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Reversed-phase high-performance liquid chromatography of anionic and ethoxylated non-ionic surfactants and pesticides in liquid pesticide formulations

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ABSTRACT

A procedure has been developed for the chromatographic separation of anionic (linear alkylbenzene sulphonates) and non-ionic (ethoxylated nonylphenols, ethoxylated fatty acids and ethoxylated fatty amines) surfactants and a pesticide (cypermethrine) in liquid pesticide formulations using a reversed-phase high-performance liquid chromatographic system.

INTRODUCTION

The type and ratio of ionic and non-ionic surfactants used in liquid pesticide formulations have a great effect on the quality and stability of emulsions and on the biological activity of the pesticide. The analysis of these emulsifiers and their separation from the active component is therefore an important analytical task.

The analysis of products containing several types of surfactants is complicated, as the materials used are complex mixtures of various homologues, isomers and oligomers. Thus the number of components is large and can vary depending on the origin and on the quality of the materials used.

In practice, an octylsilica stationary phase, gradient elution of an aqueous acetonitrile mobile phase and UV detection are used for the simultaneous chromatographic determination of ionic and non-ionic surfactants [1–5]. These separations are also suitable for the quantitation of these compounds.

The separation and analysis of mixtures containing fatty acid- and fatty amine-based surfactants is more complicated, as the UV absorption of these materials is much less than that of benzene derivatives in the usual wavelength range used. Ethoxylated fatty acids contain at least four types of components, *i.e.* free fatty acids, free polyethylene glycols (PEGs), PEG monoesters and PEG diesters [6]. The simultaneous separation of all four types of surfactants has not yet been reported.

Components of ethoxylated fatty acids were separated according to the length of the hydrocarbon chain and the chemical structure (free acid, PEG monoester, PEG diester) on a reversed-phase column [7,8]. Oligomers of ethoxylated fatty acids were separated on a normal-phase column [7,9–11].

Separation of the components of the ethoxylated fatty amines in liquid pesticide formulations was achieved based on the homologues and the degree of ethoxylation (EO) using reversed-phase and normal-phase separation techniques, respectively [12]. The detection system used was suitable for the sen-

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sitive and selective detection of amine-type surfactants as the detection system did not respond to alkylphenols, alcohols and esters.

Schreuder and Martijn [13] reported the simultaneous determination of alkylbenzene sulphonates (ABSs) and ethoxylated alkylphenols in liquid pesticide formulations. The separation of the two surfactant types was achieved in reversed-phase high-performance liquid chromatography (RP-HPLC) after the preliminary separation of the solvent and the active component of the formulation using cartridges filled with silica gel and aminoalkyl-modified silica gel.

EXPERIMENTAL

The chromatographic system used consisted of a pump (Model Liquopump 312, Labor MIM, Hungary), a variable-wavelength UV detector (Model OE-308, Labor MIM) and a six-port injection valve (Model 7125, Rheodyne) with a 20-μl loop and a recorder. The analytical column used contained octylsilica (Nucleosil C_8) and had dimensions of 150 × 4.0 mm I.D., 5 μm particle size. The column was maintained at ambient temperature (24 ± 1°C).

The eluents were methanol–aqueous phosphate buffers. The eluents were prepared by weighing (to ± 0.01 g) the calculated amount of methanol yielding the pre-selected percentage composition (density 0.796 mg/ml) into a calibrated flask. H_3PO_4 and NaH_2PO_4 were then added from stock solutions. Distilled water was slowly added while keeping the temperature at 25.0°C. After careful equilibration, the last few drops of water were added. This eluent preparation procedure was very reproducible.

The pH of the filtered and degassed eluents was measured by a combined glass electrode and a digital pH meter (Model OP-208, Radelkis, Hungary), calibrated with aqueous buffers at pH 4.0 and 7.0.

Selective elution of the four optical isomer pairs of the active component of the pesticide (cypermethrine) was achieved using a normal-phase HPLC system with a silica gel column (Bio-Separation Technologies, Budapest, Hungary; 150 × 4.0 mm I.D., 5 μm particle size), and an eluent of *n*-hexane–dichloro methane (85:15) containing 0.26% dioxane. The system used was a Spectra Physics gradient pump, a Rheodyne 7125 injector with a 20-μl loop, a Linear Instruments UV detector set at 233 nm, with data aquisition and integration on an IBM PS/2 Model 30 computer. Complete separation of the four isomer pairs was achieved within 12 min using this system at a flow-rate of 2.0 ml/min.

The macroporous, strongly basic ion-exchange resin used (Type Duolite IMAC HP 555, Rohm and Haas; capacity 3.00 mequiv./g) was cleaned by washing with aqueous methanol solutions of various methanol concentrations (25, 50 75%) and with 100% methanol.

RESULTS AND DISCUSSION

The retention behaviour of ABSs (C_8–C_{14} homologues), ethoxylated nonylphenols (average EO 2–30), ethoxylated fatty acids (non-specified fatty acid EO 6, oleic acid EO 7) and ethoxylated fatty amines (oleylamine EO 2 and EO 6, tallowamine EO 15 and EO 30 tallowamine hydrogenated EO 15 and EO 30) was studied as a function of the methanol concentration (65–85%) and pH (2.7–6.5) of the eluent. The alkyl chain composition of the non-specified fatty acid (EO 6) was not given by the manufacturer, but the main component of this sample was assumed to be C_{18} on the basis of retention volumes.

Selective separation of these four types of surfactants was not achieved by varying the methanol concentration of the eluent as the retention behaviour of all the surfactants examined was similar.

Table I shows the effect of eluent pH on the capacity factors (k') of the surfactants at a methanol concentration of 80%. The retention of the amines was greatly influenced by the eluent pH; the k' values increase with increasing eluent pH. However, the eluent pH does not appreciably affect the retention of the ethoxylated nonylphenols and the ethoxylated fatty acids. The ionic strength of eluents of pH 3.8 and 6.5 was different: the concentration of NaH_2PO_4 in the eluents was 0 and 25 mM, respectively. Therefore the decrease in the retention of the sulphonates is due to an increase in the concentration of the phosphate salt [14]. The retention of the sulphonates between pH 3.8 and 6.5 is approximately the same, as the concentration of the phosphate salt in the eluent is unchanged (25 mM NaH_2PO_4).

At pH 2.7 (80% methanol–60 mM H_3PO_4), peaks of homologues of the ABSs overlapped with

TABLE I

EFFECT OF ELUENT pH ON THE RETENTION OF COMPONENTS OF IONIC AND NON-IONIC SURFACTANTS IN METHANOL–AQUEOUS PHOSPHATE BUFFER ELUENTS CONTAINING 80% METHANOL

Eluent composition: pH 2.7, 80% methanol (MeOH)–60 m*M* H_3PO_4; pH 3.8, 80% MeOH–40 m*M* H_3PO_4–25 m*M* NaH_2PO_4; pH 4.4, 80% MeOH–10 m*M* H_3PO_4–25 m*M* NaH_2PO_4; pH 6.5, 80% MeOH–25 m*M* NaH_2PO_4). Column, Nucleosil C_8, 150 × 4.0 mm I.D., 5 μm particle size.

Component	*k'*			
	pH 2.7	pH 3.8	pH 4.4	pH 6.5
Alkylbenzene sulphonates				
C_{10} ABS	0.89	0.45	0.42	0.40
C_{11} ABS	1.13	0.59	0.56	0.56
C_{12} ABS	1.42	0.80	0.75	0.73
C_{13} ABS	1.80	0.95	0.94	0.93
Ethoxylated nonylphenols (NP)				
NP 1, 2, 3 EO	1.34	1.16	1.19	1.41
NP 23 EO	1.59	1.38	1.38	1.83
Ethoxylated fatty acids				
Oleylamine 2 EO	0.45	0.75	0.86	2.66
	0.53	0.94	1.08	2.81
Oleylamine 6 EO	0.60	1.00	1.12	3.00
Tallowamine 15 EO	0.81	1.31	1.38	3.28
Tallowamine 30 EO	0.91	1.39	1.47	3.40
Ethoxylated fatty amines				
Fatty acid 6 EO[a]	2.88	2.44	2.50	3.80
Oleic acid 7 EO	2.84	2.44	2.45	3.38

[a] The alkyl chain composition of fatty acid 6 EO was not given by the manufacturer, but the main component of this sample was assumed to be C_{18} on the basis of retention volumes.

peaks of ethoxylated amines and alkylphenols, thus selective separation was not achieved. However; complete separation of ABSs and ethoxylated fatty acids, or all three types of ethoxylated non-ionic surfactants, was achieved at this pH. At pH values between 3.8 and 4.4 (80% methanol–40 m*M* H_3PO_4–10–25 m*M* NaH_2PO_4), complete separation of ABSs, ethoxylated tallowamines and ethoxylated fatty acids, or the selective elution of ethoxylated nonylphenols and fatty acids, was obtained. The simultaneous separation of all four types of surfactants was achieved when ethoxylated oleylamine was present in the mixture at pH 6.5 (80% methanol–25 m*M* NaH_2PO_4) [15].

The selective separation of ethoxylated tallowamines and ethoxylated fatty acids was not possible because their retention was similar at pH 6.5 (Table I). The selective separation of ethoxylated tallowamines from ethoxylated fatty acids and ethoxylated nonylphenols was obtained only after the preliminary separation of ABSs from the mixture containing all four types of surfactants by ion exchange.

A 2-ml volume of a mixture containing 1.75–20.0 g/l ABS, 2.0–40.0 g/l ethoxylated nonylphenol, 20.0–62.5 g/l ethoxylated fatty amine and 7.5–40.0 g/l ethoxylated fatty acid was pipetted onto an ion-exchange column containing 10 ml of resin (Cl^-). The non-ionic surfactants were eluted quantitatively with 25 ml of methanol and were separated chromatographically with the pH 2.7 eluent (Fig. 1). Regeneration of the anion-exchange resin and the quantitative elution of ABSs was achieved with 50 ml of a mixture containing 80% methanol–0.5 *M* hydrochloric acid. Selective separation of ABS homologues was achieved with the eluent containing 65–70% methanol (65–70% methanol–40 m*M* H_3PO_4–25 m*M* NaH_2PO_4, pH $\approx$ 3.5) (Fig. 2). Note that if C_8, C_9 and C_{14} homologues are present in the mixture of the four types of surfactants, selective separation can be achieved using the separa-

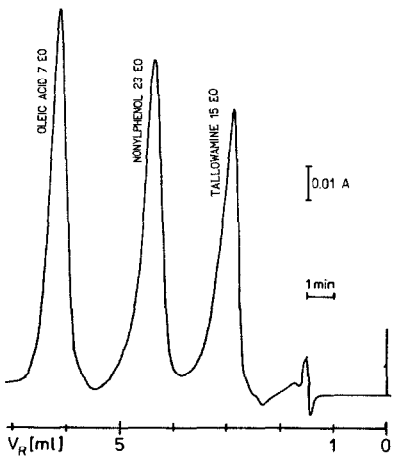

Fig. 1. Simultaneous separation of nonylphenol, tallowamine and oleic acid ethoxylates. Column, Nucleosil C_8 (150 × 4.0 mm I.D., 5 μm particle size); eluent, 80% methanol–60 m*M* H_3PO_4, pH 2.7; eluent flow-rate, 0.5 ml/min; $\lambda = 220$ nm; sample, 20 μl of 9.1 g/l tallowamine 15 EO–170 mg/l NP 23 EO–4.5 g/l oleic acid 7 EO.

tions described above (ion exchange, elution with hydrochloric acid–aqueous methanol, RP-HPLC separation).

Using RP-HPLC, selective separation of all the eight isomers of the pesticide (cypermethrine) was not achieved; all the isomers eluted as one symmetrical peak with the methanol–aqueous phosphate buffer eluent containing 80% methanol. Selective elution of the four optical isomer pairs was achieved using a normal-phase HPLC system (see under Experimental).

Such a good separation of the isomers was not achieved in the RP-HPLC system used, even with water–acetonitrile as the eluent. All the isomers of cypermethrine were eluted as one peak with the eluent containing 80% methanol. The retention of the components eluted as this peak was about the same ($k' = 1.28$) as that of nonylphenols of low EO (see Table I and Smedes *et al.* [15]). The retention of the isomers of cypermethrine was unaffected by eluent pH in the range 2.7–6.5.

Cypermethrine is stable in apolar solvents but is unstable in polar solvents, *e.g.* in aqueous methanol eluents. The active matter can be hydrolysed by even trace amounts of water in technical-grade xylene, which is the solvent in the pesticide formulation. The rate of hydrolysis is relatively low, and is about 1% per day (Antal Gajáry, Chinoin, Hungary, unpublished results). Several molecules, such as amines, can react with cypermethrine with the formation of new esters and amides. These reactions are fast enough to occur within a couple of minutes while dissolving the active component and the surfactants (ethoxylated fatty amines first) in methanol or in the eluent. The simultaneous separation of ABSs, cypermethrine, ethoxylated oleylamine and ethoxylated oleic acid is shown in Fig.

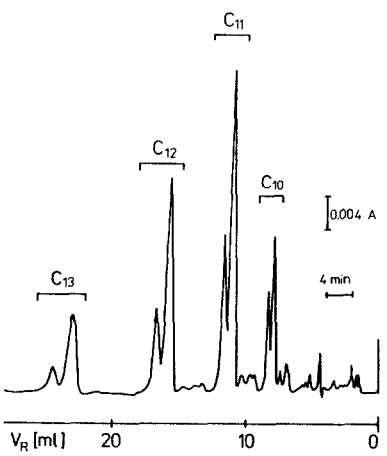

Fig. 2. Complete separation of ABS homologues and partial separation of positional isomers of homolgues. Column as in Fig. 1. Eluent, 70% methanol–25 m*M* NaH_2PO_4–40 m*M* H_3PO_4, pH 3.5; eluent flow-rate, 0.5 ml/min; $\lambda = 224$ nm; sample, 20 μl of 115 mg/l ABSs.

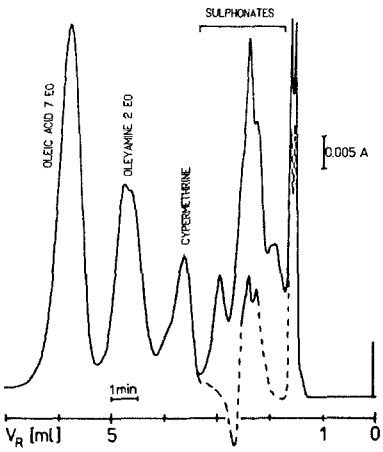

Fig. 3. Simultaneous separation of ABSs, cypermethrine, ethoxylated oleylamine and ethoxylated oleic acid before (solid line) and after (broken line) the separation of ionic compounds from the mixture using an ion-exchange resin. Column as in Fig. 1. Eluent, 80% methanol–25 m*M* NaH_2PO_4, pH 6.5; eluent flow-rate, 0.5 ml/min; $\lambda = 220$ nm.

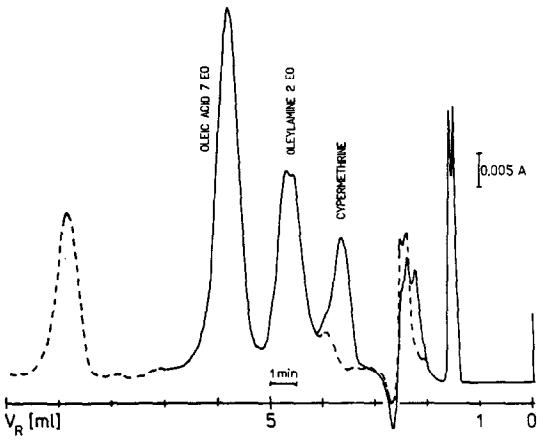

Fig. 4. Reaction of cypermethrine in the presence of ethoxylated oleylamine before (solid line) and after (broken line) the reaction between amine and cypermethrine. Column as in Fig. 1. Eluent, 80% methanol–25 m*M* NaH_2PO_4, pH 6.5; eluent flow-rate, 0.5 ml/min; $\lambda = 220$ nm.

3. The shapes of the peaks of the homologues of ABSs are assymetric, which shows the presence of the products of the reactions described above. The chromatogram marked with the broken line was obtained after the preliminary separation of sulphonates from the mixture by the anion-exchange resin. Besides this reaction, other reactions occur which produce compounds with long retention times. As a consequence of this reaction, cypermethrine disappears from the mixture (Fig. 4, broken line). Fig. 4 shows that the area and shape of the peak of the ethoxylated oleylamine does not vary while cypermethrine is reacting, so, although the amines do not react with cypermethrine, they act as catalysts. It is not surprising that oleylamine (EO 2) is divided into two peaks, as the technical-grade sample analysed contained isomers and several ethoxylated oligomers.

Cypermethrine is stable in weakly acidic solvents at about pH 4 [16]. In acidic solvents, however, ethoxylated fatty amines rapidly react with the acid and the absorbances of the products of this reaction differ strongly from those of the amines; quantitation of them is therefore impossible. Cypermethrine itself remains unchanged and can be quantified.

CONCLUSIONS

It is concluded that the simultaneous and isocratic separation of components of mixtures containing ionic (ABSs) and non-ionic (nonylphenol, fatty acid and fatty amine ethoxylates) surfactants can be achieved using a RP-HPLC technique with an octylsilica stationary phase and methanol–aqueous phosphate buffer eluents. Separation of the four types of surfactants when ethoxylated tallowamines were present in the mixture was achieved only after the preliminary separation of ionic compounds on an anion-exchange resin. The quantitative elution of non-ionic surfactants from the resin was carried out with methanol; regeneration of the resin, *i.e.* the quantitative elution of ionic components, was obtained with a methanol–hydrochloric acid mixture. The simultaneous separation of non-ionic compounds of the first fraction containing the ethoxylates was achieved in the described RP-HPLC system. Complete separation of the homologues and even partial separation of the positional isomers of homologues of the second fraction was also achieved.

In the presence of cypermethrine the separation of ABSs, ethoxylated fatty amines, ethoxylated fatty acids and the active component of the pesticide was achieved after the preliminary separation of ionic surfactants on an anion-exchange resin.

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