Effects of Solute Characteristics and Concentration on a Lyotropic Liquid Crystal: Solute-Induced Phase Change

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We investigated the effects of increased concentrations of the solutes, salicylic acid, benzoic acid, and *o*-, *m*-, and *p*-methoxy benzoic acids, on the anisotropic properties of a liquid crystal solvent. The lamellar liquid crystal was composed of 37% polyoxyethylene (20) isohexadecyl ether in aqueous buffer of pH 1. Phase change, transition temperature, refractive index, and specific resistance of the mesophase were studied in the presence of solutes. Transfer rates of the solutes from the bulk mesophase into aqueous buffer across a lipoidal barrier were used to determine their apparent permeability coefficients. The results indicate that a phase change occurred in the liquid crystal from a lamellar to a hexagonal structure, in the case of salicylic, benzoic, and *m*-methoxy benzoic acids. However, *o*- and *p*-methoxy benzoic acids showed no effect on the packing arrangement of the liquid crystal in the concentration range studied. The occurrence of the phase change was both solute and concentration dependent. Relative values of apparent permeability coefficients of solutes reflected the extent of solute–solvent interactions in the systems.

KEY WORDS: liquid crystals; phase changes in, release of solutes from, solute–solvent interactions in.

INTRODUCTION

Liquid crystals are reported to form in aqueous media (1), nonpolar solvents (2), and ionic environments (3). These systems are found in living organisms (4), and they proved to be useful in several industrial applications. Moreover, they are used as model media to study effects arising from an ordered environment on reaction kinetics (5) and material transport (6) and to simulate the biological membrane. For instance, Simon *et al.* (7) used liquid bilayers to study partitioning of the general anesthetic halothane.

Lyotropic liquid crystals accommodate different solutes to varying degrees. The presence of a solute dissolved in the mesophase could influence the structure of the liquid crystal when the solute concentration reaches a certain level. Solutes influenced the structure of the mesophase and caused a change in its form (8–11). Therefore, solute–solvent interactions are thought to affect changes in the molecular packing of liquid crystalline systems. The liquid crystalline structure was proposed as the primary factor for the water barrier of the stratum corneum (12). It is conceivable that drug molecules or other agents transported through the skin might interact with its polar lipid barrier and might influence its molecular packing and, hence, the absorption process through the skin. These effects should be considered when developing a therapeutic transdermal drug delivery system. The present investigation addresses the effects of solute characteristics and concentration on liquid crystals.

The two molecular packings most commonly found in lyotropic liquid crystals are lamellar and the hexagonal packings (8,13,14). These structures are described in the literature as the neat phase and the middle phase, respectively (15–17). The interactions between the functional groups of the solute molecules and those of molecules constituting the mesophase (surfactant and water) could influence the forces governing the association of the micelles in the ordered solvent and induce a change in the molecular packing of the mesophase. A ratio (R) reflecting effects of forces influencing micellar association in the ordered fluid was defined by Winsor (8–10) as

$$R = \frac{\text{(tendency of amphiphilic layer to spread out into lipophilic environment)}}{\text{(tendency of amphiphilic layer to spread out into polar environment)}}$$

In the neat lamellar structure of the lyotropic liquid crystal the tendencies of the amphiphilic monolayers to become convex toward its lipophilic environment and toward its polar environment are equally balanced, i.e., $R = 1$. Also, transition from the lamellar packing, characteristic of neat phases, to the hexagonal packing, characteristic of middle phases, is the consequence of the inequality of these two tendencies, i.e., $R > 1$ or $R < 1$.

The current study reports on effects arising from the presence of increased concentrations of salicylic acid and four other structurally related solutes on the mesomorphic properties of a lyotropic liquid crystal composed of polyoxyethylene (20) isohexadecyl ether in aqeous medium. Furthermore, release of the solute agents from the bulk mesophase across a lipoidal barrier was also studied.

MATERIALS AND METHODS

Materials. Polyoxyethylene (20) isohexadecyl ether was obtained from (ICI Americas Inc., Wilmington, DE). The surfactant is marketed under the name Arlasolve 200. Salicylic acid, USP, and benzoic acid, BP, were from Riedel de Haven, West Germany. *O*-Methoxy benzoic acid and *m*-methoxy benzoic acid were obtained from Fluka AG, Switzerland. *P*-Methoxy benzoic acid (BDH Chemical Ltd., England) and myristic acid isopropyl ester (Sigma Chemical Co., St. Louis, MO) were obtained from the given suppliers. A Type GS, 0.22-μm, 47-mm-diameter Millipore filter membrane was supplied by Millipore Corporation, Bedford, MA. Buffer reagents were analytical grade, supplied by E. Merck, West Germany. Double-distilled water was used in the study. A Hetotherm ultrathermostat Type 02 PT 623 was obtained from Heto Laboratory Equipment AS, Denmark. A P-1 peristaltic pump from Pharmacia Fine Chemicals, AB, Uppsala, Sweden, was used to circulate the receiving solu-

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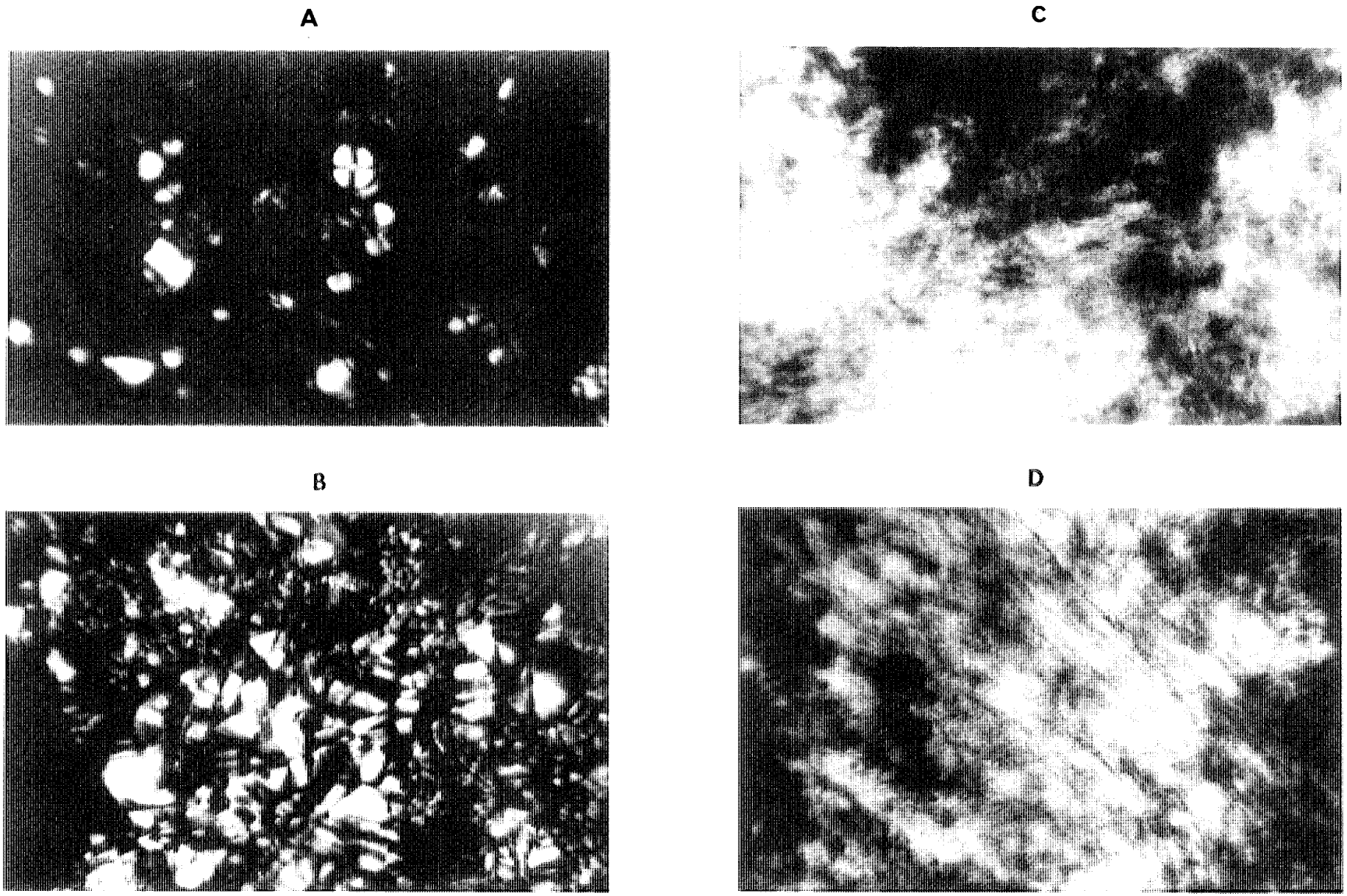

Fig. 1. Photomicrographs of liquid crystal media composed of 37% surfactant in an aqueous environment of pH 1 and $\mu = 0.168$ containing increased concentrations of salicylic acid; viewed between crossed polars at room temperature. (A) 0.05%; (B) 0.6%; (C) 1.2%; (D) 1.5%. 120×.

tion through a Uvikon-810, Kntron spectrophotometer equipped with a Uvikon recorder 21 (Switzerland).

Media Preparation and Microscope Studies. Liquid crystal media containing the solutes were prepared by dissolving weighed quantities of the solute probes in known weights of the melted surfactant. Weighed quantities of Clark-Lubs buffer (18) of pH 1 and ionic strength 0.168 were then added to the molten masses to produce the desired compositions. The buffer solution was added in one portion, swirled vigorously, and then mixed with the aid of a glass rod to ensure homogeneity. The systems were allowed to equilibrate for 48 hr, then examined for their birefringence under polarized light, using a Baush and Lomb microscope. Liquid crystal media for release studies were prepared in a similar manner. Transition temperatures of the mesophases were determined using a Kofler hot-stage from Optische Werk, Austria. Photomicrographs of the systems were taken using a Zeitz microscope fitted with a camera (Zeitz, West Germany), at a magnification of 120×. Samples for microscope work were prepared and examined as described previously (5).

Release Studies. The diffusion cell used in the study was described elsewhere (6,19). Liquid crystal media for release studies were prepared as indicated above. A 0.22-μm Millipore filter was saturated with isopropyl myristate (IPM) using the technique given by Aguiar *et al.* (19). The membrane was sandwiched between a donor compartment containing 9 g of the liquid crystal system and a receiving compartment containing 32 mL of phosphate buffer, pH 7.4. The buffer and the liquid crystals had been previously equilibrated to the experimental temperature. The receiving solution was allowed to circulate in the detection system, and UV absorption for salicylic acid, benzoic acid, and *o*-, *m*-, and *p*-methoxy benzoic acids was measured at λ_{max} of 295, 225, 279, 287, and 247 nm, respectively. No apparent change was observed in the membrane or the media at the end of the release runs. Release studies were conducted at two solute concentrations, 0.6 and 1.2%. All release experiments were carried out in duplicate at 37°C.

Conductivity Measurements. A conductivity meter, LF 530 Werkstten GMbH, West Germany, with temperature measurement capability was used to determine the conductivity of the liquid crystal media. In a typical run, 6 g of the liquid crystal medium was heated in a Block thermostat test tube, Labsco, West Germany, to 60°C. The medium was then allowed to cool and conductivity measurement was determined. Conductivity of media containing different concentrations of the solute probe was determined at 18°C, unless specified otherwise.

Refractive Index Measurements. A refractometer, Model 8800 Erma Optical Ltd., Japan, connected to a Hetotherm Ultra thermostat, was used to determine the refractive index of liquid crystal samples at 30°C. Samples were heated at a controlled rate of 1°C/min and allowed to equilibrate at the indicated temperature before readings were taken. Experiments were conducted in duplicate

RESULTS AND DISCUSSION

Figure 1 shows photomicrographs of the liquid crystal solvent with 0.05, 0.6, 1.2, and 1.5% salicylic acid, dissolved in the medium and viewed between crossed polars at room temperature. Shown in Table I is a comparison of the physical state and the microscopic appearance of the systems used in the study. Examination of Table 1 and Fig. 1 indicates that increasing the concentration of salicylic acid to a level of 1.2% brought about a change in the phase characteristics of the vehicle. While media containing 0.05 and 0.6% salicylic acid were neat mobile liquids, those containing 1.2 and 1.5% were gel-like middle phases with low flow properties. Under polarized light, the neat phases showed fan-like focal conic textures with some spherulites. The middle phases, on the other hand, showed characteristic birefringent nongeometric striations. Similar effects on the properties of the liquid crystal solvent were also induced by the presence of benzoic acid in the medium. However, it is apparent from the microscopic observations and the flow characteristics of the media that the changes brought about by salicylic acid were more pronounced than those induced by benzoic acid at the same concentrations.

Figure 2 contains photomicrographs of the mesophase with the isomer *m*-methoxy benzoic acid, dissolved in the

Table I. Comparison of Physical State, Microscopic Appearance (Under Polarized Light and at Room Temperature), and Anisotropic–Isotropic Transition Temperatures[a] of the Mesophases in the Presence of Increasing Concentrations of Different Solutes[b]

Solute conc. (%)	Salicylic acid			Benzoic acid			*o*-Methoxy benzoic acid			*p*-Methoxy benzoic acid			*m*-Methoxy benzoic acid		
	State	Phase	°C	State	Phase	°C	State	Phase	°C	State	Phase	°C	State	Phase	°C
0.05	Liquid	Neat (A)	58	Liquid	Neat (A)	62									
0.60	Liquid	Neat (B)	59	Liquid	Neat (B)	62	Liquid	Neat (A)	62	Liquid	Neat (B)	62	Liquid	Neat (B)	62
1.20	Gel	Middle (C)	53	Gel	Middle (C)	56	Liquid	Neat (A)	62	Liquid	Neat (D)	62	Gel	Middle (C)	59
1.50	Gel, viscous	Middle (C)	48	Gel	Middle (C)	53							Gel, viscous	Middle (C)	54

[a] Neat–middle transition temperatures were not reported because of the poor reproducibility of results. Anisotropic–isotropic transition temperature of mesophase with no solute is 60°C.

[b] (A) Neat phase with fan-like focal conic texture; (B) neat phase with fan-like plates; (C) middle phase with nongeometric striations; (D) neat phase with fan-like plates mixed with precipitated crystals of solute.

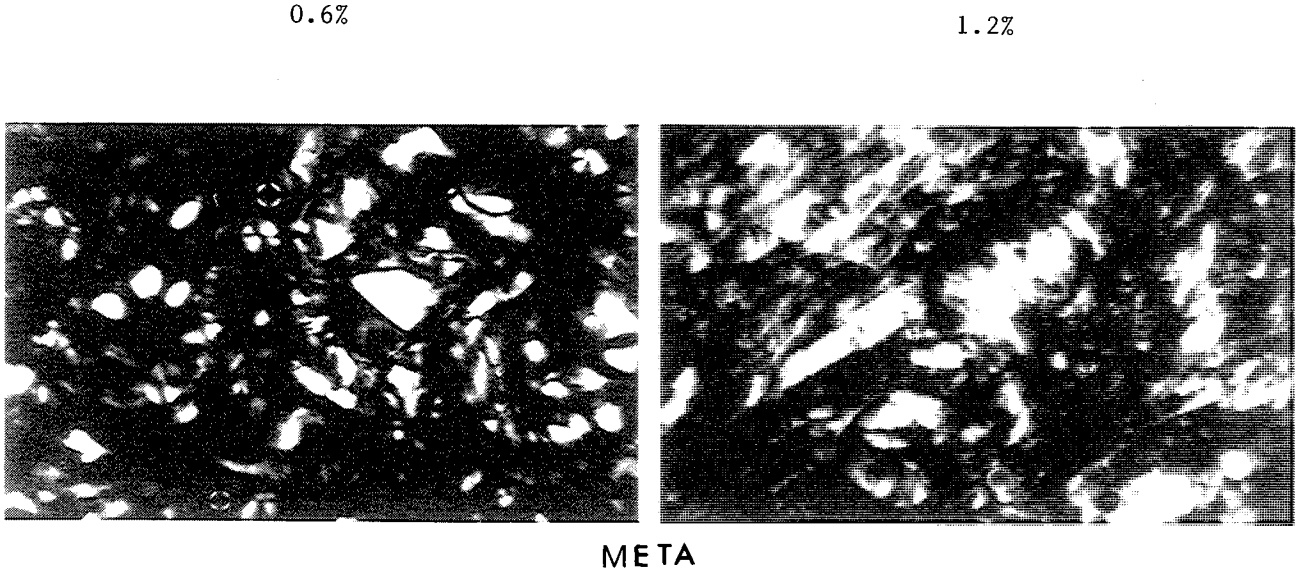

Fig. 2. Photomicrographs of liquid crystal media composed of 37% surfactant in an aqueous environment of pH 1 and μ = 0.168 containing 0.6 and 1.2% concentrations of *m*-methoxy benzoic acid; viewed between crossed polars at room temperature. 120×; reduced to 95% for reproduction.

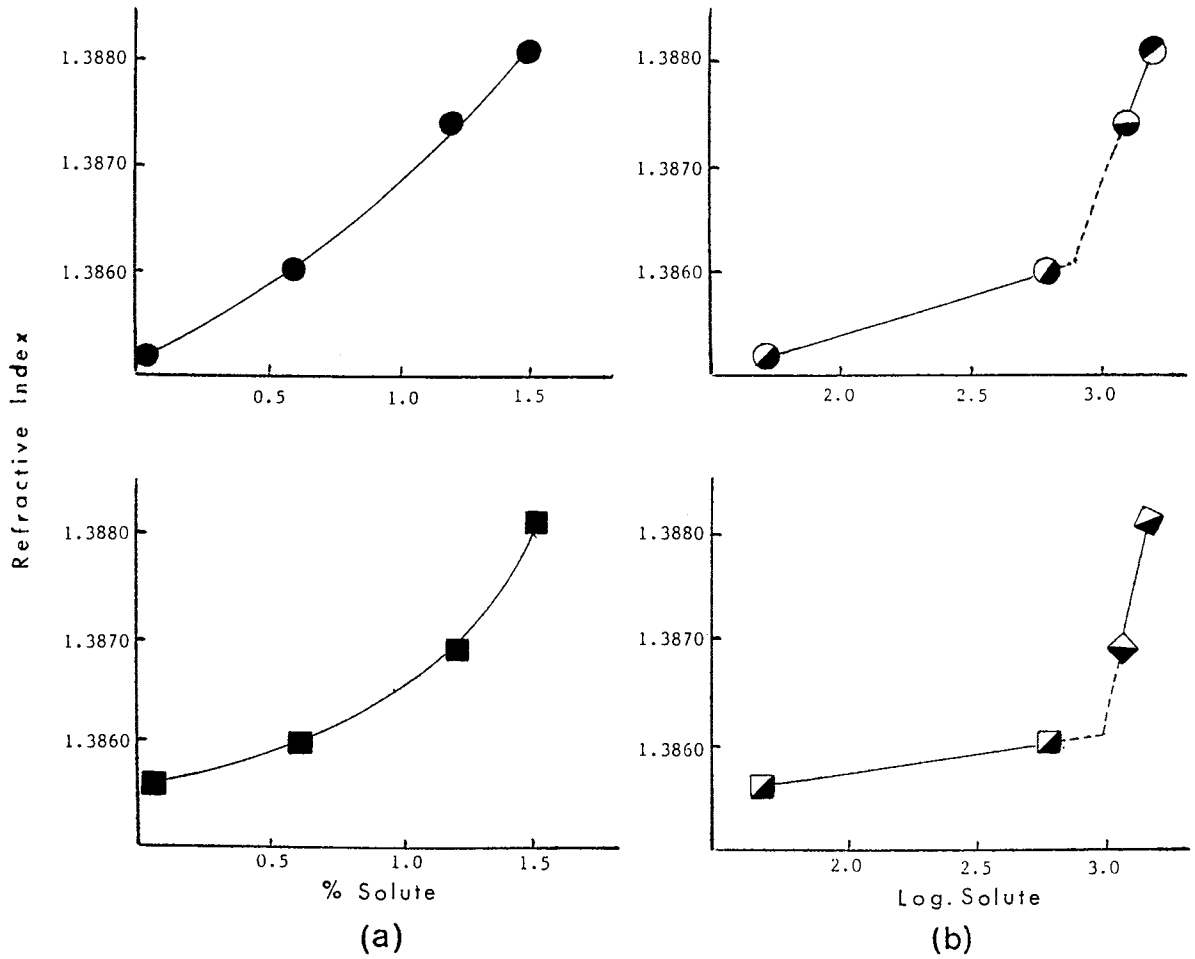

Fig. 3. Plots of the refractive index of liquid crystal media containing salicylic acid and benzoic acid as a function of (a) the solute concentration (%) and (b) the logarithm of the amount of solute (mg) per 100 g of medium, at 30°C. (●, ◕) Salicylic acid; (■, ◪) benzoic acid.

liquid crystal solvent at concentrations of 0.6 and 1.2%, respectively. Analysis of Table I and Figs. 1 and 2 indicates that *o*- and *p*-methoxy benzoic acids did not induce a change in the medium structure at the given concentration levels. The meta isomer, on the other hand, influenced the mesophase in a manner similar to salicylic and benzoic acids. Further, while *o*- and *m*-methoxy benzoic acids were easily accommodated in the mesomorphic solvent at a concentration of 1.2%, the *p*-isomer partly precipitated out of the neat liquid at that concentration.

The anistropic–isotropic transition temperatures of the different media were determined by thermal microscopy technique as described elsewhere (5). The data in Table I indicate that the formation of middle phases due to the presence of salicylic, benzoic, and *m*-methoxy benzoic acids at a concentration of 1.2% was accompanied by a lowering of the anisotropic–isotropic transition temperature of the medium. Also, higher solute concentrations, i.e., concentrations >1.2%, produced a greater drop in the transition temperature. The observed lowering of the transition temperature could be attributed to strong solute–solvent interactions. Further, the fact that the extent of temperature lowering was the highest in the case of salicylic acid suggests a stronger solute–solvent interaction compared to other solutes.

Figure 3a shows plots of the refractive index of the liquid crystal solvent as a function of increasing concentrations of salicylic and benzoic acids at 30°C. The curves were continuous, with no apparent break points, characterized from similar plots by Yakhmi *et al.* (20). Nevertheless, presentation of data as a linear-logarithmic relationship as shown in Fig. 3b revealed the biphasic behavior of the refractive index of the mesophase. The plots in Fig. 3b concur with the findings in Fig. 1 and Table I.

Although the number of data points in Fig. 3b is limited, the data in each plot could be separated into two straight lines: one corresponding to changes in the refractive index of the neat phase and the other to changes of the middle phase. An approximate estimate of the transition concentrations of the respective solutes, i.e., concentrations at which transformation from the neat to the middle phase occurs, could be obtained from Fig. 3b by extrapolation. The calculated values of the transition concentrations for salicylic acid and benzoic acid at 30°C were 0.75 and 1.0%, respectively. The smaller value of the transition concentration of salicylic acid lends support to the aforementioned suggestion of stronger solute–solvent interactions in the case of salicylic acid compared to benzoic acid.

Electrical conductivity measurements were used by Winsor (21) to demonstrate changes in the specific resistance of micellar media, which were caused by changes in media structure. In the current study the specific resistance of media as a function of increased concentrations of salicylic and benzoic acids was studied. As the solute concentration increased, the resultant middle phases showed higher specific resistance than the corresponding neat phases. These effects could be attributed to changes in media structure (21) as well as to the increased viscosity accompanying the observed phase changes.

Release rates of the solute probes from the liquid crystal across the lipoidal barrier and into the aqueous buffer were determined at 37°C. Rates of drug transfer across the barrier were calculated as indicated elsewhere (6,19). Slopes of linear segments of plots of amount transferred versus time were used to obtain the rates and the corresponding permeability coefficients according to the following relationship:

$$\frac{dQ}{dt} = PAC$$

where dQ/dt is the rate of drug transfer, P is the apparent permeability coefficient, A is the surface area, and C is the concentration in the donor compartment. Release data are presented in Table II. The data correspond to media containing solute concentrations of 0.6 and 1.2%.

The observed differences in the release rates of the solutes could be attributed to differences in their thermodynamic activities in the media. The latter could arise from different interactions between the solutes and their microsurroundings. Furthermore, the differences suggest a transfer process controlled largely by entropy contributions. Likewise, the observed increased permeability of benzoic acid indicates more favorable entropy factors influencing the transfer process in the middle phase compared to the neat phase.

The presence of the solute probes in the neat liquid crystal influenced the attractive forces at the interfaces of the amphiphilic–hydrophobic environment and the amphiphilic–polar environment of the lamellae to varying degrees. As the concentration of salicylic acid, benzoic acid, or *m*-methoxy benzoic acid reached 1.2%, it is likely that the energies of interactions per unit area of interface were in favor of the amphiphilic–polar environment i.e., the R ratio was not equal to one (8–10). Also, it is probable that molecules of these three solutes resided in proximity to the polar environment of the mesophase. Consequently, one would expect a reasonable interaction with the polar groups of the

Table II. Release Rates (±SD) of Various Solute Probes and Their Apparent Permeability Coefficients from Liquid Crystal Systems Containing 0.6 and 1.2% Solutes at 37°C

	Liquid crystal[a] containing 0.6% solute		Liquid crystal[a] containing 1.2% solute	
Solute	Release rate (mg/hr)	Permeability coefficient (cm/hr)	Release rate (mg/hr)	Permeability coefficient (cm/hr)
Salicylic acid	1.226 (±0.099)	17×10^{-3}	2.110 (±0.177)	15×10^{-3}
Benzoic acid	2.008 (±0.174)	28×10^{-3}	5.283 (±0.340)	37×10^{-3}
o-Methoxy benzoic acid	1.962 (±0.105)	27×10^{-3}	3.611 (±0.235)	25×10^{-3}
m-Methoxy benzoic acid	1.443 (±0.183)	20×10^{-3}	3.248 (±0.253)	23×10^{-3}
p-Methoxy benzoic acid	1.202 (±0.141)	16×10^{-3}	2.524 (±0.258)	18×10^{-3}

[a] Liquid crystal composed of 37% polyoxyethylene (20) isohexadecyl ether in an aqueous environment of pH 1 and $\mu = 0.168$.

monolayer and an increase in the mean area populated by one polar group at the interface of the lamellae. These combined factors favored a change in the packing of the micelles in the liquid crystal system from the lamellar to the hexagonal packing.

The proximity of the two polar groups of the *o*-methoxy isomer (carboxylic group and methoxy group) created a situation where steric factors restricted their availability for the needed interaction with the monolayer of the mesophase. Molecules of the *p*-isomer, on the other hand, being rod-like, probably were embedded within the hydrocarbon-tail strata of the lamellae away from the polar groups of the monolayers. These factors caused a weak influence on the forces at the interfaces of the amphiphilic–polar and amphiphilic–hydrophobic environment of the mesophase. Thus, in the presence of *o*- and *p*-isomers, the aforementioned interfaces remained planar over the concentration range studied, i.e., $R = 1$, and the media did not exhibit a change in phase.

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