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COMMUNICATIONS

Unusual Cholesterol Solubility in Water/Glyceryl-1-monooctanoate Solutions

Keyphrases □ Cholesterol—unusual solubility in water/glyceryl-1-monooctanoate solutions □ Glyceryl-1-monooctanoate—aqueous solutions, unusual solubilities of cholesterol □ Gallstones—cholesterol, solubility in water/glyceryl-1-monooctanoate solutions

To the Editor:

Glyceryl-1-monooctanoate (monooctanoin) (I) has been recently used in humans for dissolution of cholesterol gallstones in the common bile duct (1, 2). The solvent is slowly infused into the bile duct for several days, usually *via* a T-tube left in place following cholecystectomy. The high cholesterol solubility in I, 11.7% (w/v) at 37°, was reported in a systematic study of cholesterol solubility in organic solvents by Flynn *et al.* (3). Optimum cholesterol solubility appeared to occur when the solvent (*n*-alkanols or fatty acid ethyl esters) had a total carbon chain length of about seven atoms.

The present study was initiated to determine if cholesterol was involved in formation of liquid crystalline phases in aqueous I solutions. Larsson found that highly purified I and water formed a lamellar liquid crystalline phase at 37° when the water content was between 8 and 45% (4). Such equilibria could be important in gallstone dissolution since I would be in contact with bile during the infusion procedure and with moisture during handling. But when water/I mixtures were prepared with the same type of I used in the reported gallstone dissolution studies[1], only isotropic phases were observed by polarizing microscopy[2]. This apparent discrepancy is thought to be caused by the presence of about 30% of the corresponding diglyceride in the commercial material (1, 5). Diglycerides or triglycerides are more hydrophobic and do not form lyotropic mesophases (4). The solubility of water in the commercial sample of I was determined visually to be ~18–20% (w/w) at 37°. An exact value is not meaningful, since each batch will vary somewhat in its fatty acid distribution and diglyceride content. Above this concentration simple emulsions were formed rather than liquid crystalline phases.

Cholesterol is known to crystallize in anhydrous and monohydrate forms (6) and the anhydrous form is ~50% more soluble in aqueous bile salt solutions (7). Since either of these crystalline forms could possibly exist in aqueous I solutions, the cholesterol solubility in such solvent mixtures was determined (Fig. 1). Suspensions of anhydrous cholesterol[3] or cholesterol monohydrate (recrystallized from aqueous ethanol) were prepared in aqueous I solutions and equilibrated using a vibratory mixer[4] in a constant-temperature bath. The suspensions at equilibrium were observed with the polarizing microscope and quickly filtered through 0.45 μm membranes[5] which had been equilibrated at the test temperature. The two crystal forms were microscopically identified by their characteristic habits (6). The filtrates were analyzed for cholesterol by HPLC (8) with detection at 205 nm and for water content by Karl Fischer titrimetry[6].

Cholesterol solubility increased to a maximum and then decreased over the range of water concentration studied. The solubility was independent of the sampling time and crystal form initially present, indicating that equilibrium had been attained. An explanation cannot be offered for the higher solubility found in the present investigation compared to previous reports (3).

Immediate microscopic inspection of the suspensions showed that at water concentrations below the apparent

[1] Capmul 8210, Capitol City Products, Columbus, Ohio.
[2] Zetopan, Reichert, Vienna, Austria.
[3] Sigma Chemical Co., St. Louis, Mo.
[4] Vibromixer E1, Chemapec, Woodbury, N.Y.
[5] Millipore, Bedford, Mass.
[6] Auto-aquatrator, Precision Scientific, Chicago, Ill.

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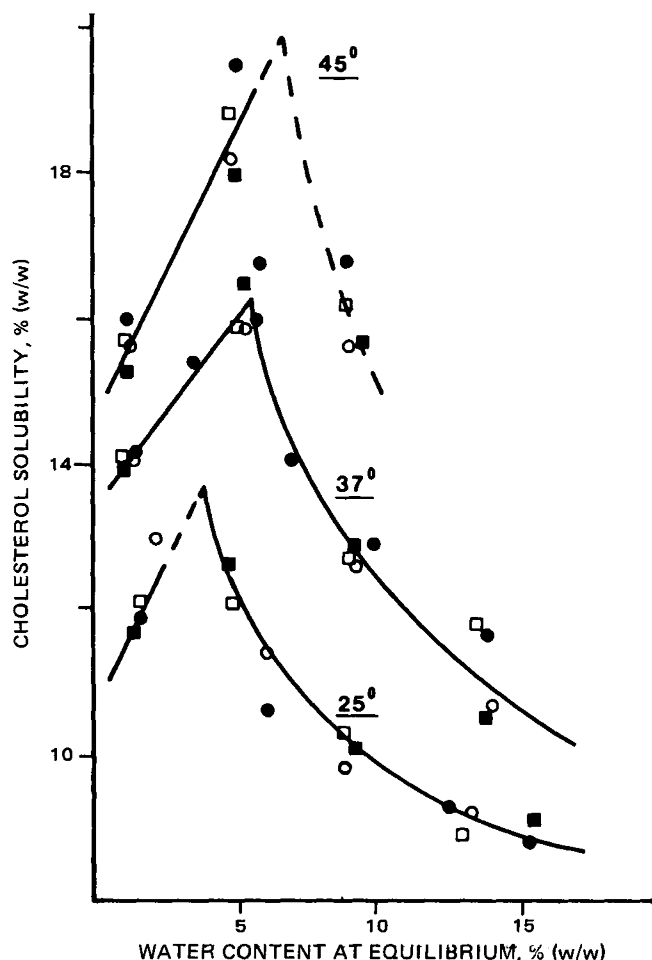

Figure 1—*Solubility of cholesterol in aqueous I solutions at 25, 37, and 45°. Key: Circles, initial solid phase was cholesterol monohydrate; squares, initial solid was anhydrous cholesterol; open symbols, 5 days; closed symbols, 7 days equilibration.*

solubility maxima, anhydrous cholesterol was present. Conversely, cholesterol monohydrate was present at water concentrations above the solubility maxima. In samples at 37° with 5.2% water, both forms were simultaneously observed. The maxima at 25 and 45° were not directly observed and the profiles are indicated by dashed lines.

When suspensions initially containing anhydrous cholesterol and about 5% water at 37 or 45° were cooled to room temperature, cholesterol monohydrate crystallized. The conversion process took place slowly on the microscope slide for several days until the anhydrous crystals were completely eliminated. Suspensions containing 1–2% water, however, remained as the anhydrous form when treated identically.

The concentration units used in this study may be misleading. A concentration of 5% (w/w) water would correspond to ~2.8 *M* and cholesterol concentrations of 14–16% are 0.36–0.41 *M*. The mole fractions for a system containing 5% water and 16% cholesterol are 0.44 and 0.07, respectively. The comparisons show that on a mole fraction basis the systems contain more water than indicated by simple concentration units.

The progression of the apparent maxima to higher water concentrations with increasing temperature (Fig. 1) is related to the heats of solution of the two crystalline forms. The ΔH^s_{anh} = 2.8 kcal/mole for the anhydrous form was estimated from the average of the slopes of ln (solubility) *versus* T^{-1} plots at water concentrations of 0 (extrapolated), 1.0, and 3.0% according to the van't Hoff relationship. For cholesterol monohydrate ΔH^s_{hydr} = 4.1 kcal/mole was estimated from the data at 9% water. The difference in these values represents the enthalpy of hydration of the crystalline anhydrous form to the monohydrate in aqueous I:

$$\text{anhydrous} + H_2O \rightleftharpoons \text{hydrate}, \Delta H = -1.3 \text{ kcal/mole}$$

This value is consistent with literature data for enthalpies of hydration which range from 1–4 kcal/mole for monohydrates (9–11). Therefore, the solubility of the monohydrate is more sensitive to temperature than is that of the anhydrous form.

The maximum cholesterol concentration occurs at the unique point at which the solubilities of the two forms are equal. As temperature increases, more added water is necessary to compensate for the greater effect of temperature on the monohydrate solubility. In this way the solubility maxima occur at higher water concentrations as temperature increases. This process is illustrated by a suspension of the two forms at 25° (solubility = 13.4%, water ≃3.5%) that is warmed to 37°. If excess solid is present and the forms behave independently, the solubility of the monohydrate should increase to 17.5%, the anhydrous form would increase to 16.1%, and the concentration of water would be slightly higher. Dissolution of the monohydrate causes the cholesterol concentration to be above the solubility of the anhydrous form and crystallization occurs. This process continues until the solid monohydrate is exhausted and the system is a suspension of anhydrous cholesterol. Addition of water to the anhydrous cholesterol suspension would cause solubility to increase slightly until the water causes crystallization of the monohydrate. The system would then be at the solubility maximum at 37°. Conversely, cooling of a suspension of both forms from 37 to 25° would result in a suspension containing only cholesterol monohydrate.

Igimi and Carey (7) reported solubility–temperature data for the two forms of cholesterol in aqueous sodium chenodeoxycholate solutions. From van't Hoff plots of these data, ΔH^s_{anh} (0.71 kcal/mole), ΔH^s_{hydr} (1.04 kcal/mole), and a difference (heat of hydration) of −0.33 kcal/mole were calculated. The poor agreement between the data in water/I and chenodeoxycholate solutions can not be explained at present, although the enthalpies for the bile salt solutions appear to be unusually low.

These preliminary data show that the maxima of cholesterol solubility in aqueous I solutions and its temperature dependence are due to the thermodynamics of interconversion of anhydrous and monohydrate crystalline forms. However, the reason for the increase in solubility of anhydrous cholesterol by small amounts of added water is not known. Addition of water to solutions containing cholesterol generally would be expected to *decrease* solubility. Further studies are in progress to investigate this behavior and determine the effect of water on dissolution rates of cholesterol and gallstones in aqueous I solutions.

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Joseph B. Bogardus
College of Pharmacy
University of Kentucky
Lexington, KY 40506

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Noncompartmental Determination of the Steady-State Volume of Distribution for Any Mode of Administration

Keyphrases ☐ Pharmacokinetics—noncompartmental determination of the steady-state volume of distribution for any mode of administration ☐ Volume of distribution—steady-state, noncompartmental determination for any mode of administration

To the Editor:

The analysis of concentration–time data by pharmacokinetic methods traditionally involves the use of compartmental models. The interpretation of this analysis, represented by a linear equation in the form of a sum of coefficient and exponential terms, provides useful insight into drug disposition. In recent years, however, there has been a move away from the traditional approach to an alternative method referred to as model-independent data analysis. There are reasons to recommend the latter approach; there is no need to ascribe the data to a specific model, and as a result it is not necessary to have a sophisticated computer and nonlinear regression programs available. The model-independent approach assumes only that all dispositional processes may be described by first-order kinetics with elimination occurring from the rapidly equilibrating or central compartment. This approach may also be termed an area analysis, since the useful parameters of clearance and volumes of distribution (V_{ss} and V_β or V_{area}) are based on determination of the total area under the plasma concentration–time curve (AUC) and total area under the first moment of the plasma concentration–time curve ($AUMC$). The areas generally are determined using the linear or logarithmic trapezoidal rule and extrapolation techniques. The elimination rate constant and half-life are determined from linear regression of the terminal (*i.e.*, post-absorption, post-distribution) concentration–time data.

Benet and Galeazzi (1) applied techniques of tracer kinetics, and used moment analysis (2, 3) to obtain the volume of distribution at steady state, V_{ss}, following an intravenous bolus injection. The purpose of this communication is to extend their analysis to permit calculation of V_{ss} for any mode of administration.

The mean transit time for a drug in the body, $\bar{t}_b$, is a function of the mean transit time for the response to the input (in), usually measured as plasma concentration, $\bar{t}_{b+\text{in}}$, and the mean transit time of the input, $\bar{t}_{\text{in}}$ (4):

$$\bar{t}_b = \bar{t}_{b+\text{in}} - \bar{t}_{\text{in}} \qquad \text{(Eq. 1)}$$

Mean transit or residence time for the response to the input, *i.e.*, plasma concentration, is given by:

$$\bar{t}_{b+\text{in}} = \int_0^\infty tC\,dt \Big/ \int_0^\infty C\,dt = AUMC/AUC \qquad \text{(Eq. 2)}$$

while the mean transit time for the input is given by (5):

$$\bar{t}_{\text{in}} = \int_0^\infty X\,dt/\text{dose} \qquad \text{(Eq. 3)}$$

where dose is the dose administered, and $\int_0^\infty X\,dt$ is the total area under the amount *versus* time curve for the input. For example, if a drug is administered as a zero-order infusion:

$$X = \text{dose} - k_0 t \qquad \text{(Eq. 4)}$$

In Eq. 4, X is the amount remaining to be infused at time t, and k_0 is the zero-order infusion rate. Administration by a first-order process (*e.g.*, extravascular administration) results in the following expression for X, the amount remaining to be administered:

$$X = F\,\text{dose}\,e^{-k_a t} \qquad \text{(Eq. 5)}$$

where k_a is an apparent first-order rate constant, and F is the fraction of the administered dose ultimately reaching the systemic circulation. Integration of Eqs. 4 and 5 yields:

$$\int_0^T X\,dt = k_0 T^2/2 \qquad \text{(Eq. 6)}$$

and

$$\int_0^\infty X\,dt = F\,\text{dose}/k_a \qquad \text{(Eq. 7)}$$

respectively. In Eq. 6, T is the duration of the infusion and is the upper limit of the integral, *i.e.*, T is equivalent to infinity.

Substitution for $\bar{t}_{b+\text{in}}$ and $\bar{t}_{\text{in}}$, according to Eqs. 2 and 3, respectively, in Eq. 1 gives the following expression for drug transit time in the body:

$$\bar{t}_b = AUMC/AUC - \int_0^\infty X\,dt/\text{dose} \qquad \text{(Eq. 8)}$$

Since V_{ss} is equal to the product of clearance (dose/AUC) and transit time (1), that is:

$$V_{ss} = \frac{\text{dose}}{AUC}\bar{t}_b \qquad \text{(Eq. 9)}$$

Equations 8 and 9 can be readily used to calculate V_{ss} following any mode of administration. Where there is a single mode of administration, Eqs. 8 and 9 can be readily solved for V_{ss}. For the case where drug is administered as a single bolus, $\int_0^\infty X\,dt = 0$,

$$V_{ss} = \frac{\text{dose}}{AUC}\bar{t}_b = \frac{\text{dose}}{AUC}\left(\frac{AUMC}{AUC}\right) = \text{dose}\frac{AUMC}{AUC^2} \qquad \text{(Eq. 10)}$$

This is the same equation as derived by Benet and Galeazzi

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