Kinetics and thermodynamics of the formation of mixed micelles of egg phosphatidylcholine and bile salts

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Abstract The kinetics of dissolution of dispersions of egg phosphatidylcholine (lecithin) by bile salts was studied by observing the decrease in turbidity as mixed micelles of lecithin and bile salt were formed. The rate of dissolution of lecithin corresponding to the formation of mixed micelles was studied in the presence of dihydroxy bile salts, sodium deoxycholate, sodium chenodeoxycholate, sodium ursodeoxycholate, and one trihydroxy bile salt, sodium cholate. The rate of dissolution of lecithin and mixed micelle formation decreased in the order: chenodeoxycholate > deoxycholate > cholate > ursodeoxycholate. Kinetic solvent isotope studies in D2O, along with measurement of enthalpies of mixed micelle formation both in H2O and D2O, appear to indicate that the formation and stabilization of mixed micelles is related to "hydrophobicity" as estimated by high performance liquid chromatography retention factors.—Rajagopalan, N., and S. Lindenbaum. Kinetics and thermodynamics of the formation of mixed micelles of egg phosphatidylcholine and bile salts. J. Lipid Res. 1984. 25: 135-147.

Supplementary key words lecithin • solvent isotope effect

It is well known that bile salts are essential for the dissolution of cholesterol and for its maintenance in the solubilized state in the gallbladder (1). Whereas bile salts are required for the solubilization of cholesterol, bile salts alone in aqueous solution increase cholesterol solubility only slightly, 30–100 bile salt molecules being required to solubilize one cholesterol molecule (2). It is well established (3) that phosphatidylcholine (lecithin) is an essential component of bile and that the combination of bile salts with lecithin is required to solubilize cholesterol and to provide an effective detergent system for lipid solubilization and dispersion. The addition of lecithin to bile salt solutions dramatically increases the amount of cholesterol solubilized; approximately 1 mmol of cholesterol is solubilized for every 3 mmol s of lecithin added (4).

On the basis of phase equilibrium and X-ray diffraction studies, a generally accepted model for the bile salt-lecithin mixed micelle was proposed by Small and Dervichian (2, 5, 6). According to this model, a disc-shaped micelle is formed on the association of lecithin with bile salt. The disc core consists of the hydrocarbon alkyl chains of the lecithin molecules surrounded by a ring of bile salt molecules. Thus, the disc-shaped micelle exterior presents to the aqueous solvent only the hydrophilic end groups of the lecithin and the hydrophilic sides of the bile salt.

Whereas it is generally agreed that mixed micelles occur in these solutions, there remains considerable controversy concerning their detailed structure. Mazer and coworkers (7) have suggested that their quasielastic light-scattering data (QLS) can best be interpreted in terms of a mixed disc model. This model preserves the disc-shaped mixed micelle of Small and Dervichian, but also requires the presence of some bile salt molecules within the interior of the micelle. These bile salt molecules within the micelle interior are presumed to exist as hydrogen-bonded dimers. Calorimetrically derived exothermic heats of mixed micelle formation have also been interpreted to support this model (8). Recently, Müller (9) has proposed another modification to the mixed micelle model. On the basis of X-ray small-angle scattering data, Müller suggests that structural dimorphism exists in bile salt-lecithin mixed micellar solutions with different types of micelles. This model supports the mixed disc model of Mazer, Benedek, and Carey (10) for solutions of bile salt-lecithin ratios smaller than 1.8:1. Similar conclusions were reached by Mazer et al. in recent publications (10, 11). At higher ratios, another structure inconsistent with the mixed disc model is inferred from the X-ray data. For micelles richer in bile salt than 2:1, an isometrical particle of globular shape having a centrosymmetric arrangement is consistent

Abbreviations: CDC, chenodeoxycholate; DC, deoxycholate; C, cholate; UDC, ursodeoxycholate; TLC, thin-layer chromatography; CMC, critical micellar concentration; HPLC, high performance liquid chromatography.

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with the X-ray data and is also supported by the differential scanning calorimetry data of Claffey and Holzbach (12).

The purpose of this study was to attempt to shed further light on the process of mixed micelle formation by measuring the rate of dissolution of lecithin in bile salt solutions.

MATERIALS AND METHODS

The bile salts used in this study were of the highest purity available commercially (13). The purity of sodium deoxycholate (Calbiochem) was confirmed by TLC and titration with perchloric acid in glacial acetic acid. The purity was found to be better than 99%. Cholic acid was purchased as the free acid (Baker and Calbiochem). The sodium salt was prepared by neutralizing the acid dissolved in 95% ethanol with a stoichiometric amount of aqueous sodium hydroxide solution. The solution was concentrated under vacuum and the salt was recrystallized from an ethanol-ethyl acetate mixture. Purity (>99%) was confirmed by TLC and nonaqueous titration. Chenodeoxycholic acid was obtained from Sigma Chemical Co. The sodium salt was prepared as described for sodium cholate; the purity was confirmed by TLC and nonaqueous titration (98% pure). Sodium ursodeoxycholate was prepared in the same way from ursodeoxycholic acid (Tokyo Tanabe Company, Ltd., Japan). Purity determination gave a single spot on TLC, and nonaqueous titration yielded 99% purity.

L-α Phosphatidylcholine (lecithin) was prepared from fresh eggs in our laboratory by the method of Singleton et al. (14). Purification of the lecithin was carried out chromatographically on an alumina column. The final purified sample showed a single spot on TLC after visualization with iodine vapor. Deuterium oxide (99.7 atom % D) was obtained from Bio-Rad and was used as received. Water was doubly deionized and degassed before use. All other chemicals were reagent grade. All studies were carried out in 0.02 M Tris (Tromethamine) buffer, pH 9.0.

The kinetics of mixed micelle formation was followed by studying the time-dependent decrease in turbidities (optical density at 600 nm, Cary 18 Double Beam Spectrophotometer) of a dispersion of lecithin (L) after addition of a given bile salt (BS) solution, as described by Lichtenberg et al. (15). In the course of the reaction multilamellar L interacts with BS to form mixed micelles M

\[ L + BS \rightleftharpoons M. \]

The turbidity of mixed micellar solutions at 600 nm was not detectable with the instrumentation used in the present study. The equilibrium optical density of all mixed micellar solutions thus returned to zero provided there was enough bile salt present to solubilize all the lecithin present. Hence the time-dependent decrease in optical density at 600 nm which reflects reduction in size of the lecithin aggregates can serve to follow the kinetics of the solubilization process. A typical trace of the time-dependent change in turbidity (optical density at 600 nm) of the lecithin dispersion after addition of the bile salt solution for two of the bile salts studied is shown in Fig. 1.

Four different bile salts CDC, DC, C, and UDC were chosen to study the comparative kinetics of solubilization of lecithin by the different bile salts. The lecithin dispersion was prepared by gently mixing the dry lipid with buffer solution, followed by sonication for 10 min in a bath sonicator (Branson, 80 watts). The sonication bath was cooled to 20°C. All dispersions after preparation were hydrated in the refrigerator for 16–18 hr prior to use and were used within 12 hr after hydration. Preliminary observations indicated that, on storage over a period of time (2–3 days), the particle size of these dispersions changed to some extent, as evidenced by changes in turbidity. Hence, it was decided to use all dispersions within 12 hr after the initial hydration period. It has been demonstrated that hydration equilibrium is obtained within 12 hr (16).

It was assumed for this work that careful control of the conditions used in the preparation of these dispersions (i.e., sonication and hydration) would lead to reproducible particle size distribution. The validity of this assumption was confirmed by the good reproducibility observed in the kinetic data when carried out with different batches of the lecithin dispersion (Fig. 2). It was also shown that no appreciable changes in turbidity occurred in these dispersions when allowed to stand for 150 min, suggesting that the particle size distribution remained stable for at least this long.

In order to standardize the turbidimetric method, six batches of the lecithin dispersion were prepared under carefully controlled conditions for construction of the calibration curve in the range 0–1.5 mM (Fig. 3). The best polynomial fit of the experimental data (solid line in Fig. 2) was used as the standard calibration curve.

\[ \text{The abscissa values in Fig. 2 refer to solutions prepared by dilution of a stock dispersion. It was pointed out, correctly, by a reviewer that in a heterogeneous system with particles of different sizes, absorbance values may not be simply related to total lecithin concentration. However, this assumption does not alter the kinetic order among the bile salts. Since careful control of the particle size distribution was maintained, any error arising out of this assumption would uniformly apply to all the bile salts and yield the same kinetic order. Further evidence that this is not a serious problem over most of the concentration range used in this study is found in the near linear behavior of Fig. 2 in the 0-1A region used for most of the calculations.} \]
In the first part of the study the concentration dependence of the bile acid on the kinetics of solubilization of lecithin was studied by varying the concentration of the bile acid from the premicellar to the postmicellar region. The concentration of lecithin in the dispersion in all these studies was maintained constant at 1.5 mM. In a typical experiment, 2 ml of a 1.5 mM dispersion of egg lecithin was mixed with 2 ml of an appropriate bile salt solution of a given concentration, and the optical density at 600 nm was monitored as a function of time.

Kinetic studies were also carried out in both pure D$_2$O and in a mixture of D$_2$O–H$_2$O (1:1) in order to determine

\[ \text{Absorbance at } 600 \text{ nm} \]

\[ \text{time (min)} \]

Fig. 1. Absorbance versus time to demonstrate the reproducibility of the kinetic data for the bile salts DC ($\Delta$, A) and CDC (O, •) with two separate experiments (open and closed symbols) in each case.

Fig. 2. Plot of absorbance versus the concentration of lecithin dispersion. The different symbols represent different preparations of lecithin dispersion. The solid line represents the best polynomial fit to the data.\(^5\)
the effect of change in isotopic solvent medium on the kinetics of mixed micelle formation.

The extent of counterion (Na⁺) binding to the mixed micelle was determined using a sodium ion selective electrode. The experimental procedure used was essentially the same as that described previously for the study of sodium ion binding to bile salt micelles (17). Briefly, a sodium ion electrode (Orion Research, Inc.) was used to monitor the change in EMF as a standard bile salt solution was titrated into a 5 mM lecithin dispersion. The solution was stirred and the temperature was maintained at 25°C during the course of the titration.

ANALYSIS OF THE KINETIC DATA

The time-dependent decrease in the concentration of lecithin undissolved in the dispersion did not fit a single exponential equation. This apparently was due to the existence of heterogeneity in particle size of the dispersion used. Takeguchi et al. (18) in their studies on the kinetics of amino acid permeability of liposome dispersions also observed multieponential kinetic data which they have attributed to the polydisperse nature of the liposome dispersion used. Also, the rate of clearance of drugs from blood encapsulated in liposomes has been shown to be predominantly controlled by the liposome size. Juliano and Stamp (19) have demonstrated that the biphasic nature of clearance observed for drugs entrapped in liposomes reflects liposome size heterogeneity. Thus, they demonstrated that when a homogeneous population of liposome containing the drug was injected into the rat, the rate of plasma clearance followed a simple exponential decay suggesting a single rate and mechanism of clearance.

In preliminary studies it was observed that, in the time range of the experiment, a sum of two exponential terms could simulate the data in most cases. The simulation would have been justified if the dispersion had been composed of two representative components, one component with a larger particle size distribution and the other with the smaller. The actual size distribution of the lecithin dispersion was, however, very broad as observed under the electron microscope. Hence, the data would be more accurately represented with a sum of a large number of exponential terms. Instead of using a sum of exponential terms, we have used a cumulative expansion for the polydisperse system as proposed by Koppel (20) and defined by Equation 1 which

\[ Y = A e^{-kt} \left( 1 + \frac{\mu_{2w} t^2}{2} \right) \]

is applicable to any experiment which yields a sum of, or distribution of, exponentials. Y in this case is the concentration of the undissolved lecithin in the dispersion; \( \mu_{2w} \), the second expansion coefficient; k, the cumulative rate constant for the solubilization process; and t, the time. The value of \( \mu_{2w}/k^2 \) is the polydispersity index for the rate constant. A value of zero for the polydispersity
index denotes a monodisperse system. The constants A, \( \mu_{gw} \), and k were obtained by a non-linear regression analysis of the kinetic data.

Whereas it is evident that the cumulative rate constant k does not refer to the rate of a single reaction, it does provide a single constant for the comparison of kinetic data obtained with different bile salts. This method is therefore particularly well suited to the present study. An example of the use of the method of cumulants for one set of kinetic data is shown in Fig. 3.

RESULTS AND DISCUSSION

The kinetics of solubilization of egg lecithin by bile salts was observed to be strongly dependent on the type of bile salt used, and its concentration (Figs. 4–7). In the premicellar concentration region the kinetics of solubilization were slow and solubilization was incomplete. The reported critical micellar concentrations (CMC) of the four bile salts CDC, DC, C, and UDC as determined using a bile acid anion selective electrode are 6.0, 6.1, 16.0, and 23.0 mM, respectively (21). In the first 3 min of the kinetic runs, the rates of solubilization were rapid. This is probably due to a burst effect generated by the smaller particles in the dispersion (probably unilamellar vesicles) dissolving almost instantly. Due to the problems encountered in curve-fitting the data in this initial time interval, all analysis of the data was carried out from 3 min onward only.

The dependence of the rate of dissolution of lecithin on the concentration of ursodeoxycholate does not follow the same regular sequence as the other bile salts. This difference is reproducible and was observed in repeated trials. A possible explanation for the maximum in the dissolution rate at approximately 25 mM may be due to the behavior of UDC as a normal 1:1 electrolyte at low concentration. UDC is a relatively poor surfactant and tends to self-associate to a lesser extent. The fully ionized UDC will therefore tend to salt out the lecithin.

The effect of the structure of the bile salt on the observed rate constant for solubilization is dramatic (Fig. 8). UDC, which differs structurally from CDC only in the configuration of the hydroxyl group at C7, reveals a strikingly different behavior in its rates of solubilization compared to CDC. As observed earlier, the rate constant for solubilization was low for all the bile salts studied in the premicellar region and rose sharply beyond the CMC. In the concentration range of this study the following order in kinetics (decreasing value of k) prevails: CDC > DC > C > UDC.

The enthalpy of mixed micelle formation between bile salts and lecithin has been reported previously (8). It is interesting to note that the values of the observed exothermic heats of mixed micelle formation decrease in the same order as the observed rates of dissolution of lecithin in the kinetic study, i.e., CDC > DC > C > UDC. With the bile salt, UDC, no measurable heats of mixed micelle formation were observed, and in nearly all cases the solubilization was incomplete in the concentration range.
studied. At every ratio of CDC to lecithin, CDC evolved more heat than any other bile salt. Thus, the position of hydroxyl groups at C3 and C7 as in CDC appears to be particularly favorable for enhancing the rate of mixed micelle formation and making the reaction more favorable energetically. Changing the position of one of the hydroxyl groups from C7 to C12 (as in DC which is 3α,12α-dihydroxy-) diminishes this ability, as does changing the
configuration of the hydroxyl group at C7 from $\alpha$ to $\beta$ in UDC. It would seem reasonable to expect that the rates of solubilization of lecithin with bile salts would correlate approximately with the hydrophobicity of the bile salt. The more hydrophobic bile salt would be less hydrated and therefore would be able to penetrate the lipid and effect its dissolution by mixed micelle formation.

The retention of bile salts by reverse phase high performance liquid chromatography (HPLC) has been used as a measure of relative hydrophobicity (22). According
to this scheme, the order of decreasing hydrophobicity is DC > CDC > C > UDC. Except for the interchange of the order for DC and CDC, this is the order found for the relative rates of effecting dissolution of lecithin. Whereas hydrophobicity as measured by HPLC retention may correlate well with many properties, the formation of a mixed micelle appears to depend also on the position and orientation of hydrophilic groups. In this case, other properties may well overshadow hydrophobicity in determining the ability of a bile salt to form a mixed micelle with lecithin.

Electrostatic effects are also expected to play an important role in effecting the rate of formation and stability of mixed micelles. The negative charge on a simple bile salt micelle or mixed bile salt-lecithin micelle would prevent further association. For this reason, it was of interest to examine the extent to which counterions (Na⁺) were bound to the micelle. Previous studies have provided data on counterion binding to simple bile salt micelles as observed with a Na⁺-specific electrode (17). Counterion binding to mixed micelles as inferred from Na⁺ ion-specific electrode measurements is shown in Figs. 9 and 10. Data for the EMF as a function of concentration for simple bile salt micelles are also included in the graphs to show the dramatic increase in the extent of counterion binding by the mixed micelles in the presence of added lecithin. In the premicellar region for both the simple and mixed micelles, near Nernstian slopes were observed ranging from 60.5 to 61.8 mV for a tenfold change in concentration. The observed break in the EMF vs concentration curves may be assumed to represent concentrations at which micelles first begin to form with consequent binding of counterions.

As can be observed in Figs. 9 and 10, the extent of counterion binding is highly concentration-dependent. It was observed that in the concentration range studied, CDC bound significantly more counterions than DC. When the binding studies were carried out with UDC, no measurable binding of the counterion to the mixed micelle was observed, while with C a small extent of binding was observed which was significantly lower than that observed with DC and CDC. Hence qualitatively from this study, the following order in counterion binding was deduced: CDC > DC > C > UDC. Thus if one presumes, as proposed by others (23, 24), that the enthalpy of mixed micelle formation primarily arises out of counterion binding to the mixed micelle, then the observed order in enthalpy measured correlates with the counterion binding data. A relationship between the observed order in the kinetic data and the fractions of counterions bound to the bile salt micelle is to be expected also. A higher fraction of counterion bound could progressively shield the net negative charge on the bile salt micelles, promoting access of the micelles from the bulk to the lecithin-bile salt solution interface. The faster rates of dissolution observed for cholesterol in bile salt micellar solutions with increasing counterion concentration has also been attributed to charge-shielding effects (25, 26). Thus, to be able to correlate the kinetic data with the fraction of counterions bound to the bile salt micelles, the following

![Graph showing EMF vs log molality](image-url)

**Fig. 9.** Plot of EMF vs log molality of sodium chenodeoxycholate (O) without lecithin; and (∆) in the presence of lecithin.
order in binding must prevail CDC > DC > C > UDC. As reported earlier (17) the observed counterion binding order was CDC = DC > C > UDC. Within the experimental uncertainty of the counterion binding measurements we are unable to distinguish between the extent of counterion binding by DC and CDC.

It appears from these studies that the enthalpy and kinetics of mixed micelle formation are related to the hydrophobicity of the bile salt as measured by the HPLC retention factor, the extent of counterion bound by mixed micelles, as well as the position and orientation of the hydroxyl groups on the bile acid micelles. As bile salts micellize by hydrophobic forces, it is evident that the more hydrophobic bile salt will form larger micelles and consequently bind a larger fraction of counterions.

Most of the measurements of this study were carried out at bile salt-lecithin molar ratios greater than 2:1. The change from the mixed disc to the centrosymmetric micelle structure was therefore not observable (9, 12).

Increasing the concentration of counterion in a micellar solution is known to decrease the surface charge on the micelle (2, 25, 26). Accordingly, it would be expected that an increase in counterion concentration would facilitate the interaction between micelle-forming species since mutual electrostatic repulsion would be diminished. Therefore, the rate of lecithin solubilization by bile salt should be enhanced in the presence of excess NaCl. This prediction is borne out as seen from the data in Fig. 11 which shows the remarkable increase in the rate of lecithin dissolution on the addition of 50 mM NaCl.

It has been suggested that bile salts bind to protein via electrostatic interaction of the anionic acid group with the positively charged amino acid residues (27). If charge interactions between the bile salt and zwitterionic lecithin are important, then increasing the ionic strength should lessen these effects.

Previous measurements of the enthalpy of mixed micelle formation (8) with sodium cholate show that the effect of adding sodium chloride is almost negligible within experimental error. However, with dihydroxy bile salts such as DC and CDC whose aggregation number is enhanced in the presence of added NaCl, the enthalpy of mixed micelle formation is also more negative at low system ratios (Fig. 12). This would suggest that the exothermic heat of interaction is due to the enhancement of counterion binding as a larger micelle is formed. It was suggested earlier (8) that the enthalpy of mixed micelle formation was due to the hydrogen-bonded association of bile salts within the mixed micelle interior. The present results, however, would suggest that, at least in part, this exothermic heat is due to enhanced counterion association. The charge neutralization brought about by counterion binding thereby promotes hydrophobic association of bile salt and lecithin to form mixed micelles.

Solvent isotope effects (studies in which the solvent H2O is replaced with D2O) have been used to investigate hydrophobic bonding in other systems. Lee and Berns (28) have suggested that the tendency of deuterium oxide to increase the state of aggregation of certain proteins is due to enhanced hydrophobic interactions in heavy water.
In the case of oligomeric proteins, this increased stability in D_2O has been attributed to the capacity of the solvent to prevent the native multimeric form from dissociating into subunits (29). Berns, Lee and Scott (30) in their studies on the aggregation of phyocyanin observed an increased association in D_2O which they attributed to the enhanced hydrophobic bonding in D_2O versus H_2O. A frequently cited argument for the enhancement of hydrophobic forces in D_2O is based on the more structured nature of D_2O (31). The term “hydrophobic forces” refers to the favorable entropic effect of squeezing together apolar molecules. With a more ordered structure, a greater ΔS contribution is expected.

However, the use of D_2O as a method of testing for the relative importance of hydrophobic bonding has been questioned by some workers. Baghurst, Nichol, and Saw-

**Fig. 11.** Plot showing the effect of added sodium chloride on the kinetics of lecithin solubilization: O, no NaCl; ●, 50 mM NaCl.

**Fig. 12.** Dependence of the enthalpy of mixed micelle formation on the mole ratio of bile salt to lecithin. O, No NaCl; ●, 150 mM NaCl.
yer (32) in their studies on the association of β-lactoglobulin A have shown that the enhanced aggregation observed in D₂O, which is accompanied by a large enthalpy change, is primarily due to the hydrogen-deuterium exchange affecting the strength of hydrogen (deuterium) intermolecular bonds and much less due to hydrophobic bonding. From a computation of the thermodynamic parameters for the association process (in H₂O ΔH = −64 Kcal/mol, ΔS = −182 eu and in D₂O ΔH = −69 Kcal/mol, ΔS = −188 eu) they have shown that the association process is enthalpy-driven in both solvents and is opposed by a large unfavorable entropy change which does not exhibit any significant isotope effect. The polymerization of flagellin (33) which is also enhanced in D₂O has been shown to be an enthalpy-driven process opposed by a large unfavorable entropic contribution.

The enthalpy of mixed micelle formation in the isotopic solvent D₂O has been measured in our laboratory (8) with the finding that the enthalpy of mixed micelle formation is identical in both the isotopic solvents, indicating that little or no enthalpy change occurs when the reaction medium is changed from H₂O to D₂O. A large part of the observed negative enthalpy of mixed micelle formation in both solvents is probably due to the process of counterion binding to the mixed micelle.

As indicated in Fig. 13, the rate of mixed micelle formation is enhanced in D₂O, the rate increasing as the mole fraction of D₂O is increased. The rate constants in D₂O are 5.2- and 3.6-fold greater than in H₂O for DC and CDC, respectively.

In this work the solvent isotope effect studies have been carried out only for the rate constant and not for the equilibrium constant. However, based on the rate enhancement in D₂O and the fact that the CMC of ionic micelles is lower in D₂O (31), it appears reasonable to assume that the equilibrium constant for mixed micelle formation should also be enhanced in D₂O. Since the enthalpy for the reaction is unchanged in going from H₂O to D₂O, the reaction must exhibit a larger positive entropy change in D₂O. Consequently, these results suggest that entropy effects, i.e., hydrophobic forces, are sufficient to stabilize the mixed micellar structure.

We have established in these studies that the kinetics of solubilization of lecithin is a strong function of the bile salt structure, its concentration, and electrolyte concentration. The rank ordering of solubilization kinetics which is CDC > DC > C > UDC is the same rank order that has been observed for the dissolution kinetics of cholesterol monohydrate in bile salt micellar solutions (25). This would suggest that the same combination of properties, i.e., hydrophobic interaction and counterion binding, which optimize the rate of formation of bile salt-lecithin micelles, is also required for enhancing the equilibrium solubility of cholesterol in this mixed micellar system.

CONCLUSIONS AND PHYSIOLOGICAL IMPLICATIONS

Investigation of the dissolution of both cholesterol monohydrate and human gallstones by both bile salts and
bile salt-lecithin mixed media by Higuchi and co-workers (34–36) has shown that the dissolution is interfacially controlled. It was observed in these studies that the addition of lecithin to the dissolution media containing the bile salt reduced the dissolution rates even though it increased the equilibrium solubility of cholesterol monohydrate in the dissolution media (35). Hoelgaard and Frøkjaer of lecithin to the dissolution media containing the bile and so determine the rate and extent of the gallstone controlled. It was observed in these studies that the addition in their studies have shown that the decrease in dissolution rates observed in the presence of added lecithin is primarily due to the absorption of lecithin on the surface of cholesterol crystals. They have proposed that this layer of adsorbed lecithin may act as an effective interfacial barrier leading to lower rates of cholesterol dissolution. This interfacial barrier is believed to be clinically important in gallstone dissolution (34). Thus the interaction between bile salt and lecithin may be of fundamental physiological importance, as the strength of this interaction may govern the extent of the interfacial barrier and so determine the rate and extent of the gallstone dissolution in vivo. These results provide further support for the conclusion that CDC is most effective in the dissolution of gallstones because it is most effective in solubilizing the lecithin adsorbed on the cholesterol surface.

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References


