International Journal of Pharmaceutics, 6 (1980) 137–146

SOLUBILIZATION OF DIAZEPAM IN BILE SALTS AND IN SODIUM CHOLATE–LECITHIN–WATER PHASES

MORTON ROSOFF* and ABU T.M. SERAJUDDIN*

*College of Pharmaceutical Sciences**, *Columbia University, 115 West 68th Street, New York City, N.Y. 10023 (U.S.A.)*

(Received March 13th, 1980)
(Revised version received May 26th, 1980)
(Accepted June 9th, 1980)

SUMMARY

The solubility of a poorly water-soluble drug, diazepam, in different bile salt solutions and in mixed micellar solutions, liquid crystals and emulsions of various sodium cholate–lecithin compositions was investigated. The solubilization capacities of the bile salts are in the relative order: sodium cholate = sodium deoxycholate > sodium taurocholate > sodium glycocholate >> sodium dehydrocholate. In mixed micellar solutions, the solubilization capacity of the system increases as the lecithin–sodium cholate molar ratio increases. At low concentrations of sodium cholate plus lecithin (<0.3 mmol/g), the solubilization capacity of the combination at any ratio between them is higher than the solubilization capacity of sodium cholate alone. At higher concentrations of lecithin + sodium cholate, lecithin initially decreases the solubilization capacity of sodium cholate, but as its proportion increases, the molar solubilization capacity of the combination ultimately becomes higher than that of sodium cholate alone. The solubility of diazepam in various phases of the sodium cholate–lecithin–water system is in the following order: lamellar > cubical > hexagonal > micellar.

INTRODUCTION

Normal human gall bladder bile contains about 85% water, 7.6% bile salts, 3% lecithin, 0.5% cholesterol and 3.5–4% other components (bile pigments, proteins, inorganic ions, etc.) (Dam et al., 1966). Although bile salt and lecithin have distinct regions of opposing hydrophilic and lipophilic tendencies and can function as surfactants, lecithin is water-insoluble and is solubilized in the bile by the formation of bile salt–lecithin mixed

* Present address: USV Laboratories, Pharmacy Research and Development Department, 1 Scarsdale Road, Tuckahoe, N.Y. 10707, U.S.A.
** Phased out in June, 1976.

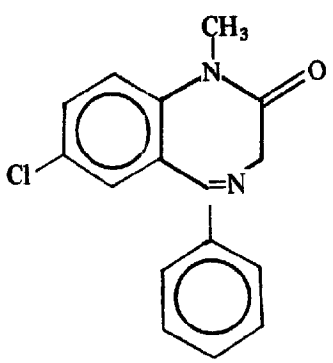
micelles (Carey and Small, 1970). According to Small (1970), 'a mixed micelle is any micelle made up of more than one lipid-like chemical species. At least one of the chemical species must be able to form micelles alone in aqueous solutions'. Small et al. (1966) studied the interactions of lecithin with sodium cholate and bile salt mixtures in water and observed 3 paracrystalline phases and an isotropic solution (micellar phase). In the range of concentrations found in the bile, bile salts alone may solubilize about one-third as much cholesterol as might be found in human gall bladder (Neiderhiser and Roth, 1968). The bile salt–lecithin–water system accounts for not only the complete solubilization of cholesterol in the bile, but also for the maintenance of bile in a sufficiently unsaturated state with respect to cholesterol in normal physiological conditions, presumably to prevent possible formation of gall stones.

Bourgès et al. (1967) studied the solubility of cholesterol in sodium cholate–water and sodium cholate–lecithin–water systems. Verzar (1933) showed that bile salts were capable of solubilizing a large number of poorly water-soluble organic and inorganic compounds. Ekwall et al. (Ekwall and Sjöblom, 1949; Ekwall and Sjöblom, 1950; Ekwall et al., 1951) studied the solubilization of various steroid hormones in bile salt solutions. Bates et al. (1966a and b) showed that the solubilities and dissolution rates of several unrelated poorly water-soluble drugs were increased by the presence of bile salts. Little work has been reported on the solubility of drugs in bile salt–lecithin mixed micellar solutions and in bile salt–lecithin–water paracrystalline phases.

In the present investigation, we have studied the solubility of a poorly water-soluble drug in solutions of different kinds of bile salts, in bile salt–lecithin–water micellar and paracrystalline phases, and also in the emulsion region of bile salt–lecithin–water mixtures. Since bile is secreted into the duodenum through the biliary duct, a knowledge of the drug-solubilizing properties of bile salts and bile salt–lecithin mixtures in micellar solutions is of great importance in understanding the role of these biological surfactants in the gastrointestinal absorption of poorly water-soluble drugs. In addition, drugs solubilized in bile salt–lecithin–water liquid crystals and emulsions have potential applications as new dosage forms of drugs.

MATERIALS AND METHODS

Drugs and chemicals. Diazepam (Hoffman–LaRoche), the drug used in this investigation as shown in Structure I, was found to be devoid of any impurity by TLC and was used as received. The following bile salts (K and K Labs) were also used as received: sodium salts of cholic acid, deoxycholic acid, dehydrocholic acid, glycocholic acid, and taurocholic acid.



STRUCTURE I

The lecithin (phosphatidylcholine) was prepared from fresh chicken egg yolks by the procedure of Singleton et al. (1965) and was analyzed by thin-layer chromatography to ensure the absence of cholesterol contamination and to determine the purity of lecithin. For detecting cholesterol, the solvent system used was benzene–ethyl acetate (2 : 1) and the detector was 10% phosphomolybdic acid in ethanol, with charring at 150°C for 10 min (Marsh and Holzbach, 1973). For detecting lecithin, the solvent system used was chloroform–methanol–water (65 : 25 : 4) and detection was obtained with a modified Dittmer-Lester spray (Vaskovsky and Kostetsky, 1968). The lecithin was free from cholesterol and any other phospholipid, and on the basis of phosphorus and nitrogen content, its molecular weight was found to be 773.

Equilibrium solubilization of diazepam in micellar solutions. Bile salt and bile salt–lecithin micellar solutions were prepared by dissolving appropriate quantities of these substances in double-distilled water without any adjustment of the natural pH of the solution (7.5–8.3). An excess of diazepam was added to the solution in appropriate volumetric flasks and shaken on a wrist action shaker (Burrell) at 30°C until equilibrium was attained (24–36 h). Each time the flasks were sampled, the shaker was turned off to allow most of the excess solids to settle to the bottom of the flasks. The supernatant liquid was then rapidly filtered through a 0.22-μm pore size membrane (Millipore). To eliminate any temperature differential during the filtration step, precautions were taken to maintain filtration equipment in an oven at 30°C. An aliquot, generally 1 ml or 1 g, according to the purpose of the experiment, was then analyzed for the drug content.

Diazepam was analyzed by a spectrophotometric method. The peak absorbance of diazepam in methanolic solution was observed to be at the wavelength of 230 nm. The position of the peak remained unchanged in the presence of bile salts and lecithin. Moreover, in the range of concentrations of diazepam in methanolic solutions, the absorbances of bile salts and lecithin were very low in comparison with that of diazepam itself (bile salts $< 5\%$, lecithin $< 20\%$). Studies at different concentrations of bile salts and lecithin showed that when a reference solution for UV analysis was made with exactly the same concentration of the surfactant as present in the diazepam solution, there was no difference in the absorbance of diazepam compared to that of a diazepam solution of the same concentration in methanol alone. For the purpose of our experiments, a quantity of surfactant solution equivalent to the aliquot taken was retained in a separate volumetric flask before the addition of diazepam for equilibrium solubilization and was appropriately diluted with methanol for use as a reference solution.

Lecithin-in-water emulsions were prepared by mixing lecithin, sodium cholate and water on a weight/weight basis and stirring in a shaker until there was no residue of lecithin present. Generally, 10 g. of the emulsion was prepared for each solubilization experiment. One gram was saved in a separate flask for subsequent use in making the reference solution for analysis and an excess of diazepam was added to the rest of the emulsion and shaken on a wrist-action shaker at 30°C until equilibrium was attained (24–36 h). This procedure was, however, not applicable to very thick emulsions.

Since filtration was not appropriate for separating the excess diazepam particles from the emulsion, a novel procedure was developed for this purpose. The emulsion was centrifuged in a super-speed centrifuge (Sorvall ss-1) at 12,000 rpm for 30 min. The supernatant emulsion was separated from the solid diazepam which settled to the bottom of the

centrifuge tube, mixed and examined with the polarizing lenses of a microscope for detecting traces of any solid diazepam remaining in the emulsion. The diazepam crystals could be readily distinguished by their characteristic birefringence. If any diazepam particles were present, the centrifugation was repeated until all the solid diazepam separated out. The emulsion was then homogenized by stirring, 1 g of aliquot taken and analyzed by the spectrophotometric method. The procedure gave highly reproducible results. Some of these experiments were repeated by a separate procedure, discussed in the following section in connection with the solubilization of diazepam in thick emulsions and liquid crystals, and gave identical results.

Equilibrium solubilization of diazepam in thick emulsions and liquid crystals. The equilibrium solubilization of diazepam in bile salt–lecithin–water thick emulsions and liquid crystals was also determined by a novel procedure. Two different volumes of 5% methanolic solutions, one of lecithin and the other of sodium cholate, were mixed to give the desired weight ratio of lecithin to bile salt. At least 10 identical mixtures of each lecithin–sodium cholate ratio were prepared and placed in specially designed glass ampules with conical bottoms. A 2.5% methanolic solution of diazepam was added to these ampules in ascending order of additions. The solvents of the final mixtures were evaporated in a stream of nitrogen and the contents were dried by continuous evacuation at room temperature over P_2O_5. After 48–72 h of evacuation, the mixture was considered dry as no weight was lost in a preliminary experiment after heating a few mg at 110°C for 30 min. The appropriate quantity of distilled water was added to each ampule using a 100 µl syringe. The quantities of lecithin, bile salt and water were so adjusted that their combined weight was 200 mg in each ampule; only the quantity of diazepam varied. The ampules were then filled with water-saturated nitrogen, sealed and shaken for 72 h.

The ampules were opened at the end of the equilibration period, the contents of each ampule were mixed manually with a glass rod and a small quantity from each ampule was removed and examined with a microscope fitted with polarizing lenses. The amount of diazepam in the particular ampule of the series where the traces of diazepam particles just disappeared was taken as the saturation solubility of diazepam in the sample of thick emulsion or liquid crystal.

This procedure was rather tedious. Several preliminary experiments had to be performed to discover the range of diazepam concentrations to be used with each particular sodium cholate–lecithin–water ratio. Each of the experiments was repeated at least 3 times and reproducible results were obtained.

RESULTS

Effect of bile salts on the solubilization of diazepam. The solubilization curves at 30°C for diazepam in a number of bile salts of various concentrations are shown in Fig. 1. It is evident from each of these curves that the solubility of diazepam increases with an increase in the bile salt concentration and the curve attains linearity after a certain intial concentration of the bile salt. From the slopes of the linear portions of the curves, the solubilization capacities of bile salts were calculated. The saturation solubility of diazepam in water at 30°C as determined in our investigation, is 0.04 mg/ml. The solubilization capacities of various bile salts are given in Table I. These values, in relative order, are:

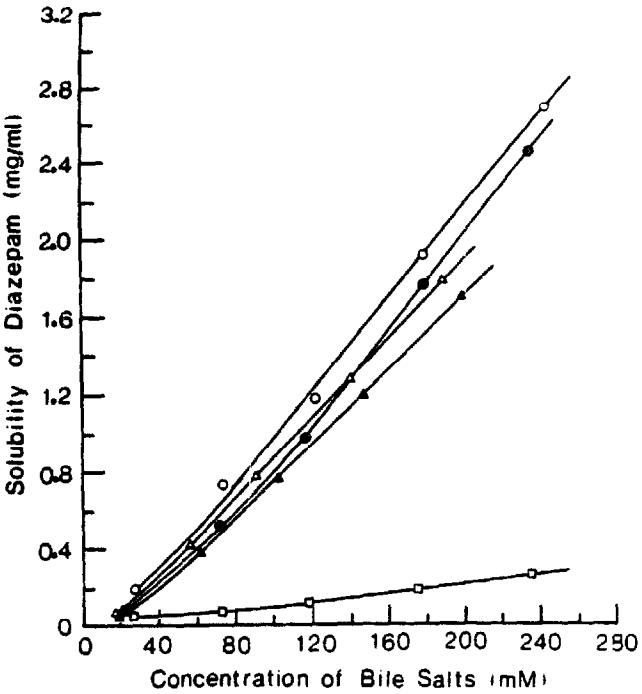


Fig. 1. Equilibrium solubility of diazepam in bile salt solutions at 30°C. ○, sodium deoxycholate; △, sodium taurocholate; ●, sodium cholate; ▲, sodium glycocholate; □, sodium dehydrocholate.

sodium cholate = sodium deoxycholate > sodium taurocholate > sodium glycocholate >> sodium dehydrocholate.

Solubility of diazepam in sodium cholate–lecithin mixed micellar solutions. The saturation solubilities of diazepam in different regions of the micellar phase (Phase IV) are shown in Fig. 2 and the corresponding regions are indicated in Fig. 2 (inset). Each curve in Fig. 2 represents the solubility of diazepam in the mixed micellar solutions with a fixed sodium cholate–lecithin ratio, only the proportion of water varies. The concentration of diazepam (per gram of solution) in the mixed micellar solution was plotted against the total millimoles of sodium cholate and lecithin present in a gram of the solution. It

TABLE I

SOLUBILIZATION CAPACITIES OF BILE SALT SOLUTIONS FOR DIAZEPAM AT 30°C

Bile salts	Solubilization capacities* (mg of diazepam/mol of bile salt)
Sodium cholate	1.25×10^4
Sodium deoxycholate	1.25×10^4
Sodium dehydrocholate	0.14×10^4
Sodium taurocholate	1.02×10^4
Sodium glycocholate	0.88×10^4

* Solubility of diazepam in water = 0.72 mg/mol.

can be observed that with the increase in concentration of sodium cholate and lecithin, the solubility of diazepam increases. The curves of higher lecithin–sodium cholate ratio (3 : 2, 1 : 1, 2 : 3 and 1 : 2) are almost linear, while the curves for lower lecithin–sodium cholate ratio (1 : 3, 1 : 5 and 1 : 10) show a decrease in the slopes as the concentration of sodium cholate plus lecithin increases.

It can also be observed from Fig. 2 that as the lecithin–sodium cholate molar ratio increases in the mixed micellar solutions (curves 2–8), the initial slopes of the curves increase, which implies that the solubilization capacity for a particular concentration of sodium cholate plus lecithin increases with an increase in the molar proportion of lecithin.

Curve 1 in Fig. 2 gives the equilibrium solubilization of diazepam by sodium cholate alone while curves 2–8 represent the solubilization in sodium cholate plus lecithin solutions. It is interesting to note that below the concentration of approximately 0.3 mmol/g, the slopes of curves 2–8 are higher than the slope of curve 1. At concentrations above 0.3 mmol/g, the slopes of the curves 2–6 become less than the slope of curve 1. Below the total surfactant concentration of 0.3 mmol/g, the presence of lecithin increases the

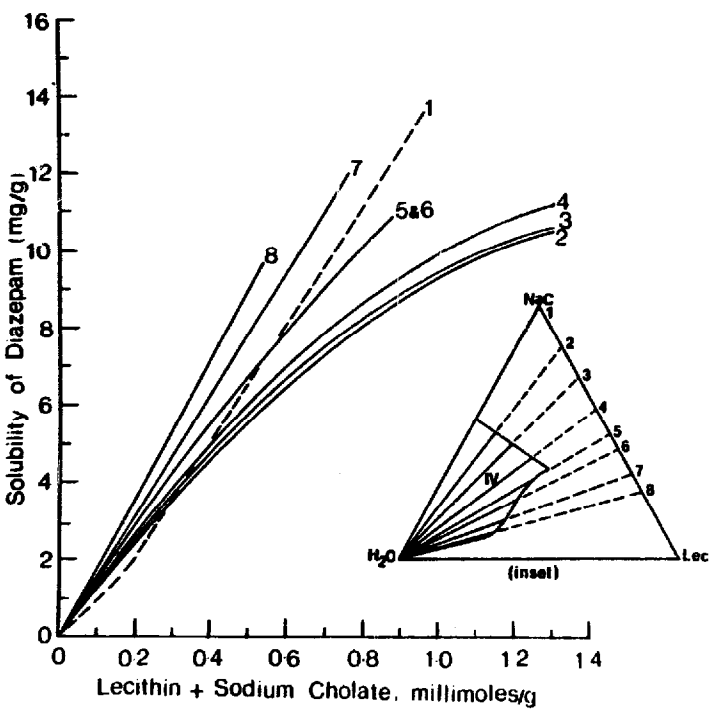


Fig. 2. Equilibrium solubility of diazepam in sodium cholate micellar solutions and in sodium cholate–lecithin mixed micellar solutions at 30°C. The solubility curves 1 through 8 correspond to the lecithin–sodium cholate molar ratios represented by solid portions of lines 1 through 8, respectively, in the phase diagram (Small et al., 1966) shown in the inset. The data points in the solubility curves are omitted for the sake of clarity. In the inset, line 1 (H_2O–NaC) represents sodium cholate alone in water while lines 2 though 8 represent lecithin–sodium cholate combinations in water. The proportions of lecithin and sodium cholate are as follows: line 2 = 1 : 10, line 3 = 1 : 5, line 4 = 1 : 3, line 5 = 1 : 2, line 6 = 2 : 3, line 7 = 1 : 1, line 8 = 3 : 2.

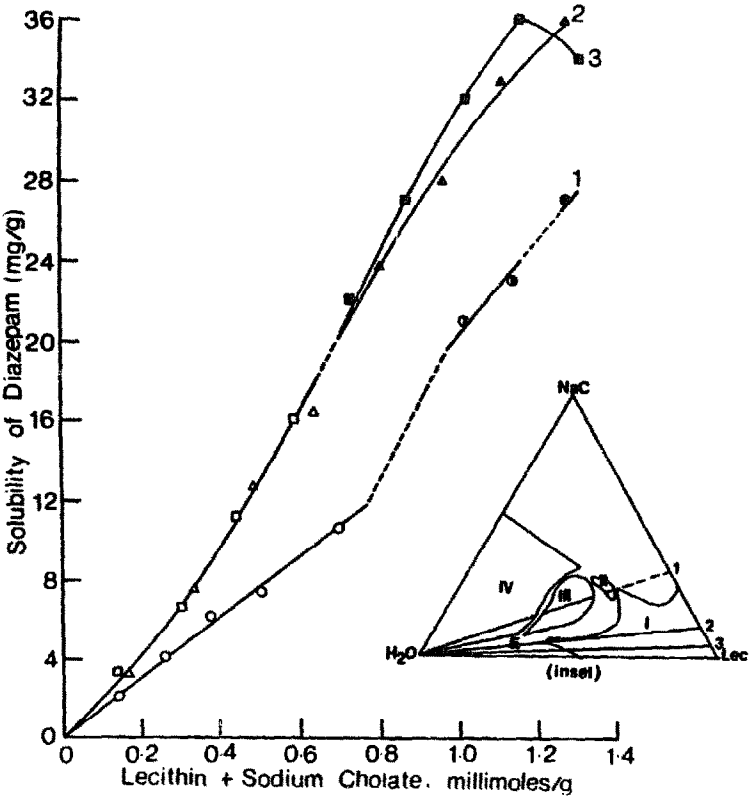


Fig. 3. Equilibrium solubility of diazepam in lecithin–sodium cholate mixed micellar solutions, liquid crystals and emulsions at 30°C. The solubility curves 1, 2 and 3 correspond to the lecithin–sodium cholate molar ratios represented by lines 1, 2 and 3 respectively, shown in the phase diagram (Small et al., 1966) in the inset. The ratios are: line 1 = 1 : 1, line 2 = 4 : 1, line 3 = 8 : 1. The micellar phase (IV), hexagonal (III), cubical (II) and lamellar (I) liquid crystalline phases and emulsion region (E) are also indicated in the inset. All these phases and regions are denoted in the solubility curves by the symbols: ○, phase IV; ◍, phase III; ●, phase II; △ and □, emulsion region; ▲ and ■, phase I. Dotted portions of the solubility curves indicate the regions of phase transition.

solubilization capacity, but at different ranges above this concentration, the solubilization capacities of the systems, represented by curves 2–6 in Fig. 2, are lower than the solubilization capacity of equimolar sodium cholate micelles. However, the solubilization capacities of the systems having lecithin–sodium cholate ratios of 1 : 1 or 3 : 2 (curves 7 and 8) are at any concentration higher than those of sodium cholate micelles.

Solubility of diazepam in bile salt–lecithin–water emulsions and liquid crystals. Fig. 3 shows the solubility of diazepam in phases I–IV and in emulsion regions of the sodium cholate–lecithin–water phase diagram and Fig. 3 (inset) denotes the regions corresponding to the solubility curves. In curve 1 of Fig. 3 the initial linear portion corresponds to curve 7 of Fig. 2. It can be seen that the solubilization of the emulsion over the same concentration range of lecithin–bile salt is greater than that of micellar phase IV. As one proceeds from phase IV to phase III (hexogonal liquid crystalline phase) there is a sharp jump in solubilization. The solubility change in the transition from phase III to phase II

(cubical phase), however, appears to be continuous with no change in slope. Curves 2 and 3 indicate that the solubility of diazepam gradually increases in going from the emulsion region to the lamellar liquid crystalline phase I. Among all the phases, phase I exhibits the maximum solubilization.

DISCUSSION

The common bile salts are steroids possessing a non-polar rigid cyclopentanophenanthrene nucleus, on one side of which there are two or 3 hydroxyl groups. Protruding from one end of the steroid nucleus is a short branched aliphatic chain terminating in a strong hydrophilic group (Carey and Small, 1970). The molecules aggregate to form small micelles which are held together by hydrophobic bonding between the hydrophobic surfaces while the hydrophilic sides and polar end groups thrust outward into the aqueous phase. Sodium cholate and sodium deoxycholate are, respectively, trihydroxy and dihydroxy bile salts. Table I shows that the molar solubilization capacities and the micellar–aqueous partition coefficients of these two types of bile salts are identical. Unconjugated bile salts are not normally present in human bile. Conjugation lowers the pK_a values of the unconjugated bile salts ($pK_a \approx 6$); the pK_a of glycine and taurine conjugates, for example, are approximately 4 and 2, respectively. This lowering of pK_a values enables the salts to remain soluble in the slightly acidic pH of the duodenum. Despite the great importance of the conjugation process for the proper physiological functioning of bile salts, Table I shows that conjugation lowers the solubilization capacity of bile salts relative to that of the unconjugated species.

Shankland (1970) observed that with an increase in the lecithin–sodium cholate ratio, the size of the mixed micelle increased gradually from the lowest molecular weight of 6×10^3, to the highest molecular weight of 150×10^3. Fig. 2 shows that with an increase in the molar ratio of lecithin, the solubility of diazepam also increased. Consequently, there appears to be a correlation between the size and the solubilization capacity of the mixed micelle. A disc-shaped bimolecular leaflet of lecithin molecules forms the core of the mixed micelles (Carey and Small, 1970; Small 1970). The hydrocarbon sides of the bile salt molecules associate hydrophobically with the alkyl chains of the lecithin disc. Bile salt molecules, however, may also be present in the core (Mazer et al., 1976, 1977) in the same molar ratio as that of the phase limit. For low values of the lecithin–sodium cholate ratio, the micellar volume will be smaller and the solubilization capacity for diazepam less than that for the higher ratios.

It was observed earlier from Fig. 2 that, at higher concentrations of lecithin plus sodium cholate (>0.3 mmol/g), addition of lecithin in lower proportion decreased the solubilization capacity of the system compared to the solubilization capacity of sodium cholate alone. This may be due to the presence in the system of simple bile salt micelles and mixed micelles of small size. With increasing lecithin–sodium cholate ratios the mixed micelles grow in size, particularly as the phase limit is approached and the solubilization capacity is enhanced.

Comparison of the initial parts of the solubilization curves in Fig. 2 (sodium cholate + lecithin < 0.3 mmol/g) shows that the net solubilization capacity of lecithin plus sodium cholate at any ratio between them is higher than that of a comparable amount of sodium

cholate alone. This is due to the fact that the CMC of mixed micellar solutions are lower than that of the sodium cholate solution. Thus, at the physiological ranges of concentrations (<40 mM), the net effect of sodium cholate–lecithin mixed micelle formation is always an increase in the solubility of water-insoluble substances.

The continuity and linearity of curves 2 and 3 in Fig. 3 from the region of thick emulsions to the region of lamellar liquid crystalline phase implies that there is no intrinsic difference in the mode of solubilization within these phases. This can be understood from the phase diagram where the emulsion bordering the phase I region contains liquid crystalline phase. The curvature in the dilute emulsion region may be due to the presence of mixed micelles with lower solubilization capacity.

The discontinuity in the solubilization curve between phase IV and phase III suggests a different mechanism for solubilization because of the differences in structure of the sodium cholate–lecithin complexes in the two phases. The absence of a discontinuity in the solubilization through the transition phase III to phase II, on the other hand, implies a similarity in the mode of solubilization.

REFERENCES

Bates, T.R., Gibaldi, M. and Kanig, J.L., Solubilizing properties of bile salt solutions I. Effect of temperature and bile salt concentration on solubilization of glutethimide, griseofulvin and hexestrol J. Pharm. Sci., 55 (1966a) 191–199.

Bates, T.R., Gibaldi, M. and Kanig, J.L., Rate of dissolution of griseofulvin and hexestrol in bile salt solutions. Nature (London), 210 (1966b) 1331–1333.

Bourgés, M.C., Small, D.M. and Dervichian, D.G., Biophysics of lipid associations. III. The quarternary systems lecithin–bile salt–cholesterol–water. Biochim. Biophys. Acta, 144 (1967) 189–201.

Carey, M.C. and Small, D.M., The characteristics of mixed micellar solutions with particular reference to bile. Amer. J. Med., 49 (1970) 590–608.

Dam, H., Kruse, I., Kallehauge, H.E., Hartkopp, O.E. and Jensen, M.K., Human Bile. I. Composition of bladder bile from cholelithiasis patients and surgical patients with normal bile compared with data for bladder bile of hamsters on different diets. Scand. J. Clin. Lab. Invest., 18 (1966) 385–404.

Ekwall, P. and Sjöblom, L., Solubilization of steroid hormones by association colloids. Acta. Chem. Scand., 3 (1949) 1179–1180.

Ekwall, P. and Sjöblom, L., Aqueous solutions of steroid hormones. Acta Endocrinol., 4 (1950) 179–191.

Ekwall, P., Lundsten, T. and Sjöblom, L., Polarographic determination of ketosteroids solubilized in aqueous solution of associated colloids. Acta Chem. Scand., 5 (1951) 1383–1392.

Marsh, M. and Holzbach, R.T., Chromatographic separation of pure phosphatidylcholine from crude egg lecithin. Clin. Chim. Acta, 43 (1973) 87–90.

Mazer, N.A., Benedek, G.B. and Carey, M.C., The size and shape of bile salt (BS), BS–lecithin (L) and BS–L–Cholesterol (Ch) micelles using quasielastic light scattering spectroscopy. Gastroenterology, 70 (1976) 998.

Mazer, N.A., Kwasnick, R.F. and Carey, M.C., Quasielastic light scattering spectroscopic studies of aqueous bile salt, bile salt–lecithin and bile salt–lecithin–cholesterol solutions. In Mittal, K.L. (Ed.), Micellization, Solubilization and Microemulsions, Vol. 1, Plenum, New York, 1977, pp 383–402.

Neiderhiser, D.H. and Roth, H.P., Cholesterol solubilization by solutions of bile salts and bile salts plus lecithin. Proc. Soc. Exp. Biol. Med., 128 (1968) 221–225.

Shankland, W., The equilibrium and structure of lecithin–cholate mixed micelles. Chem. Phys. Lipids, 4 (1970) 109–130.

Singleton, W.S., Gray, M.S., Brown, M.L. and White, J.L., Chromatographically homogeneous lecithin from egg phospholipid. J. Amer. Oil Chemists Soc., 42 (1965) 53–56.

Small, D.M., The physical chemistry of cholanoic acids. In Nair, P.P. and Kritchevsky, D. (Eds.), The Bile Salts, Vol. 1, Plenum, New York, 1970, pp. 249–356.
Small, D.M., Bourgés, M.C. and Dervichian, D.G., The biophysics of lipid associations. The ternary systems lecithin–bile salt–water. Biochim. Biophys. Acta, 125 (1966) 563–580.
Vaskovsky, V.E. and Kostetsky, E.Y., Modified spray for the detection of phospholipids in thin-layer chromatograms. J. Lipid Res., 9 (1968) 396.
Verzar, F., The absorption of fats. Nutr. Abstr. Rev., 2 (1933) 441–450.