Soft Matter

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/softmatter

Journal Name

COMMUNICATION

Spontaneous Vesicle Formation in a Deep Eutectic Solvent

Saffron J. Bryant [a], Rob Atkin[b] and Gregory G. Warr[a]

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Solvent penetration experiments and small-angle X-ray scattering reveal that phospholipids dissolved in a deep eutectic solvent (DES) spontaneously self-assemble into vesicles above the lipid chain melting temperature. This means DESs are one of the few nonaqueous solvents that mediate amphiphile self-assembly, joining a select set of H-bonding molecular solvents and ionic liquids.

Deep eutectic solvents (DESs) are mixtures of salts or, more commonly, salts and molecular H-bond donors, that form a eutectic with a melting point much lower than the individual components.[1] DESs are rapidly emerging as viable alternatives to conventional ionic liquids (ILs) as green solvents for a wide range of applications because of their low toxicity, ease of preparation, and low cost.[2, 3] The most widely studied DES is a 1:2 mol:mol mixture of choline chloride-urea (ChCl-U, Figure 1).[1, 4]

DESs are promising solvents for the formation of amphiphile self-assembly phases because, on the basis of their polarity, solvophobic interactions with hydrophobic amphiphile tail groups are anticipated. Despite their superficial resemblance, ChCl-U and related DESs do not exhibit the amphiphilic nanostructure characteristic of most ILs.[5] This reduces the solubility of non-polar alkyl chains, which makes selection of polar groups of the amphiphile critical. Spontaneous amphiphilic self-assembly into lamellar phases or vesicles has yet to be reported in deep eutectic solvents. Pal et al. reported that amphiphiles did not dissolve in the choline chloride-urea DES unless at least 5 wt% (43 mol%) water was added;[6] sodium dodecyl sulfate micelles were then identified in these choline chloride/urea/water mixtures, although Arnold et al. have more recently demonstrated micelle formation by sodium dodecyl sulfate in pure choline chloride-urea systems.[7]

[a] School of Chemistry, The University of Sydney, Sydney, NSW, Australia, 2006.
[b] Discipline of Chemistry, The University of Newcastle, Newcastle, NSW, Australia, 2308

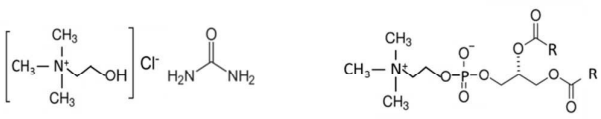

Figure 1. Structure of phosphatidylcholine lipids and the DES choline chloride-urea. R = $C_{13}H_{27}$ (DMPC); $C_{15}H_{31}$ (DPPC); $C_{17}H_{35}$ (DSPC); or a mixture (egg PC).

Large unilamellar vesicle (LUV) dispersions of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) in a DES have been reported, formed by freeze-drying aqueous solutions containing choline chloride-urea.[8] However, as these vesicles were pre-existing in an aqueous medium, this raises the question as to whether the vesicles in the DES are thermodynamically stable or rather a suspension of the aqueous structures that are long lived due to the insolubility of the lipid in the DES, akin to enzyme suspensions in hydrophobic solvents.[9] Thus, in this work we address three key questions: (1) can lipids be solubilised in the choline chloride-urea DES when the water concentration is negligible? (2) Do lipids spontaneously self-assemble in the DES? (3) Do their characteristic properties vary with lipid chain structure in a way similar to aqueous systems?

Vesicles are formed when a bilayer membrane encapsulates solvent, separating it from the external environment.[10] Phospholipids (Figure 1) self-assemble in water[11] and other polar solvents[12, 13] due to solvophobic interactions between their hydrocarbon chains and the solvent.[11, 14] Phospholipid phase diagrams are dominated by bilayer structures,[15-18] including lamellar and bicontinuous cubic phases, as well as vesicles, because their two tail groups lead to flat packing geometries.[11] Vesicles are thus excellent models for examining compartmentalisation and hence for living cells and prebiotic structures.[19-22] Phosphatidylcholine lipids are chosen as model amphiphiles as they contain a choline moiety, which should optimise the solvophilicity by mimicking the cationic component of the DES.

The choline chloride-urea DES was prepared using previously described methods.[1, 2] Briefly, urea (Merck, 99%) and choline chloride (Sigma Aldrich, 98%) were dried separately under vacuum and then mixed in a 2:1 ratio (urea:choline chloride, denoted ChCl-U). The components were mixed by heating to 70°C and then cooled to room temperature, and checked for water content using Karl-Fischer titration (<1 wt%). The solution was also checked for purity and mole ratio using NMR. Four different phosphatidylcholine (PC) lipids were used as shown in Figure 1. Three contained saturated alkyl chains of differing lengths; 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), as well as egg PC, which is a mixture containing 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) as its majority constituent. All lipids were purchased from Sigma-Aldrich with ≥99% purity and were used as received. Solvent penetration experiments were performed as previously described[23-25] to examine lyotropic phase formation over a range of concentrations and temperatures. This method is well established for identifying vesicle formation.[26-28] A spot of lipid, approximately 40µm thick, was placed on a microscope slide with a cover slip. A drop of the solvent was then placed on the outer edge of the cover slip where it was drawn into contact with the lipid via capillary action. This creates a concentration gradient. A heating stage was then used to determine the effect of temperature on phase formation.

At ambient temperature (23 °C) the DES penetrated the lipids and the characteristic optical texture of a (non-swelling) lamellar phase was observed under crossed polarisers.[9, 11-12] Above a minimum penetration temperature, a highly swollen and dispersible lamellar phase spontaneously formed, which produced the myelenic features shown in Figure 2.After just a few minutes the myelenic features develop into Maltese cross textures which are the signature of vesicle formation (Figure 2).[23, 25] The penetration temperatures of the lipids are presented in Table 1, along with the aqueous values for comparison. In both water and the DES, the penetration temperature increases with lipid alkyl chain length, but penetration temperatures for the long chain DPPC and DSPC lipids are notably higher in the DES than water.

The increase in penetration temperature with saturated chain length is consistent with the phase behaviour of aqueous phospholipids. In water, phospholipids undergo a phase transition upon warming from the highly ordered L_β phase (in which the alkyl chains are crystalline and lie perpendicular to the bilayer) into an L_α phase where the chains have liquid-like organisation.[29, 30] The resultant flexibility of the L_α phase allows solvent penetration and swelling, ultimately leading to vesicle formation. The same effects are expected here.

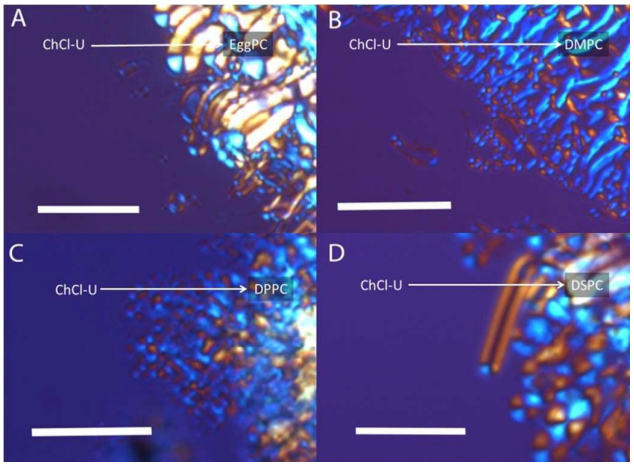

Figure 2. Polarizing optical microscopy images of phospholipids (A: EggPC, B: DMPC, C: DPPC, D: DSPC) with choline chloride-urea (ChCl-U) DES, showing vesicle formation above their transition temperatures (Table 1). Scale bars = 50µm.

Lamellar phase formation is confirmed by SAXS patterns, gathered on a line-collimated SAXSess (Anton Parr). The example given in Figure 3, 67 wt% DPPC in ChCl-U, clearly shows a series of peaks with spacings in the ratio 1:2:3:4 at 25°C.[31-33] Heating the sample above its transition temperature caused the peaks to broaden and shift to higher q, corresponding to melting of the chains in the bilayers and decreasing order.

The bilayer thickness, t, was calculated from the primary peak position $D^* = 2\pi/q$, and lipid volume fraction, ϕ, using $t = \phi D^*$.[34] This gives bilayer thicknesses of 51 Å at 25°C and 40 Å at 70°C, which are comparable to those found in water by the same method.[35, 36] As the acyl chains melt and become less rigid, going from an L_β to an L_α phase, the DPPC bilayer shrinks in ChCl-U, just as it does in water.[37, 38]

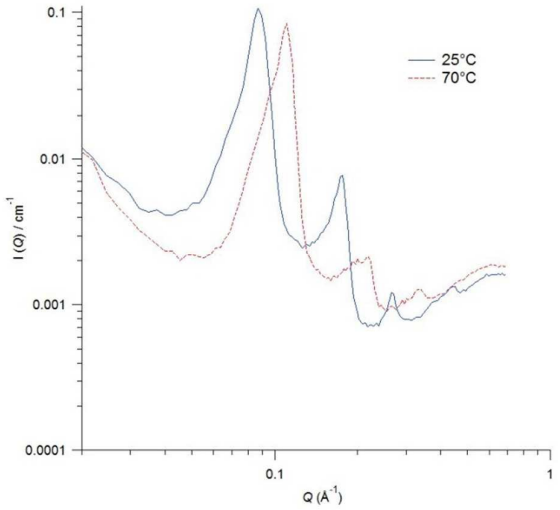

Figure 3. SAXS patterns of 67wt% DPPC in ChCl-U, above and below the transition temperature.

Table 1. Temperatures (°C) where lamellar swelling and spontaneous vesicle formation by lipids in water and DES was observed by polarizing optical microscopy flooding experiments.

	Egg PC	DMPC	DPPC	DSPC
Water	< 23*	23 (23)[15]	41 (41)[15]	56 (54)[15]
DES	< 23*	26	52	62

* Phase behaviour below room temperature was not investigated.

In water, the chain-melting temperature of phospholipids, and consequently the penetration temperature, depends primarily on the alkyl chain length and saturation of the hydrocarbon tail. The *cis* unsaturation in one alkyl chain of egg PC dramatically reduces the chain melting temperature. This is moderated by head-group structure as interactions between head-groups can stabilise either solid or liquid order in the chains.[15, 39] The same qualitative trends with chain length and saturation are seen for lipids swollen in ChCl-U. The chain-melting temperatures for the saturated phospholipids are markedly higher in the ChCl-U DES than in water (Table 1). Preliminary results indicate that a similar effect also occurs in other DESs with malonic acid and ethylene glycol in place of urea, implicating the ionic component of the DES. This may thus be a result of the chloride ion binding to the lipid membrane; this has been reported to (weakly) affect phase transition temperatures in aqueous systems through a change in Hamaker constant.[40] In the highly-screened and high ionic-strength environment of an ionic liquid or DES, and especially in zwitterionic lipids, ion-specific effects are expected to be more pronounced. A more systematic study of this phenomenon and a Hofmeister-like effect is in progress.

These results demonstrate that the choline chloride–urea DES is able to penetrate and solubilise phosphatidylcholine-based lipids even when the water concentration is negligible.

Above the penetration temperature, the DES swells the lipids, and an L_α lamellar phase spontaneously forms, which transforms to vesicles with time. As in water, the penetration temperature depends on the length of the lipid alkyl chain, but penetration temperatures are higher in the DES than water for long alkyl chain lipids. This is attributed to chloride ions, which are present at high concentration, binding to the lipid membrane.

Until now, only water, a select set of H-bonding molecular solvents, and some ionic liquids,[11, 12, 15, 41, 42] were known to support amphiphile self-assembly. The capacity of choline chloride–urea (and other similar DESs) to mediate vesicle formation means that DESs must be added to this small set of solvents that engender solvophobic self-assembly. The formation of lipid vesicles (or other self-assembled structures) in biocompatible DESs paves the way to new possibilities in industry, including compartmentalised or isolated reaction systems or substrate delivery,[6] and spontaneous self-assembly broadens the potential range of extreme environments in which life may arise.[43]

Acknowledgements

This work was supported by an Australian Research Council Discovery Grant. RA thanks the ARC for a Future Fellowship. The authors thank Mr Ajantha Abey for assistance with screening additional DES systems.

References

1. A. P. Abbott, G. Capper, D. L. Davies, R. K. Rasheed and V. Tambyrajah, *Chem. Commun.*, 2003, 70-71.
2. A. P. Abbott, D. Boothby, G. Capper, D. L. Davies and R. K. Rasheed, *J. Am. Chem. Soc.*, 2004, **126**, 9142-9147.
3. E. L. Smith, A. P. Abbott and K. S. Ryder, *Chem. Rev.*, 2014, **114**, 11060-11082.
4. Q. Zhang, K. De Oliveira Vigier, S. Royer and F. Jerome, *Chem. Soc. Rev.*, 2012, **41**, 7108-7146.
5. R. Hayes, G. G. Warr and R. Atkin, *Chem. Rev.*, 2015, **115**, 6357-6426.
6. M. Pal, R. Rai, A. Yadav, R. Khanna, G. A. Baker and S. Pandey, *Langmuir*, 2014, **30**, 13191-13198.
7. T. Arnold, A. J. Jackson, A. Sanchez-Fernandez, D. Magnone, A. E. Terry and K. J. Edler, *Langmuir*, 2015, **31**, 12894-12902.
8. M. C. Gutiérrez, M. L. Ferrer, C. R. Mateo and F. del Monte, *Langmuir*, 2009, **25**, 5509-5515.
9. A. M. Klibanov, *Nature*, 2001, **409**, 241-246.
10. B. A. Cornell, G. C. Fletcher, J. Middlehurst and F. Separovic, *Biochim. Biophys. Acta, Biomembr.*, 1982, **690**, 15-19.
11. C. Tanford, *Science*, 1978, **200**, 1012-1018.
12. F. Gayet, J.-D. Marty, A. Brûlet and N. L.-d. Viguerie, *Langmuir*, 2011, **27**, 9706-9710.
13. T. J. McIntosh, A. D. Magid and S. A. Simon, *Biochemistry*, 1989, **28**, 7904-7912.
14. D. J. Hanahan, *A Guide to Phospholipid Chemistry*, Oxford University Press, USA, 1997.
15. R. Koynova and M. Caffrey, *Biochim. Biophys. Acta, Rev. Biomembr.*, 1998, **1376**, 91-145.
16. P. Stano and P. L. Luisi, *Chem. Commun.*, 2010, **46**, 3639-3653.
17. A. C. Woodka, P. D. Butler, L. Porcar, B. Farago and M. Nagao, *Phys. Rev. Lett.*, 2012, **109**, 058102.
18. B. J. Ravoo, W. D. Weringa and J. B. F. N. Engberts, *Langmuir*, 1996, **12**, 5773-5780.
19. M. Bitbol and P. L. Luisi, *J. R. Soc., Interface*, 2004, **1**, 99-107.
20. R. A. Black, M. C. Blosser, B. L. Stottrup, R. Tavakley, D. W. Deamer and S. L. Keller, *Proc. Natl. Acad. Sci. U.S.A.*, 2013, **110**, 13272-13276.
21. P. Carrara, P. Stano and P. L. Luisi, *ChemBioChem*, 2012, **13**, 1497-1502.
22. I. A. Chen and P. Walde, *Cold Spring Harbor Perspect. Biol.*, 2010, **2**, a002170.

23. R. G. Laughlin, *The Aqueous Phase Behaviour of Surfactants*, Academic Press, San Diego, CA 92101, 1994.
24. M. U. Araos and G. G. Warr, *J. Phys. Chem. B*, 2005, **109**, 14275-14277.
25. R. G. Laughlin, *Adv. Colloid Interface Sci.*, 1992, **41**, 57-79.
26. L. S. Hirst, in *Fundamentals of Soft Matter Science*, Taylor and Francis, Boca Raton, FL, 2013, pp. 52-55.
27. F. B. Rosevear, *Journal of the American Oil Chemists' Society*, 1954, **31**, 628-639.
28. W. J. Benton, in *Physics of Amphiphilic Layers*, eds. J. Meunier, D. Langevin and N. Boccara, Springer Berlin Heidelberg, 1987, vol. 21, ch. 28, pp. 207-209.
29. C. Grabielle-Madelmont and R. Perron, *J. Colloid Interface Sci.*, 1983, **95**, 471-482.
30. A. Tardieu, V. Luzzati and F. C. Reman, *J. Mol. Biol.*, 1973, **75**, 711-733.
31. T. Imura, S. Ikeda, K. Aburai, T. Taira and D. Kitamoto, *Journal of Oleo Science*, 2013, **62**, 499-503.
32. J.-H. Ahn and W.-C. Zin, *Macromol. Res.*, 2003, **11**, 152-156.
33. C. Rodriguez, M. H. Uddin, H. Furukawa, A. Harashima and H. Kunieda, in *Trends in Colloid and Interface Science XV*, ed. P. Koutsoukos, Springer Berlin Heidelberg, 2001, vol. 118, ch. 12, pp. 53-56.
34. T. Nylander and B. Lindman, *Lipid and Polymer-Lipid Systems*, Springer Berlin Heidelberg, 2003.
35. L. J. Lis, M. McAlister, N. Fuller, R. P. Rand and V. A. Parsegian, *Biophysical Journal*, 1982, **37**, 657-665.
36. J. F. Nagle, R. Zhang, S. Tristram-Nagle, W. Sun, H. I. Petrache and R. M. Suter, *Biophysical Journal*, 1996, **70**, 1419-1431.
37. Z. V. Leonenko, E. Finot, H. Ma, T. E. S. Dahms and D. T. Cramb, *Biophysical Journal*, 2004, **86**, 3783-3793.
38. W. J. Sun, S. Tristram-Nagle, R. M. Suter and J. F. Nagle, *Biophysical Journal*, 1996, **71**, 885-891.
39. D. Chapman, R. M. Williams and B. D. Ladbrooke, *Chem. Phys. Lipids*, 1967, **1**, 445-475.
40. F. Tölgyesi, S. Györgyi and I. P. Sugár, *Mol. Cryst. Liq. Cryst.*, 1985, **128**, 263-275.
41. C. R. López-Barrón, D. Li, L. DeRita, M. G. Basavaraj and N. J. Wagner, *J. Am. Chem. Soc.*, 2012, **134**, 20728-20732.
42. S. C. Sharma, R. Atkin and G. G. Warr, *J. Phys. Chem. B*, 2013, **117**, 14568-14575.
43. L. Ojha, M. B. Wilhelm, S. L. Murchie, A. S. McEwen, J. J. Wray, J. Hanley, M. Masse and M. Chojnacki, *Nat. Geosci.*, 2015, **advance online publication**, DOI: 10.1038/ngeo2546.

Graphical Abstract: