

## New Insights into the Interactions between Dendrimers and Surfactants: 2. Design of New Drug Formulations Based on Dendrimer–Surfactant Aggregates

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The interactions between dendrimers and surfactants led to the formation of aggregates dispersed in aqueous solutions. The potential of the resulting dendrimer–surfactant aggregates as new drug formulations was evaluated. The size, morphology, and stability of the aggregates and the localization of drugs in them were determined by dynamic laser light scattering, atomic force microscopy, agarose gel electrophoresis, and nuclear magnetic resonance studies. The drug-loaded aggregates have a spherical shape and an average size of 40 nm. The drug-loading efficiency of dendrimers is significantly influenced in the presence of surfactants. The release rate of the drugs from the dendrimer–surfactant aggregates can be modulated by varying the amount of surfactant in the aggregates. The dendrimer–surfactant aggregates are promising carriers for hydrophobic drugs in transdermal administration routes.

### 1. Introduction

Incorporation of surfactants provides a lot of opportunities for optimizing polymeric drug delivery systems, such as enhancing drug loading efficiency and bioavailability, allowing controlled release of drugs from the polymeric matrixes, minimizing drug degradation and side-effects, and improving the rheological properties of drug formulations.<sup>1–4</sup> The dramatic changes in the physicochemical properties of polymers in the presence of surfactants are attributed to three types of polymer–surfactant interactions: (1) electrostatic interactions between the charged groups of the polymer and the oppositely charged group of the surfactant; (2) hydrophobic interactions between the backbone of the polymer and the hydrophobic tail of the surfactant; (3) hydrogen-bonding interactions or van der Waals interactions. As a result of the polymer–surfactant interactions, the critical micellization concentration (CMC) of the surfactant is significantly decreased to a much lower concentration, usually defined as critical aggregation concentration (CAC).<sup>5,6</sup> The resulting polymer–surfactant aggregates create a hydrophobic microenvironment in an aqueous medium, which is responsible for encapsulating hydrophobic guests and making the aggregates particularly useful in the pharmaceutical sciences.

Dendrimers are synthetic macromolecules with a well-defined treelike branching structure that radiates from a central core.<sup>7</sup> Large numbers of functional groups on the surface of dendrimers and the relative nonpolar cavities in the interior of them endow these promising materials perfect candidates as hosts for a list of guest molecules.<sup>8,9</sup> Surfactants with amphiphilic properties are ideal guests for dendrimers because of the long hydrophobic tails and the polar heads in their structures.<sup>6</sup> The structure, nature, and interaction mechanism of the dendrimer–surfactant

aggregates were reported by several groups.<sup>6,10–15</sup> The aggregates create a template-assisted supramolecular assembly consisting of a dendrimer at the core and amphiphilic surfactants on the surface by electrostatic interactions.<sup>14,15</sup> Surfactants can also penetrate the surface charge barriers of dendrimers and localize in their interior cavities by hydrophobic interactions or hydrogen-bonding interactions.<sup>6</sup> Although solubilization of fluorescent dyes such as pyrene and prodan by the dendrimer–surfactant aggregations was carried out to investigate the guest partition within the supramolecular assemblies,<sup>10,16</sup> no study reported the applications of the aggregates as drug carriers. It was reported that both dendrimers and surfactants can help drugs to penetrate through skin barriers, which is the main challenge to transdermal drug delivery.<sup>17–20</sup> The dendrimer–surfactant aggregates are expected to increase the drug loading efficiency, adhesive property, and penetration ability of dendrimers, modulate the drug release behaviors.<sup>21</sup> In a recent study from our group, the interactions between dendrimers and surfactants were investigated by two-dimensional nuclear Overhauser enhancement spectroscopy (2D-NOESY), and several dendrimer–surfactant aggregate models were proposed.<sup>6</sup> In this study, we further evaluated the possibilities of these aggregates as carriers of hydrophobic drugs. The nonsteroidal anti-inflammatory drug phenylbutazone (PBZ), the antibacterial drug sulfamethoxazole (SMZ), and the anticancer drug methotrexate (MTX) were employed as model drugs (Scheme 1). The structure of drug-loaded dendrimer–surfactant aggregates were well characterized by agarose gel electrophoresis (AGE), dynamic laser light scattering (DLS), atomic force microscopy (AFM), and 2D-NOESY techniques. The release behaviors of drugs from the aggregates and the biocompatibility of the aggregates were also evaluated.

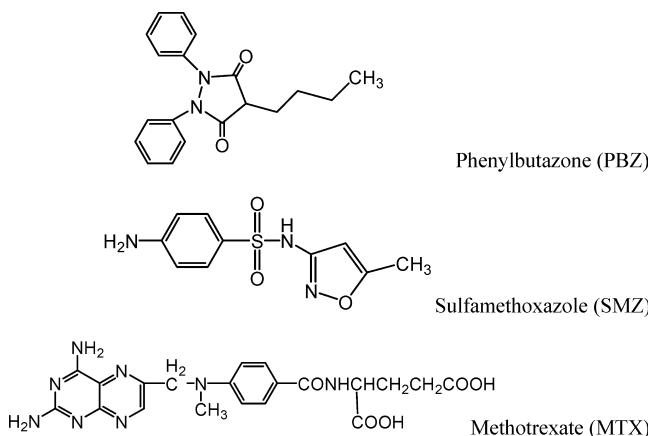
### 2. Experimental Section

**2.1. Materials.** Generation 3–5 (G3–G5) ethylenediamine (EDA)-cored and amine-terminated polyamidoamine (PAMAM) dendrimers were purchased from Dendritech, Inc. (Midland,

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**SCHEME 1: Molecular Structures of the Model Drugs PBZ, SMZ, and MTX in This Study**


MI). PBZ was obtained from Shangqiu Tiankang Fine Chemical Co. Ltd. (Henan, China). SMZ was obtained from Shouguang Fukang Pharmacy Factory (Shandong, China). MTX, sodium dodecyl sulfate (SDS), dodecyl trimethyl ammonium bromide (DTAB), and polyoxyethylene sorbitan monolaurate (Tween-20) were obtained from Shanghai BBI Co. Ltd. (Shanghai, China). Deuterium oxide ( $D_2O$ ) was purchased from Beijing Chongxi High-Tech Incubator Co., Ltd. (Beijing, China). The PAMAM dendrimers stored in methanol solutions were distilled to remove the solvents before use. Other chemicals were used as received. Double-distilled water was used in the aqueous solubility and release studies.

**2.2. Aqueous Solubility Studies.** The solubilities of PBZ, SMZ, and MTX were determined using the equilibrium solubility method described as follows.<sup>8,21</sup> Excess drugs were added to 500  $\mu L$  of each of the dendrimer–surfactant aggregate solutions (dendrimer–surfactant aggregates were prepared by adding an exact amount of surfactant into 0–4 mg/mL dendrimer solutions). The test mixtures were mechanically shaken for 24 h at room temperature to ensure that the drugs reached saturation in each tube, and then the solutions in test tubes were centrifuged at 10 000 rpm for 5 min. The saturated drug solutions were diluted by 400 times with double-distilled water, and the drug concentrations in each diluted solution were determined from the absorbance at their characteristic wavelengths (264 nm for PBZ, 265 nm for SMZ, and 303 nm for MTX) using a Perkin-Elmer UV-vis spectrophotometer (The aggregate solutions without drugs give low absorbance at these wavelengths, and the absorbance was subtracted from the total absorbance before the calculation of drug concentration). Three repeats were conducted for each sample.

**2.3. NMR Studies.** Two-dimensional-NOESY spectra for the formulations consisting of dendrimers, surfactants, and drug molecules were performed with standard pulse sequences.<sup>6,21</sup> The spectra were obtained with a 500.132 MHz NMR spectrometer (Bruker, Germany) at  $298.2 \pm 0.1$  K. The experimental data consisting of eight transients were collected over 2048 complex points. A mixing time of 300 ms, a relaxation delay of 2 s, an acquisition time of 205 ms, and a  $90^\circ$  pulse width of  $8.2 \mu s$  were used. The data were processed with a Lorentzian-to-Gaussian window function and zero filling in both dimensions to display data on a  $2048 \times 2048$  2D-matrix. All data were processed with NMRPipe software on a Linux workstation.

**2.4. DLS and AFM Studies.** DLS measurements to determine the size of the aggregates and drug-loaded aggregates were performed using a commercial spectrometer (ALV/DLS/SLS-

500F, Germany) equipped with a multi- $\tau$  digital time correlator (ALV5000) and a Fiber-Optical Detection System (CGS-5000). All the measurements were made at a scattering angle of  $90^\circ$ . A cylindrical 22 mW UNIPHASE He–Ne laser was used as the light source. The size of the aggregates was determined from their diffusion using the Stoke-Einstein equation.

AFM studies to determine the size and morphology of the drug-loaded dendrimer–surfactant aggregates were carried out with a commercial atomic force microscope (Nanoscope IIIA, Digital Instrument Inc., Santa Barbara, CA), using tapping mode under ambient conditions. Silicon tapping probes having a spring constant of 30 N/m and a radius of 5–10 nm were used for tapping scans. The drug-loaded aggregate samples were diluted to a concentration of 1–10  $\mu g/mL$  in aqueous solutions before determination. A proper amount of the solution was dropped onto a 1 cm  $\times$  1 cm glass and dried under nitrogen gas before the AFM experiments.

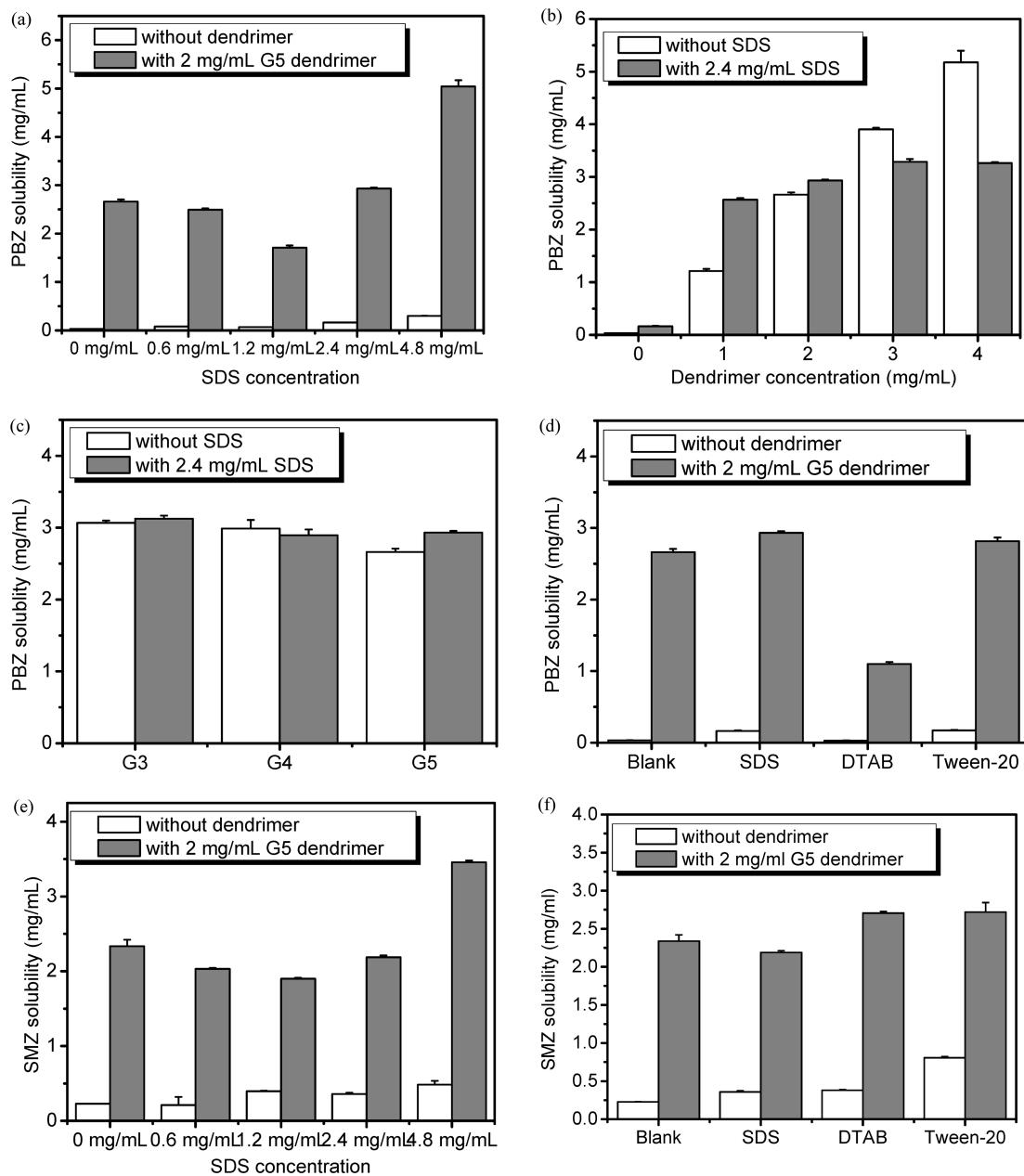
**2.5. AGE Studies.** The dendrimer–surfactant aggregates and drug-loaded dendrimer–surfactant aggregates were electrophoresed on 1% (w/v) agarose gel at 75 V for 20 min (DYCP-31BN, China). The obtained gel was stained with Coomassie brilliant blue for 30 min followed by destaining in acetic acid–ethanol solution for 1–2 h and observed with a UV transilluminator in order to localize the dendrimers.

**2.6. Release Studies.** The in vitro release behaviors of drugs from dendrimer–surfactant aggregates were investigated.<sup>21</sup> The PBZ-containing aggregates in double-distilled water were transferred to a dialysis bag (molecular weight cut off, MWCO  $\sim$  3500 Da) and immediately dialyzed against aqueous mediums containing surfactants. The outer phases were stirred and maintained at 25 °C during the experiment. At different intervals, 3 mL solution in the outer phase was withdrawn, and the outer phase was immediately replenished with fresh medium. The accumulative amount of PBZ released from the matrixes was calculated by the determination of sample absorbance at 264 nm. Three repeats were conducted for each sample.

**2.7. Cytotoxicity Assay.** The cytotoxicity of dendrimer–surfactant aggregates was evaluated by a well-established MTT colorimetric assay. Briefly, HeLa cells seeded in 96-well plates at a density of  $10^4$  cells/well were incubated at 37 °C, and 5% CO<sub>2</sub> with Dulbecco's modified Eagle's medium (DMEM) supplemented with streptomycin (100  $\mu g/mL$ ), penicillin sulfate (100 units/mL), and 10% heat-inactivated fetal calf serum (FCS) and grown overnight. One hundred microliters of fresh DMEM containing different concentrations of dendrimers, surfactants, and dendrimer–surfactant aggregates were then added to each well. The cells were incubated for 24 h, after which the culture medium was replaced with 100  $\mu L$  of DMEM containing MTT and the cells were further incubated for 3 h. Then, MTT containing DMEM was replaced with 100  $\mu L$  of DMSO to dissolve the formazan crystals. Absorbance of the products in each well was measured at 570 nm using a microplate reader (BioTeK ELX808, U.S.A.).

### 3. Results and Discussion

**3.1. Drug Loading Ability of the Dendrimer–Surfactant Aggregates.** The solubility of hydrophobic drugs PBZ and SMZ in dendrimer–surfactant aggregates were evaluated. The results shown in Figure 1a suggested that the amount of SDS in the aggregates modulate the drug loading ability of PAMAM dendrimers. For a specific SDS concentration, the aggregates solubilized considerably more PBZ than the surfactant solutions. As the concentration of SDS in the aggregates increased from 0 to 4.8 mg/mL, the solubility of PBZ first decreased from 2.66

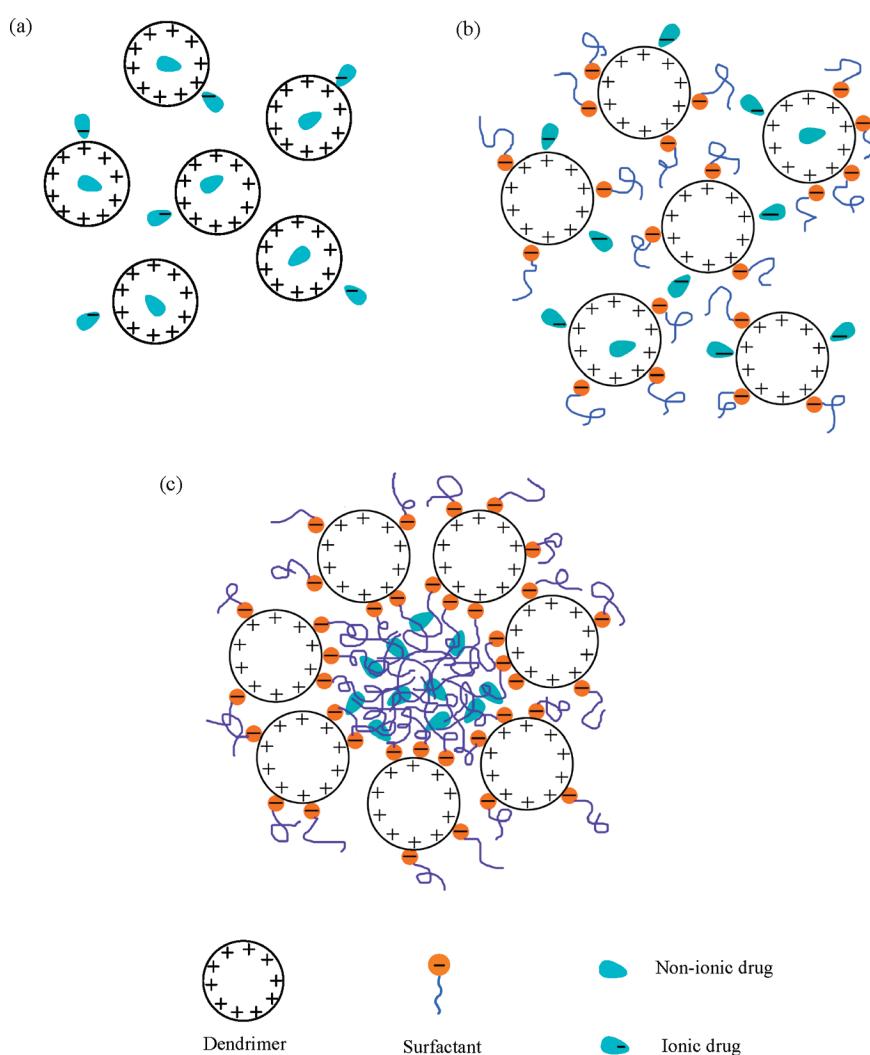


**Figure 1.** Solubilization behaviors of drugs in dendrimer–surfactant aggregates: (a–d) PBZ and (e,f) SMZ. Dendrimer concentration in the aggregates ranges from 1 to 4 mg/mL, while SDS concentration ranges from 1.2 to 4.8 mg/mL. In the aggregate consisted of 2 mg/mL G5 dendrimer and 2.4 mg/mL SDS, the molar ratio of primary amine groups on the surface of dendrimers and the surfactant is 1:1. The molar concentration of the surfactants (SDS, DTAB, and Tween-20) in (d) and (e) is 8.8 mM (equal to 2.4 mg/mL SDS). The dendrimer concentration is being constant (2 mg/mL) with an exception in (b).

(loading efficiency 57.1%) to 1.70 mg/mL (34.7%), with the lowest solubility at 1.2 mg/mL SDS, and then gradually increased to 5.04 mg/mL (42.6%) as the SDS concentration further increased. In previous studies, we have demonstrated that the enhanced solubility of PBZ in cationic dendrimers is due to (1) electrostatic interactions between the cationic surface of dendrimers and the negatively charged PBZ molecules and (2) hydrophobic or hydrogen-bonding interactions between the interior cavities of dendrimers and the drug molecules.<sup>8,21</sup> Also, it is reported that positively or negatively charged dendrimers can form different types of dendrimer-templated aggregates with oppositely charged surfactants above the CAC of the surfactant.<sup>10,14,15</sup> These aggregates have much more hydrophobic microenvironments in the interior than the naked dendrimers,<sup>10</sup> which is beneficial to the hydrophobic encapsulation of drugs such as PBZ molecules. However, the assembly of surfactants

on the surface of dendrimers decreases the charged amine groups thus decreases the chances for electrostatic attachment of drugs. Since the exterior electrostatic interaction contributes much more to the solubility enhancement of drugs than the interior encapsulation,<sup>8</sup> the addition of SDS (0–1.2 mg/mL) causes a decrease of PBZ solubility in dendrimer solutions. Further addition of SDS (1.2–4.8 mg/mL) in the dendrimer solution forms larger aggregates, in which surfactant is in bilayer or micelle forms.<sup>13,22</sup> The resulting aggregate can encapsulate hydrophobic guests in the bilayers and micelles, and finally increase the solubility of PBZ to a much higher level (Figure 1a). Since the pure SDS solution even at a high concentration of 4.8 mg/mL has poor solubilization ability toward PBZ, the significantly enhanced PBZ solubility is not due to the increased SDS concentration. The proposed encapsulation mechanism of

**SCHEME 2: Cartoon Models of Drug-Loaded Dendrimer–surfactant Aggregates with Different Ratio of Surfactants and Dendrimers<sup>a</sup>**



<sup>a</sup> (a) Dendrimer–drug aggregates without surfactants, (b) drug-loaded aggregates with low concentration of surfactants, and (c) drug-loaded aggregates with high concentration of surfactants.

PBZ molecules by the dendrimer–surfactant aggregates at different stages is shown in Scheme 2.

The effects of dendrimer concentration and generation in dendrimer–surfactant aggregates on the solubilization of PBZ are shown in Figure 1b,c, respectively. In the absence of SDS, the solubility of PBZ linearly increased with the dendrimer concentration. However, the dendrimer concentration (1–4 mg/mL) in the aggregates scarcely influenced the solubility of PBZ, suggesting that dendrimer in the aggregates mainly act as a scaffold rather than a carrier, and that drug molecules localize in the bilayer or micelle of surfactants rather than in the interior or on the surface of dendrimers. Similarly, dendrimer generation in the aggregates caused slight changes on drug loading ability of the aggregates (Figure 1c).

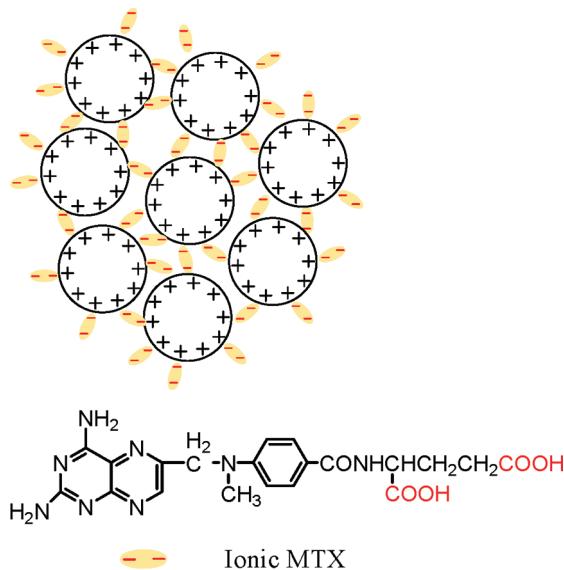
The solubility of PBZ in different surfactant and dendrimer–surfactant aggregates are shown in Figure 1d. Three kinds of surfactants with the same length of hydrophobic chain (C12) were used, SDS, an anionic surfactant, DTAB, a cationic surfactant, and Tween-20, a nonionic surfactant.<sup>14</sup> At a molar concentration of 8.8 mM, SDS and Tween-20 scarcely influenced the solubility of PBZ in dendrimer solutions, while DTAB reduced the PBZ loading ability of dendrimers. The decreased

PBZ solubility in dendrimer–DTAB solution is probably due to the precipitation of larger aggregates consisted of dendrimer, DTAB, and PBZ.

The loading ability of dendrimer–surfactant aggregates toward the antibacterial drug SMZ is shown in Figure 1e,f. Similar results were obtained when SMZ was used as the model drug. The only difference between the two drugs is their solubilization behavior in dendrimer–DTAB solutions caused by different molecular structures of PBZ and SMZ. Surprisingly, cationic dendrimers were not able to solubilize the anticancer drug MTX in the absence of SDS, while the addition of SDS to the dendrimers considerably increased the amount of MTX solubilized (data not shown). The MTX molecule has two carboxylic acid groups that may form cross-linking structures with the cationic surface of dendrimers and cause the precipitation of dendrimer–MTX complexes (Scheme 3). However, the presence of anionic surfactant SDS can protect the surface of dendrimers from cross-linking with MTX molecules. Therefore, MTX molecules can be encapsulated in the SDS bilayers or micelles in the aggregates.

In general, dendrimer–surfactant aggregates showed strong encapsulation/complexation ability toward hydrophobic drugs

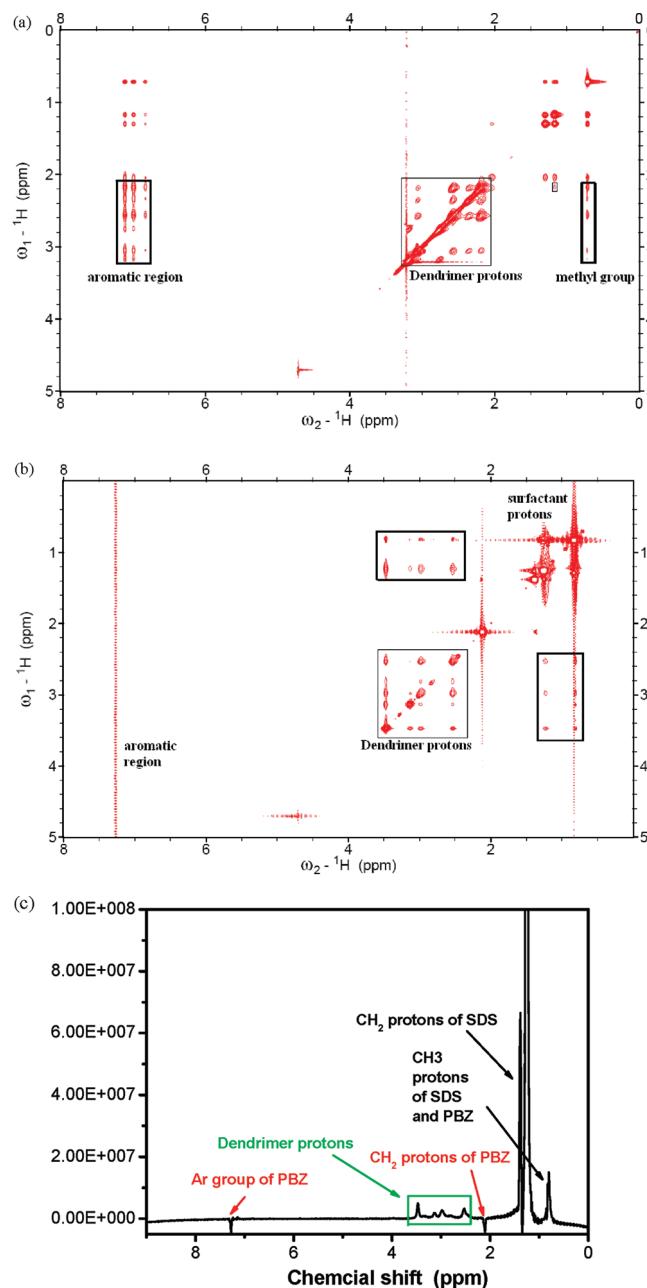
**SCHEME 3: Proposed Structures of the Insoluble Aggregates Formed by MTX and Cationic Dendrimers**



such as PBZ, SMZ, and MTX. We can optimize the ability of the aggregates to host guest molecules by altering dendrimer and surfactant concentrations.

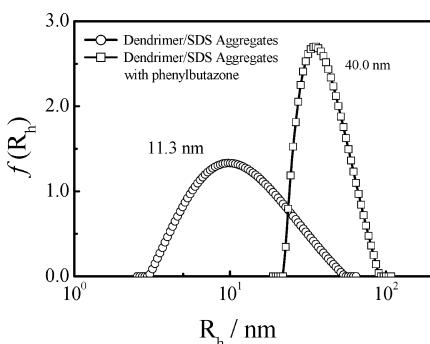
**3.2. Structure Characterization of the Drug-Loaded Dendrimer–Surfactant Aggregates.** The structure of drug-loaded aggregates was characterized by 2D-NOESY, DLS, AFM, and AGE studies to give further insights into interactions between the drug and the aggregate. The 2D-NOESY technique has been proved to be an effective tool in the investigation of host–guest interactions.<sup>5,6,8,21,23–25</sup> It is capable of revealing a spatial relationship between protons in a complex of molecules. This technique was employed in this study to investigate the localization of drug molecules in dendrimers and dendrimer–surfactant aggregates. The 2D-NOESY spectra of dendrimer/PBZ and dendrimer/SDS/PBZ are shown in Figure 2. In the case of dendrimer/PBZ (Figure 2a), strong NOE interactions are observed between PBZ protons (aromatic protons and methyl protons) and dendrimer scaffolds (methylene protons), indicating close proximity between these protons and the encapsulation of PBZ molecules in the interior cavities of dendrimers. However, these cross-peaks became much weaker in the spectrum of dendrimer/SDS/PBZ as shown in Figure 2b than that shown in Figure 2a. This is due to the presence of surfactant bilayers or micelles on the surface of dendrimers in the aggregates,<sup>13,22</sup> which reduce the encapsulation of guests in the cavities of dendrimers as well as the electrostatic attachment on the surface of dendrimers. In addition, the presence of NOE interactions between surfactant protons (middle nine  $\text{CH}_2$  groups) and PBZ protons (aromatic groups and  $\text{CH}_2$  groups) in Figure 2c indicates the localization of PBZ molecules near the surfactant hydrophobic chains. Furthermore, the downfield shift of the methylene protons of dendrimers in the aggregates compared to the dendrimer–drug complexes was found in Figure 2b, suggesting the presence of strong electrostatic interactions between dendrimers and ionic SDS molecules. Therefore, the guests solubilized by the aggregates are located in the bilayers and micelles of surfactants rather than in the interiors of dendrimers (Scheme 2c).

Further characterization of the structure of the drug-loaded aggregates was carried out to investigate their size change before and after the drug was loaded. Figure 3 shows the size distributions of dendrimer–surfactant aggregate and PBZ-loaded

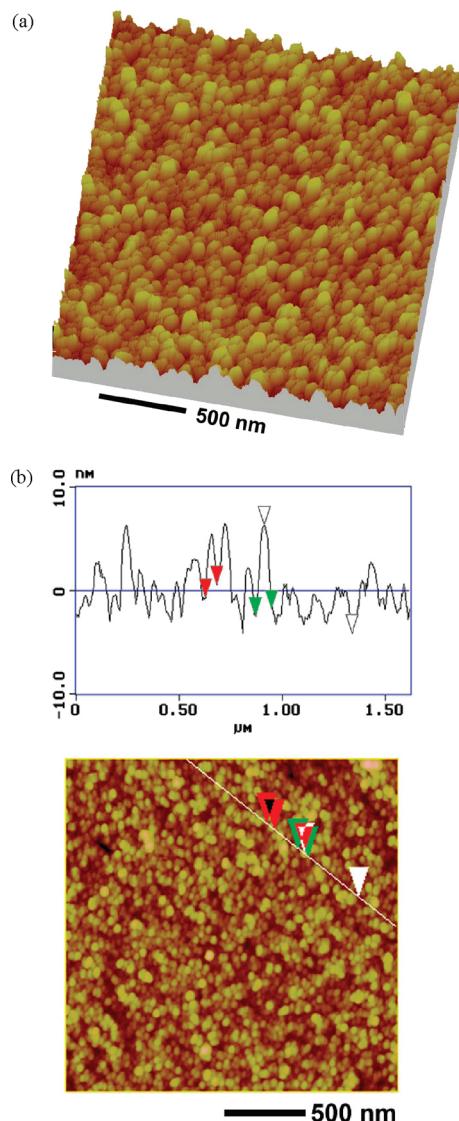


**Figure 2.**  $^1\text{H}$ – $^1\text{H}$  NOESY spectra of G5 dendrimer/PBZ/ $\text{D}_2\text{O}$  solution (a) and G5 dendrimer/SDS/PBZ/ $\text{D}_2\text{O}$  solution (b) at a mixing time of 300 ms. (c) Cross-section taken at the position of the  $\text{CH}_2$  protons of SDS at 1.25 ppm along  $\omega_1$  from the NOESY spectra in Figure 2b. For the samples, 2 mg dendrimer, 2 mg PBZ, and 4.8 mg SDS was dissolved in 1 mL  $\text{D}_2\text{O}$ . The cross-peaks between dendrimer protons and PBZ protons or surfactant groups are indicated by rectangles.

aggregate. The mean diameter of the dendrimer–SDS aggregate increased from 11.3 to 40.0 nm as a result of loading PBZ into the aggregates. It was reported that the aggregation process in solutions of dendrimer and surfactant depends on the charge and hydrophobic property of both species.<sup>10,11,13,22</sup> The addition of a hydrophobic drug into the aggregate solution may destroy the hydrophilic–hydrophobic balance in the solution and lead to the formation of new aggregates with larger sizes (Scheme 2). The morphology of the aggregates was further investigated by AFM studies. In Figure 4, one can see that the drug-loaded aggregates have uniform spherical shapes with a diameter of 50–60 nm. Dendrimers with surface amine groups are easily spread on a mica surface like a drop of water in order to maintain

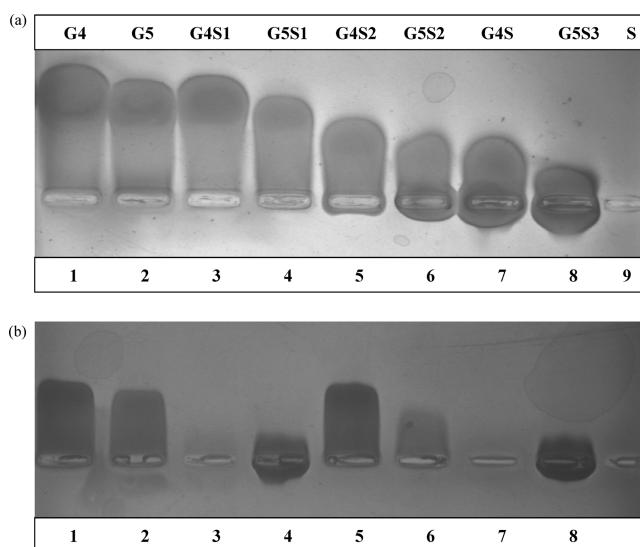


**Figure 3.** The size distribution of dendrimer–SDS aggregate and PBZ-loaded dendrimer–SDS aggregate determined by dynamic laser light scattering. The G5 dendrimer and SDS concentration in the aggregate is 2 and 4.8 mg/mL, respectively. For the drug-loaded sample, 2 mg PBZ was dissolved in the aggregate solution.

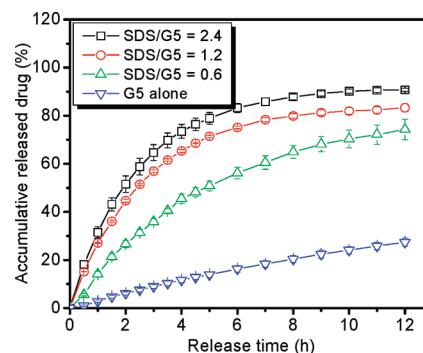


**Figure 4.** AFM image of PBZ-loaded dendrimer–SDS aggregate (a) and profile section of the drug-loaded aggregates (b) on mica surface. The drug-loaded aggregate solution was prepared as described in DLS studies and diluted into 1–10  $\mu$ g/mL before AFM studies.

lower surface tension.<sup>26</sup> Therefore, the size of the drug-loaded aggregate obtained by AFM studies are slightly larger than that from DLS studies. The DLS and AFM results are in accordance with the assumed structure of drug-loaded aggregates illustrated in Scheme 2.

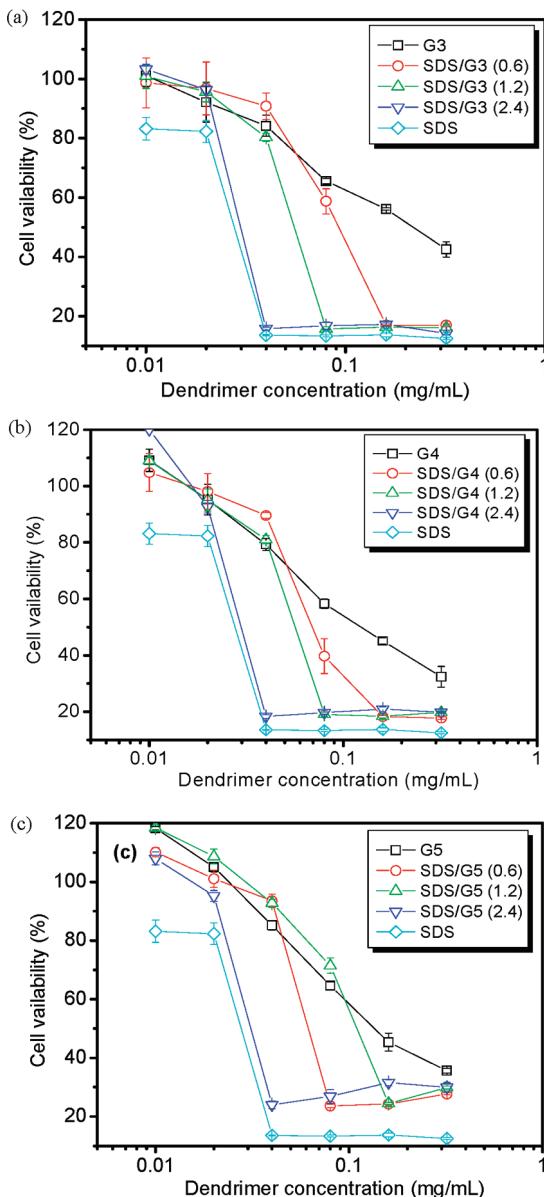


**Figure 5.** Identification of dendrimer–surfactant aggregates (a) and drug-loaded aggregates (b) using agarose gel electrophoresis. (a) lane 1, 2 mg/mL G4; lane 2, 2 mg/mL G5; lane 3, G4 + 1.2 mg/mL SDS (G4S1); lane 4, G5 + 1.2 mg/mL SDS (G5S1); lane 5, G4 + 2.4 mg/mL SDS (G4S2); lane 6, G5 + 2.4 mg/mL SDS (G5S2); lane 7, G4 + 4.8 mg/mL SDS (G4S3); lane 8, G5 + 4.8 mg/mL SDS (G5S3); lane 9, 2.4 mg/mL SDS. (b) lane 1, G5 + 2 mg/mL SMZ; lane 2, G5 + SMZ + 1.2 mg/mL SDS; lane 3, G5 + SMZ + 2.4 mg/mL SDS; lane 4, G5 + SMZ + 4.8 mg/mL SDS; lane 5–8, PBZ-loaded dendrimers and dendrimer–SDS aggregates. The samples were run in the 1% agarose gel and stained with Coomassie brilliant blue.



**Figure 6.** Release behaviors of PBZ from dendrimer–SDS aggregates. The dendrimer concentration in the release studies is constant (2 mg/mL before transferred into dialysis bag). In the dendrimer–SDS aggregates, the surfactant concentration ranges from 1.2 to 4.8 mg/mL.

The stability of the drug-loaded formulations was characterized by AGE studies. It was reported that dendrimers with large numbers of amine groups can be easily stained by anionic dyes,<sup>27</sup> such as Coomassie brilliant blue. The addition of anionic surfactant and drugs into dendrimers reduces the surface amine groups of dendrimers, which causes the reduced motility of the aggregates in the electrical field (Figure 5a). The nature of the aggregates changed from positive charge to negative charge as the SDS concentration increased from 0 to 4.8 mg/mL. Therefore, part of the dendrimer–SDS aggregates even moved to the anode in lane 7 and lane 8 in Figure 5a. The dendrimer–surfactant aggregates seemed to be stable even in electrical field. However, the drug-loaded aggregates are less stable than the free aggregates with an exception of the drug-loaded aggregates consisted of 2 mg/mL G5 and 4.8 mg/mL SDS (Figure 5b). The results are in accordance with solubility studies.



**Figure 7.** Cytotoxicity of dendrimer–surfactant aggregates by MTT assay. (a) G3, (b) G4, and (c) G5. The weight ratio of SDS and dendrimer in the aggregates is 0.6, 1.2, and 2.4, respectively. For free SDS, the surfactant concentration is the same as SDS concentration in the aggregate (SDS/dendrimer = 2.4).

**3.3. Release Behavior and Cytotoxicity of the New Drug Formulations.** The in vitro release of drug from dendrimer–surfactant aggregates was investigated and the results are shown in Figure 6. PAMAM dendrimer exhibited sustained drug release for PBZ with 27.4% of the entrapped drug released from G5 dendrimer during the period of 12 h. However, the release rate of PBZ from the dendrimer–SDS aggregates is significantly faster than that from the naked dendrimers. The higher the concentration of SDS in the aggregates, the faster the release rate of PBZ is obtained. In previous studies, electrostatic interaction between dendrimer scaffolds and drugs was found to be the major mechanism for drug complexation by dendrimers.<sup>8</sup> We can expect that electrostatic interaction plays an important role in drug release from the dendritic matrixes.<sup>21</sup> As demonstrated in Section 3.2, the drug molecules are mainly encapsulated in the surfactant bilayers or micelles in the aggregates, leading to a rapid release from the drug formulations.

In traditional drug administration routes such as intravenous and oral routes, rapid release of a drug is not a good physicochemical property of the drug formulation.<sup>17</sup> Rapid-release formulation will lose most of its drugs during circulating in blood, decrease the therapeutic efficiency, and cause systemic toxicity of the administrated drugs. Transdermal drug delivery (TDD) is a noninvasive method of penetrating therapeutic agents through the skin and has already revolutionized the pharmaceutical industry.<sup>18</sup> It provides a steady drug concentration in the blood, simplifies the dosing schedule, improves patient compliance, eliminates the hepatic first-pass effect, and thus offers a significant potential for safe administration of therapeutic agents. However, TDD is limited because of the slow rate of transdermal delivery, chiefly attributable to the barrier functions of the skin, which limits this promising technology in clinical practice.<sup>17</sup> The most common method to improve drug penetration through the skin is to use transdermal enhancers that directly react with the skin, and thus increase its permeability. Therefore, rapid release of the drugs from the formulations after the transdermal enhancers have transiently opened the skin channels is essential for dermal delivery of drugs. Previous studies have reported that both the surfactants and cationic dendrimers with amphiphilic properties are excellent transdermal enhancers.<sup>18–20</sup> The cytotoxicity of the aggregates on HeLa cells is between that of dendrimers and surfactants, indicating that the new formulation is as safe as the clinical ointments consisting of surfactants (Figure 7). In general, the new drug formulations consisted of dendrimers and surfactants in this study may effectively facilitate skin penetration of drugs and have the potential applications for the development of new transdermal formulations.

#### 4. Conclusions

Dendrimer–surfactant aggregates are of great potential interest in the design of new drug formulations, especially in transdermal delivery routes. They can significantly enhance the solubility of hydrophobic drugs and make it possible to optimize the drug loading of the formulations by altering the surfactant concentration. The existence of effective transdermal enhancers such as dendrimer and surfactant in the formulation and the rapid release of drugs from the aggregate make it suitable for applications in transdermal delivery routes.

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