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Note

A note on the thin-layer chromatography of mixed surfactant systems

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Phosphated surfactants have been introduced in recent years for the emulsification of pesticides and wetting activity under a wide range of environmental conditions since, although they are themselves acidic, they are relatively unaffected by pH or the presence of electrolytes. Two of these interesting materials, phosphated nonylphenol ethoxylate (PNE) and phosphated fatty alcohol ethoxylate (PFE), have recently been investigated in our laboratory since they may find application in the formation of spontaneous emulsions[1–6] and we were presented with the problem of the separation and quantitative estimation of mixtures of these compounds.

The thin-layer chromatography (TLC) of surfactants has been investigated by others[7,8], and it seemed to us that the method should be applicable to our compounds. A large number of developing systems were investigated to cover a wide range of polarities and pHs. In the majority of cases the individual compounds "tailed" or failed to run satisfactorily. However, as described by Mustafa[1], a mixture consisting of equal parts of butanol, glacial acetic acid and water resulted in the development of adequate spots, and using the method described in this paper, the individual compounds were shown to be substantially homogeneous in identity[2,3]. Mixtures of the two compounds resulted in the unexpected detection of additional spots, depending upon the constitution of the mixture, and this observation led to the demonstration of complex formation between the two compounds[5].

Of direct interest to us was the fact that additional spots could be detected when quaternary mixtures of the two surfactants, *n*-hexane and water were run on the plates since these were known to form different types of liquid crystalline structure, again depending on the constitution of the system.

Since the association between surfactants is well documented[9], our observations may be relevant to other investigations involving the detection of components in mixtures of surfactants.

EXPERIMENTAL

Materials

PNE: Phospholan PNP-9 (Lankro Chemicals, Manchester, Great Britain)[2].
PFE: Phospholan PDB-3 (Lankro Chemicals)[3].

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Butanol, chloroform, glacial acetic acid and *n*-hexane were all laboratory grade reagents (Fisons, Loughborough, Great Britain).

Water: triple glass-distilled.

Method

TLC was based on the method of Patterson *et al.*[7] and Mansfield and Locke[8]. Silica gel (Kieselgel, E. Merck, Darmstadt, G.F.R.) was spread at a thickness of 0.25 mm on 20 × 20 cm glass plates and dried. Using a 5-μl pipette with drying between each application 30-μl volumes of the surfactants dissolved in chloroform (10 mg·ml^{-1}) were applied as spots at the origin.

After development with a mixture of equal parts of butanol, glacial acetic acid and water, the plates were dried and organic materials detected by spraying with sulphuric acid and heating in a hot-air oven at 110°. Similar results were obtained by exposing the plates to iodine vapour or by spraying with Burger's Reagent, as modified by Patterson *et al.*[7].

RESULTS

Results obtained in replicate experiments are summarized in Table I.

TABLE I

TLC OF COMBINATIONS OF PFE AND PNE (SILICA GEL, DEVELOPED WITH BUTANOL–GLACIAL ACETIC ACID–WATER)

LC = liquid crystal.

System composition (%, w/w)				*Identity*	R_f *(mean of 7)*	R_{ST}*
PNE	*PFE*	*n-Hexane*	*Water*			
100	—	—	—		0.66	93
—	100	—	—		0.75	106
90	10	—	—	mixture	0.66 (0.71)**	93 (100)
10	90	—	—	mixture	0.75 (0.71)**	106 (100)
50	50	—	—	complex	0.71	100
5	5	40	50	middle LC	0.60	84.5
15	15	20	50	neat LC	0.81	114
13.75	13.75	22.5	50	neat LC	0.81	114
12.5	12.5	25.0	50	neat LC	0.81	114

* Values from ref. 10, taking the complex (50:50) as 100%.

** Minor spot.

DISCUSSION

Using other physical methods the two compounds under investigation have been shown to form a definable complex in the presence of water in approximately equal parts by weight[4,5]. It was not altogether unexpected therefore when a 50:50 mixture was run under the same conditions with a polar development system that only a single spot could be detected with an R_{ST} roughly halfway between those of the spots of the individual components. In addition, it was noted that mixtures outside this ratio would give two spots, one corresponding to that of the complex alone and the other corresponding to whichever component was in excess. Clearly the water in the developing solvent is able to enter into the mixture at the origin and assist *in situ* in the formation of a complex which, in itself, must evidently be tightly bound together. It is believed that the complex forms by electrostatic attraction between the two molecules, and Yalabik (unpublished results) has demonstrated that complex formation is suppressed at high pH, suggesting that the ionization of the phosphate polar head groups on the individual molecules causes them to repel each other. This observation may well account for the fact that the compounds themselves can only be developed on a silica gel plate under very acid, polar conditions.

A detailed investigation of the phase relationships in the quaternary system PNE–PFE–*n*-hexane–water showed[3] that the system is characterized by the presence of extensive areas of anisotropic liquid crystalline material. Two different types of liquid crystal were identified, the predominant type being described as "neat" with a smaller amount of "middle phase" liquid crystal also being present. When the equilibrium phase diagram is drawn out, it is evident that the complex system described above is, in some way, involved in the formation of liquid crystals since they tend to form along the tie line corresponding to a 50:50 mixture of PNE–PFE. However, both water and *n*-hexane are also involved in the formation of the liquid crystals which evidently have different structures, one form (the "neat") changing to the "middle phase" structure as the proportion of the non-polar *n*-hexane is increased. What was suprising was that the liquid crystals appeared to behave as if they were new entities without disrupting into their component parts and, indeed, with properties quite different from those of the components. For example, it will be noted from Table I that the middle liquid crystal moves much slower than the slowest of the two surfactants, whereas the neat material moves appreciably faster than the fastest of the two components.

Two alternative mechanisms can be put forward to account for the separation of materials by a chromatographic process[11]. The first mechanism depends on the separation produced by the compounds being distributed between the solvents developing the plate. Under the highly polar conditions used in this experiment it might be anticipated that the PFE, being substantially fat-soluble, would be retained to some extent in the butanol fraction in preference to the PNE which is highly water-soluble, and will therefore run with the solvent front. This is the experimental situation and is compatible with the fact that the complex has an R_{ST} intermediate between those of the two components. However, it does not explain why the liquid crystalline materials, with identical ratios of PFE and PNE, behave anomalously.

The alternative mechanism takes into account the nature of the supporting medium, in this case silica gel, and suggests that material will tend to be retained if polar groups are bound by the silica groups of the gel matrix. The gel itself is acidic and the developing system is also acidic so that there is unlikely to be any significant degree of ionization of the phosphate groups of the surfactant molecules, which will therefore be separated on the basis of their relative hydrophilicity. PNE, although having twice the molecular weight of PFE, has a greater number of ethylene oxide groups per molecule and is more hydrophilic. It will have a greater tendency to be bound onto the carrier matrix, allowing the PFE to move more rapidly. Formation of the complex appears to have a net effect of increasing the polarity of the PFE molecules and this may account for the fact that the R_F is not an exact intermediate value between those of its constituents.

By analogy, the structure of the neat phase material is thought to consist of lamellar plates of alternate layers of hydrophobic and hydrophilic molecular groups[12] and this would be likely to have a less polar character than the individual components. This would account for this material running more readily in the developing solvent and explain why three different samples, differing only in their hexane contents, had very similar R_F values.

On the other hand, since the middle phase liquid crystal contains more *n*-hexane than the neat material, it would be predicted on the basis of Winsor's R Theory[13] that the middle phase would have its hydrophilic groups projecting outwards and be, therefore, more polar in character so that it would be the slowest running of all these materials.

This evidence for the relative hydrophilicity of the surface of the liquid crystalline phases is supported by other evidence[4-5], but clearly requires further investigation since it is possible that TLC might provide an interesting method for the examination of these materials.

However, the properties of these two surfactants are by no means unique. The formation of complexes and liquid crystalline phases are common features of many surface-active materials. Since it appears that, at least under some conditions, these resultant materials may move as separate entities, chromatographically they will be detected as artifacts or "false" spots on the developed plate. Complex molecules could well be produced in mixtures of surfactants and they are likely to be detected at low concentrations. On the other hand, liquid crystals are more likely to form at relatively high concentrations and their presence in a dilute system such as may be encountered during an analytical investigation would be unlikely. However, the possibility of liquid crystals forming will exist if there is any stage of the manipulation involving extraction and concentration before placing on the TLC plate. The observations reported here are therefore relevant to the TLC examination of systems likely to contain mixtures of surfactants.

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