Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl16

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Version of record first published: 21 Mar 2007.

To cite this article: R. Thomas Holzbach & Mitsuko Marsh (1974): Transient Liquid Crystals in Human Bile Analogues, Molecular Crystals and Liquid Crystals, 28:1-2, 217-222

To link to this article: http://dx.doi.org/10.1080/15421407408083166

*Mol. Cryst. Liq. Cryst.*, Vol. 28, pp. 217-222

Printed in Dordrecht, Holland



# Transient Liquid Crystals in Human Bile Analogues

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(*Received February 14, 1974*)

The process of phase transition and equilibration in bile analogue systems was studied because of the recent demonstration of an unstable liquid crystalline state in metastably supersaturated human bile. We found similar transient mesophases in model solutions of physiological relevance. Observed transition rates were strong functions of lecithin concentration. The composition boundaries of these transitional systems are defined.

Recent demonstration of an unstable liquid crystalline state in metastably supersaturated human bile[1] suggested the need for a detailed examination of the process of phase transition and equilibration in bile analogue systems. As cholesterol supersaturation has now been shown to occur frequently in the bile of healthy man[2], such observations seem especially relevant. Accordingly, the aims of the present work were the following: first, to measure time scales for transition rates of this mesophase; second, to assess the effect of variations in lipid concentrations on transition rates; and third, to define lipid concentration boundaries for phase transition phenomena in these systems.

Bile analogue solutions containing lecithin, cholesterol, and bile salts were prepared as previously described[2]. Mixed bile salts were in proportions found in human bile[3]. The solutions were shaken for 24 hours, and then incubated for 21 days at 37°C. The appearance of each solution was examined daily for opacity, stratification and phase transition phenomena during the 21 day period. Aliquots were examined periodically by standard and polarizing microscopy at

37°C (Zeiss Photomicroscope II, Carl Zeiss, 444 5th Ave., N. Y., N. Y. 10018).

A total of 30 bile analogue solutions were prepared. Thirteen of which were constructed to conform to the entire inner perimeter of the boundary for maximum cholesterol solubility derived from the phase diagram of Admirand and Small[4]. The remaining 17 solutions were constructed in ranges outside this boundary where relative lecithin concentrations were greater than 30 percent. We have previously shown that accurate phase separations at true equilibrium can be accomplished within the range of zero to 30 moles percent lecithin concentration by microfiltration of solutions initially supersaturated with cholesterol[2]. As microfiltration failed to separate micellar from two-phase systems containing an excess of 30 moles percent of lecithin, the standard phase separation technique of microscopy was used. Final lipid concentrations were directly measured by methods previously described[2].

Liquid crystalline mesophases were not seen in bile analogue solutions containing less than 14 moles percent lecithin (Table 1, Solutions 1–4). Microfiltration at 72 hours effected complete removal of cholesterol crystals. This indicated that these solutions had quickly reached equilibrium, enabling accurate phase separations. Where lecithin concentration ranged between 14 to 30 moles percent, (Table 1, Solutions 5–9) a liquid crystalline mesophase was observed to develop in the absence of cholesterol microcrystallites. With further

TABLE 1

Aqueous mixtures of bile salts, lecithin and cholesterol describing the boundary in a triangular coordinate system for maximum equilibrium micellar cholesterol solubility for 10 percent solutions

| No. | Percent total moles | | |
|---|---|---|---|
| | Bile salts | Lecithin | Cholesterol |
| 1 | 96.8 | 0 | 3.2 |
| 2 | 93.1 | 3.6 | 3.3 |
| 3 | 88.2 | 7.9 | 3.9 |
| 4 | 86.0 | 9.8 | 4.2 |
| 5 | 82.0 | 13.5 | 4.5 |
| 6 | 77.0 | 17.4 | 5.6 |
| 7 | 72.3 | 21.1 | 6.6 |
| 8 | 68.9 | 23.9 | 7.2 |
| 9 | 64.3 | 28.0 | 7.7 |
| 10 | 60.0 | 31.0 | 9.0 |
| 11 | 56.0 | 35.0 | 9.0 |
| 12 | 51.0 | 41.0 | 8.0 |
| 13 | 46.0 | 46.0 | 8.0 |
| 14 | 40.0 | 54.0 | 6.0 |
| 15 | 38.0 | 58.0 | 4.0 |
| 16 | 38.0 | 62.0 | 0 |

incubation, we found complete disappearance of the mesophase (Figure 1, A and B) and appearance of cholesterol microcrystallites. For example, an initially supersaturated solution containing 21 moles percent lecithin exhibited pleomorphic mesophase patterns which gradually developed into liquid crystals during 14 days of incubation at 37°C. These suddenly disappeared and within a few hours cholesterol crystals formed. Transition rates were found to be strong functions of the lecithin concentrations present: 14 to 18 moles percent = 7 days; 18 to 22 moles percent = 14 days; and 22 to 30 moles percent = 21 days. Finer gradations between these intervals were not examined. Such spontaneous transitions have not previously been reported in lyotropic systems.

Solutions containing an excess of 30 moles percent of lecithin just outside the perimeter of the previously published definition of maximum cholesterol solubility[4] (Figure 2, dotted line) initially were visually turbid due to the presence of mesophase but became clear and microscopically isotropic by 21 days. In contrast, such changes did not occur even after prolonged incubation in solutions further outside the perimeter of this boundary. With incubation beyond 21 days and for as long as 8 weeks, the droplets enlarged in diameter, at least two to three fold, probably by coalescence. Such nonphase transitional changes were not considered relevent to supersaturation. In order to plot the phase boundary for the micellar zone, equilibrated solutions containing more than 30 moles percent of lecithin were mapped according to their visual and microscopic characteristics at 21 days (Table 1, Solutions 10–16). The micellar boundary for solutions of relative lecithin concentration between 35 to 65 percent extends slightly above and beyond the previous definition[4]. The entire range of maximum equilibrium micellar cholesterol solubility from present observations combined with those we have recently published[2] is shown in Figure 2.

Abnormal (gallstone-associated) and normal (absence of biliary tract disease) human bile specimens were obtained by direct needle aspiration of the gallbladder during abdonimal surgery. When examined microscopically in the fresh state, occasional normal biles contained liquid crystals[1], but they were more commonly observed in abnormals. Sequential microscopic observations of these specimens revealed spontaneous phase transition phenomena identical to those seen in compositionally similar bile analogue solutions. As in our previous study[2], analysis of lipid composition of these samples showed prevalent supersaturation and great overlap between normals and abnormals.

We conclude from this study that, a transient liquid crystalline mesophase observed in human bile is seen in physiologically relevant lipid concentrations; phase transition rates are strong functions of lecithin concentration; the composition of these transitional systems is defined by the presence of supersaturation and a lecithin concentration of approximately 14 to 30 moles percent; and finally, that prolonged equilibration is necessary to derive accurate phase boundaries when phase transitions are slow.

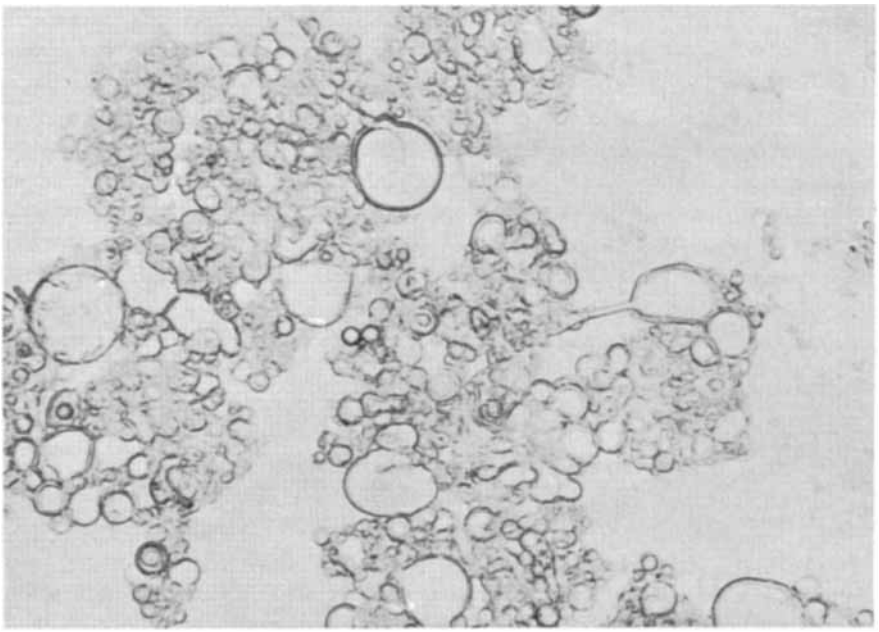
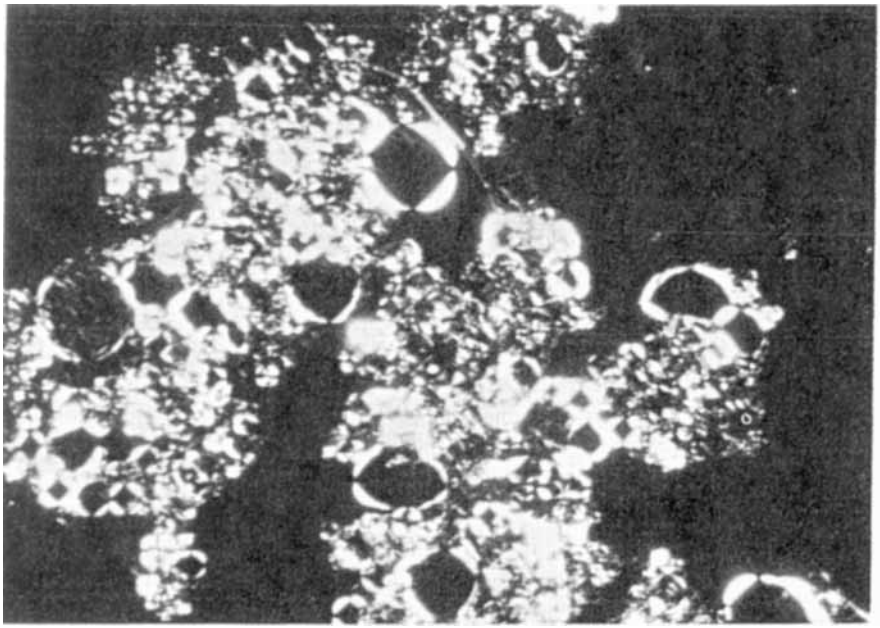


FIGURE 1 (A) Liquid crystalline mesophase droplets in the metastable supersaturated state of bile analogue solutions. (Original magnification = 400x).(B). Birefringence of liquid crystalline mesophase droplets shown in Figure 2 (A) with polarizing microscopy.



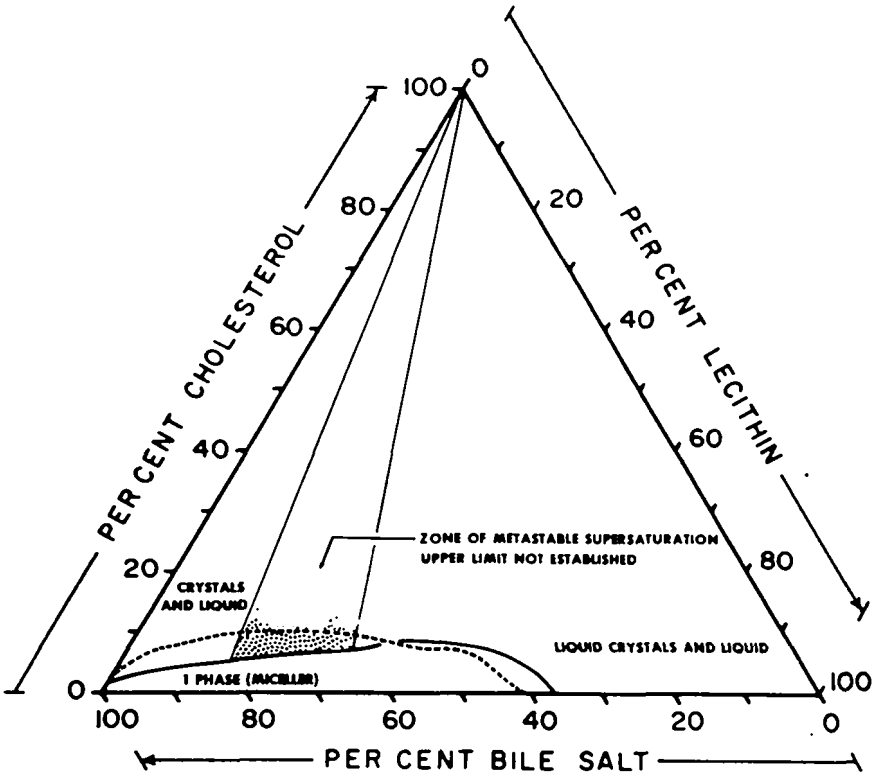



FIGURE 2 Boundaries for the metastably supersaturated (shaded area) and equilibrium solubility state (solid line) for the bile analogue system. Dotted line represents the boundary for cholesterol solubility limit of Admirand and Small (4).

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