

Rapid report

Liquid-liquid immiscibility in lipid monolayers

John P. Hagen, Harden M. McConnell *

Department of Chemistry, Stanford University, Stanford, CA 94305-5080, USA

Received 27 May 1997; accepted 29 May 1997

Abstract

Some binary lipid mixtures form coexisting liquid phases when spread at the air/water interface. This work describes the pressure–composition phase diagrams of binary mixtures of four unsaturated phosphatidylcholines with dihydrocholesterol. These four binary mixtures have critical compositions of approximately fifty mole percent, and average critical exponents of 0.25 ± 0.07 . The data can also be approximated by a regular solution thermodynamic model, yielding parameters for the non-ideality of these mixtures. © 1997 Elsevier Science B.V.

Keywords: Phase diagram; Fluorescence microscopy; Critical point

Fluorescence microscopy has been used to study lipid monolayers at the air/water interface [1–3]. Monolayers composed of mixtures of cholesterol and phospholipids are of particular interest as biological cell membranes contain these lipids [4]. Some of these mixtures exhibit liquid-liquid immiscibility [5,6]. It is of interest to know what molecular features give rise to liquid-liquid phase separation [7,8]. This paper presents the room-temperature surface pressure–composition phase diagrams for binary mixtures of dimyristoleoylphosphatidylcholine (DMoPC), dipalmitoleoylphosphatidylcholine (DPoPC), dioleoylphosphatidylcholine (DOPC), and dieicosenoylphosphatidylcholine (DEPC) with dihydrocholesterol

(DChol). The acyl chains are 14, 16, 18, and 20 carbon atoms in length, respectively. These acyl chains are *cis*-unsaturated at position 9 with the exception of DEPC which is *cis*-unsaturated at position 11. The phospholipids were chosen to probe the effect of phospholipid acyl chain length and unsaturation on the binary phase diagrams with DChol.

Phase diagrams for binary mixtures of DMoPC, DPoPC, DOPC, and DEPC with DChol were mapped using epifluorescence microscopy. A fluorescent probe was incorporated into the monolayer. The probe partitions unequally into the two phases, providing contrast. Under the experimental conditions described, low concentrations of the fluorescent probe (0.1–1 mol%) have a negligible effect on the derived phase diagram.

Materials and methods. The phospholipids DMoPC, DPoPC, DOPC, and DEPC were purchased from Avanti Polar Lipids, Inc. The fluorescent lipid probe *N*-(Texas Red sulfonyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (TR-DPPE) was purchased from Molecular Probes, Inc. The synthetic cholesterol analog dihy-

Abbreviations: DChol, dihydrocholesterol; DEPC, dieicosenoylphosphatidylcholine; DMoPC, dimyristoleoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; DPoPC, dipalmitoleoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; TR-DPPE, *N*-(Texas Red sulfonyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt

* Corresponding author. Fax: +1 (415) 723-4943.

drocholesterol (DChol) was purchased from Sigma Chemical Company. All chemicals were used without further purification. The experiments were carried out at 20–22°C. DChol rather than cholesterol itself is used to minimize air oxidation of the steroid.

The monolayers were spread from 1 mM chloroform solution containing 0.25 mol% TR-DPPE dye onto a subphase of distilled, deionized water. The monolayers were blanketed with argon to minimize oxidation of the lipids. Compression and expansion of the film were carried out with a movable barrier and the surface pressure was measured with a Wilhelmy plate. The monolayer was viewed with a Zeiss epifluorescence microscope fitted with a Cohu low-light-level video camera. After spreading, the monolayers were compressed while under observation. Phase boundaries were determined by noting the pressure at which the two phases become homogeneous. For the lipid mixtures described the compression–expansion of the monolayer giving rise to the

disappearance–appearance of the two phases is reversible and reproducible in the vicinity of the phase boundary, to within less than plus or minus one mN/m.

Results and discussion. The derived phase diagrams for the binary systems of DMoPC, DPOPC, DOPC, and DEPC with DChol are shown in Fig. 1. These binary phase diagrams reveal several trends when compared to each other and with the phase diagrams of other systems. To make these comparisons it is useful to fit the data to models that characterize liquid-liquid immiscibility with critical points.

The data can be fit to the following critical exponent equation:

$$|X - X_c| = F(\pi_c - \pi)^\beta \quad (1)$$

Here X is the mole fraction of dihydrocholesterol in either phase, X_c is the mole fraction of dihydro-

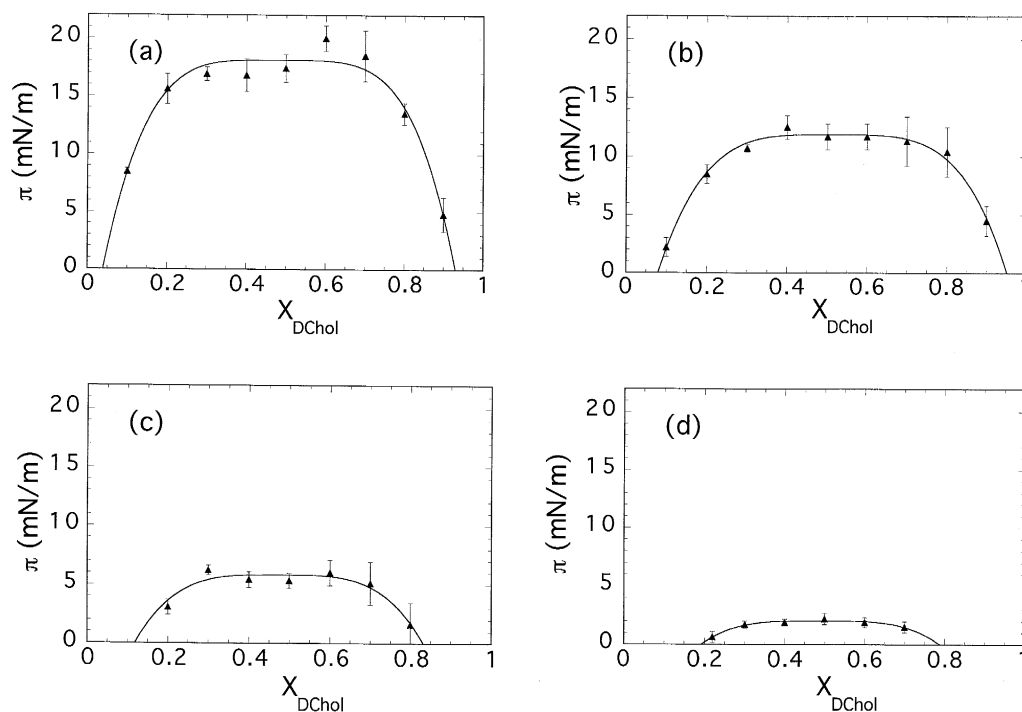


Fig. 1. The pressure–composition phase diagrams for four phospholipids with dihydrocholesterol. The data are fit to the equation $|X - X_c| = F(\pi_c - \pi)^\beta$ where X is the mole fraction of dihydrocholesterol in either phase, X_c is the mole fraction of dihydrocholesterol of the critical mixture, β is the critical exponent, and F is an adjustable parameter. (a): The DMoPC-DChol phase diagram. $X_c = 0.48 \pm 0.01$, $\pi_c = 18 \pm 1$, $\beta = 0.23 \pm 0.06$, and $F = 0.2 \pm 0.4$. (b): The DPOPC-DChol phase diagram. $X_c = 0.52 \pm 0.01$, $\pi_c = 12 \pm 1$, $\beta = 0.25 \pm 0.03$, and $F = 0.2 \pm 0.2$. (c): The DOPC-DChol phase diagram. $X_c = 0.48 \pm 0.01$, $\pi_c = 6 \pm 1$, $\beta = 0.27 \pm 0.07$, and $F = 0.2 \pm 0.3$. (d): The DEPC-DChol phase diagram. $X_c = 0.49 \pm 0.01$, $\pi_c = 2.0 \pm 0.4$, $\beta = 0.3 \pm 0.1$, and $F = 0.2 \pm 0.6$.

cholesterol at the critical composition, β is the critical exponent, and F is an adjustable parameter. The average value of β for these systems was determined to be 0.25 ± 0.07 . For comparison, the classical theoretical value of β for mathematically two-dimensional systems is 0.125 while that of three-dimensional systems is 0.32 [9]. Although these monolayers are only one molecule in thickness the intermolecular forces are not strictly two-dimensional [10]. The measured value of β is thus plausible.

The values of the critical pressure π_c given for these unsaturated phosphatidylcholines contrast with those known for similar saturated phosphatidylcholines. The saturated analog of DMOPC is dimyristoylphosphatidylcholine, DMPC. Its mixtures have a critical pressure, π_c , of 10.2 mN/m [11], while we see in Fig. 1 that π_c for DMOPC is 18 mN/m. The saturated analog of DPOPC is dipalmitoylphosphatidylcholine, or DPPC. Its mixtures with DChol have a π_c of approximately 2 mN/m (S. Perković, unpublished data). Other work suggests that these mixtures with cholesterol have a π_c of approximately 4 mN/m [7]. However, Fig. 1 shows that π_c for DPOPC is 12 mN/m. Unsaturation at position 9 in phosphatidylcholines therefore raises π_c by 8–10 mN/m.

The values of the critical composition X_c , given by the fits for these unsaturated phosphatidylcholines also contrast with those known for similar saturated phosphatidylcholines. The saturated phosphatidylcholines DMPC and DPPC have critical compositions X_c of approximately 0.3 [11], while all four of the

unsaturated phosphatidylcholines used in this study have critical compositions X_c of approximately 0.5.

It is also instructive to compare the values of π_c for the various unsaturated lipids with each other. In Fig. 2, π_c is plotted as a function of phospholipid acyl chain length. Note that there is a linear trend towards greater miscibility with increasing phospholipid acyl chain length. A similar trend of greater miscibility with increasing acyl chain length was found by Slotte [7] in mixtures of *saturated* phosphatidylcholines with cholesterol.

These phase diagrams can be accounted for with the following simple thermodynamic model. We begin by considering a binary mixture of liquids that can be modeled as a regular solution [12]. The chemical potential of component 1 is written

$$\mu_1 = \mu_1^0 + RT \ln X_1 + (A_1 + aX_2^2)(\pi - \pi_0) + 2RT_c^0 X_2^2 \quad (2)$$

Here μ_1^0 is the chemical potential of pure component 1 at the pressure $\pi = \pi_0$, X_1 and X_2 are the mole fractions of components 1 and 2, T_c^0 is the critical temperature when the pressure is π_0 , A_1 is the molar area of component 1, and a is an area contraction parameter. A similar equation holds for component 2 with the subscripts 1 and 2 reversed. Mixtures of phosphatidylcholines and cholesterol are known to contract in area when mixed at constant pressure, with contraction parameters of the order of minus 40 square Ångströms per molecule. From Eq. (2) it follows that the critical temperature of the mixture when the pressure is π is $T_c(\pi) = T_c^0 + (\pi - \pi_0)a/2R$. When the pressure π is equal to the critical pressure π_c the critical temperature is room temperature, $T_c(\pi_c) = T$. Thus $T_c(\pi) = T + (\pi - \pi_c)a/2R$. When the pressure is above the critical pressure, room temperature is above the critical temperature and the system is one phase, $T > T_c(\pi > \pi_c)$. When the pressure is below the critical pressure, room temperature is below the critical temperature and the monolayer shows two immiscible liquid phases $T < T_c(\pi < \pi_c)$. The simple thermodynamic chemical potential in Eq. (2) gives rise to a symmetric phase diagram for which the equation is

$$\pi = \pi_c + (2RT/a)[\ln(X_1/X_2)/2(X_1 - X_2) - 1] \quad (3)$$

Eq. (3) was used to fit the data for the four binary

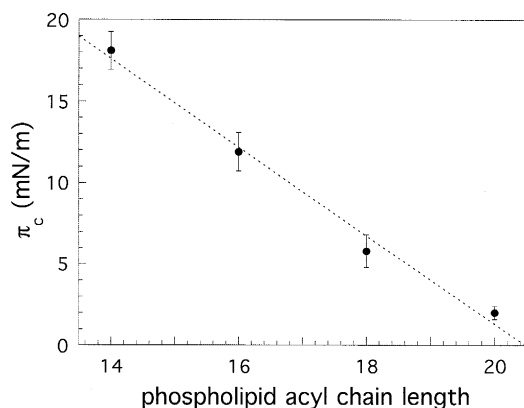


Fig. 2. The critical pressures of phospholipid/dihydrocholesterol mixtures vs. phospholipid acyl chain length. The dashed line is a linear fit.

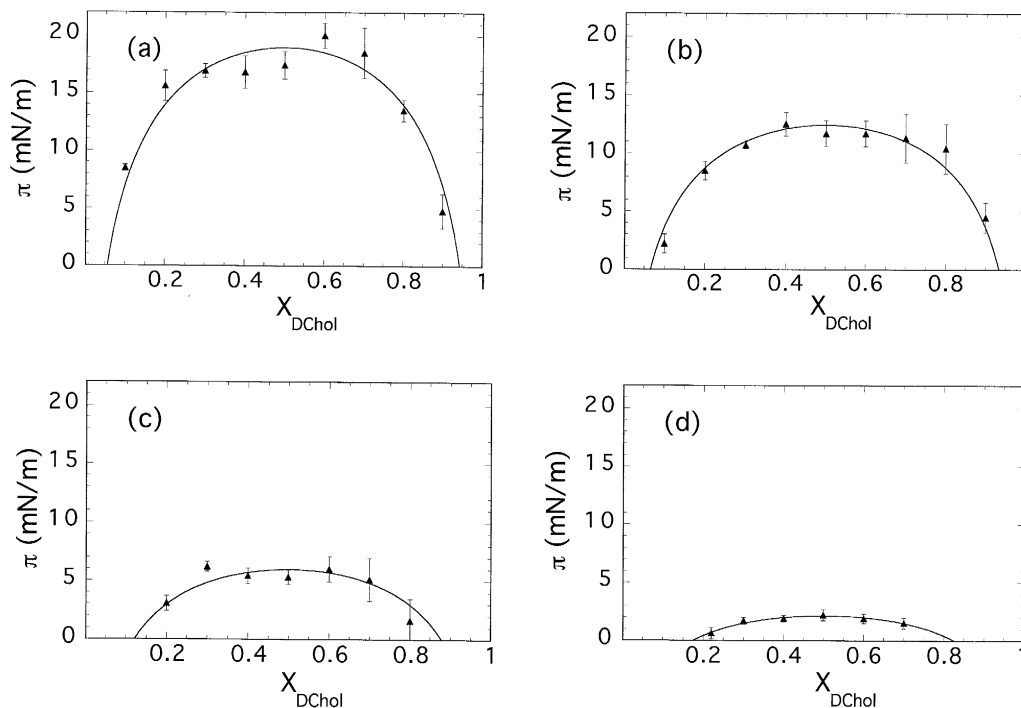


Fig. 3. The pressure–composition phase diagrams for four phospholipids with dihydrocholesterol. The data are fit to Eq. (3). (a): The DMOPC-DChol phase diagram. $\pi_c = 17.5 \pm 1$ mN/m and $a = -26 \pm 3$ Å²/molecule. (b): The DPDChol phase diagram. $\pi_c = 12.4 \pm 1$ mN/m and $a = -35 \pm 3$ Å²/molecule. (c): DOPC-DChol phase diagram. $\pi_c = 6.15 \pm 1$ mN/m and $a = -37 \pm 6$ Å²/molecule. (d): The DEPC-DChol phase diagram $\pi_c = 2.18 \pm 1$ mN/m and $a = -74 \pm 8$ Å²/molecule. The critical pressures used here were judged to give the best fit to the data using Eq. (3), and are only slightly different from the critical pressures given in Fig. 1.

mixtures. As will be seen the fits are rather good. The critical exponent required by Eq. (3) is 0.5, substantially larger than the value of β derived by fitting the data to Eq. (1). The derived values of the contraction parameter are given in the legend to Fig. 3 and are consistent with earlier work [11,13].

The most surprising result from the present work is the symmetry of the phase diagrams about a critical composition of 0.5 mole fraction. This implies that in each binary mixture the phosphatidylcholine and dihydrocholesterol molecules act equivalently to one another in their physical properties. This should provide a significant simplification in the molecular modeling of these mixtures.

A central problem in this field is the question of the existence of critical point transitions in lipid bilayers, and the possible relevance to biological membranes [14]. Phase diagrams for monolayers such as those given here may facilitate the search for critical point phase separations in bilayers.

We thank Dr. Sara Perković (in this laboratory) for helpful discussions concerning her unpublished observations on binary mixtures of DChol and DPPC. This work was supported by National Science Foundation Grant No. NSF MCB 9316256.

References

- [1] C.M. Knobler, *Science* 24a (1990) 870–874.
- [2] H. Möhwald, *Annu. Rev. Phys. Chem.* 41 (1990) 441–476.
- [3] H.M. McConnell, *Annu. Rev. Phys. Chem.* 42 (1991) 171–195.
- [4] D.B. Datta, *Membrane Biochemistry*, Floral Publishing, 1987, ch. 3, pp. 72–76.
- [5] S. Subramaniam, H.M. McConnell, *J. Phys. Chem.* 91 (1987) 1715–1718.
- [6] C.L. Hirschfeld, M. Seul, *J. Phys. Fr.* 51 (1990) 1537–1540.
- [7] J.P. Slotte, *Biochim. Biophys. Acta* 1238 (1995) 118–126.
- [8] J.P. Slotte, *Biochim. Biophys. Acta* 1237 (1995) 127–134.

- [9] J.S. Rowlinson, B. Widom, *The Molecular Theory of Capillarity*, Clarendon Press, Oxford, 1982, ch. 9, p. 265.
- [10] D.J. Keller, H.M. McConnell, V.T. Moy, *J. Phys. Chem.* 90 (1986) 2311–2315.
- [11] K.Y.C. Lee, H.M. McConnell, *Biophys. J.* 8 (1995) 1740–1751.
- [12] J.G. Kirkwood, I. Oppenheim, *Chemical Thermodynamics*, McGraw-Hill, New York, 1961, ch. 11.
- [13] M.C. Phillips, *Progress in Surface and Membrane Science*, Academic Press, New York, 1972, vol. 5.
- [14] J.P. Hagen, H.M. McConnell, *Biochim. Biophys. Acta* 1280 (1996) 169–172.