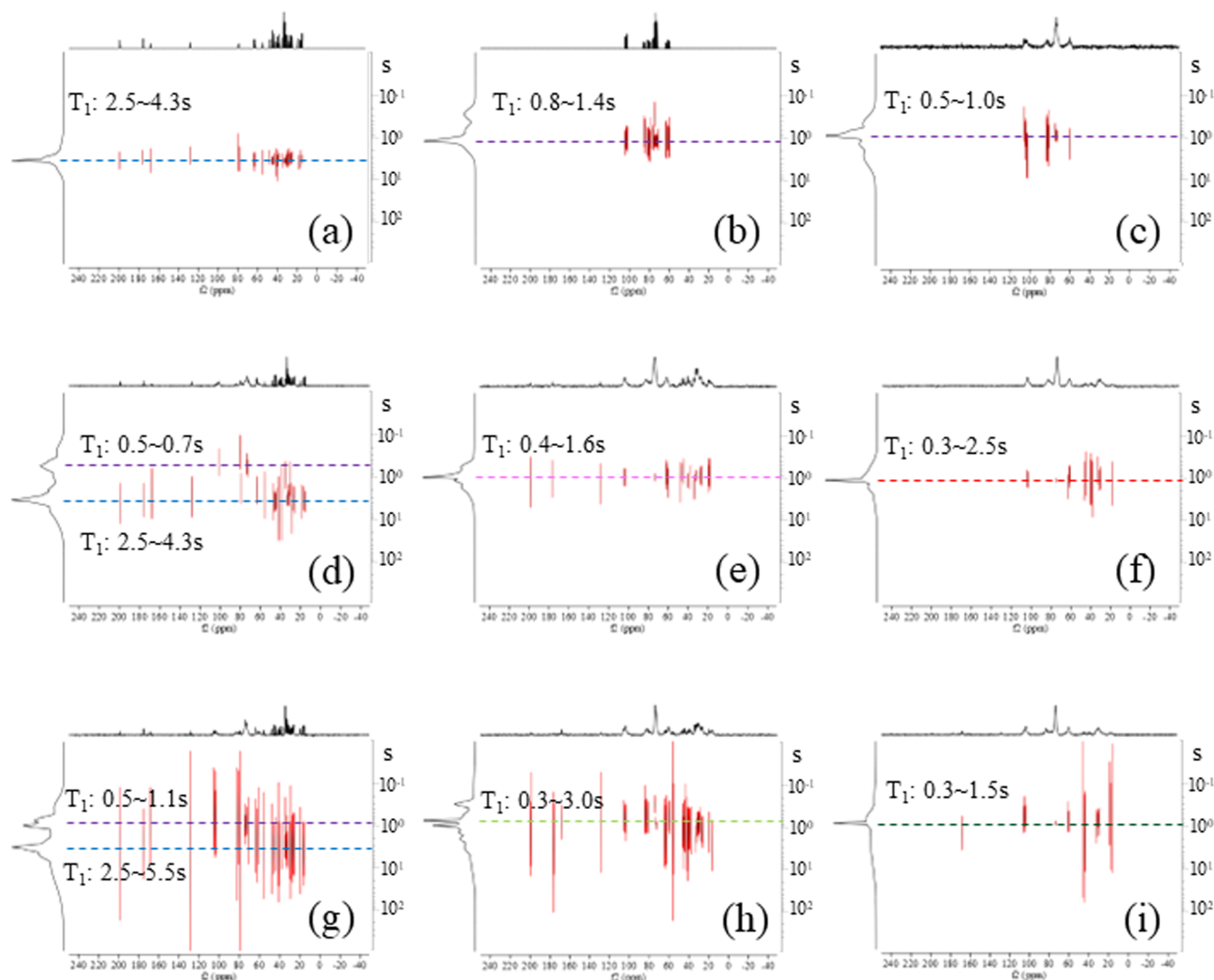
intermolecular interactions occur between the  $-\text{CH}$  groups of SG and the  $-\text{OH}$  groups located within the CD cavity in the



**Figure 4.**  $^{13}\text{C}$  CP/MAS NMR spectra of (a) SG/ $\gamma$ CD and (b) SG/CD-MOF-1 systems: (a) SG intact, (b) host material ( $\gamma$ CD, CD-MOF-1), (c) PM, (d) EVP (1/1), and (e) EVP (1/2).

SG/ $\gamma$ CD and SG/CD-MOF-1 systems. This indicates that the inclusion complex formation proceeds in the solid state. To investigate the spatial arrangement and chemical environment of the components in the solid state,  $^{13}\text{C}$  cross-polarization magic-angle spinning nuclear magnetic resonance ( $^{13}\text{C}$  CP/MAS NMR) spectroscopy was performed.  $^{13}\text{C}$  CP/MAS NMR is a powerful technique for characterizing the interaction between CDs and guest molecules and elucidating inclusion

modes. In particular, chemical shift changes in the carbon atoms of the glucose units in CDs—especially at the C-2, C-3, and C-4 positions—are widely recognized as indicators of inclusion complexation.<sup>31,32</sup> To evaluate the molecular states of the SG/ $\gamma$ CD and SG/CD-MOF-1 complexes, solid-state  $^{13}\text{C}$  CP/MAS NMR spectra were recorded. The peak assignments of SG were based on the comparison with the  $^1\text{H}$  NMR spectra of glycyrrhetic acid (GA) and SG and the  $^{13}\text{C}$  CP/MAS



**Figure 5.**  $T_1$ -NMR measurement of SG/ $\gamma$ CD and SG/CD-MOF-1 systems: (a) SG intact, (b)  $\gamma$ CD, (c) CD-MOF-1, (d) PM (SG/ $\gamma$ CD = 1/1), (e) EVP (SG/ $\gamma$ CD = 1/1), (f) EVP (SG/ $\gamma$ CD = 1/2), (g) PM (SG/CD-MOF-1 = 1/1), (h) EVP (SG/CD-MOF-1 = 1/1), and (i) EVP (SG/CD-MOF-1 = 1/2).

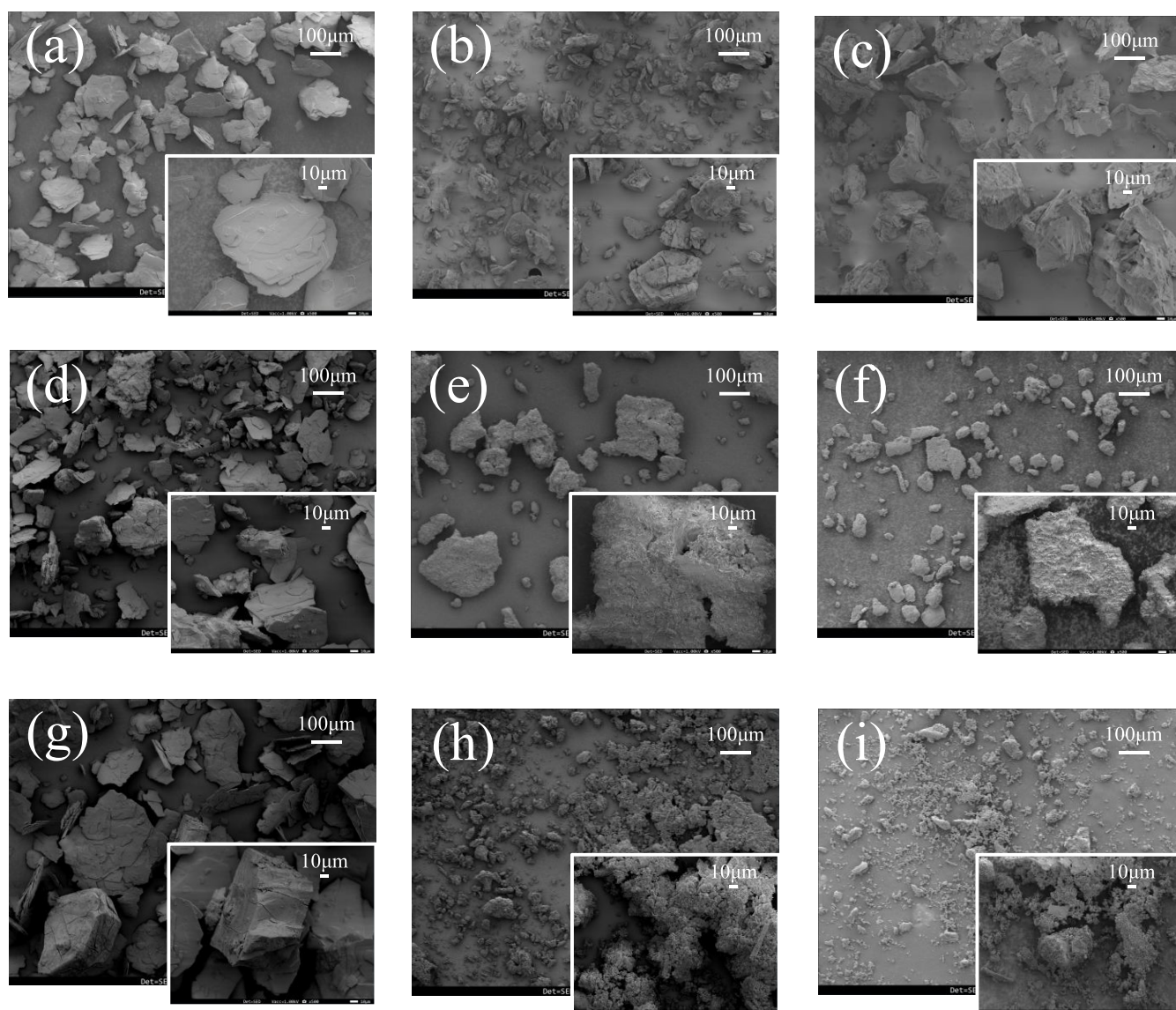
NMR data of SG itself. The interpretation of SG spectra was supported by comparison with previously reported  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of glycyrrhetic acid, which exhibit the characteristic signals of the triterpenoid backbone.<sup>33</sup>

In the spectrum of SG, characteristic peaks derived from SG were identified at the following chemical shifts: C-A (199.1 ppm), C-B (175.5 ppm), C-C (168.5 ppm), C-D (128.4 ppm), C-E (79.7, 79.1 ppm), C-F (63.7 ppm), C-G (63.3 ppm), C-H (55.7 ppm), C-I (48.0–35.0 ppm), and C-J (34.5–15.3 ppm) (Figure 4a:a). For  $\gamma$ CD, characteristic peaks were observed at C-1 (100.0–105.0 ppm), C-4 (86.0–79.0 ppm), C-3 (77.0–74.0 ppm), C-2,5 (74.0–71.0 ppm), and C-6 (64.0–59.5 ppm) (Figure 4a:b). In the physical mixture (PM), peaks from both the SG and  $\gamma$ CD were detected (Figure 4a:c).

In contrast, for the EVP (SG/ $\gamma$ CD = 1/1) sample, significant peak shifts and broadenings were observed for the SG-derived peaks: C-A (199.1–200.0 ppm), C-B (175.5–177.3 ppm), C-C (168.5–169.8 ppm), and C-D (128.4–129.4 ppm). The peaks for C-I (48.0 and 35.0 ppm) and C-J (34.5 and 15.3 ppm) were also broadened. Additionally, the signals corresponding to C-E, C-F, C-G, and C-H disappeared (Figure

4a:d). In the EVP (SG/ $\gamma$ CD = 1/2) sample, the SG-derived peaks C-I and C-J exhibited significant broadening and partial disappearance. Peaks corresponding to C-A, C-B, C-C, C-D, C-E, C-F, C-G, and C-H could no longer be detected (Figure 4a:e). For both EVP (SG/ $\gamma$ CD = 1/1 and 1/2) samples, peaks derived from  $\gamma$ CD showed broadening across all regions (Figure 4a).

Similarly, in the case of CD-MOF-1, characteristic peaks were observed at C-1 (100.0–105.0 ppm), C-4 (86.0–79.0 ppm), C-3 (77.0–74.0 ppm), C-2,5 (74.0–71.0 ppm), and C-6 (64.0–59.5 ppm). In the PM of SG and CD-MOF-1, signals from both components were retained. In EVP (SG/CD-MOF-1 = 1/1), SG-derived peaks exhibited peak shifts and broadenings: C-A (199.1–198.7 ppm), C-B (175.5–175.6 ppm), C-C (168.5–168.1 ppm), C-F (63.7–64.4 ppm), C-G (63.3–63.1 ppm), C-H (55.7–55.8 ppm), and C-J (broadening). No noticeable changes were detected for C-D or C-E (Figure 4b:d). In EVP (SG/CD-MOF-1 = 1/2), SG-derived peaks C-I and C-J exhibited significant broadening with the disappearance of multiple peaks. Signals corresponding to C-A, C-B, C-C, C-D, C-E, C-F, C-G, and C-H were absent (Figure



**Figure 6.** SEM morphology of SG/ $\gamma$ CD and SG/CD-MOF-1 systems: (a) SG intact, (b)  $\gamma$ CD, (c) CD-MOF-1, (d) PM (SG/ $\gamma$ CD = 1/1), (e) EVP (SG/ $\gamma$ CD = 1/1), (f) EVP (SG/ $\gamma$ CD = 1/2), (g) PM (SG/CD-MOF-1 = 1/1), (h) EVP (SG/CD-MOF-1 = 1/1), and (i) EVP (SG/CD-MOF-1 = 1/2).

4b:e). Furthermore, peaks derived from CD-MOF-1 were broadened throughout all spectral regions in both EVP (SG/CD-MOF-1 = 1/1 and 1/2) samples (Figure 4b).

The C-3 and C-6 carbon atoms of cyclodextrin are located on the wider and narrower rims of the cavity, respectively, and their chemical shifts are key indicators for evaluating the orientation and depth of guest inclusion.<sup>34,35</sup> The broadening of these signals in EVP (SG/ $\gamma$ CD = 1/2) and EVP (SG/CD-MOF-1 = 1/2) suggests that structural deformation of the CD cavity occurs to accommodate the SG molecules. This deformation is likely attributed to host–guest interactions, particularly involving the  $-\text{CH}$  groups of SG, which might have caused slight contraction or rearrangement of the CD ring. Collectively, these findings indicate that in EVP (SG/ $\gamma$ CD = 1/1), the stearyl side chain or steroidal framework of SG (e.g., C-E, C-I) may be inserted into the  $\gamma$ CD cavity from the wider rim (C-3) toward the narrower rim (C-6). In EVP (SG/ $\gamma$ CD = 1/2) and EVP (SG/CD-MOF-1 = 1/2), both the stearyl chain and the steroidal core appear to be simultaneously

encapsulated. In contrast, EVP (SG/CD-MOF-1 = 1/1) seems to involve the inclusion of only the stearyl moiety within the CD-MOF-1 cavity. Overall, the findings suggest that EVP (SG/ $\gamma$ CD = 1:2) and EVP (SG/CD-MOF-1 = 1/2) exhibit similar inclusion modes, while EVP (SG/ $\gamma$ CD = 1/1) and EVP (SG/CD-MOF-1 = 1/1) are likely to adopt different modes of inclusion.

**Solid-State  $T_1$ -NMR Analysis for Evaluating the Molecular Mobility of SG/ $\gamma$ CD and SG/CD-MOF-1 Inclusion Complexes.**  $T_1$  relaxation time measurements in the solid state were applied to characterize the molecular motion of SG within inclusion complexes formed with  $\gamma$ CD and CD-MOF-1. This technique utilizes differences in the  $^1\text{H}$  spin–lattice relaxation times ( $T_1$ ) among the individual components within a physical mixture to selectively obtain the corresponding  $^{13}\text{C}$  CP/MAS NMR spectra. Unlike solution-state NMR, each compound in the solid state exhibits a distinct  $T_1$  value due to its inherent molecular dynamics. Therefore, when  $T_1$  significantly differs between the

components, this method enables spectral separation without dissolution, preserving structural information specific to the crystalline state. As such,  $T_1$ -based solid-state NMR is a valuable tool for assessing the structure and molecular mobility of inclusion complexes in the solid state.<sup>36</sup>

In the solid-state NMR measurements, neat SG exhibited relatively long  $T_1$  values (2.5–4.3 s), attributed to its high crystallinity and restricted molecular motion (Figure 5a). In contrast,  $\gamma$ CD alone showed shorter  $T_1$  values (0.8–1.4 s), likely due to the presence of hydrated structures and enhanced molecular flexibility that facilitate efficient relaxation (Figure 5b). In the PM of SG and  $\gamma$ CD (1/1 molar ratio), distinct  $T_1$  values for both components were independently observed (Figure 5d), indicating the absence of significant molecular interactions. Conversely, the evaporated complex (EVP) of SG/ $\gamma$ CD (1/1) exhibited shortened  $T_1$  values ranging 0.4–1.6 s, suggesting accelerated relaxation compared with neat SG and approaching the  $T_1$  values of  $\gamma$ CD (Figure 5e). A similar trend was observed in the EVP sample with a 1/2 SG/ $\gamma$ CD ratio, where  $T_1$  values ranged 0.3–2.5 s (Figure 5f). These reductions in  $T_1$  suggest that SG is incorporated into the hydrophobic cavity of the  $\gamma$ CD, altering its molecular dynamics. Notably, the shorter relaxation times and convergence toward the  $T_1$  values of  $\gamma$ CD imply that SG resides in a more flexible, less rigid environment, likely due to its inclusion within the host cavity. These findings indicate that genuine inclusion complex formation rather than simple physical mixing occurs in the EVP samples.

For CD-MOF-1 alone,  $T_1$  values ranged 0.5–1.0 s (Figure 5c), which may reflect the porous architecture and relatively high-water content that promote efficient spin–lattice relaxation. In the physical mixture of SG/CD-MOF-1 (1/1), the  $T_1$  values for SG and CD-MOF-1 were distinguishable (Figure 5g), suggesting the coexistence of unassociated components. In contrast, the EVP sample (SG/CD-MOF-1 = 1/1) displayed shortened  $T_1$  values of 0.3–3.0 s, indicating a marked reduction in relaxation time compared with neat SG and a convergence toward the  $T_1$  range of CD-MOF-1 (Figure 5h). A similar trend was observed in the 1/2 EVP sample with  $T_1$  values ranging from 0.3 to 1.5 s (Figure 5i). These reductions in  $T_1$  suggest that SG molecules are accommodated within the cavities of CD-MOF-1 and exposed to a more hydrophilic and dynamic environment, resulting in enhanced molecular motion and efficient relaxation.

These  $T_1$  changes are an important indirect indicator of inclusion complex formation in the EVP samples. The present study achieved a multifaceted approach to evaluating inclusion complexation and its mode by combining  $T_1$  relaxation time analysis with conventional techniques such as PXRD, DSC, and NIR spectroscopy, along with  $^{13}\text{C}$  CP/MAS NMR chemical shift analysis.

This methodology enabled a more detailed characterization of molecular dynamics and host–guest interactions within the cyclodextrin cavities, which are typically elusive when relying solely on conventional physicochemical techniques. Thus, the combined use of chemical shifts and  $T_1$  relaxation times as NMR indicators provides valuable insights into the structure and dynamics of inclusion complexes and is a potential analytical strategy for elucidating supramolecular systems in the solid state.

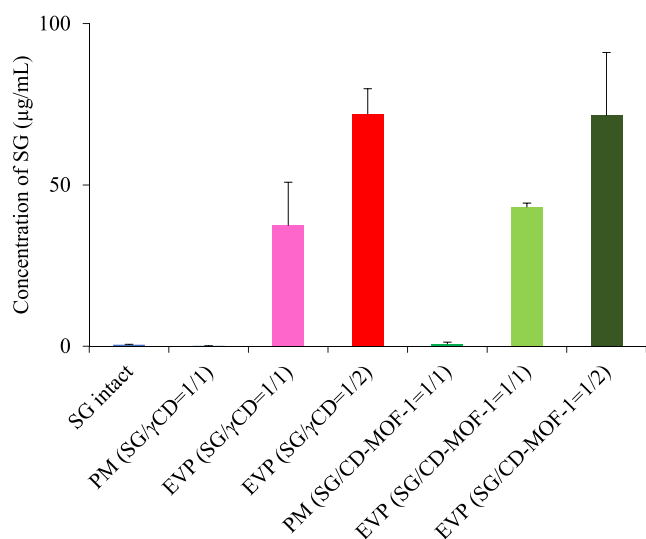
**Scanning Electron Microscopy (SEM).** The formation of inclusion complexes in both EVP (SG/ $\gamma$ CD) and EVP (SG/CD-MOF-1) was supported by PXRD, DSC, NMR, and NIR

analyses. Additionally, PXRD patterns revealed alterations in the crystal state upon complexation. To investigate morphological characteristics and surface features of the samples, SEM was conducted (Figure 6). SEM provides valuable insights into the physical appearance and surface texture of particulate materials. SEM micrographs were obtained to qualitatively assess the morphology and surface characteristics of raw SG, SG/ $\gamma$ CD, and SG/CD-MOF-1 samples. The observed changes in surface texture and particle shape provide visual confirmation of the inclusion complex formation. It should be noted that the SEM analysis in this study was intended for qualitative morphological assessment rather than quantitative particle size distribution. The surface of intact SG appeared flat, with small thin fragments adhering to larger ones, and particle sizes were approximately 100  $\mu\text{m}$  (Figure 6a).  $\gamma$ CD showed smooth surfaces with angular shapes, also approximately 100  $\mu\text{m}$  in size (Figure 6b). CD-MOF-1 exhibited smooth surfaces with square-shaped particles that were approximately 60  $\mu\text{m}$  in size (Figure 6c). The cubic packing structure of CD-MOF-1 has been previously reported;<sup>37</sup> herein, the same cubic morphology is confirmed, suggesting that the preparation method used was appropriate. CD-MOF-1 partially collapses into fine particles upon neutralization.<sup>38</sup> No notable changes in surface morphology were observed for physical mixtures SG/ $\gamma$ CD and SG/CD-MOF-1, as evidenced by the SEM images (Figure 6d,g).

In contrast, EVP (SG/ $\gamma$ CD) displayed roughened particle surfaces with fine fragments adhering to larger particles, while the overall particle size remained at approximately 100  $\mu\text{m}$  (Figure 6e,f). For EVP (SG/CD-MOF-1), surface aggregation of cubic crystals smaller than 2  $\mu\text{m}$  was observed, with the entire particles measuring up to approximately 50  $\mu\text{m}$  in size (Figure 6h,i). Although SEM analysis provided useful qualitative information about morphological differences between raw SG and its inclusion complexes, we acknowledge that quantitative evaluation of particle size distribution, specific surface area, and dispersion stability would further strengthen the understanding of their influence on dissolution behavior. These aspects are considered to be valuable directions for future investigations. The absence of particles with the characteristic morphologies of SG,  $\gamma$ CD, or CD-MOF-1 in the EVP (SG/ $\gamma$ CD) and EVP (SG/CD-MOF-1) samples indicates that the original particle structures underwent substantial morphological transformation due to complex formation.

**Solubility of SG/ $\gamma$ CD and CD-MOF-1 Systems in Fasted-State Simulated Intestinal Fluid (FaSSIF).** Solid-state characterization confirmed that EVP (SG/ $\gamma$ CD) and EVP (SG/CD-MOF-1) formed inclusion complexes at molar ratios of 1/1 and 1/2. To evaluate the solubility of SG/ $\gamma$ CD and SG/CD-MOF-1 under physiologically relevant conditions, dissolution studies were conducted using fasted-state simulated intestinal fluid (FaSSIF), which reflects the bile salt, phospholipid, and pH composition of the intestinal fluids. FaSSIF contains, among others, sodium taurocholate (NaTC) and lecithin, which may compete with the drug for the hydrophobic cavity of CDs, potentially displacing it. Notably, the rapid release of SG from the EVP complexes in FaSSIF may be influenced by competitive interactions with bile salts. Previous reports have described that bile salts can effectively displace guest molecules from cyclodextrin cavities and facilitate their incorporation into micelles, thereby enhancing solubilization.<sup>35,39</sup> In agreement with these findings, our

dissolution data suggest that bile-salt-mediated displacement and subsequent micellar solubilization may contribute to the enhanced dissolution of SG from both  $\gamma$ CD and CD-MOF-1 complexes. While our results are consistent with this mechanism, we acknowledge that the present study did not include direct competitive binding or bile salt-free release assays, and thus, the precise contribution of bile salts requires further confirmation in future work. After 72 h in FaSSIF, the solubility of intact SG was 0.37  $\mu\text{g/mL}$ , whereas EVP (SG/ $\gamma$ CD = 1/1) and EVP (SG/ $\gamma$ CD = 1/2) exhibited significantly enhanced solubilities of 37.41 and 71.93  $\mu\text{g/mL}$ , respectively. Similarly, EVP (SG/CD-MOF-1 = 1/1) and EVP (SG/CD-MOF-1 = 1/2) showed solubilities of 43.08 and 71.46  $\mu\text{g/mL}$ , respectively (Figure 7). Compared to intact SG, these values

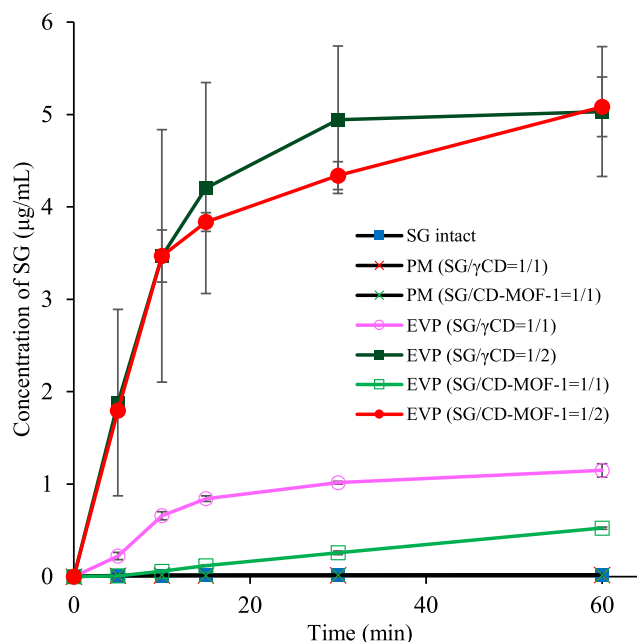


**Figure 7.** Solubility of SG/ $\gamma$ CD and SG/CD-MOF-1 systems in FaSSIF (72 h). Data are expressed as mean  $\pm$  SD ( $n = 3$ ).

represent approximately 101-fold (1/1) and 194-fold (1/2) increases for SG/ $\gamma$ CD and 116-fold (1/1) and 193-fold (1/2) increases for SG/CD-MOF-1, respectively.

These marked improvements in solubility are attributed to the formation of inclusion complexes with  $\gamma$ CD or CD-MOF-1, likely driven by differences in host–guest interactions and inclusion behavior specific to each system.

**Dissolution Profiles of SG/ $\gamma$ CD and CD-MOF-1 Systems in FaSSIF.** The formation of inclusion complexes in the solid state was suggested for EVP (SG/ $\gamma$ CD) and EVP (SG/CD-MOF-1). To evaluate their dissolution behavior, *in vitro* dissolution tests were conducted using SG alone, PM (SG/ $\gamma$ CD), EVP (SG/ $\gamma$ CD), PM (SG/CD-MOF-1), and EVP (SG/CD-MOF-1) (Figure 8). At 15 min following test initiation, the dissolution rate of the intact SG was approximately 0.014% (0.014  $\mu\text{g/mL}$ ). In contrast, EVP (SG/ $\gamma$ CD = 1/1) exhibited a dissolution rate of approximately 0.804% (0.843  $\mu\text{g/mL}$ ), while EVP (SG/ $\gamma$ CD = 1/2) showed a significantly higher rate of approximately 3.894% (4.203  $\mu\text{g/mL}$ ). EVP (SG/CD-MOF-1 = 1/1) and EVP (SG/CD-MOF-1 = 1/2) showed dissolution rates of approximately 0.097% (0.118  $\mu\text{g/mL}$ ) and 3.114% (3.836  $\mu\text{g/mL}$ ), respectively. In EVP (SG/ $\gamma$ CD = 1/2) and EVP (SG/CD-MOF-1 = 1/2), a rapid release was observed immediately following test initiation. This is likely due to competitive interactions between bile salts and CDs, which displaced SG from the



**Figure 8.** Dissolution profiles of SG/ $\gamma$ CD and SG/CD-MOF-1 systems in FaSSIF. Results were expressed as mean  $\pm$  SD ( $n = 3$ ).

hydrophobic cavity of CD and facilitated its incorporation into bile salt micelles. This suggests that SG is transferred from the SG/ $\gamma$ CD and SG/CD-MOF-1 inclusion complexes to the bile salt micelles, leaving the CD moieties free in solution. Furthermore, the formation of micelle-like structures around the SG by taurocholic acid (TCA) might have further enhanced dissolution. The enhanced dissolution of SG observed in FaSSIF can likely be attributed to a combination of amorphization and inclusion complexation. PXRD and DSC results indicated a reduction in crystallinity and the presence of amorphous phases upon complex formation, while NMR analyses supported molecular-level interactions between SG and the cyclodextrin hosts. These findings suggest that both amorphization and inclusion contribute synergistically to improving the solubility. However, the precise contribution of amorphization versus inclusion could not be distinguished within the scope of the present study. Future work involving advanced techniques, such as solid-state 2D NMR, synchrotron PXRD, or dissolution testing under conditions that decouple amorphous and inclusion effects, will be required to clarify their respective roles. The differing inclusion modes of SG with  $\gamma$ CD and CD-MOF-1 may also contribute to the distinct release profiles observed. In particular, the release behavior is likely influenced by the competitive displacement of SG from the CD cavity by NaTC in FaSSIF, as previously reported.<sup>40</sup> While the dissolution profiles of SG/ $\gamma$ CD and SG/CD-MOF-1 were broadly similar, the two carriers differ in their practical characteristics.  $\gamma$ CD complexes are advantageous due to their simplicity of preparation, established safety, and regulatory acceptance, making them attractive for straightforward pharmaceutical applications. In contrast, CD-MOF-1 provides a rigid three-dimensional framework with a unique potential for enhanced guest stability, controlled release behavior, and multifunctional loading. However, CD-MOF-1 may also pose potential risks associated with potassium ion release and *in vivo* stability, which warrant careful evaluation in future studies.  $\gamma$ CD is composed of toroidal cavities with

flexible cyclic oligosaccharide frameworks, whereas CD-MOF-1 features a rigid three-dimensional microporous network constructed from  $\gamma$ CD units bridged by potassium ions ( $K^+$ ) and stabilized by oxide ions ( $O^{2-}$ ). These structural differences give rise to distinct channel architectures:  $\gamma$ CD provides relatively flexible hydrophobic cavities that can accommodate guest molecules through dynamic conformational adjustment, while CD-MOF-1 maintains rigid channels that may promote more uniform encapsulation and controlled release. Despite these differences, both complexes exhibited similarly rapid dissolution in FaSSIF, which may be attributed to the competitive displacement of SG by bile salts and subsequent micellar solubilization, overriding the structural variations in the carriers. In addition, the presence of  $K^+$  and  $O^{2-}$  in CD-MOF-1 may influence the local microenvironment by modulating ionic strength and basicity, potentially enhancing the destabilization of host–guest interactions and accelerating SG release. These considerations underscore the importance of both the host framework structure and the inorganic components of CD-MOF-1 in dictating its dissolution behavior. In this study, inclusion complexes of SG, a lipophilic derivative of glycyrrhizic acid, with  $\gamma$ -cyclodextrin ( $\gamma$ CD) and CD-MOF-1 were successfully prepared using a solvent evaporation (EVP) method. PXRD and DSC analyses confirmed the disappearance of the crystalline peaks and the melting point of SG, indicating amorphization or inclusion complex formation. In addition, NIR and  $^{13}C$  CP/MAS NMR analyses revealed chemical shift changes and peak broadening of the C–H and carbon signals, indicating intermolecular interactions and differences in inclusion modes between  $\gamma$ CD and CD-MOF-1.

Notably, CD-MOF-1 possesses a rigid 3D crystalline framework, which differs substantially from the flexible structure of  $\gamma$ CD in terms of molecular diffusion and solvent interactions. This structural distinction may yield different drug-retention capabilities, dispersion behaviors in aqueous media, and dissolution profiles.<sup>41,42</sup>

SG possesses anti-inflammatory and hepatoprotective properties; however, its poor water solubility limits its gastrointestinal absorption and clinical efficacy. This study demonstrated that inclusion complexation with  $\gamma$ CD and CD-MOF-1 markedly improved the solubility of SG. Since SG conventionally requires high oral doses, the improved absorption achieved by these inclusion complexes may enable a substantial reduction in the required dosage. This would reduce dose-dependent adverse effects and improve patient adherence and, ultimately, treatment outcomes.<sup>43</sup> The findings of this study support the potential utility of  $\gamma$ CD- or CD-MOF-1-based formulations for developing effective oral delivery systems of SG for chronic liver disease and hepatic disorder treatment and prevention. From a safety perspective,  $\gamma$ CD is generally regarded as a pharmaceutically acceptable excipient; however, the *in vivo* stability and potential toxicological concerns of CD-MOF-1 warrant careful consideration. Recent studies have reported the feasibility of CD-MOFs as drug carriers and provided preliminary evidence of acceptable biocompatibility.<sup>44,45</sup> Nevertheless, issues such as potassium ion release, framework degradation in the gastrointestinal tract, and possible accumulation in tissues cannot be excluded. Therefore, comprehensive toxicological investigations, including both *in vitro* and *in vivo* studies, will be essential to confirm the clinical applicability of CD-MOF-1-based formulations. It should also be mentioned that the solubility of SG in water was

too low to allow reliable quantification by HPLC. Likewise, although solution-state NMR in  $D_2O$  would have been valuable for probing SG–CD interactions, the lack of solubility precluded such experiments. These limitations highlight the practical challenges of working with SG and further support the rationale for exploring solid-state and inclusion complex strategies.

**Limitations and Future Work.** This study was confined to *in vitro* physicochemical and FaSSIF dissolution assessments aimed at establishing proof of concept for inclusion-driven solubility enhancement of SG. Future studies should evaluate the *in vivo* pharmacokinetics and bioavailability of these inclusion complexes using animal models to validate their pharmaceutical applicability.<sup>8</sup> Although the EVP (SG/ $\gamma$ CD = 1/2) and EVP (SG/CD-MOF-1 = 1/2) samples showed  $\sim 194$ - and  $\sim 193$ -fold higher apparent solubility after 72 h and rapid initial release in FaSSIF, respectively, *in vivo* pharmacokinetic confirmation remains necessary to determine whether these gains translate into improved oral exposure ( $C_{max}$ , AUC). As a critical next step, we plan to conduct comparative oral PK studies in rodents across intact SG, SG/ $\gamma$ -CD, and SG/CD-MOF-1, alongside the evaluation of systemic potassium exposure and the structural integrity/biotransformation of CD-MOF-1 in the gastrointestinal tract. These investigations will also help decouple the respective contributions of amorphization and inclusion to the observed dissolution behavior.

## CONCLUSION

In this study, the inclusion complexes of SG with  $\gamma$ CD and CD-MOF-1 were successfully prepared in the solid state at molar ratios of 1/1 and 1/2 using the evaporation method.

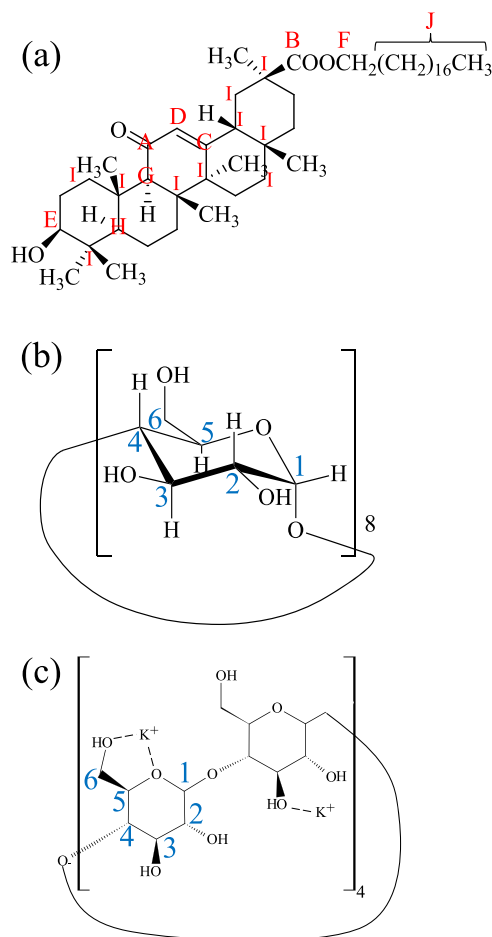
The disappearance of characteristic crystalline and melting peaks derived from SG in the PXRD and DSC measurements confirmed the formation of inclusion complexes and their amorphization.  $^{13}C$  CP/MAS NMR and NIR analyses revealed chemical shifts and a broadening of C–H and carbon signals, indicating clear molecular interactions between SG and  $\gamma$ CD and CD-MOF-1. In the solubility studies, EVP (SG/ $\gamma$ CD = 1/2) and EVP (SG/CD-MOF-1 = 1/2) exhibited approximately 194-fold and 193-fold improvements, respectively, compared with intact SG. In dissolution tests, both complexes demonstrated rapid initial release, with the 1/2 molar ratio samples showing particularly high initial dissolution rates. These results indicate the high aqueous dispersibility and efficient drug release of the complexes in bile salt-containing simulated intestinal fluid (FaSSIF). Moreover, CD-MOF-1, with its rigid three-dimensional framework, exhibited distinct inclusion behavior and dispersion properties compared to  $\gamma$ CD. These characteristics highlight the potential of CD-MOF-1 as a novel host carrier for poorly water-soluble drugs.

The findings of this study demonstrate that inclusion complexation with  $\gamma$ CD and CD-MOF-1 significantly enhances the solubility and dissolution performance of SG, providing an effective pharmaceutical strategy for the oral formulation development of SG. This approach offers promising potential for the treatment and prevention of chronic liver diseases using low-dose orally administered SG.

While the present work provides an *in vitro* foundation for SG formulation using  $\gamma$ -CD and CD-MOF-1, confirmation of oral bioavailability improvement through *in vivo* pharmacokinetic studies is warranted as a critical next step.

## MATERIALS AND METHODS

**Materials.** SG ( $C_{48}H_{76}O_4$ , purity >98%, Lot QQH8A-IQ) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).  $\gamma$ -Cyclodextrin ( $\gamma$ -CD; Lot 801250) was kindly provided by CycloChem Co., Ltd. (Tokyo, Japan) and used after storage at 40 °C and 82% relative humidity for 7 days (Figure 9). All other reagents were obtained from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan) or Kanto Chemical Co., Inc. (Tokyo, Japan).



**Figure 9.** Chemical structures of SG/ $\gamma$ CD and SG/CD-MOF-1: (a) SG ( $C_{48}H_{76}O_4$ ), (b)  $\gamma$ CD, and (c) CD-MOF-1.

**Preparation of Cyclodextrin-Based Metal–Organic Frameworks (CD-MOF-1).** The cyclodextrin-based metal–organic frameworks (CD-MOF-1) were prepared as previously described.<sup>37</sup>  $\gamma$ -Cyclodextrin ( $\gamma$ CD; 1 mmol) and KOH (8 mmol) were dissolved in distilled water (approximately 20 mL), and ethanol (50 mL) was used for vapor diffusion over 1 week. The resulting crystals were collected via filtration, washed with ethanol, and air-dried at room temperature. To maintain a constant water content, CD-MOF-1 was stored under ambient conditions in a desiccator. The molecular composition of CD-MOF-1 was represented as  $\gamma$ CD $\cdot$ (KOH)<sub>2</sub> (Figure 9).

**Preparation of PM and EVP.** PM was prepared by weighing SG and  $\gamma$ CD or CD-MOF-1 at a molar ratio of 1/1 and then mixed with a vortex mixer for 1 min. For EVP preparation, SG was dissolved in 12.5 mL of ethanol and mixed with CD-MOF-1 premoistened with 100  $\mu$ L of distilled water.

The mixture was then subjected to solvent evaporation using a rotary evaporator (Rotavapor R-215, Büchi, Switzerland) under conditions of 47 °C and 58 mbar to yield the EVP.

**FaSSiF.** FaSSiF was prepared as previously described.<sup>46</sup> A stock buffer solution (500 mL) was prepared by dissolving 31.526 mM sodium dihydrogen phosphate dihydrate and 116.6 mM sodium chloride in distilled water, and the pH was adjusted to 6.5 using sodium hydroxide solution. From this stock solution, 125 mL was used to prepare a NaTC solution (12 mM), ensuring a final concentration of NaTC of 3 mM in FaSSiF when added to 450 mL of the stock solution.

To prepare 500 mL of FaSSiF, 0.2856 g of lecithin for biochemical use was weighed to yield a final concentration of 0.75 mM. The weighed lecithin was dissolved in 3 mL of dichloromethane in a round-bottomed flask. Then, 125 mL of the NaTC solution was added dropwise to the lecithin solution under ultrasonication. The resulting mixture was subjected to solvent evaporation using a rotary evaporator (Rotavapor R-215, Nihon Buchi, Tokyo, Japan) at 40 °C under reduced pressure (50 mbar). The concentrate was transferred to a 500 mL volumetric flask. The original round-bottomed flask was rinsed with the remaining 325 mL of the stock solution, and the rinses were combined into a volumetric flask. Finally, the volumetric flask was brought to volume with distilled water, while the glassware used in the preparation was rinsed, yielding 500 mL of FaSSiF.

**Quantitative Analysis of SG Using High-Performance Liquid Chromatography (HPLC).** Quantitative analysis was performed using an HPLC (e2695, Waters, Tokyo, Japan) under detection at a wavelength of 254 nm. The chromatographic separation was carried out using an Inertsil ODS-3 column (4.6  $\times$  150 mm, 5  $\mu$ m particle size, Serial No. 19J0056789). The injection volume was 20  $\mu$ L, and the column temperature was maintained at 40 °C. The mobile phase consisted of a methanol/ethanol mixture (75/25, v/v); the retention time of SG was adjusted to approximately 9 min.

**PXRD.** PXRD analysis was performed by using a MiniFlex II diffractometer (Rigaku Corporation, Tokyo, Japan). The diffraction intensity was detected with a NaI scintillation counter. Cu K $\alpha$  radiation was used as the X-ray source at 30 kV and 15 mA. The measurements were conducted by using a scan rate of 4°/min over a  $2\theta$  range of 5–40°. Powder samples were packed onto glass sample holders in a manner that ensured a flat and uniform surface for measurements.

**DSC.** Thermal analysis was conducted by using a Thermo Plus EVO high-sensitivity differential scanning calorimeter (Rigaku Corporation, Tokyo, Japan). Approximately 2 mg of each sample was sealed in an aluminum pan and heated at a rate of 10.0 °C/min under a nitrogen gas flow of 60 mL/min. Prior to measurement, the instrument was calibrated using indium as a standard.

**NIR Absorption Spectroscopy.** NIR spectroscopy was performed using a UV–vis–NIR spectrophotometer (V-770 EX, JASCO, Tokyo, Japan). The measurements were conducted in the wavenumber range of 10,000–4000  $\text{cm}^{-1}$ , with a scan time of 8 s and at a measurement temperature of 25 °C. Each sample was loaded into a sample cup and scanned at 1 nm intervals along the optical path. The acquired spectra were further processed via a second-derivative transformation.

**Solid-State NMR Spectra.** Solid-state NMR measurements were performed using a digital NMR spectrometer (AVANCE NEO 400 system, Bruker, Kanagawa, Japan). A 4 mm iProbe CP/MAS probe was used with resonance

frequencies of 400.13 MHz for  $^1\text{H}$  and 100.62 MHz for  $^{13}\text{C}$ . The MAS spinning rate was set at 10 kHz, and the measurements were conducted at 25 °C. The  $^{13}\text{C}$  chemical shifts were externally referenced to the carbonyl carbon peak of solid glycine (176.46 ppm).

- (1) Measurement of  $^{13}\text{C}$  CP/MAS NMR spectra: The spectral width was set at 30,120.48 Hz with an acquisition time of 0.0425 s. The recycle delay was 10 s, the contact time was 2 ms, and 256 scans were accumulated.
- (2)  $T_1$  relaxation measurement (saturation recovery,  $^{13}\text{C}$  detection via indirect  $^1\text{H}$  observation):  $T_1$  measurements were performed using a saturation recovery method with an acquisition time of 0.0425 s, a recycle delay of 5 s, and a contact time of 2 ms.

**SEM.** Field-emission SEM (FE-SEM) was performed using a JSM-IT800 system (SHL configuration, JEOL Ltd., Tokyo, Japan). Prior to imaging, each sample was mounted on an aluminum stub by using carbon tape and coated with a thin layer of gold (approximately 5 nm) for 60 s. SEM images were acquired under an acceleration voltage of 1 kV by using a secondary electron detector. Observations were conducted at room temperature under a high vacuum. Representative fields were selected to ensure reproducibility and capture the characteristic morphology of each sample.

**Solubility of SG/ $\gamma$ CD and SG/CD-MOF-1 Systems in FaSSIF.** SG, PM(SG/ $\gamma$ CD), PM(SG/CD-MOF-1), EVP (SG/ $\gamma$ CD), or EVP (SG/CD-MOF-1) was added to 10 mL of FaSSIF at a concentration of 10 mg per sample. The mixtures were shaken at 37 °C for 6, 24, and 72 h using a shaking incubator (Taitec Co., Ltd., Saitama, Japan). After each time point, the samples were filtered through a 0.45  $\mu\text{m}$  membrane filter, and the resulting filtrates were analyzed by using HPLC.

**Dissolution Profile.** Dissolution testing was conducted using a dissolution tester (NTR-VS6P, Toyama Sangyo Co., Ltd., Osaka, Japan) in accordance with the paddle method described in the Japanese Pharmacopoeia, 17th Edition. The test was performed using 300 mL of FaSSIF maintained at 37  $\pm$  0.5 °C and stirred at 50 rpm. SG was accurately weighed to obtain a dose of 30 mg and then introduced into the vessel.

Aliquots of 10 mL were withdrawn at 5, 10, 15, 30, and 60 min, and each sample was filtered through a 0.20  $\mu\text{m}$  membrane filter (DISMIC 2SAS, ADVANTEC, Tokyo, Japan). To maintain a constant volume of the dissolution medium, an equal volume of prewarmed FaSSIF at the same temperature was added immediately after each sampling. The resulting filtrates were analyzed under the specified HPLC conditions.

## ■ ASSOCIATED CONTENT

### Data Availability Statement

The data underlying this study are available upon reasonable request from the corresponding author. All data supporting the findings are included in the manuscript and [Supporting Information](#).

### SI Supporting Information

The Supporting Information is available free of charge at <http://pubs.acs.org/doi/10.1021/acsomega.5c07202>.

Additional PXRD and DSC patterns (Figures S1 and S2) for SG/ $\gamma$ CD and SG/CD-MOF-1 systems, along with supporting data to confirm the formation of inclusion complexes at different molar ratios ([PDF](#))

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Conceptualization: M.U., A.O., J.T., Y.Is., D.N., K.T., and Y.In.; Methodology: M.U., A.O., J.T., and Y.In.; Software: M.U., A.O., J.T., Y.Is., D.N., K.T., and Y.In.; Validation: M.U., A.O., J.T., and Y.In.; Formal analysis: M.U., A.O., J.T., and Y.In.; Investigation: M.U., A.O., J.T., and Y.In.; Resources: M.U., A.O., J.T., and Y.In.; Data curation: M.U., A.O., J.T., and Y.In.; Writing—original draft preparation: M.U., A.O., J.T., and Y.In.; Writing—review and editing: M.U., A.O., J.T., and Y.In.; Visualization: M.U., A.O., J.T., and Y.In.; Supervision: M.U., A.O., J.T., and Y.In.; Project administration: M.U., A.O., J.T., and Y.In.; Funding acquisition: Y.Is., D.N., K.T., and Y.In. All authors have read and agreed to the published version of the manuscript.

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### Notes

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The authors declare no competing financial interest.

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## ■ ABBREVIATIONS

CD, cyclodextrin; CD-MOF-1, cyclodextrin-based metal-organic framework-1; CP/MAS NMR, cross-polarization/magic-angle spinning nuclear magnetic resonance; DSC,

differential scanning calorimetry; EVP, evaporation method; FaSSIF, fasted-state simulated intestinal fluid; NIR, near-infrared spectroscopy; PXRD, powder X-ray diffraction; SEM, scanning electron microscopy; SG, stearyl glycyrrhetinate; TCA, taurocholic acid; NaTC, sodium taurocholate

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