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RESEARCH PAPER

Solubility and Stability of Lorazepam in Bile Salt/Soya Phosphatidylcholine–Mixed Micelles

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ABSTRACT

In the present study, the solubility and stability of the drug lorazepam, which was solubilized in bile salt/soya phosphatidylcholine–mixed micelles (BS/SPC-MMs), were investigated. The solubility of lorazepam could be enhanced substantially in different bile salts and also in sugar ether, whereas the solubility in Pluronic F68 (Pl.F68) was of lower order. Moreover, the addition of SPC to different BS solutions greatly enhanced their solubilizing capacities toward lorazepam; this could be correlated with the ability of the formed MM to reduce the surface tension. The stability study showed that lorazepam degradation followed apparent first-order degradation kinetics in phosphate buffer, as well as in the BS/SPC-MM, with highly enhanced stability in the latter system. The stabilizing effect of BS/SPC-MM was higher in the case of trihydroxy BS than for dihydroxy BS. From an Arrhenius plot with degradation constants in a temperature range from 30°C to 60°C, a shelf stability of about 10 months could be calculated for BS/SPC-MM at 5°C. The solubility studies in BS/SPC-MM showed a recrystallization and a polymorphic transition from modification II to I.

Key Words: *Arrhenius Plot; BS/SPC-MM; Lorazepam; Polymorphism; Solubilization; Stability; Surface tension; X-ray diffraction.*

INTRODUCTION

Lorazepam is a benzodiazepine with anticonvulsant, anxiolytic, sedative, muscle relaxant, and amnesic properties. Its poor aqueous solubility and stability exclude the use of water alone as a solvent for formulating lorazepam in solution form suitable for intravenous administration, which is the most suitable route in emergency cases.

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The introduction of bile salt/phosphatidylcholine–mixed micelles (BS/PC-MM) in pharmaceutical formulations as a vehicle for drugs that are not soluble in water has eliminated many disadvantages associated with the other cosolvents often used. Mixed micelles of this type offer the advantage of utilizing components known to occur in the body. The preparation, shape, and size of BS/PC-MM have been extensively studied (1–7). In addition, the safety of BS/PC-MM with respect to different tissues in animals was also reported (8). Due to their safety and solubilizing capacity, BS/PC-MMs were accepted as a vehicle for insoluble drug substances such as diazepam (Valium MM) (9,10). The possibility of using these systems as solubilizers for widely different drugs was reported (11–13). However, the stability behavior of unstable drugs solubilized in these systems was not reported. The main aim of this study was to enhance the aqueous solubility and stability of lorazepam by using bile salt/soya phosphatidylcholine–mixed micelles (BS/SPC-MM) as a vehicle.

MATERIALS AND METHODS

Materials

Lorazepam USP 23/BP 93 was purchased from Welding GmbH (Hamburg, Germany). Sodium glycocholate (SGC), sodium deoxycholate (SDC), and soya phosphatidylcholine 95.8 % (SPC) as Phospholipon 100 were a gift from Nattermann Phospholipid GmbH (Cologne, Germany). Acetonitrile (high-performance liquid chromatography [HPLC] grade), methanol (HPLC grade), chloroform, sodium cholate (SC), NaH_2PO_4, and Na_2HPO_4 were obtained from Merck AG (Darmstadt, Germany). Sugar ether as Glucoside 81s (Gl.81s; the alkyl residue C8-10 and degree of glucosidation from 1.1 to 3) was a gift from Hüls AG (Witten, Germany). Poloxamer 188 as Pluronic F68 (Pl.F68) was obtained from Erbslöh GmbH (Krefeld, Germany). The study was carried out with double-distilled water.

Methods

Preparation of Mixed Micelles

The BS/SPC-MMs were prepared by the coprecipitation method (7). BS and SPC (at different mole fractions [MFs]) were dissolved in a mixture of methanol-chloroform (1:1 v/v). A film was formed after evaporation of the organic solvents at room temperature under vacuum until constant weight was obtained (48–72 hr). The resulting films were dispersed in a given amount of the dispersion medium (phosphate buffer pH 7.4, 0.067 M) to give clear micellar solutions with the required concentration.

High-Performance Liquid Chromatography Analysis of Lorazepam

An acidified aqueous acetonitrile mobile phase was reported to be capable of separating the degradation products of lorazepam (14). In the present study, a mobile phase consisting of a mixture of double-distilled water: acetonitrile:acetic acid (55:45:1 v/v) at a flow rate of 1 ml/min using a wavelength of 254 nm for detection was developed for the HPLC analysis of lorazepam. The instruments used consisted of an RP-18 5-μm column (150 ∗ 4.6 mm) (Merck, Darmstadt, Germany); a high-precision pump, Gyncotek 300C (Gyncotek, München, Germany); an autosampler, Kontron 360, an ultraviolet (UV) detector, Kontron 742 (Kontron Instruments, München, Germany); and an integrator, Shimadzu C-R6A chromatopac (Shimadzu, Kyoto, Japan).

Solubility Study

Excess amounts of lorazepam were added to 10 ml of each of the different micellar solutions in vials, which were then tightly closed under N_2 and shaken in a thermostated water bath (SW-20C, Julabo Labortechnik, Seelbach, Germany) at 25°C until equilibrium was reached, which was determined by repetitive sampling (24–48 hr). Nondissolved lorazepam was separated by 5-min centrifugation at 13,000 rpm in a centrifuge (Biofuge A, Heraeus Instrument GmbH, Hanover, Germany). Of the supernatant solutions, 0.5 ml was properly diluted with a mixture of methanol:water (80:20 v/v) and then subjected to HPLC analysis. Each run was repeated at least twice. For the calibration curve, different concentrations (at least 5) in a range from 1 to 10 μg/ml were prepared by dilution from a stock solution of lorazepam in methanol:water (80:20 v/v), and the dilution was made with the same solvent mixture. The concentration-absorption relationship obeyed the Beers-Lambert law (r^2 not less than 0.999).

X-Ray Diffraction Study

For determination of the X-ray diffraction pattern, a certain amount of the recrystallized lorazepam in SGC/SPC-MM solution was separated and dried under vacuum at room temperature. The X-ray diffraction pattern was determined by a powder X-ray diffractometer with rotating anode (STOE Cie, Darmstadt, Germany).

Surface Tension Measurements

The surface tension at the air-water interface was measured with a tensiometer K12 (Krüss, Hamburg, Germany) employing a platinum plate as in the Wilhelmy plate principle.

Different concentrations from all of the systems were prepared from stock solutions by dilution with phosphate buffer pH 7.4 (0.067 M). Each concentration was prepared twice in a volume of 40 ml, from which 30 ml were poured into the measurement glass container of the apparatus. Each sample was equilibrated at 25°C for 10 min in the tensiometer before starting the measurement. The apparatus is connected to K121 and K122 software for adjustment of the measurement parameters and for calculation of the surface tension from the measured force. The measurement parameters were adjusted as follows: immersion depth, 2.0 mm; sensitivity, 0.001 g; number of measurements, 10; and measurement interval, 10 s.

During the measurement, the glass container containing the sample was raised until the lower margin of the platinum plate was immersed in the liquid. The force needed to detach the platinum plate from the liquid surface was recorded and converted to γ. Between the measurements, the plate was rinsed thoroughly and heated to incandescence in a flame to remove all organic substances.

Stability Study

Certain amounts of lorazepam were added as a methanolic solution during the first stage of MM preparation to give a final concentration of 0.5 mg/ml. In the case of the buffer, the required amount of clonazepam was added as a methanolic solution (200 µl) to each 20 ml of the buffer to give a concentration of about 5 µg/ml. All solutions in the case of either MM or buffer alone were prepared at least in duplicate. Different solutions to be studied were filled in 2-ml ampoules under N_2. The solutions were stored at the required temperatures. At suitable time intervals, samples were taken, properly diluted, and subjected to HPLC analysis.

RESULTS AND DISCUSSION

Solubility Study

Solubility Behavior and X-Ray Diffraction Study

Figure 1 shows the solubility behavior of lorazepam with time in different BS/SPC-MMs, as well as in Gl.81s,

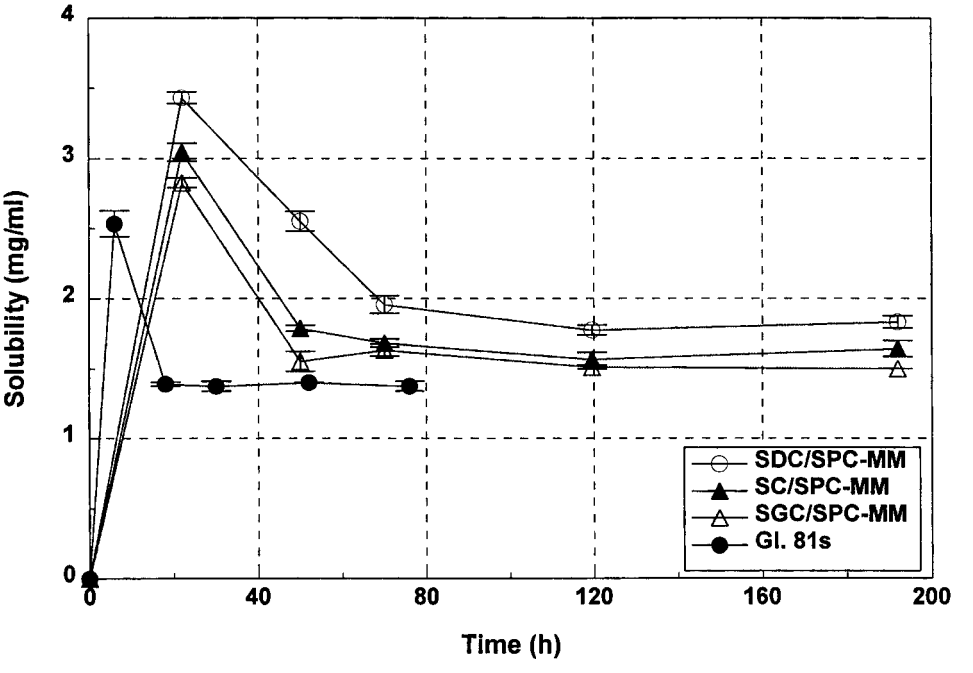

Figure 1. Change of lorazepam solubility in different BS/SPC-MM (MF 0.5), as well as in Gl.81s, at 25°C (5% solutions in phosphate buffer, pH 7.4, 0.067 M) (mean value ± SD, *n* at least 2).

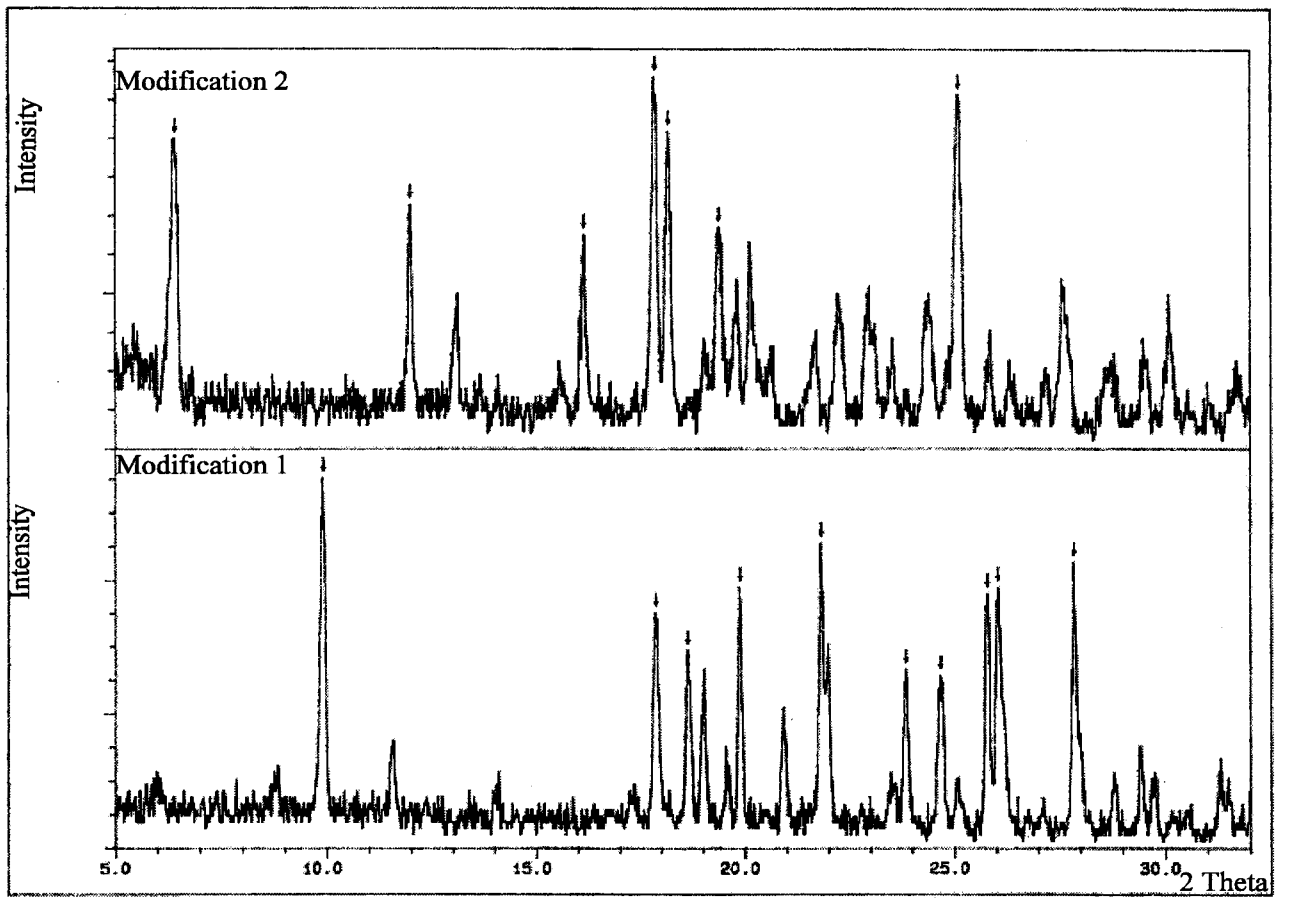

Figure 2. X-ray diffraction pattern of lorazepam powder before (modification 2) and after (modification 1) recrystallization from SGC/SPC-MM.

solutions. The solubility of lorazepam displayed an initial increase, followed by a decrease, and ultimately followed by a plateau stage. The change to the lower solubility modification occurred faster in the case of Gl.81s than in the case of the BS/SPC-MM systems. This indicates that BS interferes with the nucleation of lorazepam to a greater extent than Gl.81s. In the plateau region, the solubility of lorazepam could be arranged in the following increasing order: Gl.81s < SGC/SPC-MM < SC/SPC-MM < SDC/SPC-MM.

The observed change in the solubility behavior was accompanied by the appearance of a sediment. By means of X-ray diffraction studies, it could be shown that a change in the crystal modification occurred. Figure 2 shows the recrystallized modification as form I, while the modification used for the study is represented as polymorph II. The polymorphism of lorazepam was reported by Rutger and Shearer (14); however, such a phenomenon could occur during solubility studies in micellar systems.

Solubility Profile in Different Micellar Systems

The solubility profiles of lorazepam in different micellar systems are illustrated in Fig. 3. A linear increase in solubility with increasing concentration was observed for the different systems. The linear solubility profile is presumed to result from the parallel increase in the number of the formed micelles with increasing concentration. Lorazepam solubility could be increased from 0.048 to 0.16, 2.76, and 2.82 mg/ml, thus showing increases of 3.3-, 57.5-, and 58.8-fold for Pl.F68, Gl.81s, and SGC/SPC-MM, respectively, at a concentration of 10%. It was

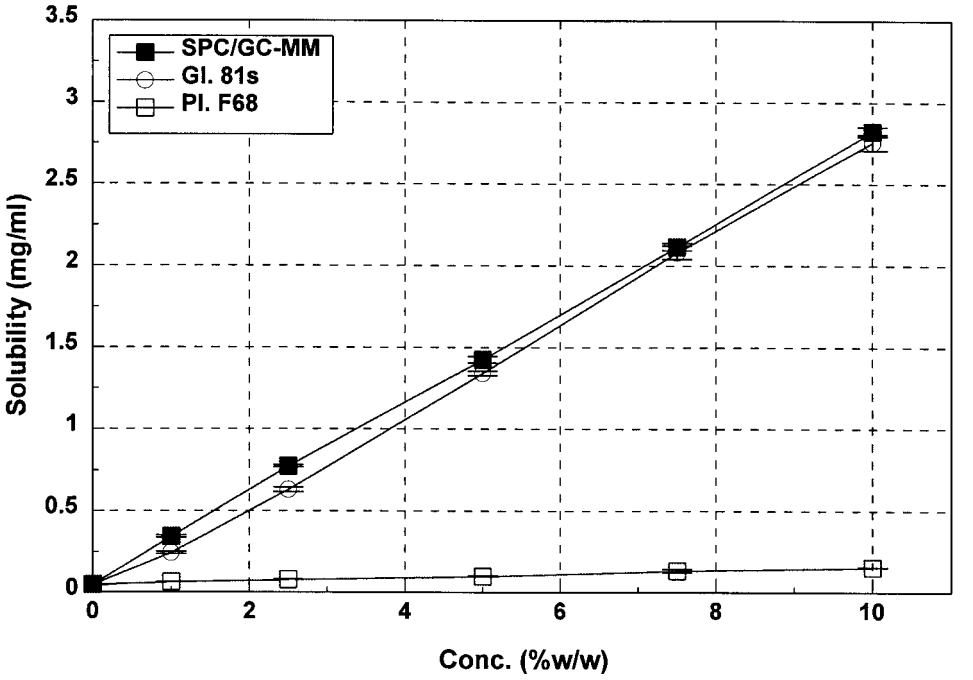

Figure 3. Solubility of lorazepam in SGC/SPC-MM (MF 0.5) compared with that in Gl.81s and Pl.F68 at 25°C (solutions in phosphate buffer, pH 7.4, 0.067 M) (mean value ± SD, *n* at least 2).

suggested that the lower solubility for Pl.F68 is due to water penetration into the oxypropylene region of the micelle (15). This effect would render this region too polar for the solubilization of unpolar molecules and hence cause a decrease in the binding to the micellar core. Incomplete micellization of Pl.F68 is also expected, which could be another reason for the decreased solubilizing capacity.

Factors Affecting Solubility in Bile Salt/Soya Phosphatidylcholine–Mixed Micelles

Table 1 gives the solubility data of lorazepam in BS/SPC-MM as a function of different formulation parameters. A partial replacement of BS by SPC resulted in an enhanced solubilizing capacity of the system, which is indicated by the solubility of the drug in BS/SPC-MM at an SPC mole fraction of 0.3 or 0.5 for SC/SPC-MM or SGC/SPC-MM, respectively. The increased solubilizing capacity of MM by incorporation of SPC is explained by the increase in the micellar size and lipophilicity. These are more proper factors for the micellar solubilization of lipophilic drugs (16).

Comparison of different BS/SPC-MMs at the same concentration and SPC mole fraction indicated that the presence of BS with lower hydrophilicity favored higher solubilization of lorazepam in MM. The solubilizing power in different BS/SPC-MMs could be arranged in the following descending order: SDC/SPC-MM > SC/SPC-MM > SGC/SPC-MM. The decreased hydrophilicity of BS results in decreased repulsion between the

Table 1

Effect of Type of BS and SPC Mole Fraction on Lorazepam Solubility in BS/SPC-MMs at 25°C

SPC Mole Fraction	System	Concentration (% w/w)	Solubility[a] (mg/ml)
0	SGC	5	0.75 ± 0.0
0.3	SGC/SPC	5	$1.08 \pm 0.7 * 10^{-2}$
0.5	SGC/SPC	5	$1.43 \pm 2.12 * 10^{-2}$
0	SC	5	$0.77 \pm 0.7 * 10^{-2}$
0.3	SC/SPC	5	$1.22 \pm 2.12 * 10^{-2}$
0.5	SC/SPC	5	$1.62 \pm 0.7 * 10^{-2}$
0.5	SC/SPC	10	$3.26 \pm 3.54 * 10^{-2}$
0.5	SDC/SPC	5	$1.73 \pm 4.24 * 10^{-2}$
0.5	SDC/SPC	10	3.54 ± 0.0

[a]Mean value ± SD, *n* at least = 2.

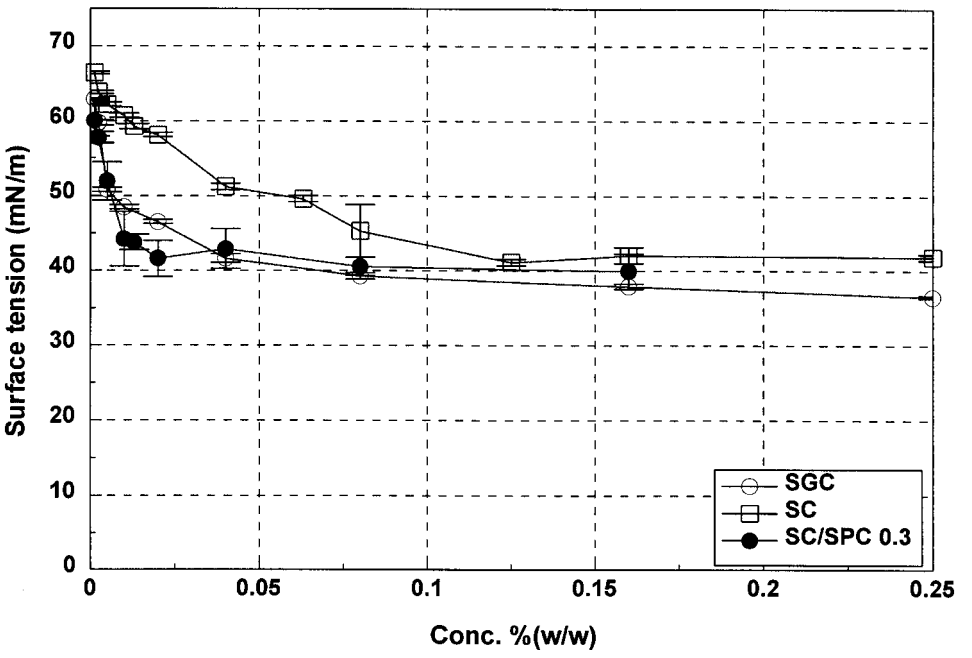

Figure 4. Surface tension reduction as a function of concentration for different BS and BS/SPC-MM (SPC mole fraction 0.3) (mean value ± SD, *n* at least 2).

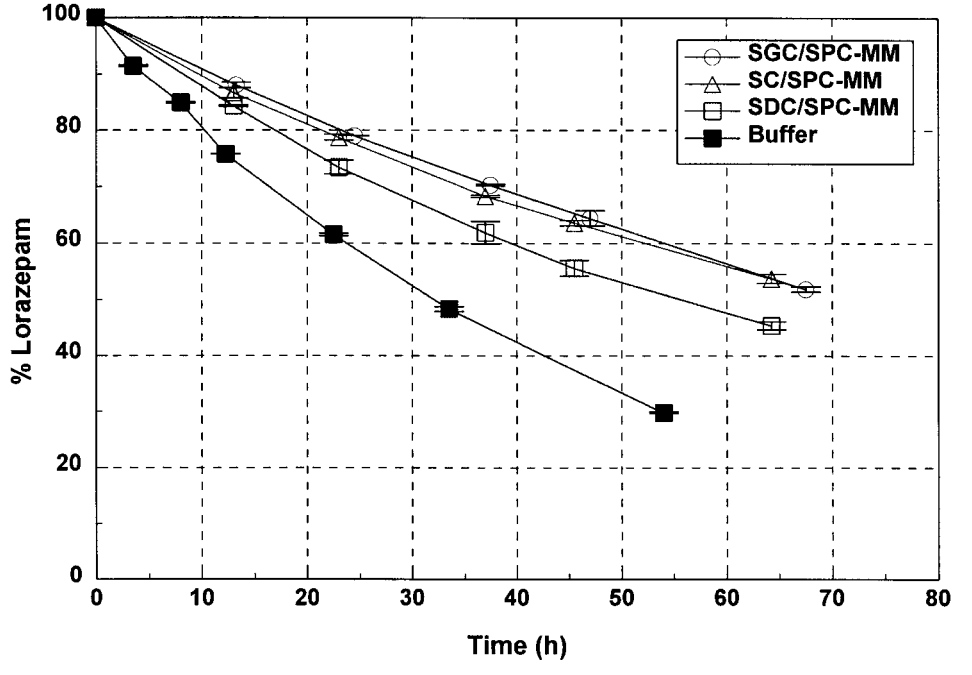

Figure 5. Degradation rates of lorazepam in different systems at 50°C (solutions in phosphate buffer, pH 7.4, 0.067 M) (mean value ± SD, *n* at least = 2).

micellar species, leading to lower critical micelle content (CMC) and larger micellar size with a concomitant increase in the solubilizing capacity.

Increasing the concentration of either SC/SPC-MM or SDC/SPC-MM from 5% to 10% led to an increase to approximately double the solubility of lorazepam. This was also observed for the linear profile for SGC/SPC-MM in Fig. 3.

Surface Tension of Bile Salt and Bile Salt/Soya Phosphatidylcholine–Mixed Micelles

The effect of the type of BS and the presence of SPC on the surface tension and CMC is illustrated in Fig. 4. The results showed that SGC was more effective than SC in reducing the surface tension. These two BSs belong to the trihydroxy BS; however, SGC has an amide group due to the conjugation of the side chain with the glycine molecule. Accordingly, SGC is expected to be anchored at the liquid-gas interface through more groups. This increases the capacity per mole of SGC for interaction at the interface; hence, a greater reduction of the surface tension is expected. The CMC of SC of approximately 2.8 mM obtained in this study is very close to the reported range of CMC in the presence of electrolyte (3–8 mM, Nagata et al., 1988). For SGC, Martis et al. (1972) reported a CMC of about 1.1 mM in water, which was slightly shifted to about 0.72 mM in this study. This is probably due to the presence of phosphate buffer, which could reduce CMC. The differences in the surface tension and CMC of these two BSs could not be correlated with the results of the solubility.

The addition of SPC with a mole fraction of 0.3 to SC resulted in a greater decrease in both surface tension and CMC. A drop in CMC from approximately 2.8 mM for SC alone to approximately 0.2 mM for SC/SPC-MM was observed. These results are in agreement with those of Naylor et al. (1993), who studied sodium taurocholate/EPC-MM solutions. This reduction in CMC in the presence of SPC could be partially responsible for the increased solubility of drugs in MM. The higher efficiency in surface tension reduction in the presence of SPC could also be correlated with the enhanced solubilizing capacity.

Stability Study

Effect of Type of Bile Salt

The degradation rates of lorazepam in MM based on different BSs, as well as in phosphate buffer, at 50°C are

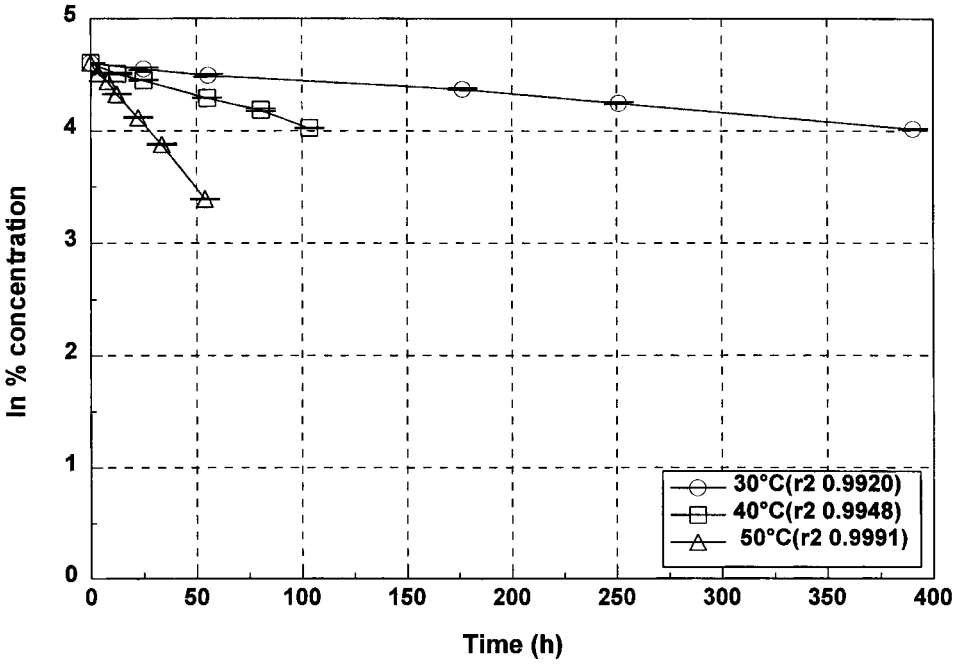

Figure 6. Degradation kinetics of lorazepam in phosphate buffer (pH 7.4, 0.067 M) at different temperatures (mean value ± SD, *n* at least = 2).

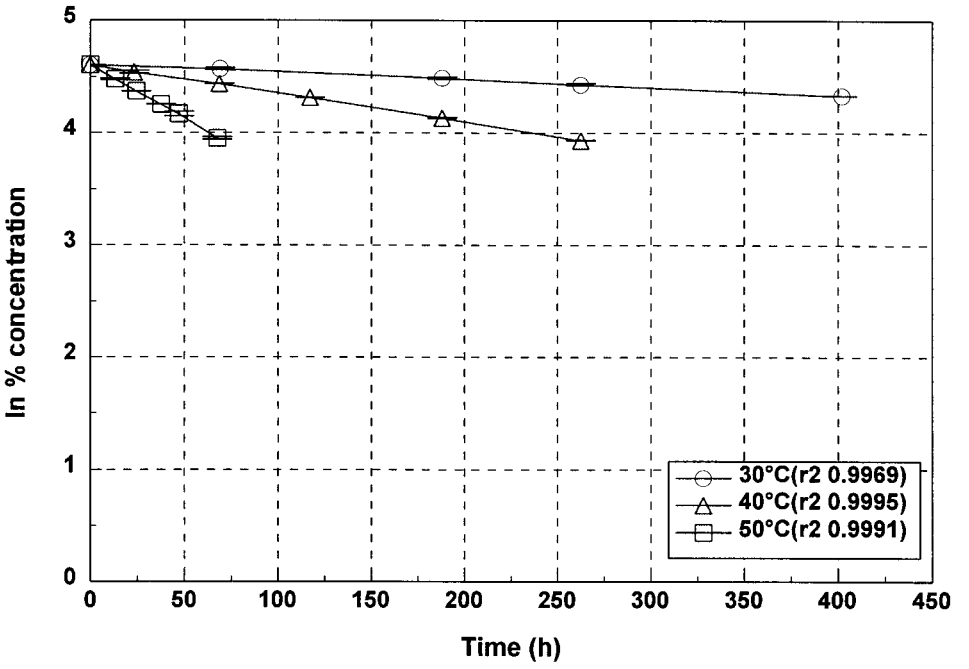

Figure 7. Degradation kinetics of lorazepam in SGC/SPC-MM at different temperatures (solutions in phosphate buffer, pH 7.4, 0.067 M) (mean value ± SD, *n* at least = 2).

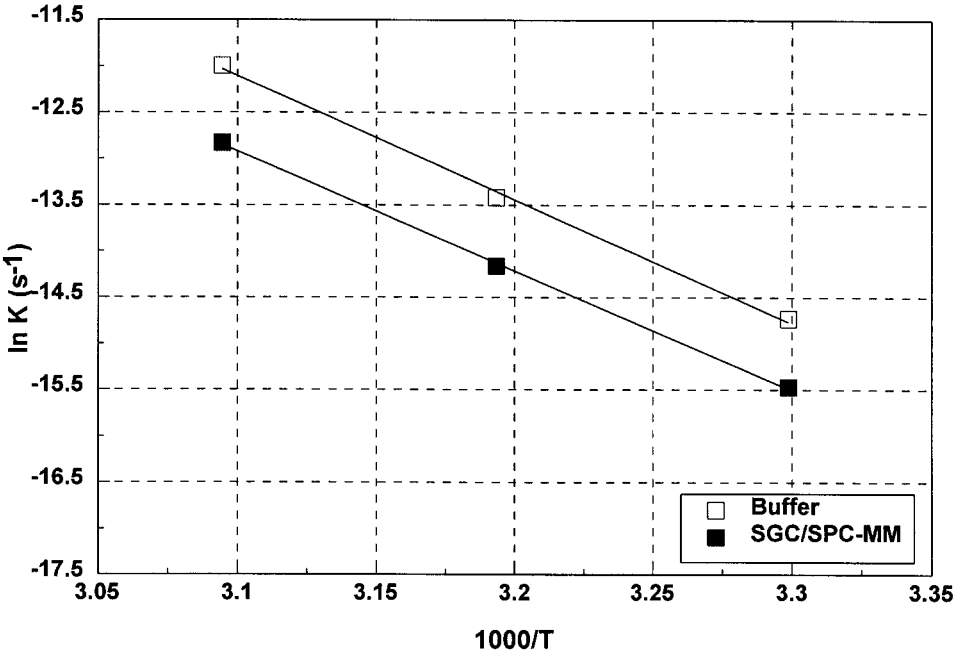

Figure 8. Arrhenius plot for lorazepam degradation in either SGC/SPC-MM or phosphate buffer (pH 7.4).

Table 2

Shelf Stability and Activation Energy E_a of Lorazepam in Phosphate Buffer and SGC/SPC-MM

		Shelf Stability t_{90} (days)	
System	E_a (kJ mol^{-1})	5°C	25°C
Buffer	111.4	166.6	6.58
SGC/SPC-MM	107.5	298.6	13.2

illustrated in Fig. 5. All the micellar systems displayed stabilizing effects for lorazepam compared with the stability in phosphate buffer alone. The results showed an insignificant difference between the degradation rates in SC/SPC-MM and in SGC/SPC-MM. On the other hand, SDC/SPC-MM showed fewer stabilizing properties for lorazepam than MM containing the trihydroxy BS. The increased stability in the case of trihydroxy BS may result from the increased protective effect that could be offered by OH groups in repulsing the attacking species and decreasing the mobility of water molecules near the micellar surface by hydrogen bond formation.

Effect of Temperature

The degradation kinetics of lorazepam in either phosphate buffer or in SGC/PC-MM showed reasonable agreement with apparent first-order degradation kinetics in a temperature range from 30°C to 60°C, as indicated by the linearity of the relation between logarithm of percentage of concentration against time (Figs. 6 and 7). The kinetic study could be described with an Arrhenius plot (Fig. 8), from which the stability at lower temperatures could be predicted by extrapolation. The activation energy values, calculated from the slope of the plot in the case of either phosphate buffer and SGC/SPC-MM (Table 2), were close to each other, indicating the same mechanism of degradation.

The obtained shelf stabilities corresponding to 10% degradation (t_{90}) at either 25°C or 5°C showed about a twofold increase in lorazepam stability when SGC/SPC-MM is added to the aqueous buffer solution. The shelf stability of lorazepam in the case of SGC/SPC-MM at 25°C is very short (about 2 weeks), whereas in a refrigerator (at 5°C), the shelf stability approaches 10 months. This improves the possibility of formulating the drug in aqueous solution.

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