Pharmaceutical Research, Vol. 9, No. 12, 1992

The Influence of Liquid Crystalline Phases on Drug Percutaneous Absorption. I. Development of a Vehicle

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Received March 5, 1992; accepted May 11, 1992

A phase diagram approach has been used to formulate topically applied vehicles containing liquid crystalline phases. The current paper describes the construction of a major portion of the polyoxyethylene(20)cetyl ether:dodecanol:water phase diagram. Known mixtures of the three components were equilibrated and centrifuged to separate the resultant conjugate phases. These were identified and analyzed quantitatively to determine phase boundaries in relevant portions of the phase diagram. Two isotropic liquid phases, several two- and three-phase regions, a solid surfactant phase, and at least three distinct liquid crystalline phases were identified. The determination of tie lines was undertaken in a two-phase region containing an aqueous isotropic micellar solution and a liquid crystalline gel. This information will be used to prepare a number of vehicles of known phase composition and concentration for a systematic evaluation of the effect of liquid crystalline phases on transdermal drug delivery.

KEY WORDS: liquid crystalline phases; percutaneous absorption; transdermal drug delivery; topical vehicle formulation; phase diagrams; tie lines.

INTRODUCTION

The relation between structure and physicochemical properties of a molecule or ion and its percutaneous absorption has been extensively reported, as has the formulation of dermatological vehicles and their influence on percutaneous absorption (1,2). Most studies in the latter area have involved single-phase systems. However, to meet the several criteria for an acceptable topical preparation, multiphase systems, such as emulsions, are frequently required. Phase equilibria in emulsions can be complex and involve the presence of liquid crystalline (LC) phases in addition to the primarily aqueous and nonaqueous phases that constitute simple emulsions (3–5). While attention has been paid to the effect of LC phases on the physical stability of emulsions (6,7), there is only limited information on the influence of such phases on drug permeation through skin (8). This is surprising, given the fact that LC phases are capable of increasing the solubilization of a molecule, can be quite viscous, and possess a structure that is not dissimilar to that possessed by intracellular lipids in the stratum corneum (9). As such, LC phases might be expected to modify the overall permeability process between a permeant and the skin.

The work reported here is part of a study that had three objectives. The first of these was to prepare, and quantitate in terms of phase equilibria, topically acceptable emulsified systems containing LC phases that could serve as suitable vehicles for studying drug delivery through the skin. The second objective was to elucidate the effect of LC phases on the percutaneous absorption of a selected permeant. The third objective was to develop a comprehensive model that would account for the factors affecting percutaneous drug absorption from emulsified vehicles containing LC phases. This paper relates to the first objective, namely, the phase diagram approach taken to develop a suitable vehicle. Subsequent publications will detail percutaneous absorption from these vehicles through excised human skin (see the following paper) and the development of a model to describe the process.

Phase Equilibria in Emulsified Systems

The basic components for an emulsion are usually water, an oil, and an emulsifying agent. Depending on the relative amounts of and interactions among these three components, a variety of conjugate phases can be formed. From a knowledge of the phase diagram, one or more of these phases can be varied systematically such that its influence on a particular parameter (e.g., permeability, physical stability) can be studied (3). The phases present in such a parameter (e.g., permeability, physical stability) can be studied (3). The phases present in such systems have been defined previously (3,4):

L_1 is an isotropic micellar solution of oil in water;
L_2 is an isotropic micellar solution of water in oil; and
LC is an anisotropic liquid crystalline phase, or mesophase.

LC phases are frequently encountered in contemporary topical emulsions and are believed to confer improved physical stability on such preparations (6).

Within a two-phase region, the compositions of the conjugate phases are joined by a tie line. Ternary component systems in these regions prepared so as to fall on a common tie line will, by definition, contain two phases having constant, but different, compositions. Moreover, the relative amount of each of these phases varies in a manner that is readily calculated. Within a three-phase region, the compositions of the separate phases are invariant; only the relative amounts of each phase varies as different systems are prepared within such a region. Knowledge of these properties of phase diagrams allows investigation of complex surfactant–oil–water systems in a logical, controlled manner.

This paper reports on the development of a suitable ternary component system to act as an emulsified vehicle for topically applied drugs. Components were sought that (i) resulted in a vehicle with substantial LC regions in the phase diagram and acceptable physical and chemical stability in the absence and presence of the active, (ii) were easy to analyze, (iii) had a good availability, and (iv) were unlikely to have any overt adverse effects when in contact with human skin. The phase diagram was determined, with emphasis on the L_1, LC, and L_1 + LC phase regions.

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MATERIALS AND METHODS

Chemicals

Polyoxyethylene(20)cetyl ether (Brij 58; Cetomacrogol; designated $C_{16}E_{20}$), industrial grade, was obtained from Emulsion Engineering, Sanford, FL. Dodecanol ($C_{12}OH$), technical grade, was obtained from Matheson-Coleman-Bell, Norwich, OH. All other chemicals were of analytical reagent or HPLC grade. Double-distilled water was used in all studies.

Construction of the Phase Equilibrium Diagram

Preparation and Equilibration of Vehicles. Appropriate amounts of the surfactant ($C_{16}E_{20}$), $C_{12}OH$, and water were weighed into screw-capped test tubes. The contents were heated to 80–90°C and the tubes agitated vigorously until a uniform mixture was obtained, a process taking 10–60 min, depending on the composition. The samples were air-cooled to 25°C with constant agitation and then stored at 25°C for 1 week.

Characterization of Phases Present. Approximately 4 g of each mixture was transferred to a polypropylene centrifuge tube and ultracentrifuged (Beckman model L5-65) at 25°C. With most samples, sufficient separation for collection of individual phases occurred within 24 hr at 60,000 rpm, although longer times were employed where necessary. The degree of separation was measured as the distance from the interface to the bottom of the tube. Separated phases were transferred to screw-cap test tubes for storage and analysis at 25°C. Liquid isotropic phases were identified as L_1 if miscible with water and L_2 if miscible with dodecanol. The presence of liquid crystalline material (LC) in any sample or phase was determined by the presence of birefringence under polarized light. Solid phases, presumably unsolubilized $C_{16}E_{20}$ or solid solutions, were characterized as S phases.

Determination of Phase Boundaries. For the L_1 phase region, $C_{12}OH$ was added dropwise to equilibrated binary mixtures of $C_{16}E_{20}$ and water until birefringence was observed, signifying the L_1 to L_1 + LC phase boundary. The L_2 to L_2 + LC phase boundary was determined by the addition of water to equilibrated $C_{16}E_{20}$–$C_{12}OH$ mixtures. Initially, these mixtures contained two phases, L_2 and solid $C_{16}E_{20}$. The phase boundary was detected when, after the addition of water, the mixture became one phase. Continued addition of water led to the L_2 to L_2 + LC phase boundary. In the L_1 + L_2 + LC region, analysis of the L_1 and LC equilibrated conjugate phases was undertaken as described under *Analysis of Samples*.

Determination of Tie Lines. Following location of the phase boundaries, tie lines in the L_1 + LC phase region were determined by preparing, equilibrating, and separating appropriate ternary component systems into L_1 and LC conjugate phases. The composition of each phase was determined by measuring the $C_{16}E_{20}$ and $C_{12}OH$ concentrations as described under *Analysis of Samples*. Particular attention was given to determining tie lines in the L_1 + LC region and preparing systems lying along each of these tie lines that contained increasing amounts of LC. In these circumstances, the composition of the L_1 and LC phases remained constant, while the amount of the LC phase present increased at the expense of its conjugate L_1 phase.

Analysis of Samples

To a known weight of equilibrated, separated conjugate L_1 or LC phase (approximately 1 g and 100 mg, respectively) was added 1 ml of an internal standard solution consisting of tetradecanol in methanol, such that the expected peak height ratios were between 0.3 and 3.0. Standard L_1 preparations (containing from 0.5 to 2.5 μg/ml $C_{12}OH$ and from 3 to 15 μg/ml $C_{16}E_{20}$) and LC preparations (containing from 7 to 40 mg/g of both $C_{12}OH$ and $C_{16}E_{20}$) were similarly treated with the tetradecanol internal standard solution. Additional water was added so that each standard contained approximately the same amount of water as the test samples. All samples were analyzed by reverse-phase HPLC using a Waters Model M45 solvent pump, Rheodyne Model 7125 injector, Alltech 10-μm C18 column, and Waters refractive index detector. The mobile phase was methanol/dichloroethane/water (200:7:3) at a flow rate of 1.25 ml/min and the injection volume was 100 μl. Retention times for $C_{12}OH$, tetradecanol, and $C_{16}E_{20}$ were 4, 5.5, and 8 min, respectively. A standard curve was calculated by regression of peak height ratio against standard concentration.

RESULTS AND DISCUSSION

Choice of Ternary System

Based on the criteria mentioned earlier, a number of different surfactants and oils were examined as potential components of the system. Nonionic surfactants as a class were chosen because of their low local and systemic toxicity (9,10). The polyoxyethylene glycol alkyl ether (Brij; C_xE_y) series was chosen for detailed investigation since a wide variety of alkyl and polyoxyethylene chain length materials is available, and there is additional evidence that these compounds do not irritate skin even at high concentrations (11). Oils investigated included isopropyl myristate and alkanols (C_xOH), in which x, the number of carbon atoms, ranged from 10 to 16. The ternary system judged best to meet the selection criteria was $C_{16}E_{20}$, $C_{12}OH$, and water.

The $C_{16}E_{20}$–$C_{12}OH$–Water Phase Diagram

The equilibrium phase diagram for this system at 25°C is shown in Fig. 1.

Binary Component Systems. When water was added to $C_{16}E_{20}$, a transparent anistropic LC gel phase was formed at about 80% $C_{16}E_{20}$. This gel became a highly viscous isotropic system as more water was added to give 61% $C_{16}E_{20}$. At 27% $C_{16}E_{20}$, a two-phase (L_1 + LC) system was formed; below 26%, $C_{16}E_{20}$ was completely soluble in water. These results are consistent with the work of Nixon *et al.* (13). With $C_{16}E_{20}$–$C_{12}OH$ mixtures, a solid phase gave way to a S + L_2 phase system when the concentration of $C_{12}OH$ was raised above 30%. This system persisted until the $C_{16}E_{20}$ concentration fell to 3%. The fact that $C_{16}E_{20}$ is not more soluble in $C_{12}OH$ reflects its hydrophilic nature (14) and suggests that this surfactant does not readily form micelles in nonpolar solvents. With water–$C_{12}OH$ mixtures, the L_1 and

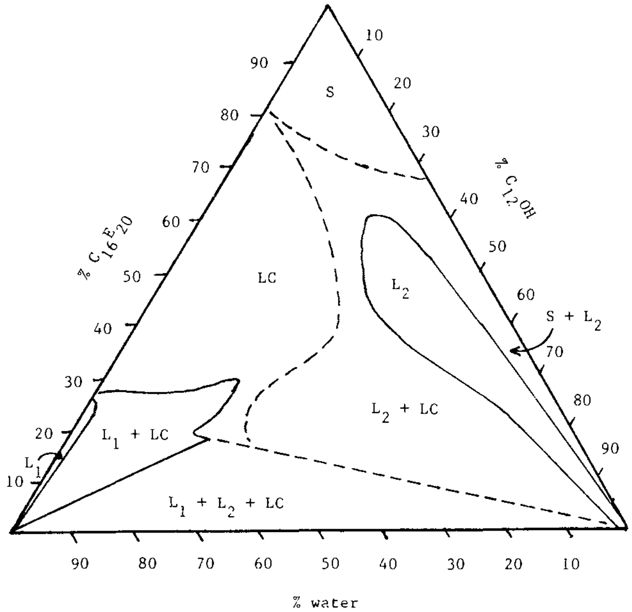

Fig. 1. Equilibrium phase diagram for the $C_{16}E_{20}$–$C_{12}OH$–water system (see text for definition of terms).

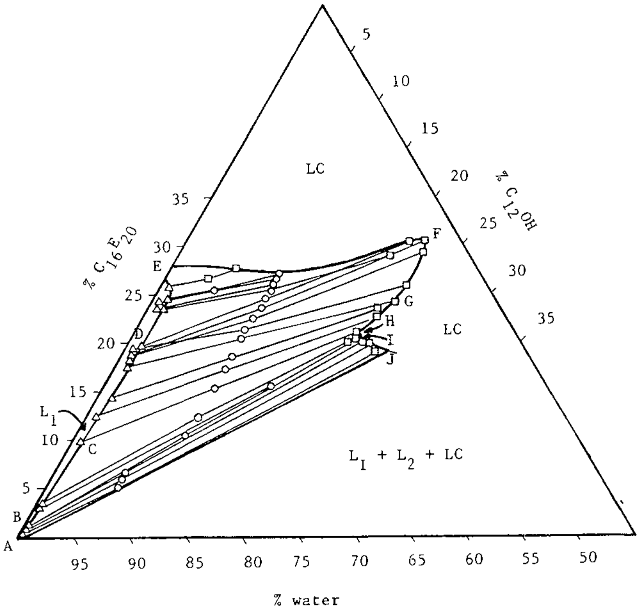

Fig. 2. Analysis of mixtures in the L_1 + LC region. (○) Composition of the original mixture; (△) composition of the L_1 phase; (□) composition of the LC phase.

L_2 phase boundaries exist at concentrations too low (99.9 and 2.2% water, respectively) to show clearly in Fig. 1.

Ternary Component Single-Phase Regions. The L_1 region was found to be wedge shaped and extends upto 26% $C_{16}E_{20}$ in water. A two-phase (L_1 + LC) region exists to the right of the L_1 phase boundary, which was produced with mixtures containing 0.2 g of $C_{12}OH$ per g of $C_{16}E_{20}$. This boundary represents the stage at which the micelles reach saturation, leading to the formation of a conjugate LC phase which can accommodate a greater ratio of hydrocarbon ($C_{12}OH$) to polyoxyethylene moiety ($C_{16}E_{20}$). The L_2 region is bounded on one side by the S + L_2 region and on the other by L_2 + LC and is able to contain almost 60% $C_{16}E_{20}$. This is in contrast to the fact that, in the absence of water, the solubility of $C_{16}E_{20}$ in $C_{12}OH$ is only 3%. This finding is consistent with the view that polyoxyethylene-type surfactants more readily form micelles in nonaqueous solutions when water is present (15). Significant visual differences were observed within different parts of the LC region shown in Fig. 1. However, it was not possible to delineate these regions accurately since no attempt was made to separate LC mixtures and identify structures by any means other than birefringence.

Ternary Component Two-Phase Regions. The L_1 + LC region determined by analysis of the separated conjugate phases is shown in Fig. 2, which is a detailed enlargement of the lower left-hand corner of Fig. 1. The compositions of the original and the associated conjugate phases, joined by a tie line, are as indicated. Systems prepared from compositions above the tie line drawn between point C and point H in Fig. 2 and containing less than 30% LC gradually separated on standing. In contrast, LC in mixtures below the tie line CH remained dispersed indefinitely. Before separation, the mixtures in the area ABIJ were similar in appearance to those in the area CDGH. When mixtures in the latter area were separated, the LC phase was somewhat translucent. In contrast, the separated LC phase from mixtures in ABIJ was opaque and textured, suggesting that the LC along the boundary GH was of a different structure from that along IJ. From a stability standpoint, the L_1 + LC systems below the tie line CH exhibited the best potential as topical vehicles.

L_2 + LC systems separated readily in all but those mixtures which were close to 100% LC. Mixtures in this region of the phase diagram were not determined by analysis of the conjugate phases. Rather, the boundaries shown in Fig. 1 were obtained by centrifuging mixtures near the LC to L_2 + LC boundary and noting whether or not an L_2 phase was present. The $L_1 + L_2$ region consisted of $C_{12}OH$–water mixtures with only trace amounts (less than 0.2%) of $C_{16}E_{20}$ and is not shown in Fig. 1. It, together with the S + L_2 and S + LC regions, was not considered further.

Ternary Component Three-Phase Regions. The only three-phase region investigated in detail was $L_1 + L_2$ + LC. Centrifugation of the systems studied indicated that the density of the phases increased in the order L_2 < LC < L_1. The concentrations of $C_{16}E_{20}$ and $C_{12}OH$ in the L_1 phase were too low to be determined, as were those of $C_{16}E_{20}$ in the L_2 phase. The concentration of water was not determined in the L_2 phase but was assumed to lie close to the solubility of water in dodecanol, namely, 2.2% (w/w).

Location of the phase boundary between the L_1 + LC and the $L_1 + L_2$ + LC phase regions was also investigated by determining the rate of sedimentation of a series of mixtures known to be on either side of the boundary. The approach was based on that of Friberg *et al.* (16), except that the water content, rather than the ratio of $C_{12}OH$ to water, was held constant at 90% (w/w). The $C_{16}E_{20}$ content was then varied from 1 to 6% (w/w). To compare the rates at which the mixtures underwent separation, the log of the degree of separation was plotted as a function of time. When the slope of the plot (equivalent to a separation coefficient) was plotted against the percentage (w/w) $C_{16}E_{20}$ (Fig. 3), a distinct break was observed at 4% (w/w) $C_{16}E_{20}$. This system corresponds to the tie line labeled AJ in Fig. 2 and confirms the phase boundary drawn in Fig. 1 between the L_1 + LC and the L_1 + L_2 + LC phase regions.

CONCLUSIONS

Construction of the phase diagram for a multicomponent system provides a means of systematically changing components and phases in order to study the effect of various phases on desired vehicle parameters such as stability, rheology, and release and/or penetration of a drug, particularly once tie lines within the phase region(s) of interest have been identified. This report illustrates how such an approach can provide information from which the effect of LC on transdermal delivery of drug from vehicle formulations can be rationally explored. Thus, determination of relevant areas of the $C_{16}E_{20}$–$C_{12}OH$–water phase diagram revealed that significant portions of the phase diagram with surfactant concentrations not exceeding 20% (w/w) contained either L_1 + LC or L_1 + L_2 + LC phase mixtures judged to be suitable vehicles from the standpoint of physical stability, appearance, and spreadability. The influence of LC phases of known composition in well-characterized ternary phase systems on the percutaneous absorption of drugs across excised human skin is the subject of the following report.

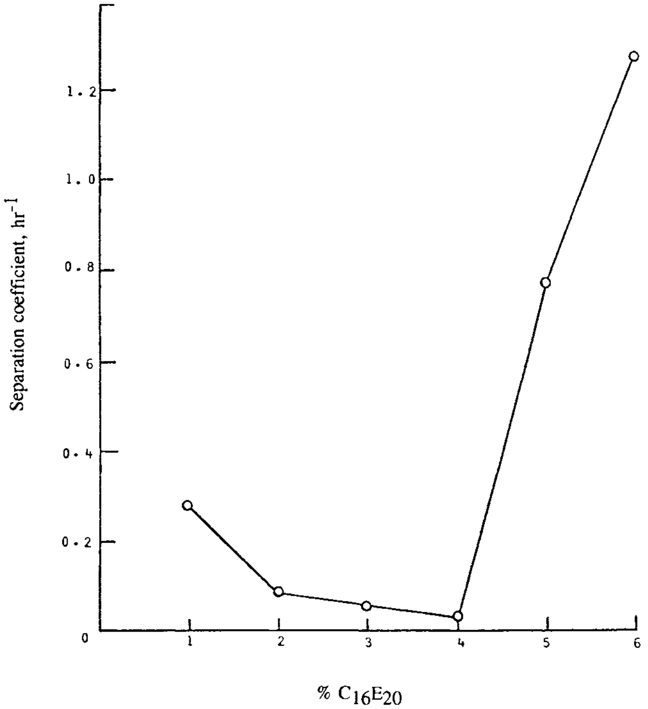

Fig. 3. Separation coefficient of $C_{16}E_{20}$–$C_{12}OH$–water mixtures as a function of percentage $C_{16}E_{20}$ in mixtures containing 90% water.

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