



Solid-state β -cyclodextrin complexes containing geraniol

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ABSTRACT

Geraniol (GO) is a natural acyclic monoterpene derived from herb oils. In the present work, the complexation of GO with β -cyclodextrin (β -CD) was performed using physical mixture (PM), slurry (SC) and paste (PC) procedures and the complexes obtained were evaluated. To prove the formation of complexes in final products, FT-IR, DSC, TG/DTG, Karl Fisher method and SEM were considered. Thermal analysis clearly indicated the formation of complexes by SC and PC methods, but the best results were obtained especially in the case of PC samples GO/ β -CD. In the IR spectra the characteristic absorption of β -CD is superposed over the geraniol ones. The new solid phase formed using the slurry and paste procedures had a crystal structure which was different from the original morphology of β -CD.

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1. Introduction

Geraniol (3,7-dimethylocta-trans-2,6-dien-1-ol) is an acyclic monoterpene alcohol with the chemical formula $C_{10}H_{18}O$ (Fig. 1) [1]. It is considered as generally-recognized-as-safe (GRAS) by FDA. Also, geraniol may be a useful chemical moiety to serve as a lead molecule for anticancer drug development [2–5]. In addition, geraniol exhibits various biochemical and pharmacological properties [6]. Researchers have shown geraniol to be an effective plant-based insect repellent and its potential as an antimicrobial agent has been highlighted in several studies [1]. Geraniol exerts *in vitro* and *in vivo* antitumor activity against murine leukemia, hepatoma and melanoma cells [7–11].

However, approximately 40% of new chemical entities exhibit poor aqueous solubility, which presents a major challenge for their use in therapeutics because it leads to low bioavailability [12]. Cyclodextrins (CDs) are water-soluble, cyclic oligomers comprising 6–8 Unit of glucopyranose bonded together by α -(1,4) linkages [13]. Among the cyclodextrins, the β -CD (which comprised 7 Unit of glucopyranose) is widely used since its cavity size is suitable for

common pharmaceutical drugs with molecular weights between 200 and 800 g mol⁻¹ and due to its reasonable price [14].

Thus, the most common pharmaceutical application of CDs is that of enhancers of solubility of hydrophobic drugs, but they can also increase the stability and bioavailability of drug molecules [15,16]. Also, CDs can be used to reduce gastrointestinal and ocular irritation, to eliminate unpleasant odors or taste, and to prevent drug-additive interactions [17].

In the present work, it seemed of interest to investigate the complexation of geraniol with β -CD. To this aim equimolar geraniol- β -cyclodextrin combinations prepared by physical mixing (PM), paste method (PC), and slurry (SC) were tested for solid-state interaction using differential scanning calorimetry (DSC), thermogravimetry/derivative thermogravimetry (TG/DTG), Karl Fisher analysis, scanning electron microscopy (SEM) and Fourier transform infrared (FT-IR) spectroscopy.

2. Materials and methods

2.1. Material

Geraniol 98% was purchased from Sigma (St. Louis, USA, lot 9446LJ-498). Pharmaceutical grade β -cyclodextrin was obtained from Sigma-Aldrich, St. Louis, USA. All other chemical reagents

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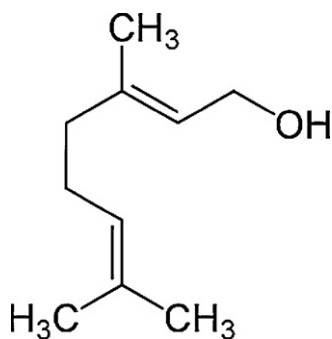


Fig. 1. Molecular structure of geraniol.

were of at least reagent grade and all materials were used as supplied.

2.2. Preparation of inclusion complexes

Inclusion complexes were prepared by three different procedures. A physical mixture (PM) was prepared by the addition of geraniol (GO) to an agate mortar containing powdered β -CD under manual agitation. The GO/ β -CD molar ratio was maintained as described for inclusion complex preparation and the mechanical mixture was stored in airtight glass containers. Paste complexation (PC) was carried out by homogenization of β -CD (1.135 g) with water (1.2:4, v/w) directly in an agate mortar. In a second step, 154 mg of GO (1:1 molar guest:host ratio) was added to β -CD paste under constant manual agitation. Then, the material was dried at room temperature (in a desiccator) till the formation of a glass film, which was removed by manual trituration and stored in airtight glass containers. Slurry complexation (SC) was carried out by the addition of water to 126 a beaker containing 1.135 g of β -CD (3:4, v/w). 154 mg of GO, which is equal to about a 1:1 molar guest:host ratio, was added to the slurry and stirred for 36 h by a magnetic stirring device operating at 400 rpm (Quimis Q 261A21, Brazil). Thereafter, the mixture was heated to 70 °C for 2 h in the same device, transferred to an agate mortar, and dried in a desiccator.

2.3. Moisture determination

The moisture contents of the mechanical mixture, slurry, and paste complexes were determined by the Karl Fisher method using a KF 1000 Analyzer (Brazil) and pyridine (Merk) as titrating solution. The analyses were carried out in triplicate.

2.4. Thermal analysis

DSC curves were obtained in a DSC-50 cell (Shimadzu) using aluminum crucibles with about 2 mg of samples, under dynamic nitrogen atmosphere (50 mL min⁻¹) and heating rate of 10 °C min⁻¹ in the temperature range from 25 to 600 °C. The DSC cell was calibrated with indium (m.p. 156.6 °C; $\Delta H_{\text{fus.}} = 28.54 \text{ J g}^{-1}$) and zinc (m.p. 419.6 °C). TG/DTG curves were obtained with a thermobalance model TGA 50 (Shimadzu) in the temperature range 25–900 °C, using platinum crucibles with ~3 mg of samples, under dynamic nitrogen atmosphere (50 mL min⁻¹) and heating rate of 10 °C min⁻¹. The TG/DTG was calibrated with calcium oxalate monohydrate, conforming to ASTM standard.

2.5. Fourier transform infrared (FT-IR) spectroscopy

The infrared spectra of geraniol (GO), β -CD, physical mixture (PM), paste complex (PC) and slurry complex (SC) were evaluated and the infrared absorption data was obtained in the range of 4000–400 cm⁻¹ in KBr pellets using an FT-IR Bomem spectrophotometer, model MB-120, at room temperature.

2.6. Scanning electron microscopy

The β -CD, physical mixture (PM), paste complex (PC) and slurry complex (SC) were mounted on aluminum stubs, coated with a thin layer of gold and visualized with a JEOL Model JSM-6360-LV scanning electron microscope, at an accelerated voltage of 20 kV.

3. Results and discussion

Thermal analyses of the GO/ β -CD particles revealed the formation of complexes. The DSC curve of GO shows an endothermic peak at nearly 150 °C corresponding to its volatilization. As can be seen in Fig. 2, the curves corresponding to GO/ β -CD complexes did not

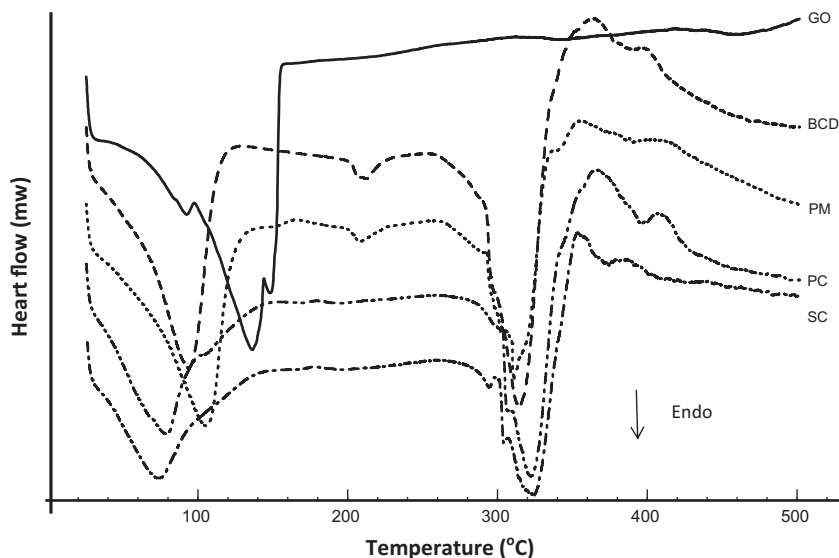


Fig. 2. DSC curves of geraniol (GO), β -CD, physical mixture (PM), paste complex (PC) and slurry complex (SC) in dynamic nitrogen atmosphere (100 mL min⁻¹) and heat rate 10 °C min⁻¹.

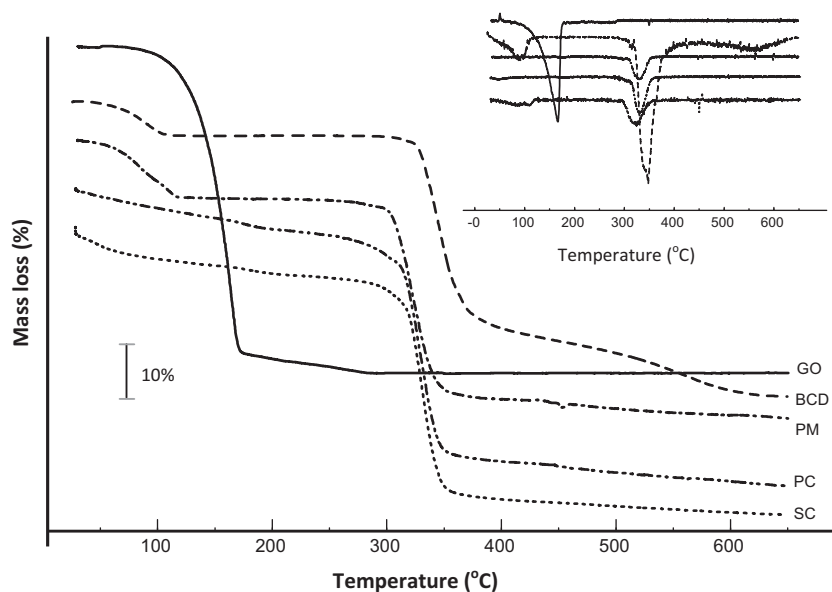


Fig. 3. TG curves of geraniol (GO), β -CD, physical mixture (PM), paste complex (PC) and slurry complex (SC) in dynamic nitrogen atmosphere (100 mL min^{-1}) and heat rate $10^\circ \text{C min}^{-1}$.

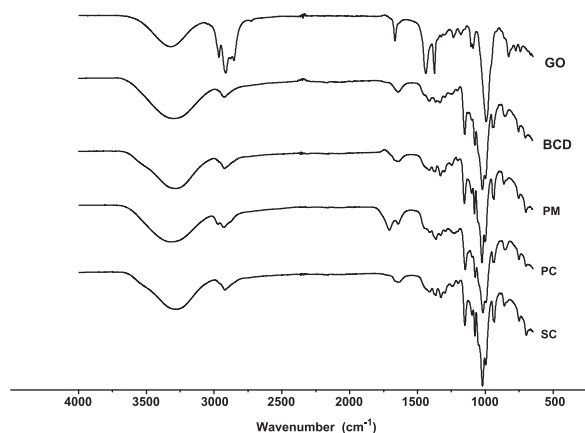


Fig. 4. The infrared spectra of geraniol (GO), β -CD, physical mixture (PM), paste complex (PC) and slurry complex (SC).

show a sharp endothermic peak in the range of the volatilization of the pure compound (150°C). The disappearance of this event is due to its encapsulation in the host β -CD.

Thus, the DSC curves of the GO/ β -CD complexes indicate endothermic peaks: the first in the range of $25\text{--}121^\circ \text{C}$ (which correspond to the release of water molecules as well as to release of

GO, probable adsorbed in the surface), the second in the range of $121\text{--}270^\circ \text{C}$, where GO strongly encapsulated is released, and at $\sim 280^\circ \text{C}$, where the decomposition of cyclodextrin molecules appears. In the case of β -CD, only the peaks corresponding to the release of water molecules (higher than in the case of complexes) and to decomposition appear (Fig. 2). Similar results were observed by Hadaruga et al. that studied the influence of the hydrophobicity of solvent mixture and the preheating temperature on the water extraction process for α - and β -CD as well as for their complexes with various essential oils using classical Karl Fischer titration method and thermogravimetric analysis [18].

The difference in the DSC curves of the PM and the complex of GO/ β -CD clearly indicate complex formation between the components. The temperature peak at 297°C (indicated in the graphic) is because of the decomposition of the formed inclusion complex.

Fig. 3 shows the TG/DTG curves of the materials and Table 1 lists the mass losses calculated from specific intervals for each material studied in the present work. By their data analysis, it can be seen that the major fraction of GO ($\Delta m = 99.14\%$) evaporates up to 230°C . The boiling point was determined according to Betts [19]. Then a continuous decomposition of GO and small amount of impurities occurs in a temperature range from 230 to about 291°C .

As shown in Fig. 3, the mass loss (%) of free β -CD at the heating rate of $10^\circ \text{C min}^{-1}$ can be divided into three consecutive processes. The sample in process a is in the temperature range

Table 1

Mass losses for geraniol, β -CD, physical mixture and geraniol/ β -CD complexes and moisture contents obtained by Karl Fisher method.

	Mass loss (%)				Karl Fisher
	1st step	2nd step	3rd step	4th step	% water
Geraniol (GO)	99.14 ^a	1.10	–	–	1.91
β -CD	11.83 ^b	–	65.00 ^e	23.12 ^f	13.19
Physical mixture (PM)	19.46 ^c	1.48 ^d	66.04 ^e	6.99 ^f	11.60
Paste complex (PC)	8.12 ^c	9.31 ^d	73.21 ^e	9.91 ^f	11.05
Slurry complex (SC)	8.94 ^c	6.13 ^d	73.19 ^e	6.10 ^f	11.81

^a Percentage of the geraniol evaporates up to 230°C .

^b Percentage of water releasing up to 120°C .

^c Mass loss related to evaporation of the geraniol and the water release up to 120°C .

^d Mass loss probably attributed to geraniol release in the interval from 120 to 270°C .

^e Thermal decomposition in the interval from 270 to 365°C .

^f Elemental carbon formation due to sample carbonization in the interval from 365 to 900°C .

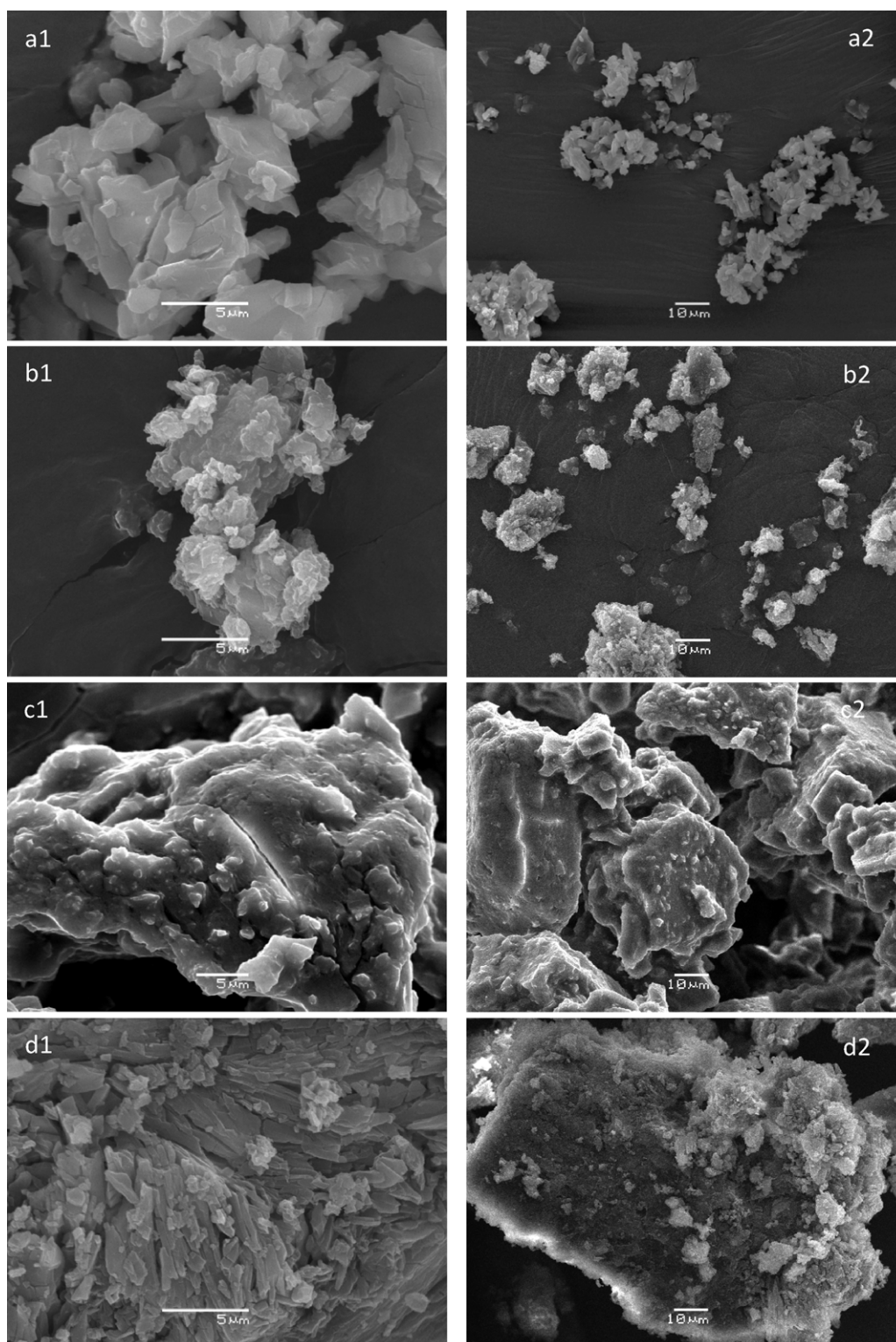


Fig. 5. SEM micrographs of cross-sections (1 and 10 μm) of (a) β -CD, (b) physical mixture (PM), (c) paste complex (PC) and (d) slurry complex (SC).

from 25 to 125 °C with a mass loss of 11.61%, which is due to the release of water molecules from the outside or/and inside of β -CD cavity. Subsequently, the sample is kept in a very wide temperature range from 125 to 270 °C. Then the sample undergoes a rapid melting/decomposition ($\Delta m = 63.54\%$, 270–391 °C). During continuous heating, the sample is carbonized and incinerated ($\Delta m = 24.16\%$, $T = 391$ –800 °C). These results are also in accordance

with those shown by Xu et al. [20] for the encapsulation of clove oil and Marreto et al. for the inclusion of *Lippia gracilis* essential oil [21].

The curve of the PM was a superposition of the guest and host curves, which indicates a lower evidence of inclusion and significant interaction between the host and guest molecules. Two overlapping steps were exhibited as causing 19.46% of mass loss,

related to the evaporation of the GO and the water release from the β -CD up to 120 °C (Fig. 3).

The curve of the complex prepared by slurry method (SC) showed a water/GO loss event from r.t. up to 125 °C. In the interval from 125 to 275 °C, a gradual mass loss (6.13%) was recorded and can be attributed to GO release. On the other hand, the curve of the complex prepared by paste method (PC) was similar to the SC curve, but showed an important difference between 125 and 275 °C. In this interval, the PC curve presents an acute mass loss event, which gives a strong indication of guest inclusion, in contrast to that observed by slurry method. For this method, between 130 and 275 °C, 9.31% further mass loss was detected because of the release of GO from its inclusion complex.

It is important to note that TG cannot distinguish between GO and water mass losses from mechanical mixture or inclusion complexes. Thus, a volumetric water determination method (Karl Fisher) was used to estimate total geraniol losses from TG curves. Table 1 lists the percentages of water calculated by the Karl Fisher method. Total oil retention of the complexes was calculated by subtracting total mass loss, up to 275 °C, from the percentage of water amount determined by the Karl Fisher method, and expressed as a function of the theoretical amount of oil added to the complexation medium [21]. According to Hadaruga et al. classical Karl Fischer water titration is a good tool for evaluation of water concentration in cyclodextrins and their micro/nanoparticles used in food industry and KFT results are in good agreement with the thermogravimetric results; the KFT chemical method is more accurate than TG due to the fact that it evaluate only water concentration and furthermore the “surface” and “strong-bonded” water molecules can be determined [18].

The FT-IR spectra of GO, β -CD, PM, PC and SC inclusion complexes are presented in Fig. 4. The FT-IR spectrum of β -CD showed prominent absorption bands at 3292 cm^{-1} (for O–H stretching vibrations), 2925 cm^{-1} (for C–H stretching vibrations), 1643 cm^{-1} (for H–O–H bending), 1152 cm^{-1} (for C–O stretching vibration) and 1020 cm^{-1} (C–O–C stretching vibration). Similar results were obtained by Wang et al. [22]. The FT-IR spectrum of the GO was characterized by principal transmission peaks at 3326 cm^{-1} , 1416 cm^{-1} (OH), 2978 cm^{-1} , 2933 cm^{-1} , 1371 cm^{-1} (CH_2), 1650 cm^{-1} , 1022 cm^{-1} , 946 cm^{-1} , 863 cm^{-1} (C=C), 1100 cm^{-1} , 1155 cm^{-1} (C–C–C) and 1707 cm^{-1} (C–O). In the FT-IR spectra of the complexes the GO bands are almost completely obscured by very intense and broad β -CD bands. The FT-IR spectra of the GO/ β -CD inclusion complexes showed no features similar to pure GO. An examination of complexes (Fig. 4) spectra revealed two small but distinct bands at 2923 and 1081 cm^{-1} . Absorption band at 1707 cm^{-1} was shifted toward this higher frequency at 1723 cm^{-1} of GO/ β -CD. These changes may be related to the formation of intra-molecular hydrogen bonds between GO and β -CD.

The SEM of PM, PC and SC complexes is shown in Fig. 5. β -CD aggregates resulting from self aggregation and associating with GO were examined. Pure β -CD appears as crystalline particles of different sizes without a defined shape. The electron microscopic pictures showed that the physical appearance and size of the complexes formed were different. As seen from Fig. 5, there were drastic changes in particle shapes and original morphologies of the inclusion complex products. The complexation between GO and β -CD at both methods (SC and PC) appeared as agglomerates. In contrast, the particle shapes and morphologies of the corresponding physical mixture were similar to those β -CD. These differences are due to the stacking interactions which appear in the case of natural cyclodextrins (cyclodextrin hydrate) in comparison with the cyclodextrin complexes. However, the morphology of the complex is not the only indicator of complex formation [23].

Choi et al. studied the physical characteristics of fish oil encapsulated by β -CD using a method described as self-assembling aggregation of β -CD, and reported that β -CD complexes show the largest size by the self-assembly aggregation of β -CD depending on the different mixing ratio between β -CD and fish oil [24]. In addition, Songkro et al. reported that inclusion complexes of citronellol appeared as agglomerates similar to those produced by citronellal oil, and no changes in shapes and morphologies of β -CD were observed in the case of physical mixtures when compared with pure β -CD [25].

4. Conclusion

IR, DSC, TG/DTG, SEM and Karl Fischer water titration techniques were used to verify complex formation, in a 1:1 molar ratio. These methods could be used to identify distinct differences among the various complexes. Thermal analysis clearly indicated the formation of complexes by slurry and paste methods used to obtain the inclusion complexes, but the best results were obtained especially for geraniol complexation with β -cyclodextrin by paste method. GO/ β -CD inclusion complexes form different aggregates under different conditions.

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