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Solubility and stability of clonazepam in mixed micelles

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Abstract

The solubility and stability of clonazepam in bile salt/soya phosphatidylcholine-mixed micelles (BS/SPC-MM) were investigated. Compared with other surfactant systems such as pluronic F68, sugar ether and BS, BS/SPC-MM proved to be superior in enhancing the solubility of clonazepam. At a concentration of 10%, 3.5-, 30-, 40- and 50-fold increases in clonazepam solubility were observed in pluronic F68, BS, sugar ether and BS/SPC-MM, respectively. Moreover, increasing SPC ratio in MM led to parallel increase in solubility. Slight differences in solubilizing capacity were observed among MM prepared from different bile salts. Furthermore, the effect of addition of alcohols with different hydrophilicity such as, ethanol, propanol, butanol, pentanol, cyclohexanol, benzyl alcohol as well as 2-phenylethanol on clonazepam solubility in MM was also studied. Additional increase in solubility of clonazepam in MM could be achieved by the presence of some of these additives. The chemical stability of clonazepam in MM was also examined. The degradation of clonazepam was shown to follow apparent first-order degradation kinetics. The type of BS in MM and pH of the dispersion medium significantly affected the degradation rate. An Arrhenius plot showed a great enhancement in clonazepam stability in the presence of MM. More than 6-fold increase in the shelf stability of clonazepam could be obtained in the presence of BS/SPC-MM which makes it possible to formulate clonazepam in aqueous mixed micellar solution with proper shelf stability.

Keywords: Bile salts/soya phosphatidylcholine-mixed micelles; Clonazepam; Solubilization; Additives; Stabilization

1. Introduction

Solubilization in surfactant solutions above the critical micelle concentration offers one approach to the formulation of poorly soluble drugs in solution form. The limiting factors in the use of solubilizers as effective formulation aids are (i) the finite capacity of the micelle for the drug, (ii) the possible short- or long-term adverse effects of the surfactant on the body, and (iii) the concomitant solubilization of other ingredients, such as preser-

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vatives, flavouring and colouring matter in the formulation, with consequent alterations in the stability and effectiveness (Attwood and Florence, 1983).

Bile salt/phosphatidylcholine-mixed micelles have been used as solubilizing vehicle for poorly soluble drugs (Bloois et al., 1987; Nagata et al., 1988; Dürr et al., 1994). Their physiological compatibility makes their application more advantageous as compared with other solvents used parenterally.

The drug substance clonazepam is a benzodiazepine with anticonvulsant properties. It has very limited aqueous solubility which precludes the use of water alone as solvent. Moreover, the stability of clonazepam with regard to hydrolytic degradation is another factor which must be taken into consideration when formulating clonazepam in aqueous preparations. The formulation of clonazepam in a vehicle which is safe and which could enhance the chemical stability of solubilized clonazepam is a factor which will be of paramount importance.

The aim of this work was, therefore: (i) to enhance the solubility of clonazepam in BS/SPC-MM, (ii) to enhance the solubilizing capacity of BS/PC-MM towards clonazepam with additives, and (iii) to enhance the chemical stability of clonazepam by using BS/SPC-MM.

2. Materials and methods

2.1. Materials

Clonazepam, USP 23 quality, was purchased from Welding GmbH (Hamburg, Germany). Degradation product DP1 (3-amino-4-(2-chlorophenyl)-6-nitro-1H-quinoline-2-one) and degradation product DP2 (2-amino-2′-chloro-5-nitro-benzophenone) were obtained from Arzneimittelwerk Dresden (Radebeul, Germany). Sodium glycocholate (SGC), sodium deoxycholate (SDC) and SPC 95.8%, phospholipone 100 were a gift from Nattermann Phospholipid GmbH (Cologne, Germany). Acetonitrile (HPLC grade), methanol (HPLC grade), chloroform, ethanol, propanol, butanol, pentanol, benzyl alcohol, 2-phenylethanol, sodium cholate (SC), NaH_2PO_4, and Na_2HPO_4 were supplied by Merck AG (Darmstadt, Germany). The sugar ether or glucoside 81s (alkyl residue C8–10 and 1.1–3 degrees of glucosidation) was a gift from Hüls AG (Witten, Germany). Pluronic F68 was obtained from Erbslöh GmbH (Krefeld, Germany). The study was carried out in double-distilled water.

2.2. Methods

2.2.1. Preparation of MM

BS/SPC-MM were prepared by the coprecipitation method (Schurtenberger et al., 1985). BS and SPC (at different mole fractions) 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 h). The resulting films were dispersed in a given amount of the dispersion medium (water or buffer) to give clear micellar solutions with the required concentration.

2.2.2. HPLC analysis of clonazepam

A mobile phase consisting of a mixture of double-distilled water and acetonitrile (55:45, v/v) at a flow rate of 1 ml/min. and wave length of 310 nm was developed for the HPLC analysis of clonazepam. The instrument which was used consisted of an RP-18 5-μm column (150 × 4.6 mm)

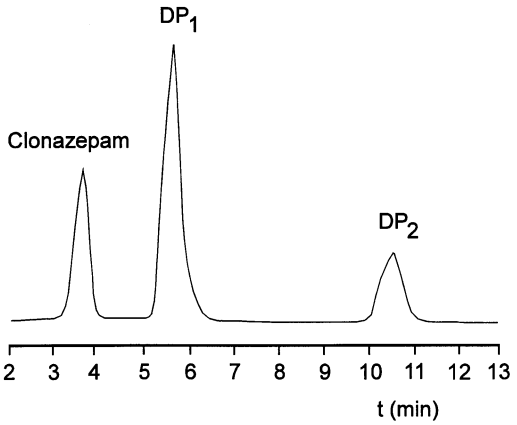

Fig. 1. HPLC chromatogram of clonazepam and its degradation products.

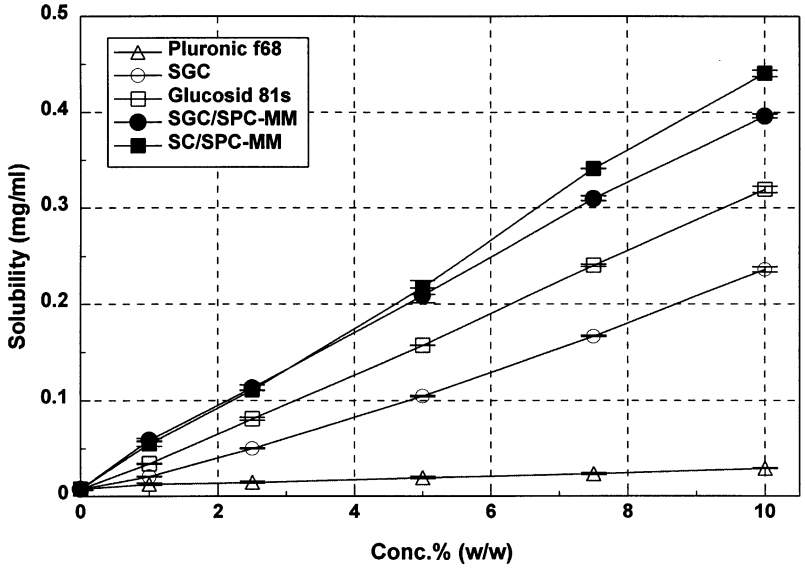

Fig. 2. Solubility of clonazepam in different micellar systems at 25°C (dispersion medium phosphate buffer, pH 7.4, 0.067 M) (mean value ± S.D., $n \geq 2$).

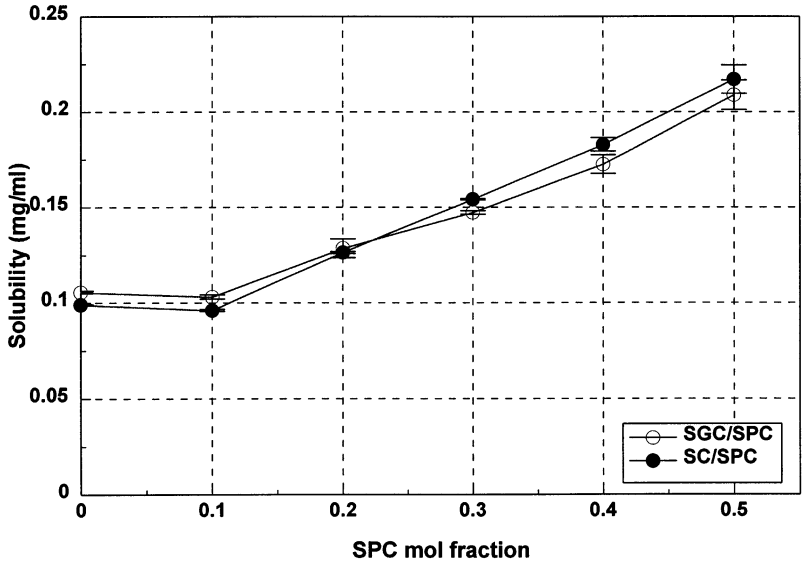

Fig. 3. Effect of SPC mole fraction on clonazepam solubility in different MM at 25°C (tota conc. 5% (w/w) in phosphate buffer, pH 7.4, 0.067 M) (mean value ± S.D., $n \geq 2$).

(Merck; Darmstadt, Germany), a high precision Gyncotek 300C pump (Gyncotek; München, Germany), an autosampler Kontron 360 and a Kontron 742 UV detector (Kontron Instruments; München, Germany), and an integrator Shimadzu C-R6A chromatopac (Shimadzu, Kyoto, Japan).

2.2.3. Solubility study

Excess amounts of clonazepam were added to 10 ml 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 which was determined by

Table 1
Effect of type of bile salt and dispersion medium on clonazepam solubility in MM (conc. 5%, w/w) at 25°C

MM[a]	Dispersion medium	Solubility (mg/ml)
SGC/SPC	Phosphate buffer, pH 6.5	$0.207 \pm 9.2 \times 10^{-4}$
SGC/SPC	Phosphate buffer, pH 7.4	$0.209 \pm 7.7 \times 10^{-3}$
SGC/SPC	Phosphate buffer, pH 8	$0.207 \pm 6.9 \times 10^{-3}$
SGC/SPC	Water	$0.215 \pm 2.4 \times 10^{-3}$
SC/SPC	Phosphate buffer, pH 7.4	$0.217 \pm 7.4 \times 10^{-3}$
SDC/SPC	Phosphate buffer, pH 7.4	$0.195 \pm 2.1 \times 10^{-3}$

[a] Mole fraction of BS and SPC, 0.5.
[b] Mean value ± S.D., $n \geq 2$.

Table 2
Solubility of clonazepam in different alcohols together with solubility of the different alcohols in water

Alcohol	Solubility of:	
	Clonazepam in alcohol (mg/ml)	Alcohol in H_2O (%, v/v)
Methanol	8.6[b]	Miscible[c]
Ethanol	4.7[b]	Miscible[c]
n-Propanol	3.39 ± 0.035[a]	Miscible[c]
n-Butanol	2.7 ± 0.00[a]	9.1[c]
n-Pentanol	2.26 ± 0.014[a]	2.7[c]
Cyclohexanol	4.31 ± 0.120[a]	3.6[c]
Benzyl alcohol	33.5 ± 0.219[a]	4[d]
2-Phenylethanol	24.5 ± 0.092[a]	2[d]

[a] Mean value ± S.D., $n \geq 2$.
[b] From Winslow (1977).
[c] From Wade (1987).
[d] From The Merck Index (1983).

repetitive sampling (24–48 h). Excess amounts of clonazepam were separated by 5 min centrifugation at 13000 rpm on a centrifuge (Biofuge A; Heraeus Instrument GmbH, Hannover, Germany). Of the supernatant solutions, 0.5 ml was properly diluted with a methanol:water mixture (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 five) in a range from 1 to 10 μg/ml were prepared by dilution from a stock solution of clonazepam in the methanol:water mixture (80:20, v/v) and the dilution was made with same solvent mixture. Addition of 20% water to methanol helped to achieve accurate pipetting of methanol. The concentration absorption relationship obeyed the Beers–Lambert law (r^2 not less than 0.999).

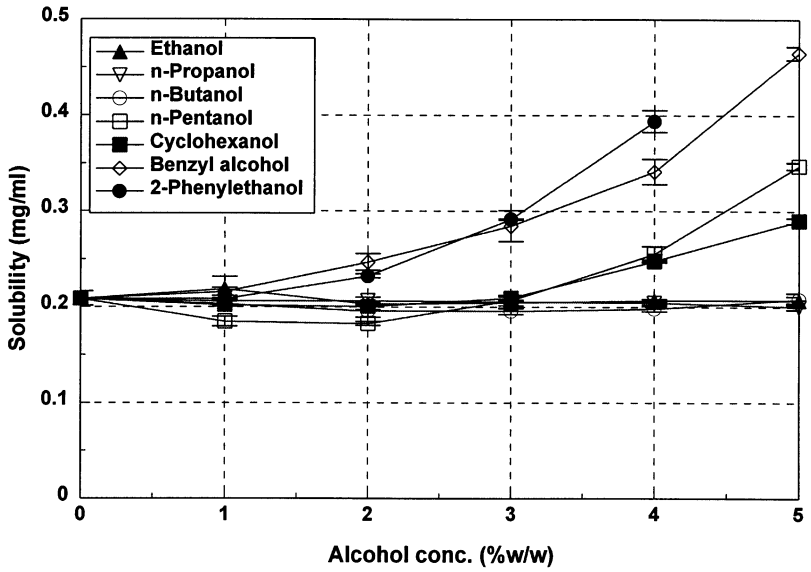

Fig. 4. Effect of different alcohols on the solubility of clonazepam in SGC/SPC-MM (mole fraction 0.5, total conc. 5% (w/w) in phosphate buffer, pH 7.4, 0.067 M) at 25°C (mean value ± S.D., $n \geq 2$).

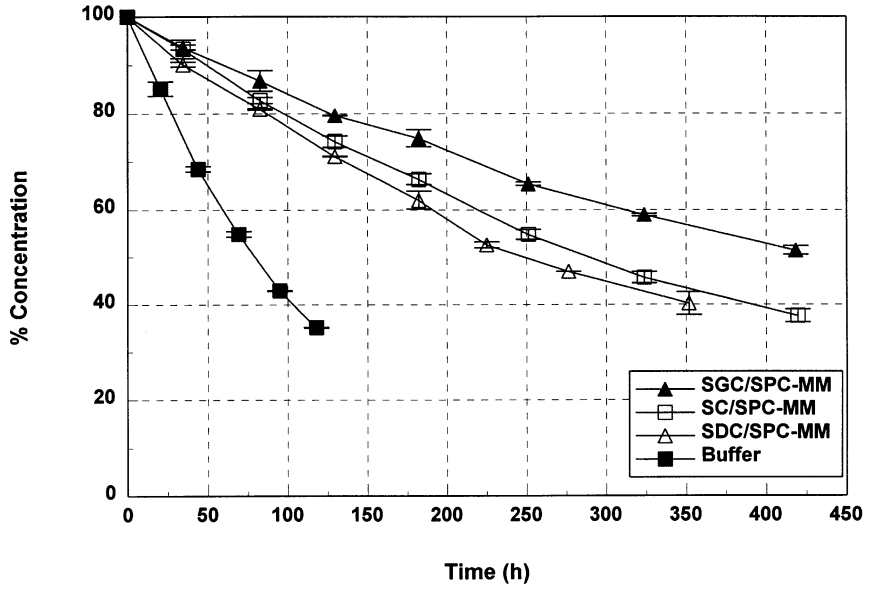

Fig. 5. Clonazepam degradation rates in different BS/SPC-MM as well as in phosphate buffer (pH 7.4, 0.067 M) at 60°C.

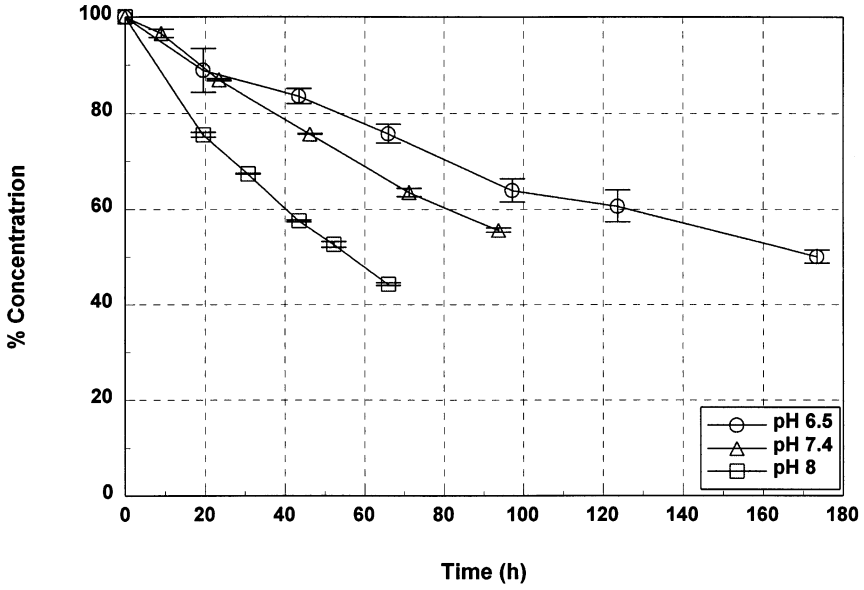

Fig. 6. Effect of pH on clonazepam degradation rate in SGC/SPC-MM at 70°C (dispersion medium, phosphate buffer, 0.067 M).

2.2.4. Stability study

Certain amounts of clonazepam were added as methanolic solution during the first stage of MM preparation to give a final concentration corresponding approximately to the saturation solubility in the system to be studied. In the case of buffer, the required amount of clonazepam was added as methanolic solution (200 μl) to each 20 ml of the buffer. All solutions in the case of either MM or buffer alone were prepared in duplicate. The solutions to be studied were placed in 2-ml ampoules under N_2. Then, they were stored at the required

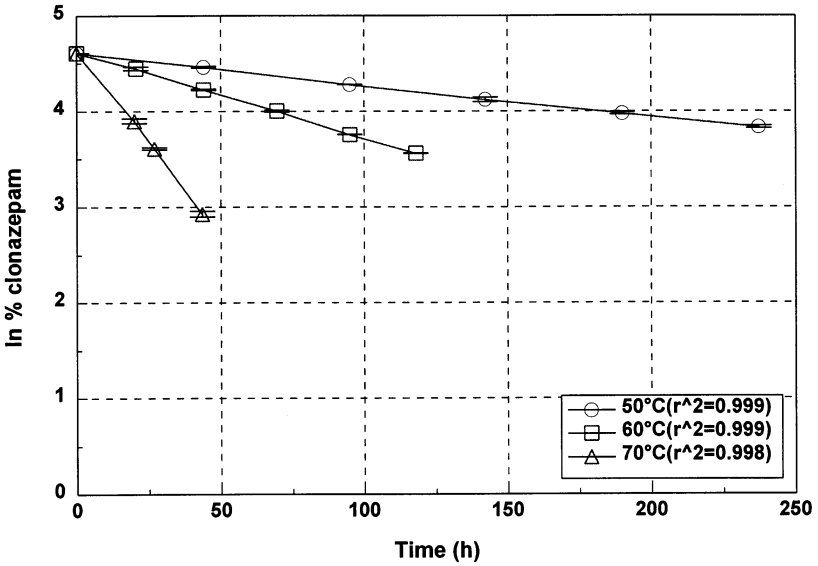

Fig. 7. Degradation kinetic of clonazepam in phosphate buffer (0.067 M, pH 7.4) at different temperatures (mean value ± S.D., $n \geq 2$).

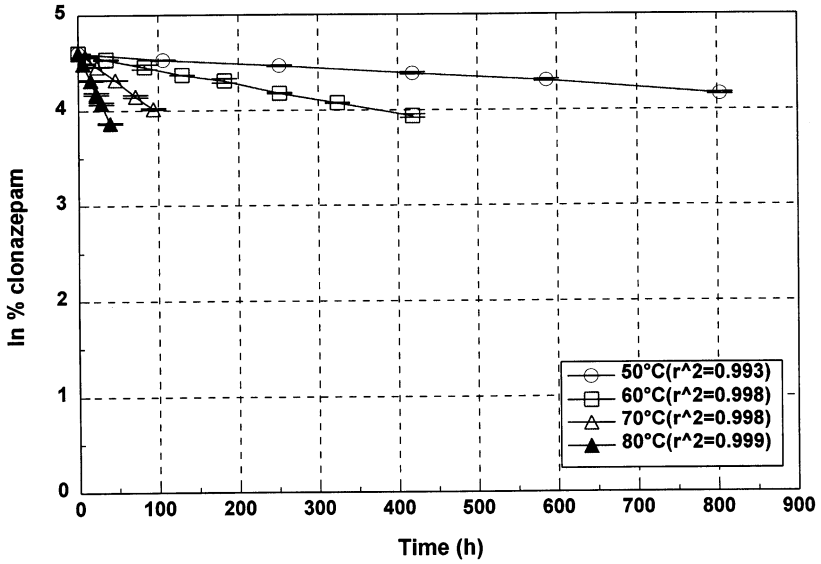

Fig. 8. Degradation kinetic of clonazepam in SGC/SPC-MM (mole fraction 0.5, total conc. 5% (w/w) in phosphate buffer, pH 7.4, 0.067 M) at different temperatures (pH 7.4) (mean value ± S.D., $n \geq 2$).

temperature. At suitable time intervals, samples were taken, properly diluted, and subjected to HPLC analysis.

Injection of clonazepam together with its degradation products, DP1 and DP2, into HPLC indicated that the developed method is capable of separating clonazepam from its degradation products (Fig. 1). The UV-spectra in the used mobile phase indicated that DP1 has higher absorption than that of clonazepam, while DP2 absorbs at a lower extent at the chosen wavelength. Because of this, and although PC does not absorb at 310 nm, the use of HPLC method for separation of the DP was necessary.

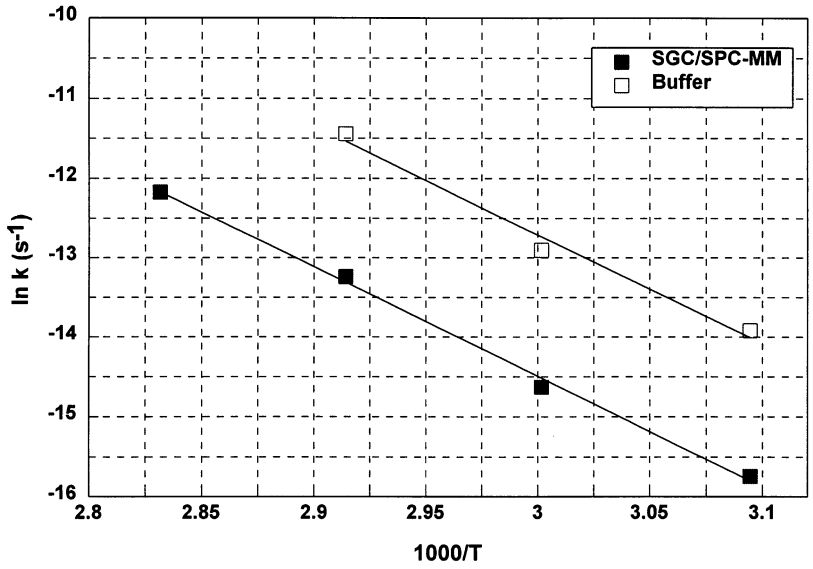

Fig. 9. Arrhenius plot for clonazepam degradation in either SGC/SPC-MM or phosphate buffer.

3. Results and discussion

3.1. Solubility study

3.1.1. Effect of type of micellar system

Fig. 2 shows the solubility of clonazepam in different micellar systems as a function of the concentration. The linear increase in clonazepam solubility is attributed to the parallel increase in the number of micellar species available to solubilize clonazepam (Alkan-Onyuksel et al., 1994). The BS/SPC-MM systems displayed the highest solubilizing capacity, while pluronic F68 showed the lowest. The lower solubilizing capacity of pluronic F68 can be explained by the fact that it has a semipolar micelle core which is not quite suitable for accommodation of the lipophilic side of clonazepam molecules that would be responsible for interaction and orientation of the drug in the micelle. Moreover, water may penetrate into the oxypropylene region of the micelle. This effect would render this region too polar for solubilizate molecules (Elworthy and Patel, 1983). In addition, incomplete micellization is expected from the structural features of pluronic surfactants which could be another reason for the decreased solubilizing capacity of these surfactants.

On the other hand, higher solubility could be achieved in MM probably due to the simultaneous presence of both charged palisade layer (because of the charges of PC and BS) and lipophilic core (because of PC fatty acids residue and the lipophilic side of BS) in the MM, which result in enhanced interaction with polar and nonpolar parts, respectively, of the clonazepam molecule. Lower solubility in SGC compared with that in BS/SPC-MM is thought to be due to its smaller micelle size and higher hydrophilicity.

Although glucoside 81s forms anisometric 'worm-like' micelles with high aggregation numbers (Balzer, 1996) which would favour higher solubilization, it has a lower solubilizing capability compared with that of BS/SPC-MM. This can be explained by lower lipophilicity of the micelle core (C8–10) compared with that of BS/SPC-MM. In addition, the bulky hydrophilic palisade layer of the glucose units is expected to hinder interaction of clonazepam molecules with the micelles. The slight difference which is observed between SC/SPC-MM and SGC/SPC-MM is accounted for by the small difference in micellar size as these two BS are trihydroxy bile salts and expected to form more or less similar micelles.

Table 3
Shelf stability and Arrhenius parameters of clonazepam in MM and in phosphate buffer at pH 7.4

System	Arrhenius parameters		Shelf stability (months) at 25°C	
	A (S^{-1})	AE (kJ M^{-1})	$t90$	$t50$
Phosphate buffer	1.957×10^{12}	113.66	1.69	11.16
SGC/SPC-MM	4.908×10^{11}	114.77	10.55	69.63

A, frequency factor; AE, activation energy.

3.1.2. Effect of formulation parameters of BS/SPC-MM

In Fig. 3 the gradual increase in clonazepam upon increasing incorporation of SPC in the system was previously reported to be due to the parallel increase of both size and lipophilicity of the formed BS/SPC-MM. This effect has also been noted during the solubility study of diazepam in MM from SC and egg phosphatidylcholine (Rosoff and Serajuddin, 1980).

Table 1 summarizes the effect of the dispersion medium and type of BS on clonazepam solubility. Slight differences were observed among MM containing different BS. Using water as dipersion medium or aqueous phosphate buffer with different pH (from pH 8 to 6.5) had insignificant influence on clonazepam solubility in MM.

3.1.3. Effect of additive

The effect of different alcohols with a wide range of hydrophilicity on clonazepam solubility in SGC/SPC-MM is depicted in Fig. 4. The solubility change of clonazepam as a result of alcohol addition could be explained considering the hydrophilicity and the chemical structure of the alcohol as well as the solubility of clonazepam in pure alcohol (Table 2). Addition of alcohols with ascending lipophilicity beginning with ethanol, propanol, and up to butanol, insignificantly affected clonazepam solubility in MM. Addition of pentanol cyclohexanol, benzyl alcohol, or 2-phenylethanol increased the solubility of clonazepam in MM to different degrees. The increase in the lipophilicity of alcohol increased its affinity to the micellar phase and hence a higher concentration of the alcohol in the micellar phase is expected.

It has been reported that water-soluble alcohols (methanol to butanol) are predominantly dissolved in the water phase and may decrease or increase the micellar aggregation number, n, depending on the alcohol concentration. Moderately soluble alcohols (pentanol, hexanol) are distributed between the aqueous and micellar phases and may increase the association number (Backlund et al., 1981). Moreover, more hydrophilic alcohols were found to increase the CMC, while more lipophilic alcohols were found to decrease the CMC (Green, 1972). Although the decrease in CMC could partially contribute to the increase in the solubilizing capacity it could also be considered as an indication of the formation of micellar species with larger size. These results furnish a basis which agrees with our explanations. In addition, Roe and Barry (1982) reported that addition of 2-phenylethanol with concentrations in the same range used in this study to different bile salt solutions (each with a concentration of 5%) showed an increase in the micellar size. The increase in the size of bile salt micelles as a function of 2-phenylethanol concentration occurred in more or less similar fashion to the increase of clonazepam solubility in MM.

Incorporation of alcohol into MM could result in a swelling of MM, and this in turn increases its solubilizing capacity. This increase in size does not appear to be the only factor which is responsible for the higher solubility. Comparing the chemical structures indicates that alcohols which have aromatic rings showed the highest potency in increasing clonazepam solubility in MM. The saturation solubility of clonazepam in these alcohols was considerably higher compared with its solubility in the other alcohols. This leads to the

assumption that the solubilization capacity of MM is influenced by the nature of the incorporated alcohol. Accordingly it could explain why benzyl alcohol is more effective than pentanol in increasing the solubility of clonazepam, even though the latter is more lipophilic.

On the other hand, 2-phenylethanol has a similar chemical structure to benzyl alcohol and hence a similar solubilizing capacity after incorporation in MM. The saturation solubility of 2-phenylethanol in the aqueous 5% MM phase is about 4%, while this is about 5% for benzyl alcohol. The slightly higher increase in the solubilizing capacity of 2-phenylethanol at 4% could result from the partitioning of this alcohol due its higher lipophilicity (see Table 2).

From the pharmaceutical point of view, the increase in clonazepam solubility in MM by addition of either benzyl alcohol or 2-phenylethanol makes their usual use as preservatives more advantageous due to the possibility of decreasing the concentration of MM.

3.2. Stability study

3.2.1. Effect of type of degradation medium

Fig. 5 shows that all BS/SPC-MM displayed a stabilizing effect for clonazepam in comparison with phosphate buffer. Incorporation of clonazepam in the micellar species where it encounters a less polar environment could be the reason for the enhancement of the stability of clonazepam against hydrolytic degradation.

The stabilizing effect of different BS/SPC-MM could be arranged in the following descending order; SGC/SPC-MM > SC/SPC-MM > SDC/SPC-MM, indicating that the presence of trihydroxy BS increases the stability of clonazepam in MM. Measurement of the pH of these mixed micellar solutions showed that the pH of either SDC/SPC-MM or SC/SPC-MM slightly exceeded that of the dispersion medium (the measured pH values of these solutions were about 7.55). On the other hand, the pH of SGC/SPC-MM was the same as that of the dispersion medium (about 7.4). Accordingly, the variation in the stability could not be totally ascribed to the variation in pH of the different mixed micellar solutions. The increased stability in case of trihydroxy BS (SC/SPC-MM and SGC/SPC-MM) may result from the ability of the hydroxyl groups to protect clonazepam against attacking species. The mechanisms of the stabilizing effect could be an immobilisation of water molecules due to hydrogen bonding to the hydroxyl groups near the micellar interface and a repulsive effect against negatively charged attacking species, such as OH^- ions. The more hydrophilic nature of SGC and the presence of longer side chains (through conjugated glycine) could offer more protective effects than in the case of SC.

3.2.2. Effect of pH

The effect of pH on clonazepam stability in SGC/SPC-MM is shown in Fig. 6. The results indicate that clonazepam degradation was significantly decreased as the pH was decreased from pH 8 to 6.5. This behaviour is in agreement with that of other benzodiazepines (Mayer et al., 1972).

3.2.3. Effect of temperature

Figs. 7 and 8 show clonazepam degradation in phosphate buffer and SGC/SPC-MM, respectively. In all cases the degradation of clonazepam was found to follow apparent first-order degradation kinetics where ln concentration (%) was a linear function of time. Construction of the Arrhenius plot (Fig. 9) indicated that MM greatly enhanced the chemical stability of clonazepam. Compared with phosphate buffer alone, more than 6-fold increase in shelf stability of clonazepam at 25°C could be achieved in the presence of MM at pH 7.4. Table 3 gives the calculated Arrhenius parameters in addition to shelf stability at 25°C.

4. Conclusions

The solubility of clonazepam could be substantially increased by BS/SPC-MM. The solubilizing capacity of BS/SPC-MM could be highly increased by addition of benzyl alcohol and 2-phenylethanol. The stability of clonazepam could be greatly enhanced in the presence of BS/SPC-MM.

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