


## ORIGINAL RESEARCH

# Why is glycocholic acid sodium salt better than deoxycholic acid sodium salt for the preparation of mixed micelle injections?

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## Abstract

Classical mixed micelle systems make excellent parenteral drug carriers for lipophilic or poorly soluble drugs, but many formulations details are not fully understood and need further study. Thus, we constructed mixed micelle systems with lecithin and either glycocholic acid sodium salt or deoxycholic acid sodium salt in order to investigate the differences between the bile salts. Vitamin K<sub>1</sub>, a lipid-soluble drug, was encapsulated in the mixed micelles, and the influence of bile salts on the quality and stability of the mixed micelle systems was analyzed. Both bile salts displayed similar profiles, and the amounts of bile salts used in formulating clear solutions did not differ. Mixed micelle systems formed from glycocholic acid sodium were physically stable at low pH levels (5.5), whereas those formed from deoxycholic acid required higher pH (>8.5). High pH levels hurt active pharmaceutical ingredients that are prone to hydrolytic and oxidative degradation. Hence, when mixed micelle systems formed from deoxycholic acid sodium were sterilized, unexpected chemical unstability occurred. Therefore, we conclude that glycocholic acid sodium salt is more suitable than deoxycholic acid sodium salt for the preparation of mixed micelle injections.

## KEYWORDS

deoxycholic acid salt, glycocholic acid salt, mixed micelle injection, physiochemical stability, Vitamin K<sub>1</sub>

## 1 | INTRODUCTION

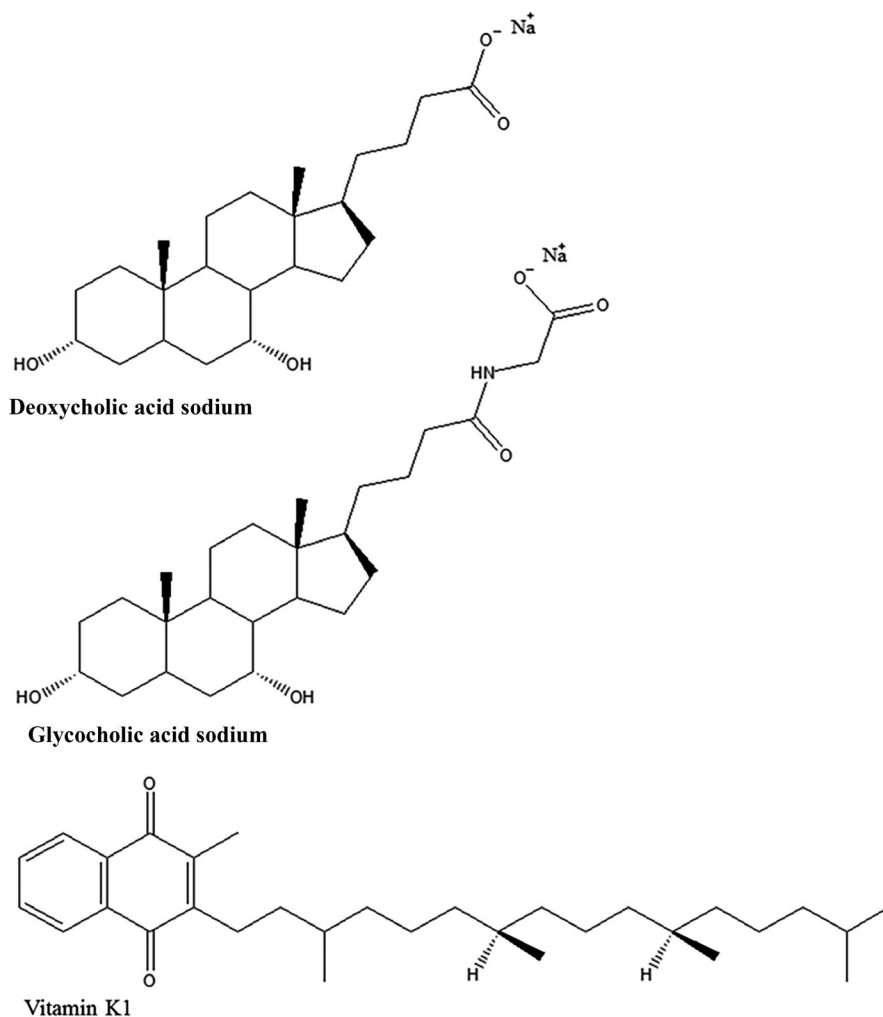
Classical mixed micelle (MM) systems, composed of natural phospholipids and bile salts, offer a nontoxic, highly biocompatible, and parenterally well-tolerated drug carrier for lipophilic and poorly soluble drugs. Researchers have attempted various approaches (Amedee-Manesme, Grüter, & Hanck, 1991; Hammad & Müller, 1998; Mrestani, Behbood, Härtl, & Neubert, 2010; Muranishi, Muranushi, & Sezaki, 1979), but only one product (Konaktion<sup>®</sup> MM

has been approved for the pharmaceutical market; while a few published results have described the influence of lecithin/bile salt ratios and the effect of total concentration on the size and shape of MMs (Krishnadas, Rubinstein, & Önyüksel, 2003; Lichtenberg, Robson, & Dennis, 1983; Shankland, 1970), many other factors are not fully understood. Therefore, more basic studies are required for elucidating the formation and stability of MM preparations.

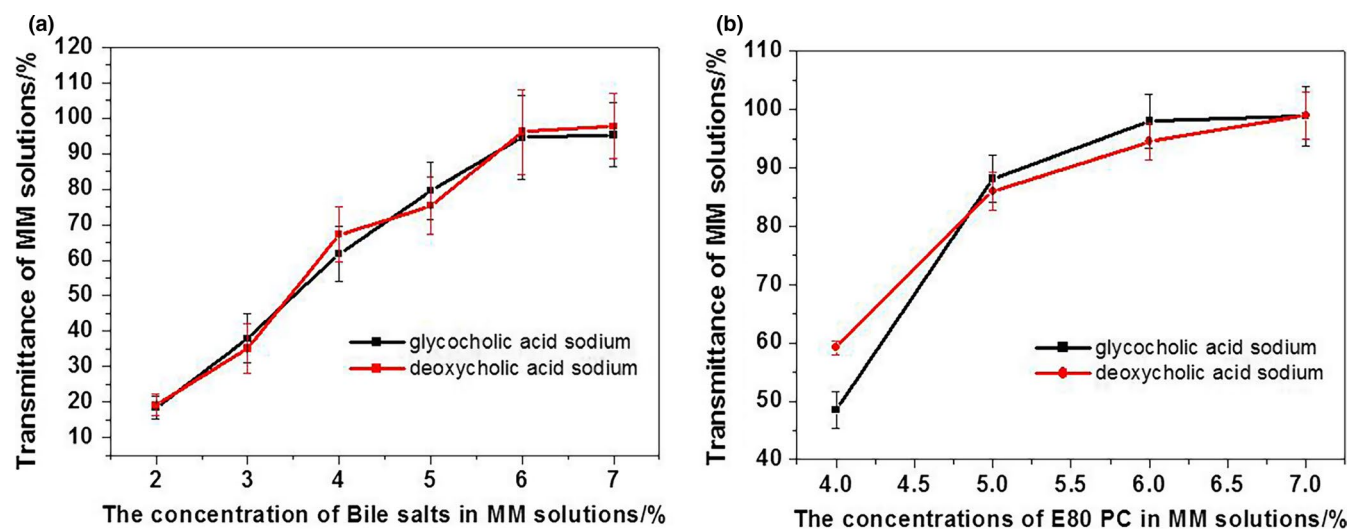
Glycocholic acid sodium (GAS) salt and deoxycholic acid sodium (DAS) salt are the most common cholic acid salts in the

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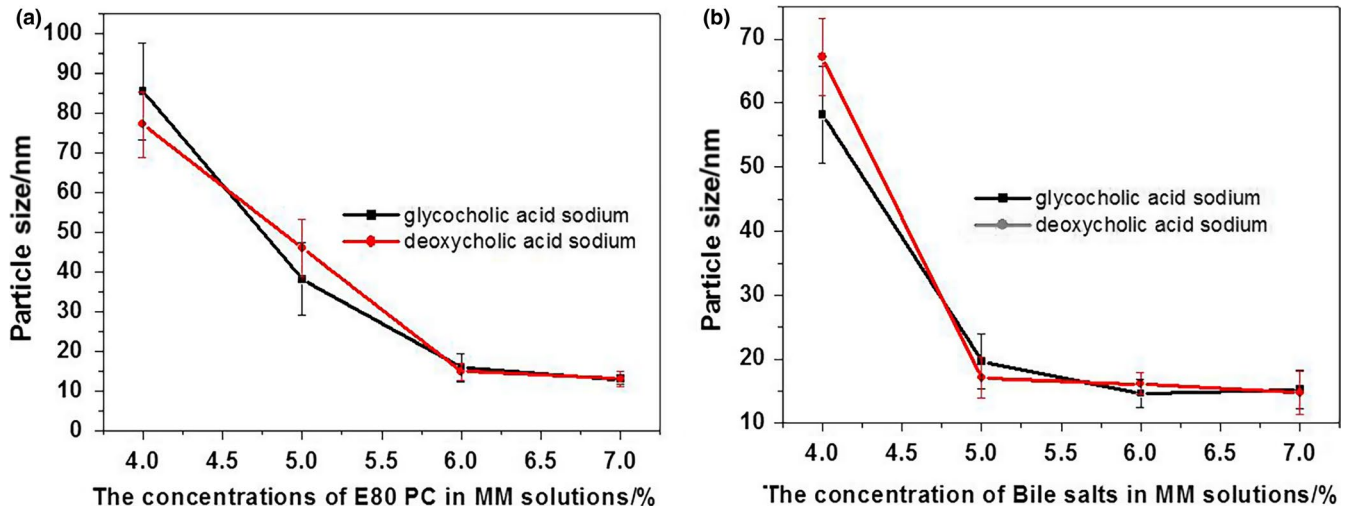
**FIGURE 1** The chemical structures of deoxycholic acid sodium salt, glycocholic acid sodium salt, and vitamin K<sub>1</sub>



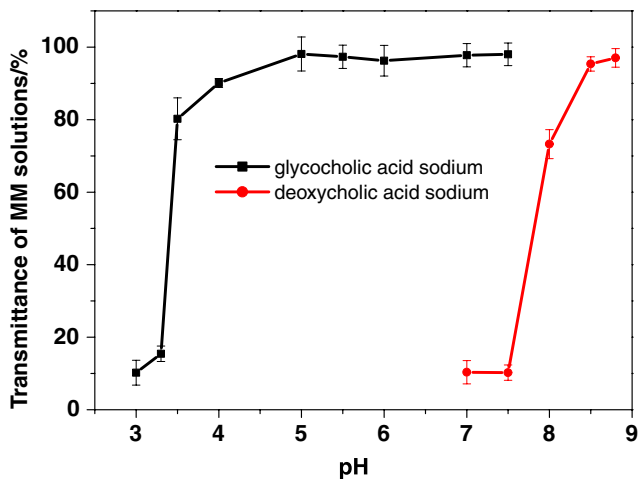
**FIGURE 2** The transmittances of mixed micelle solutions with different concentrations of phospholipid (PC) E80 and bile salts (650 nm; mean  $\pm$  SD,  $n = 3$ ): (a) the concentrations of bile salts ranged from 2.0% to 7.0% while that of PC E80 was constant (6.0%); (b) the concentrations of PC E80 ranged from 4.0% to 7.0% while that of bile salts was constant (6.0%)

pharmaceutical industry, especially for the preparation of MMs. Both are water-soluble anionic surfactants that have the same steroidal backbones (Figure 1). Their physicochemical properties are

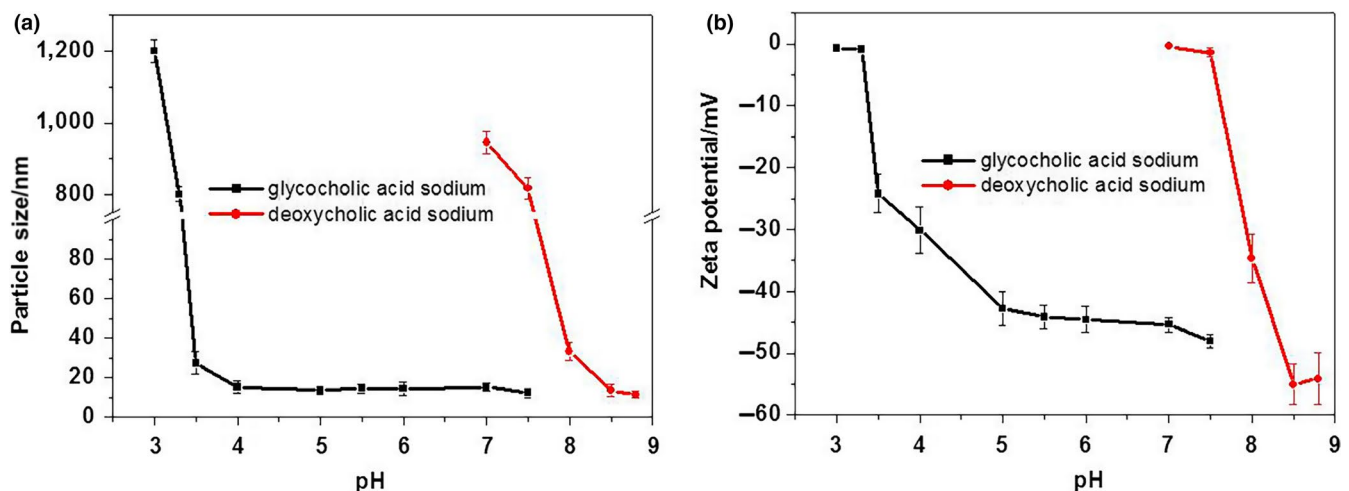
so similar that their differences are often ignored. However, these slight differences affect the quality of MM injections very strongly in some times. So it is important to know the details to investigate



**FIGURE 3** The particle sizes of mixed micelle solutions with different concentrations of phospholipids (PC) E80 and bile salts (mean  $\pm$  SD,  $n = 3$ ): (a) the concentrations of bile salts ranged from 2.0% to 7.0% while that of PC E80 was constant (6.0%); (b) the concentrations of PC E80 ranged from 4.0% to 7.0% while that of bile salts was constant (6.0%)



**FIGURE 4** The transmittances of mixed micelle solutions after adjusting the pH from high to low values (650 nm; mean  $\pm$  SD,  $n = 3$ )

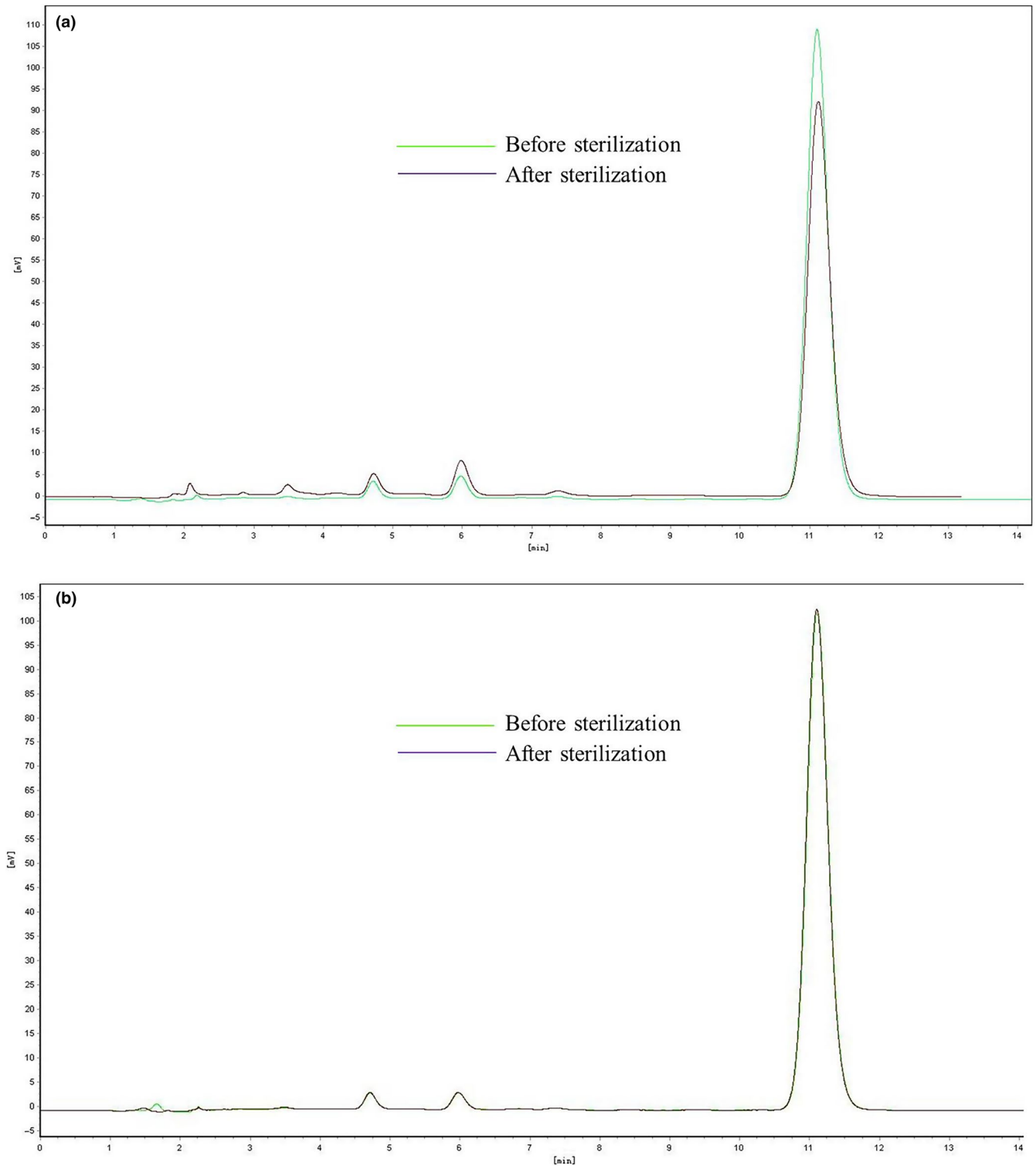


**FIGURE 5** The particle sizes and zeta potentials of mixed micelle solutions after adjusting the pH from high to low values (mean  $\pm$  SD,  $n = 3$ )

how they differ, we constructed MM systems from lecithin and bile (GAS or DAS) salts. Vitamin  $K_1$  (Figure 1), a lipid-soluble drug, was selected as the model drug for encapsulation in the MMs, after which the influence of bile salts on the quality and stability of MM formulations was analyzed.

All MM solutions or dispersions consisted of 1% vitamin  $K_1$  (Shandong Guangtongbao Pharmaceuticals Co. Ltd.), 4.0 ~ 7.0% phospholipids, and 2.0 ~ 7.0% bile salts in double-distilled water that was prepared by a film-forming or evaporation method. The egg phospholipid Lipoid E80 (Lipoid GmbH), as well as GAS or DAS (Sigma-Aldrich), and vitamin  $K_1$  were dissolved in ethanol (10 ml). After the solvent was evaporated by a rotary evaporator, pure water was added to obtain a solution of MMs loaded with vitamin  $K_1$ .

For an MM formulation to serve as a good parenteral drug carrier system, it must form an isotropically clear solution. An ultraviolet-visible spectrophotometer (U-3010, Hitachi) was used to analyze

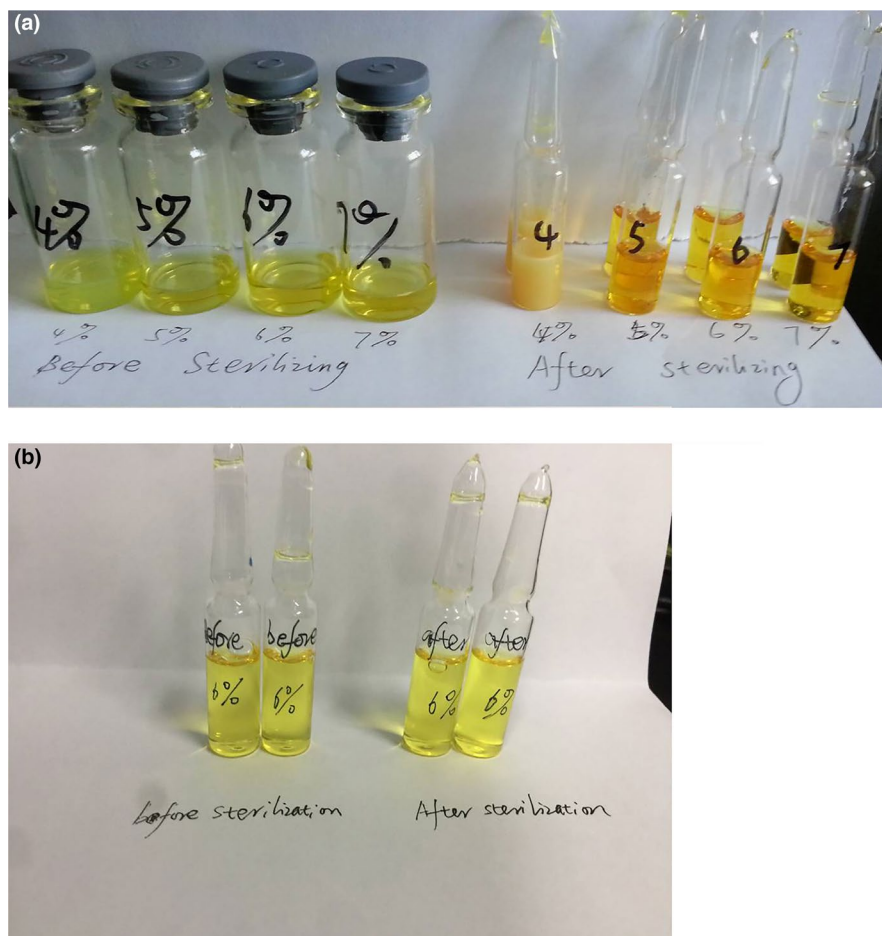


**FIGURE 6** Vitamin K<sub>1</sub> levels were determined by HPLC of mixed micelle solutions made with deoxycholic acid sodium (a) and glycocholic acid sodium (b)

the transmittance of MM systems at 650 nm in order to elucidate the influence of bile salt or phospholipid (PC) concentrations on the formation of clear solutions in the presence of 1% (m/v) vitamin K<sub>1</sub>. Clear solutions were obtained with high concentrations (>5%) of PC or bile salts (Figure 2): The more bile salts and phospholipids, the

more hydrophobic core was available to encapsulate vitamin K<sub>1</sub>. Both GAS and DAS salts exhibited similar abilities to form isotropically clear solutions with the same amounts of PC and vitamin K<sub>1</sub>. Thus, the formation of isotropically clear solutions was dependent on concentration rather than type of bile salt.

**FIGURE 7** The appearance of solutions of vitamin  $K_1$  in mixed micelles made with deoxycholic acid sodium (a) or glycocholic acid sodium (b) before and after sterilization (121°C, 15 min)



The particle sizes in MM systems were measured with a Nano ZS90 Zetasizer (Malvern Instruments). The mean sizes of MM particles rapidly decreased as the concentrations of PC or bile salts increased until a final plateau region was reached (Figure 3). In the plateau region, MM solutions with particles smaller than 15 nm were clear, which corresponds to results from the light transmittance tests. Therefore, the optimal formulation for MM systems consisted of 1% vitamin  $K_1$ , 6% PC, and 6% bile salts.

Mixed micelle solutions containing GAS and DAS salts were prepared according to the optimal formulation described above. For pH determination, all samples were measured directly with a pH meter (Mettler Toledo). The initial pH levels of the DAS and GAS MM solutions were 8.83 and 7.57, respectively.

The GAS MM solutions were physically stable at a large range of pH levels (7.5–3.5; Figure 4). In contrast, transmittance of the DAS MM solutions quickly declined when the pH was adjusted below 8.5. The difference in pH-related physical stability between the MM systems was related to the difference in pKa between the bile salts. The pKa of DAS (6.86) was much higher than that of GAS (3.77). Therefore, lowering the pH of the solutions affected the zeta potential of the DAS MM system more intensively than it did the GAS MM system (Figure 5). A high zeta potential indicates more electrostatic repulsion between the nanoparticles. When the zeta potential neared zero, particles of MMs precipitated and caused cloudiness.

From these experiments, the optimal pH levels for maintaining high physical stability in DAS and GAS MM systems were 8.5 and 5.5, respectively. However, more insensitive hydrolytic and oxidative degradation of active pharmaceutical ingredients was taken place in alkaline medium. Therefore, MM formulations with low pH values have higher chemical stability than those with high pH values.

We prepared DAS and GAS MM formulations using the methods described above, followed by adjustment of pH levels to 8.5 and 5.5, respectively. Then, they were sterilized for 15 min at 121°C. All samples were analyzed using a high-performance liquid chromatography (HPLC) system (I-7110/L-7420, Hitachi) with a C18 column (Zorbax Rx-C18, 4.6 × 250 mm, 5 μm) purchased from Agilent Instrument, Inc. The column was eluted at a flow rate of 1.0 ml/min with a 90:10 (v:v) mixture of ethanol and distilled water. The injection volume was 20 μl for samples both before and after sterilization. The detection wavelength was 215 nm, and the column was maintained at ambient temperature. For the DAS MM solutions, obvious degradation was observed following sterilization. The vitamin  $K_1$  content declined by 10% and the amount of impurities obviously increased. In contrast, both content and related substances in the GAS MM solutions remained unchanged after sterilization (Figure 6). Sterilization tended to improve the color change of MM solutions containing DAS rather than GAS (Figure 7), and PCs in the concentration range of 4 ~ 6% did not affect stability to sterilization. We believe that the GAS MM

system did not deteriorate significantly during the sterilization process because it had a much lower pH (5.5) than that of the DAS MM system.

To summarize, low pH levels improve the stability of many active pharmaceutical ingredients, especially those that are prone to hydrolytic and oxidative degradation. Although GAS MM systems were able to maintain physical stability at low pH levels (5.5), DAS MM systems underwent considerable degradation when sterilized at their optimal pH (greater than 8.5). Thus, we conclude that DAS MM systems have limited use as parenteral drug carriers and that GAS is more suitable than DAS for the preparation of mixed micelle injections.

## ACKNOWLEDGMENTS

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## CONFLICT OF INTEREST

The authors declare that there was no conflict of interests.

## ETHICAL APPROVAL

The study conforms to the Declaration of Helsinki, USA, and/or European Medicines Agency Guidelines for human subjects. The protocols and procedures in this study were ethically reviewed and approved by Ethical Committee of Beijing Institute of Pharmacology and Toxicology. This work does not involve any animal or human testing.

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