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Research paper

Host–guest interactions of 5-fluorouracil in supramolecular organogels

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ABSTRACT

Supramolecular organogels were formed by the self-assembly of gelator 1,3:2,4-di-O-benzylidene-D-sorbitol (DBS) in 1,2-propylene glycol. 5-Fluorouracil (5-Fu) was entrapped in the organogels as guest molecules. Field-emission scanning electron microscopic measurements of the organogels indicate that the diameters of the fibrillar aggregates formed by DBS self-assembly were in the range of 20–70 nm. The host–guest interactions between 5-Fu and supramolecular gel matrix were investigated by using temperature-dependent differential UV spectroscopy and differential scanning calorimetry. The red shifts of the absorption band of 5-Fu in the organogels are indicative of the interaction between 5-Fu and DBS. The red shifts were enhanced upon decreasing the temperature. Calculation of optimized geometries revealed the formation of hydrogen bonds between 5-Fu and DBS aggregates. The analysis of differential scanning calorimetric data for the organogels with and without 5-Fu further showed that the self-assembly of DBS was interfered by the 5-Fu in the organogels, resulting in a decrease in the dissociating temperature of DBS aggregates.

1. Introduction

Supramolecular gels are formed from low-molecular-mass organic gelators in organic or aqueous solvents via noncovalent self-assembly [1,2]. Their thermoreversibility, chemical sensitivity and polymorphic nanostructures offer potential applications as drug carriers [3,4], templates of nanomaterial preparation [5], gel electrolytes [6] and chiral separation [7].

Host–guest interaction is a subject of increasing attention in the field of supramolecular chemistry. This interaction occasionally plays an important role when guest molecules are introduced into supramolecular gels. For instance, van Esch et al. prepared a kind of supramolecular hydrogel formed by the gelator N′-dibenzoyl-L-cystine (DBC) and investigated the release of quinoline derivatives from the hydrogel. It was found that the release rate of 8-aminoquinoline was slow in comparison with that of 2-hydroxyquinoline due to electrostatic interactions between DBC and 2-hydroxyquinoline [8]. Montalti et al. observed energy transfer between the guest molecule propyldansylamide and the gelator 1,3,5-cyclohexyl-tricarboxamide in supramolecular hydrogels [9]. Sakurai et al. introduced plasmid DNA into the supramolecular hydrogel formed by methyl 4,6-O-(*p*-nitrobenzylidene)-α-D-glucopyranoside (*p*-NO_2Glu) as gelator and found that *p*-NO_2Glu aggregates and DNA are twisted together to form higher hierarchic fibers. It was found that the DNA in these assemblies had less expression ability compared with naked DNA [10]. Although these investigations involved host–guest interactions, little attention has been paid to the effect of temperature on the interactions because a main feature of supramolecular gels is their thermoreversibility. To the best of our knowledge, host–guest interactions, using varying temperature-dependent differential UV spectroscopy, have not been reported previously.

In the present work, hosted supramolecular organogels were prepared by the self-assembly of the gelator 1,3:2,4-di-O-benzylidene-D-sorbitol (DBS) in 1,2-propylene glycol. 5-Fluorouracil (5-Fu) was entrapped in the organogels as guest molecules. The host–guest interactions were investigated by field-emission scanning electron microscopy, polarized optical microscopy, varying temperature differential UV spectroscopy and differential scanning calorimetry. These studies not only contribute to a better understanding of the formation mechanism of supramolecular gels, but are also significant for possible applications as drug carriers, since the mass transfer behavior of drugs in supramolecular gels can be modulated by controlling the host–guest interaction process.

2. Materials and methods

2.1. Materials

1,3:2,4-Di-O-benzylidene-D-sorbitol (DBS, purity 99%) was commercially available (Hubei Huabang Chemicals Co). 5-Fluorouracil (5-Fu, purity 99.9%) was purchased from Nantong Pharmaceutics Co. 1,2-Propylene glycol was purchased from Aldrich and

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used as received. Scheme 1 shows the molecular structures of DBS and 5-Fu.

2.2. Field-emission scanning electron microscope (FESEM) and polarized optical microscopy (POM)

Designed amounts of DBS, 5-Fu and 1,2-propylene glycol were added in a test tube. The mixture was heated (ca. 120 °C) in an oil bath until the solid completely dissolved. The solution of this mixture was dropped on a glass plate and allowed to cool to room temperature. The obtained supramolecular gel (denoted as DBS gel) was freeze-dried (Freezone 6, Labconco) by liquid nitrogen and coated by gold for the measurement of FESEM (Sirion 200, FEI, accelerating voltage was 10 kV). Similarly, the DBS gel without freeze-drying was used as the sample for POM (BH-2, Olympus).

2.3. Varying temperature differential UV spectroscopy

The DBS gels with and without 5-Fu were prepared according to the above-mentioned procedure. UV absorption spectra of 5-Fu were recorded with UV–visible spectrophotometer (UV2401 PC, Shimadzu) using a matched pair of quartz cuvettes of 5-mm optical length placed in a thermostated cell holder. Herein, the UV spectra of 5-Fu were calculated based on the difference spectra [11–13] between the gels with and without 5-Fu.

2.4. Differential scanning calorimetry

Thermograms of DBS gels with and without 5-Fu were recorded on a differential scanning calorimeter (DSC 7, Perkin–Elmer) at a scan rate of 10 °C/min.

2.5. Calculation of optimized geometries

Calculations were performed utilizing the Spartan program package (Spartan 06, Wavefunction Inc.). The optimized geometries of 5-Fu/DBS and 5-Fu/DBS dimer were constructed by using the ab initio Hartree-Fock method and a 3-21G* basis set [14].

3. Results and discussion

3.1. Morphology of DBS aggregates in the supramolecular gels

The DBS is an efficient gelator for most organic solvents. The mechanism of self-assembly in organic solvent has been extensively reported in the literature [15–17]. Fig. 1A shows the FESEM image of the DBS xerogel. The fibrillar DBS aggregates with diameters ranging from 20 to 70 nm are clearly observed. Fig. 1B shows the POM image of the DBS gel. Typical Maltese cross extinction reveals that the fibrils further aggregate into spherical crystallites [18]. In our experiments, it was found that the presence of 5-Fu in the DBS gels does not influence the formation of DBS aggregates and probably exerts insignificant effects on the morphology of DBS aggregates (*vide infra*). We assume that the solvent 1,2-propylene glycol is immobilized in the three-dimensional networks, consisting of fibrillar DBS aggregates, through the capillary force [19]. 5-Fu was also entrapped in the networks as guest molecules.

3.2. Differential UV spectra of 5-Fu in DBS/1,2-propylene glycol solutions and DBS gels

Generally, the UV spectrum of guest molecules will change when the guest and host molecules interact with each other [20]. Fig. 2A shows the UV spectra of 5-Fu in 1,2-propylene glycol containing 0.25 wt% of DBS (non-gel state) and the organogel formed by 1.5 wt% of DBS, respectively.

As shown in Fig. 2A, the λ_{max} values of 5-Fu in 1,2-propylene glycol (spectrum 1) and DBS/1,2-propylene glycol solution (spectrum 2) are 267 nm and 269 nm, respectively, while the λ_{max} of 5-Fu in the DBS gel red shifts to 277 nm and the absorbance intensities are smaller. This bathochromic shift indicates that interaction between 5-Fu and DBS does occur in the DBS gels. As shown in Scheme 1, the relevant groups for hydrogen bonding are OH and NH as donors and carbonyl and oxygen atoms as acceptors on the 5-Fu and DBS. Apparently, hydrogen bonds may be formed between these moieties, resulting in a decrease in the required energy of excitation of 5-Fu and the red shift of λ_{max}.

To further characterize the effect of temperature on this kind of interaction and taking into account the thermoreversibility of the formation of organogels, varying temperature UV spectra of 5-Fu in DBS gels were measured (Fig. 2B). Upon an increase in the temperature, blue shifts of the λ_{max} of 5-Fu were observed ranging from 286 nm at 4 °C to 279 nm at 90 °C, whereas the absorbance intensities also increased. We observed that the DBS gel formed from 1.5 wt% DBS transformed into a solution state when the temperature was raised above 80 °C. Most likely, the blue shifts of the λ_{max} of 5-Fu can be attributed to a gradual dissociation of the DBS aggregates and a collapse of the three-dimensional networks within the organogels. This result also indicates that the interaction between 5-Fu and DBS is related to the self-assembly of DBS. In other words, the space conformation of the DBS aggregates may be beneficial to the formation of hydrogen bonds with 5-Fu. To further substantiate this assumption, the calculations of optimized geometries between 5-Fu/DBS and 5-Fu/DBS dimer were carried out (Fig. 3) [21].

Calculation results indicate that hydrogen bonding occurred for both 5-Fu/DBS and 5-Fu/DBS dimer. The sites for hydrogen bonding in the case of 5-Fu/DBS are the H_{30} on the 5-Fu and the O_3

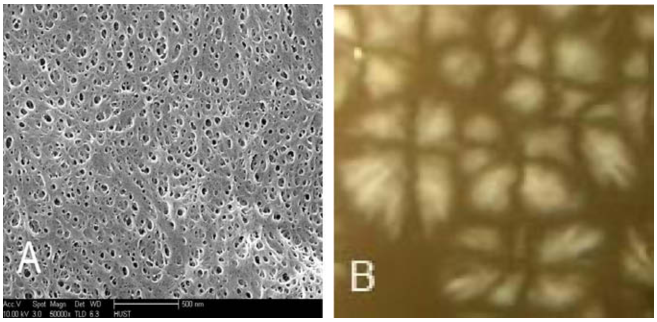


Fig. 1. FESEM (A) and POM (B) images of the organogels formed by 6 wt% of DBS in the presence of 5-Fu. The scale bar in the SEM image is 500 nm. The magnification of POM image was 200×.

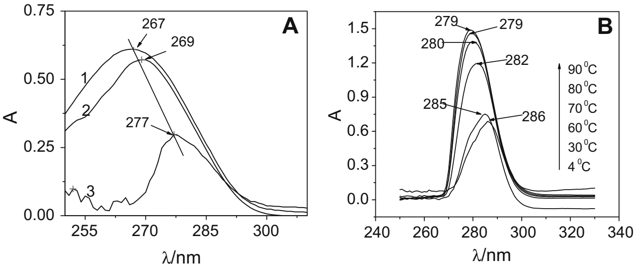


Fig. 2. Plot A: UV spectra of 5-Fu in 1,2-propylene glycol (1), 1,2-propylene glycol/DBS (0.25 wt%) solution (2) and DBS gel formed by 1.5 wt% DBS (3). The concentration of 5-Fu in the samples was 0.12 mmol/L. The measurement was carried out at room temperature. Plot B: varying temperature UV spectra of 5-Fu in a DBS gel formed by 1.5 wt% of DBS. The molar ratio of 5-Fu to DBS was 1/60.

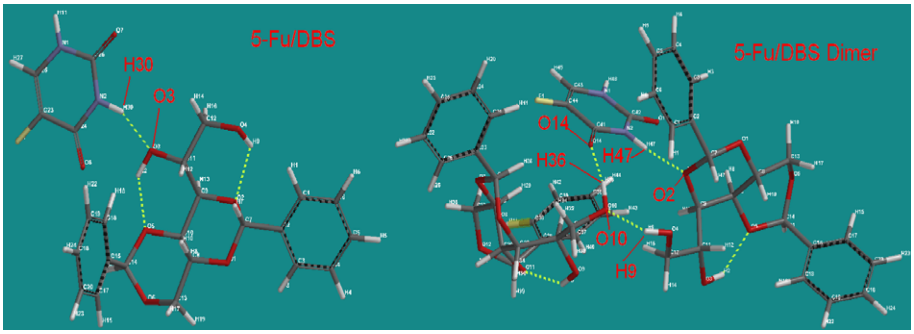


Fig. 3. Optimized geometries of 5-Fu/DBS and 5-Fu/DBS dimer.

on the DBS. The bond length is 1.752 Å. In the case of the 5-Fu/DBS dimer, however, two hydrogen bonds are found. The sites for the hydrogen bonding are the H_{47} on the 5-Fu and the O_2 on the DBS and O_{14} on the 5-Fu and the H_{36} on the DBS. The bond lengths are 1.679 Å and 1.856 Å, respectively. In addition, hydrogen bonding does also occur in the DBS dimer at the sites of $O_{10}H_9$ and $O_{14}H_{36}$. Hence, the differences of the hydrogen bond lengths and amounts of hydrogen bonds reveal that the 5-Fu/DBS dimer is more stable than 5-Fu/DBS; in other words, the interaction of 5-Fu/DBS dimer is stronger than that of 5-Fu/DBS. The calculations of optimized geometries further support the conclusions drawn on the basis of the analysis of UV spectroscopy. Thus, we assume that a part of 5-Fu dispersed in 1,2-propylene glycol and another part of 5-Fu were incorporated within the DBS aggregates as shown in Scheme 2.

3.3. The effect of concentration of 5-Fu on the interaction between 5-Fu and DBS

Generally, the host–guest interactions are related to their concentrations in the system [20]. Fig. 4A shows UV spectra of varied concentrations of 5-Fu in the 1,2-propylene glycol. Obviously, there exists a good linear relationship between absorbance intensities and concentrations in the range of experimental concentrations of 5-Fu (0.024–0.38 mmol/L). Furthermore, the λ_{max} of 5-Fu almost stays constant (266 nm) with an increase in the concentration of 5-Fu although the absorbance intensities increased, indicating that there is no interaction between the 5-Fu molecules.

Fig. 4B shows the UV spectra of varied concentrations of 5-Fu in 1,2-propylene glycol with 1.5 wt% of DBS (gel state). Herein, the molar ratios of 5-Fu and DBS were consistently used to compare the effect of concentrations of 5-Fu in the gel states. Red shifts of λ_{max} of 5-Fu were observed with an increase in concentration of 5-Fu; for instance, when the molar ratio of 5-Fu and DBS was 1/5, λ_{max} red shifts to 294 nm, indicating a significant interaction between 5-Fu and DBS aggregates. Interestingly, the absorbance peak at λ_{max} splits into two peaks when the molar ratios of 5-Fu and DBS $\geqslant$ 1/1. The peak at 296 nm no longer shifts and another peak at 299 nm still shifts to 304 nm when a 6/1 molar ratio was applied. This result is consistent with the assumption proposed in Scheme 2, namely, that the interaction of 5-Fu with DBS depends on the environment where 5-Fu is located. We propose that, for example, the λ_{max} (296 nm) was assigned to the free 5-Fu in the gels and λ_{max} (304 nm) was assigned to the 5-Fu incorporated with DBS aggregates when high concentrations of 5-Fu were applied. No peak appeared at 266 nm because the local polarity of free 5-Fu in gels is different from that in propylene glycol solution. The interaction between 5-Fu and DBS aggregates is definitely enhanced with an increase in the concentration of 5-Fu.

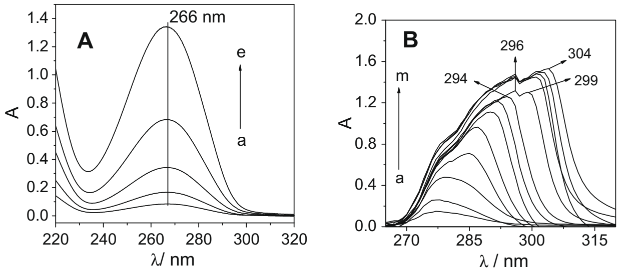


Fig. 4. Plot A: UV spectra of 5-Fu in 1,2-propylene glycol. The concentrations of 5-Fu were 0.024 mmol/L (a), 0.048 mmol/L (b), 0.096 mmol/L (c), 0.19 mmol/L (d), 0.38 mmol/L (e). Plot B: UV spectra of 5-Fu in 1,2-propylene glycol with 1.5 wt% of DBS (gel state). The molar ratios of 5-Fu and DBS were 1/750 (a), 1/300 (b), 1/150 (c), 1/60 (d), 1/30 (e), 1/12 (f), 1/10 (g), 1/5 (h), 1/1 (i), 2/1 (j), 3/1 (k), 4/1 (l) and 6/1 (m).

3.4. Thermal analysis of the DBS gels in the presence of 5-Fu

As mentioned above, supramolecular gels are thermoreversible. The sol/gel phase transition temperature is an important parameter for the characterization of their thermoreversibility. To obtain good differential scanning calorimetric data, 4 wt% of DBS was used as a gelator to prepare the DBS gels. Fig. 5 shows the thermograms of the DBS gels with and without 5-Fu. We find that the phase transition temperature of the DBS gel was about 105 °C in the absence of 5-Fu. Interestingly, this temperature decreased to about 100 °C in the

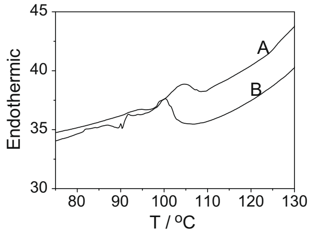
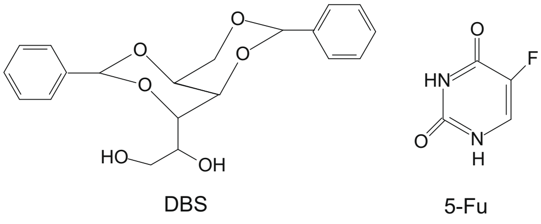
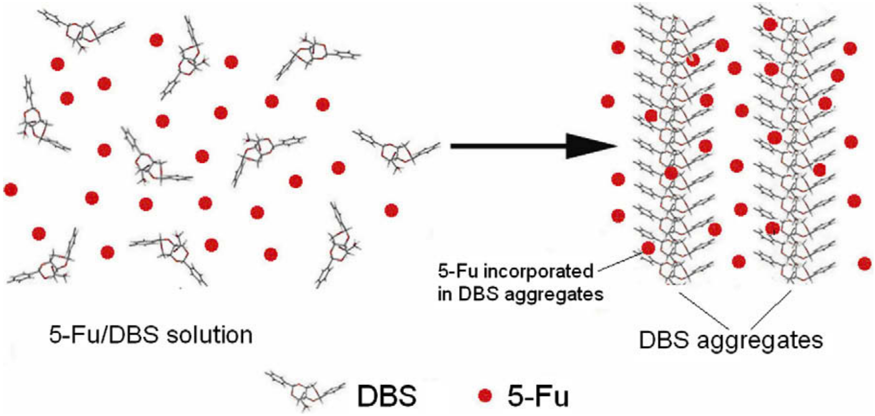


Fig. 5. DSC thermograms of 1,2-propylene glycol gel formed by 4 wt% of DBS in the absence (A) and presence (B) of 5-Fu.



Scheme 1. Molecular structures of DBS and 5-Fu.



Scheme 2. Schematic illustration of 5-Fu dispersed in the 1,2-propylene glycol gel formed by DBS.

presence of 5-Fu. It is well known that the sol–gel transition temperature is in fact the dissociation temperature of the gelator aggregates. As discussed above (Fig. 4A), almost no interaction was observed when 5-Fu was dissolved in the 1,2-propylene glycol. Therefore, the decrease in the phase transition temperature can be only attributed to a change in the morphology of the DBS aggregates. Most likely, the self-assembly of DBS is hindered by the formation of hydrogen bonds between 5-Fu and DBS aggregates as discussed above, leading to reduced order of DBS aggregates and a decrease in the dissociation temperature [22]. Similar results were also reported by Escuder and co-workers that the interaction between guest molecules (resorcinol) and gelator aggregates weakened the fibril packing, leading to a corresponding gel containing resorcinol, which is not self-sustained at room temperature [23].

4. Conclusions

The host–guest interaction was investigated for the supramolecular gels formed by the gelator 1,3:2,4-di-O-benzylidene-D-sorbitol (DBS) and 1,2-propylene glycol in the presence of 5-fluorouracil. The morphology of the xerogel reveals that the fibrillar aggregates of DBS are formed by self-assembly and that the presence of 5-Fu in the DBS gels does not affect the formation of DBS aggregates. A bathochromic shift in the UV spectra of the DBS gels was observed, indicating the hydrogen bonding interaction between 5-Fu and DBS aggregates. Upon increasing temperature, the interaction becomes weaker because the DBS aggregates gradually dissociate and the three-dimensional networks within the gels collapse. The effect of the 5-Fu concentration on the interaction indicated that the interaction between 5-Fu and DBS depends on the environment where 5-Fu is located. Thermal analysis of DBS gels further indicates that the self-assembly of DBS is hampered by the formation of hydrogen bonds between 5-Fu and DBS aggregates, resulting in reduced order of DBS aggregates and a decrease in the dissociation temperature.

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