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Microemulsion electrokinetic chromatography with different organic modifiers: separation of water- and lipid-soluble vitamins

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Abstract

Microemulsions prepared by using either sodium dodecyl sulfate (SDS) or trimethyltetradecylammonium bromide (TTAB) were tested with regard to the migration time, selectivity and number of theoretical plates using a set of both water-soluble and lipid-soluble vitamins. While in the separations where SDS was used as the surface-active agent for microemulsion preparation, better resolutions were obtained with both hydrophilic and lipophilic vitamins, with TTAB the best separations were obtained with the micelle-forming reagent only or with diethyl ether as the microemulsion-forming component. In the SDS-based separations nicotinic acid was moving with the same velocity (relative to the endoosmotic flow) regardless of the emulsion or micellar phase used. It is proposed that owing to its behaviour it can serve as an ideal internal standard in further studies on microemulsion separations.

1. Introduction

Microemulsion capillary electrophoresis (electrokinetic chromatography, MEEKC) represents a variation of micellar electrokinetic chromatography commonly applied today. Whereas in the latter the partition process occurs between the surrounding background electrolyte and the micelle, in microemulsion separations the partitioning takes place between the background electrolyte and the microemulsion droplets.

Microemulsions are microheterogeneous liquids which have characteristic properties as solvents such as optical transparency, thermodynamic stability and high solubilization power. The first attempt to apply these microemulsions for electrokinetic separations was published by Watarai [1] in 1991. In this case an oil-in-water emulsion was applied, consisting of water–sodium dodecyl sulfate (SDS)–1-butanol–heptane (89.28:3.31:6.61:0.81, w/w); 0.01 *M* phosphoric acid or 0.01 *M* hydrogen-carbonate buffer served for controlling the pH of the electrolyte. In separating a test mixture of fluorescent aromatic compounds at pH 3.0, it was observed that all of the solutes, including neutral and anionic species, migrated to the anodic end. It was concluded that the migration of the anionic microemulsion droplets is faster than the electroosmotic flow, which is in the opposite direction to the migration of the droplets. The migration order could be related to hydrophobicity, indicating that the better partitioning solutes migrate faster. Under alkaline conditions the endo-

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osmotic flow becomes faster than the electrophoretic mobility of the microemulsion and consequently the solutes migrated to the cathodic end.

More recently, fundamental characteristics of MEEKC were described by Terabe et al. [2]. A microemulsion consisting of heptane–SDS–butanol–buffer (0.81:1.66:6.61:90.92, w/w) was mainly employed.

The separation selectivity of MEEKC was compared with that of MEKC by using three different test mixtures. In microemulsion separations stronger affinity to the droplets compared with SDS micelles was observed with non-polar compounds. The migration time window could be extended by increasing the proportion of SDS in the microemulsion. It was also observed that the plate heights were higher in MEEKC.

Microemulsions prepared by mixing the organic solvent, water (buffer), surfactant and cosurfactant (such as a medium-chain alcohol) are transparent and thermodynamically stable. According to Terabe et al. [2], they consist of an organic solvent-containing core surrounded by the surfactant and cosurfactant. Their role is to stabilize the droplet.

The higher solubilization power of microemulsions has been claimed also as an advantage offering a wider dynamic range in sample concentration [3].

Despite the great potential of this technique, there have been only a few reports on the use of microemulsion systems so far.

In order to obtain some information about both lipid- and water-soluble solutes, a mixture of lipid- and water-soluble vitamins was selected as the test mixture. A mixture of similar composition has been fully separated by Ong et al. [4] using 30 m*M* SDS in 0.1 *M* borate–0.05 *M* phosphate (pH 7.6); however, addition of *β*-cyclodextrin (3 m*M*) was needed, in particular to obtain an adequate separation of the vitamin B-group members. More recently, micellar electrokinetic chromatography was applied to the separation of water-soluble vitamins by Dinelli and Bonetti [5]. The analytical procedure used the same Beckman system as in this work, but with a 70 cm × 100 μm I.D. capillary at 25°C operated with 50 m*M* sodium borate–22.5 m*M* SDS–10% (v/v) methanol (pH 8.0) at 16 kV.

In this work, we attempted to compare the efficiency of microemulsions containing different solvents as the organic phase core. The efficiency of different solvents was tested using water- and organic solvent-soluble vitamins.

2. Experimental

Electrokinetic chromatography was performed on a P/ACE System 2100 (Beckman, Palo Alto, CA, USA) with a 47 cm (40 cm to the detector) × 50 μm I.D. fused-silica capillary (untreated) (Polymicro Technologies, Tucson, AZ, USA). The device was run routinely at 10 kV (about 17.5 μA) at 25°C. UV detection at 214 nm was applied. The instrument was computer operated using the System Gold software.

All the reagents and standards (samples) were of analytical-reagent grade. The set of vitamins used for testing the different emulsions was obtained from Merck (Darmstadt, Germany) and solvents were purchased from Carlo Erba (Milan, Italy).

Both an anionic (SDS; Sigma, St. Louis, MO, USA) and a cationic surfactant [trimethyltetradecylammonium bromide (TTAB); Sigma] were used to prepare the microemulsions.

Microemulsions were prepared by mixing the organic solvent (0.81%, w/w), SDS (or TTAB) (6.62 or 3.31%, w/w, respectively) and butanol (6.61%, w/w) with 20 m*M* phosphate buffer (pH 7.0) (85.96 or 89.28%, w/w) according to the method of Watarai [1]. Alternatively, 500 m*M* SDS or TTAB (in water) were mixed 2:1 with the solvent in question, vortex mixed and titrated with 1-butanol until the mixture cleared (no addition of 1-butanol was needed in the case of 2-methyl-2-propanol). The resulting solution was diluted with 20 m*M* phosphate buffer (pH 7.0) to yield a 20 m*M* solution with respect to the surfactant. With SDS-containing background electrolytes, a high voltage was applied to the anodic end of the capillary, whereas with TTAB the polarity was reversed.

Samples were routinely prepared as solutions

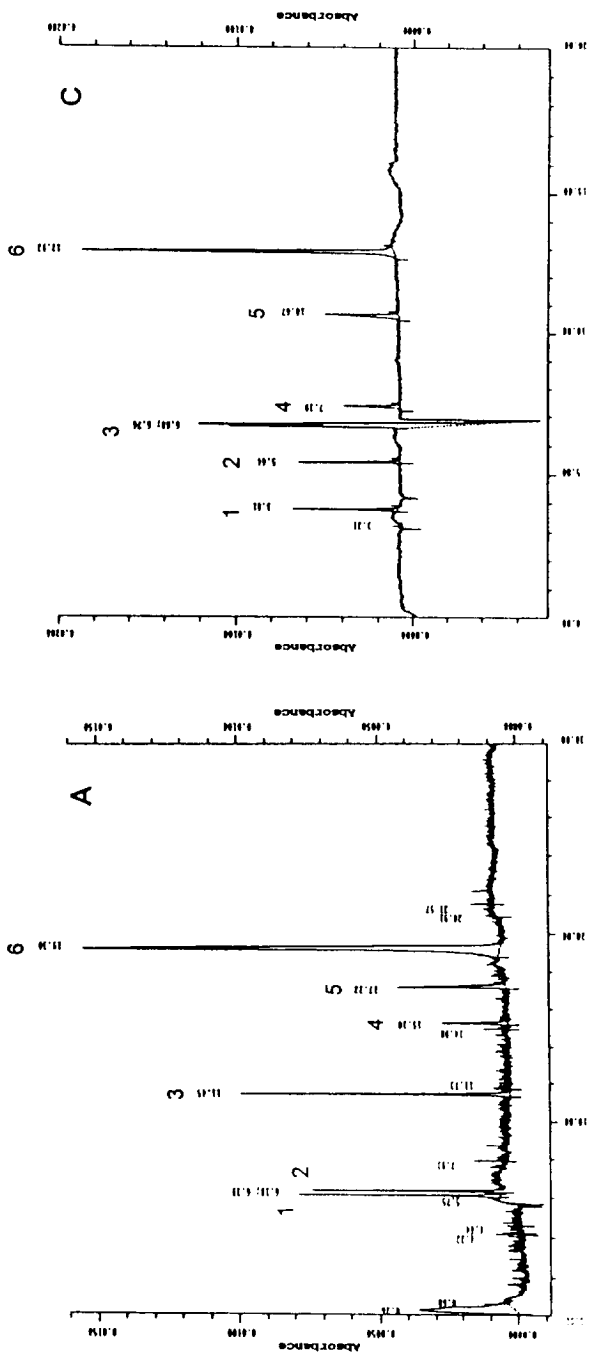

Fig. 1. Comparison of results obtained with two different methods of micellar phase preparation: (A) SDS–*n*-hexane (0.81% *n*-hexane, 6.62% SDS, 6.61% *n*-butanol, 85.96% phosphate buffer); (B) 500 m*M* SDS–n-hexane (2:1), *n*-butanol added until the solution cleared. Peaks: 1 = nicotinamide; 2 = pyridoxol; 3 = nicotinic acid; 4 = thiamine; 5 = vitamin E; 6 = vitamin A. (C) TTAB–*n*-amyl alcohol (0.81% *n*-amyl alcohol, 3.31% TTAB, 6.61% *n*-butanol, 89.28% phosphate buffer); (D) 500 m*M* TTAB–*n*-amyl alcohol (2:1), *n*-butanol added until the solution cleared. Peaks: 1 = thiamine; 2 = nicotinic acid; 3 = pyridoxol; 4 = nicotinamide; 5 = vitamin E; 6 = vitamin A.

of 100 μg/ml in methanol–water (1:1, v/v) and were applied by an overpressure of $3.45 \cdot 10^7$ Pa for 3 s.

3. Results and discussion

As shown in Fig. 1, there was a very small difference, if any, between the two methods of microemulsion preparation. Therefore, the method using dilution of concentrated surfactant was preferred in subsequent experiments. In order to visualize the effect of different microemulsions most clearly, suboptimum conditions for the separation in the background electrolyte were selected. Figs. 2 and 3 summarize the retention time changes with different organic phases in the microemulsion core with SDS and TTAB surfactants respectively. In both cases pyridoxol (vitamin B_6) and nicotinamide represented the critical combination that was difficult to separate. With TTAB-containing mobile phases the situation was further complicated by the fact that both of these vitamins were running near the endoosmotic flow peak.

3.1. SDS

Whereas no separation of pyridoxol (B_6), thiamine (B_1) and nicotinamide occurred with the background electrolyte containing SDS at 20 m*M* concentration only, on changing the micellar phase to an *n*-hexane-, *n*-heptane- or cyclohexane-containing emulsion resulted in a resolution of 1.0 or higher (in the case of cyclohexane) (Table 1). Another change observed was the reversed elution of vitamin E relative to vitamin A. In the absence of the microemulsion-forming agent vitamin A is eluted before vitamin E; this observation confirms the previously published results of Ong et al. [4]. However, in any of the organic solvents tested (including those capable of separating pyridoxol and nicotinamide), vitamin A moves more swiftly to the anode, which results in a later appearance of its peak on the electropherogram (Fig. 1) as compared with

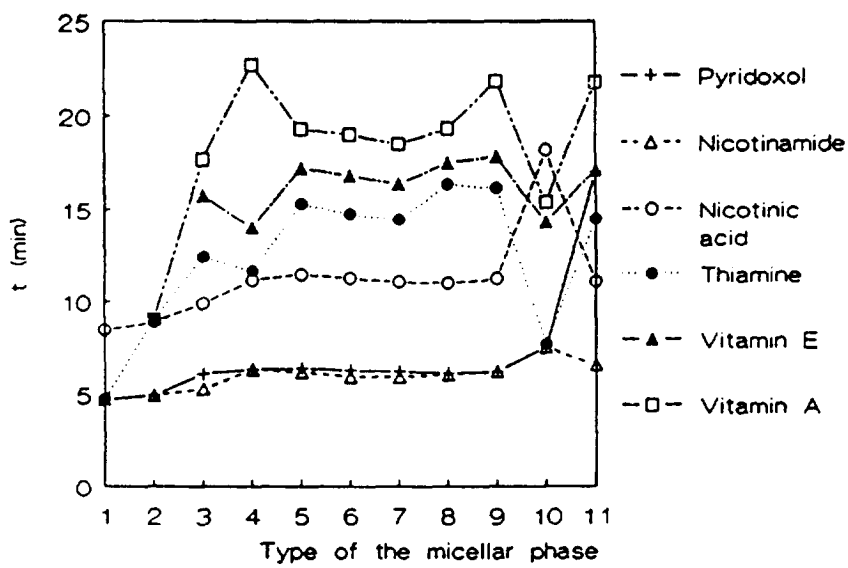

Fig. 2. Changes in electrophoretic mobility in different microemulsion phases with SDS as surfactant. (1) 20 m*M* phosphate buffer (pH 7.0); (2) 20 m*M* SDS (vitamin E did not elute within a 28-min running time; (3) 20 m*M* phosphate buffer (pH 7.0)–50 m*M* SDS, microemulsion phase prepared by mixing SDS and diethyl ether (4:1) (see Experimental); (4) 20 m*M* phosphate buffer (pH 7.0)–50 m*M* SDS, microemulsion phase prepared by mixing SDS and diethyl ether (2:1); (5) as (4), but with *n*-hexane; (6) as (4), but with *n*-heptane; (7) as (4), but with cyclohexane; (8) as (4), but with chloroform; (9) as (4), but with methylene chloride; (10) as (4), but with 2-methyl-2-propanol; (11) as (4), but SDS and octanol were mixed 10:2.

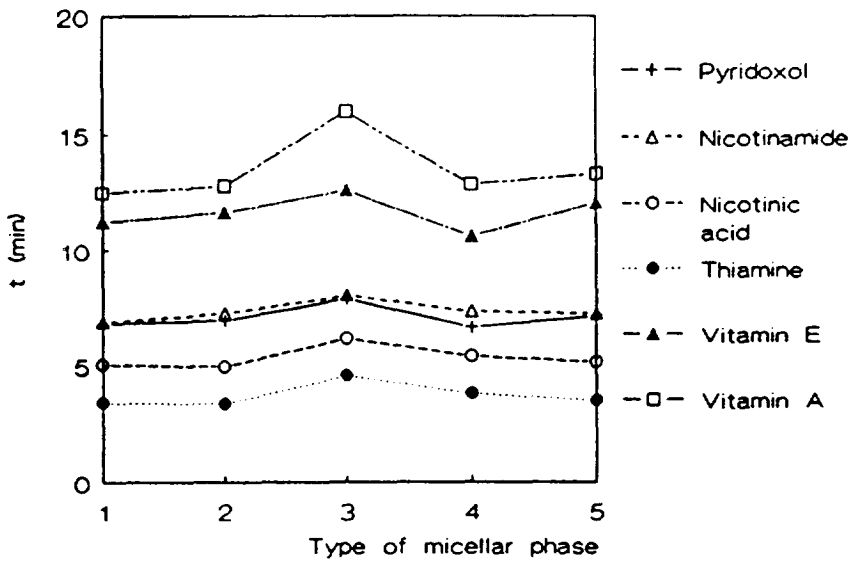

Fig. 3. Changes in electrophoretic mobility in different microemulsion phases with TTAB as surfactant. (1) 20 m*M* pnosphate buffer (pH 7.0)–10 m*M* TTAB; (2) as (1), but with diethyl ether added to concentrated (500 m*M*) TTAB in a ratio 1:2; (3) as (2), but with 2-methyl-2-propanol; (4) as (2), but with *n*-amyl alcohol; (5) as (2) but with 2-methyl-2-propanol, prepared by mixing the solvent with TTAB in a ratio 1:4.

vitamin E (the sequence of these two peaks is interchanged).

The time after which the vitamin A peak passes the detector can be efficiently influenced by the proportion of the organic solvents in the microemulsion. If the proportion of, e.g., diethyl ether in the microemulsion is increased, the time delay to the detector of vitamin A is increased in spite of the fact that all the other vitamins (including vitamin E) exhibit a shorter running time. Concomitantly, with a higher proportion of the organic solvent in the microemulsion, the resolution of the water-soluble pyridoxol–nicotinamide pair is lost.

Table 1
Resolution of the critical pair pyridoxol–nicotinamide

Surfactant used	Organic component of the emulsion	Resolution[a]
SDS	SDS only	No separation
	Diethyl ether	0.90
	n-Hexane	1.00
	n-Heptane	1.00
	Cyclohexane	1.50
	Chloroform	0.70
	Octanol	No separation
TTAB	TTAB only	0.87
	Diethyl ether	0.85
	2-Methyl-2-propanol	0.75 (divided by EOF in two peaks)
	n-Amyl alcohol	0.50

[a] $R = \Delta t / 4\sigma_t$, where Δt is the difference in time of elution between two consecutive peaks and σ_t is the standard width of a single peak.

Comparing these results with the migration times in cyclohexane-loaded micelles, one there is a much longer migration time of vitamin E compared with the diethyl ether-containing emulsion. In contrast, the running time of vitamin A is shorter, indicating that this vitamin interacts with the cyclohexane microemulsion less readily than with the emulsion prepared with diethyl ether.

Table 2 gives the numbers of theoretical plates achieved with different organic components of the microemulsion and different components of the test mixture. Generally, low plate counts were observed with the solutes moving in front of the electropherogram. However, selection of a proper organic component of the microemulsion can increase the plate count of these (water-

Table 2
Number of theoretical plates for the test mixture in microemulsions containing different organic solvents with SDS as surfactant

Vitamin	Diethyl ether	*n*-Hexane	*n*-Heptane	Cyclo-hexane	Chloroform	Octanol
Nicotinamide	17 978	82 495	77 400	215 001	180 821	} 234 327
Pyridoxol	79 847	244 992	87 117	237 383	230 552	
Nicotinic acid	585 680	283 181	280 462	271 559	266 674	272 050
Thiamine	13 500	505 634	478 356	463 988	148 640	116 478
Vitamin E	536 495	444 792	435 888	149 185	162 374	72 651
Vitamin A	100 125	196 997	200 099	190 016	91 905	65 730

No. of theoretical plates (N) calculated according to the equation $N = 5.54\ (t_r/\sigma)^2$, where t_r is the migration time and σ is the peak width at half-height [5,6]. Data obtained represent averages of three measurements; S.D. lies within 5–10% relative limits.

soluble) vitamins by a factor of more than ten (compare nicotinamide counts in diethyl ether- and cyclohexane-containing microemulsions). The highest plate counts in the mixture tested were always seen with nicotine acid. This is perhaps due to the very clear separation mechanism, based on its charge only. Indeed, the retention of nicotonic acid in our experiments varied in parallel with the endoosmotic flow and no influence was observed regarding the changing organic core of the microemulsion droplets (Table 3). This makes nicotonic acid a very suitable internal standard in future investigations on microemulsion separations. Typical runs showing the separations obtained with *n*-hexane-, *n*-heptane- and cyclohexane containing microemulsions are shown in Fig. 4.

The partition mechanism of vitamin A is the other extreme, as it is preferentially influenced by the nature of the organic component of the microemulsion. It always virtually coincides with the peak of Sudan III, whatever organic modifier is used in the emulsion. Consequently, the position of vitamin A in the electrophoregrams shown indicates the end of the separation window. In the absence of the organic component of the microemulsion (in micellar separations) this is not true, as the last-eluting component is always vitamin E, which elutes within the separation window, elution of the Sudan III peak being delayed after the vitamin E peak.

Table 3
Relative retention of the nicotinic acid peak with respect to the endoosmotic flow

Organic solvent added to SDS	Relative migration
None	1.90
Diethyl ether	1.91
n-Hexane	1.88
n-Heptane	1.92
Cyclohexane	1.90
Chloroform	1.86
Octanol	1.80

3.2. TTAB

An overview of migration differences using TTAB as surfactant for microemulsion preparation is shown in Fig. 3. Table 4 summarizes the N values for individual components of the test mixture. Data regarding resolution of the critical pair pyridoxol–nicotinamides are presented in Table 1.

In general, the effect of the organic microemulsion phase in the presence of a cationic surfactant is much less pronounced than that with an anionic surfactant. As can be be predicted, the sequence of the water-soluble vitamins (vitamin B_1, nicotinic acid, pyridoxol, nicotinamide) is reversed compared with the SDS-based separations, where nicotinamide is the first peak from the mixture to be seen in the detector. On the other hand, the lipophilic species, vitamin E and A emerge in the same sequence in

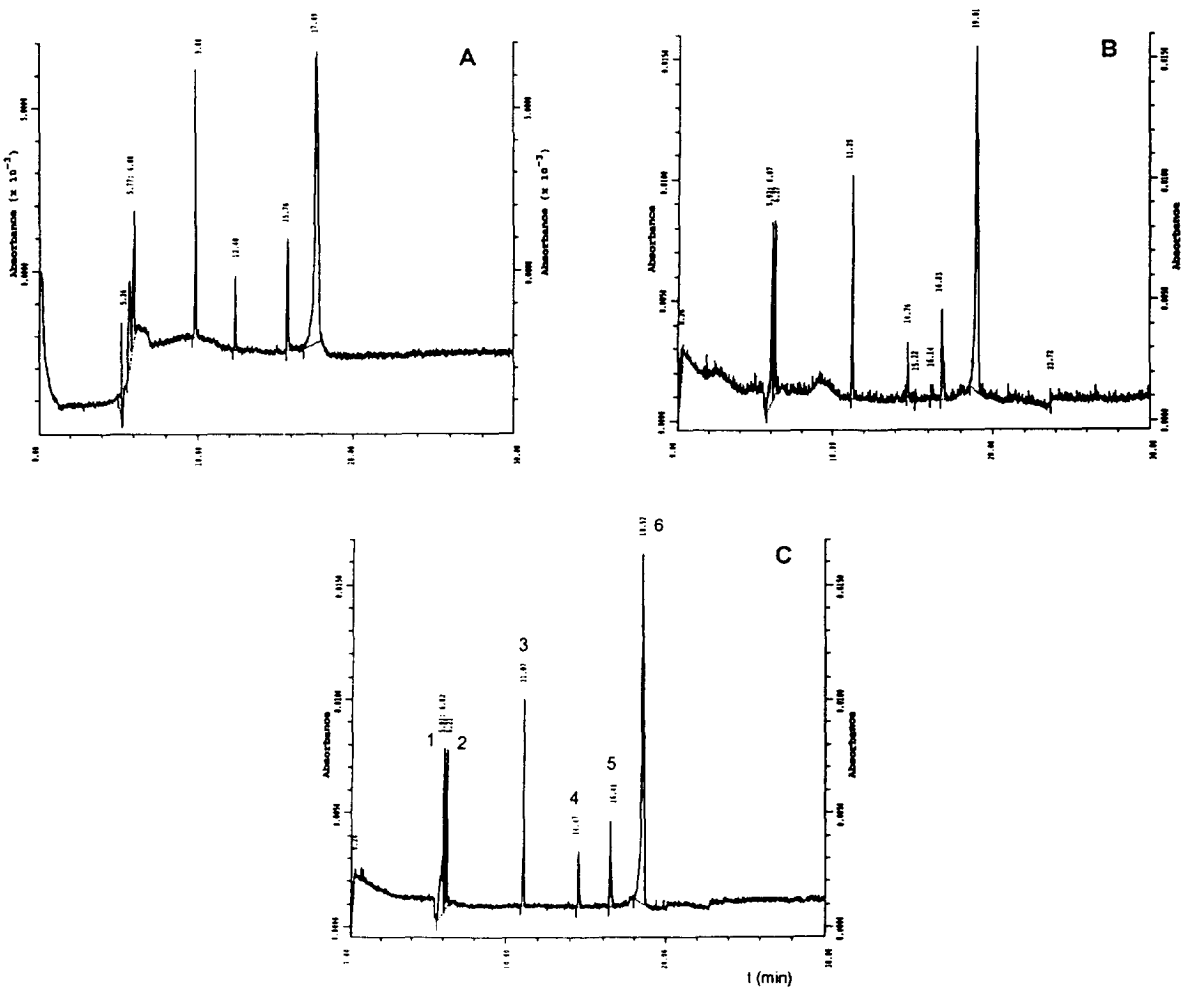

Fig. 4. Typical profiles obtained with (A) SDS–diethyl ether, (B) SDS–*n*-hexane and (C) SDS–cyclohexane. Peaks: 1 = nicotinamide; 2 = pyridoxol; 3 = nicotinic acid; 4 = thiamine; 5 = vitamin E; 6 = vitamin A. Microemulsion phase was prepared by mixing two parts of 500 m*M* SDS with 1 part of the solvent (see Experimental), except with diethyl ether, where the ratio was 4:1. No separation of nicotinamide and pyridoxol was obtained with micelles containing a lower proportion of SDS.

Table 4
Number of theoretical plates for the test mixture components in microemulsions containing different organic solvents with TTAB as surfactant

Vitamin	No organic solvent	Diethyl ether	2-Methyl-2-propanol	*n*-Amyl alcohol
Thiamine	17 685	24 869	31 495	32 167
Nicotinic acid	55 824	54 078	131 386	178 873
Pyridoxol	102 166	298 184	383 196	98 883
Nicotinamide	104 435	116 799	399 787	120 365
Vitamin E	57 689	75 190	179 738	128 694
Vitamin A	21 640	22 691	289 364	114 164

Definition of *N* and data representation as in Table 2.

both instances, indicating that the main partitioning occurs between the organic phase core in the emulsion droplet and the aqueous phase, the surfactant yielding to this system the charge and mobility towards cathode. No complete resolution of the critical pair pyridoxol–nicotinamide was obtained and in fact the best resolution was obtained either without any organic solvent added to the surfactant or addition of diethyl ether. Typical separations are shown in Fig. 5. At least, however, addition of the organic solvent to the cationic surfactant can result in a better peak shape, as indicated in Table 4.

4. Conclusions

Microemulsion separations, as tested with a set of lipid- and water-soluble vitamins, with both anionic and cationic surfactants can offer better resolution than micellar electrokinetic chromatography alone. However, in this case, SDS-based microemulsions are superior to the TTAB systems. Whereas in the former the separation can be influenced not only by the lipophilic vitamins but also by the hydrophilic vitamins, with TTAB the best separations obtained were either in the micellar mode only or in the

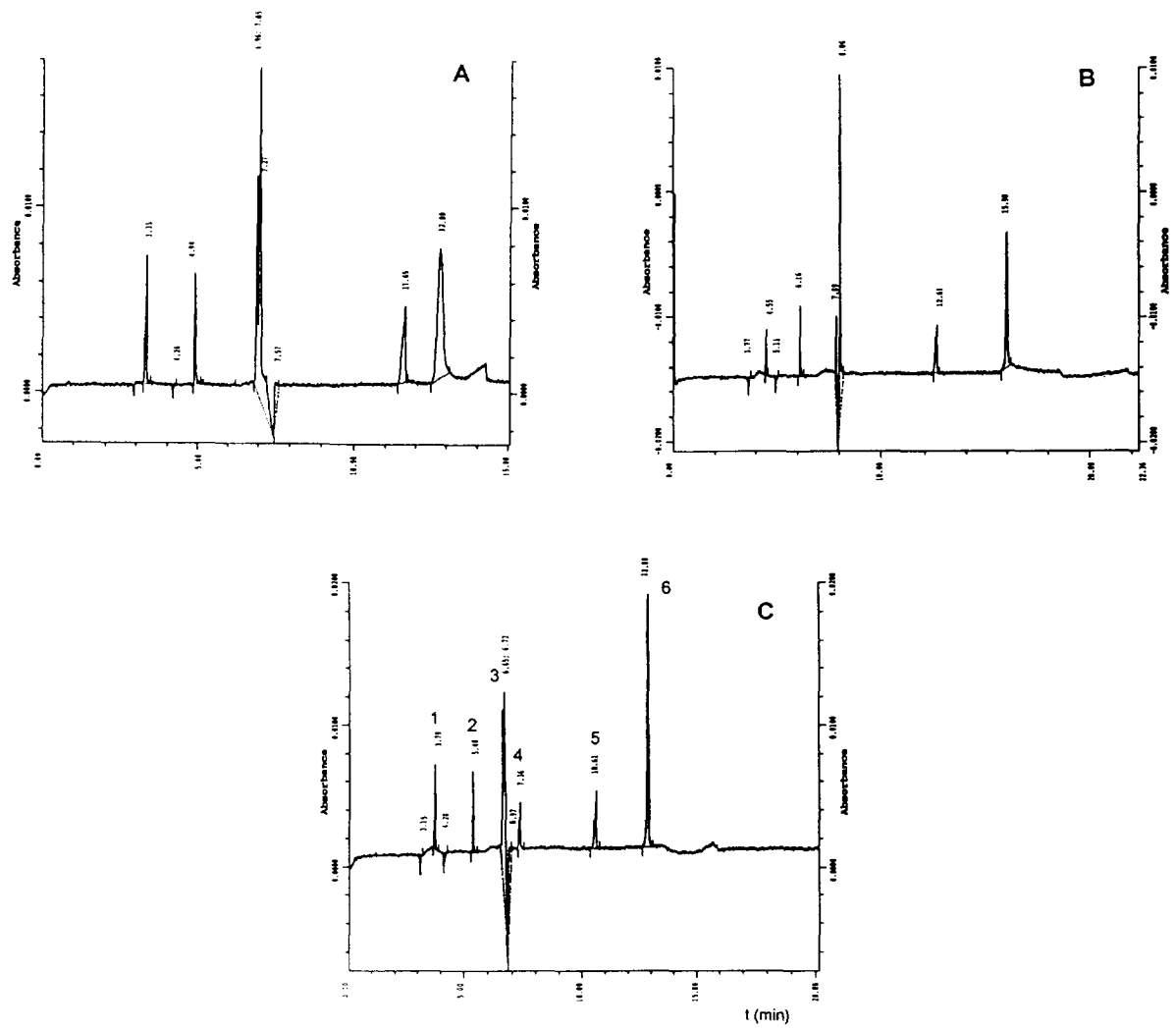

Fig. 5. Typical profiles obtained with (A) TTAB–diethyl ether, (B) TTAB–2-methyl-2-propanol and (C) TTAB–*n*-amyl alcohol. Peaks: 1 = thiamine; 2 = nicotinic acid; 3 = pyridoxol; 4 = nicotinamide; 5 = vitamin E; 6 = vitamin A.

presence of diethyl ether as the microemulsion component. In the SDS-based separations nicotinic acid can serve as an internal standard as its behaviour does not depend on the composition of the micellar phase. In fact, its relative position (with respect to the endoosmotic flow) is the same with different microemulsions or micelles or even without any surfactant present in the system. On the other hand, vitamin A interacts readily with all the microemulsion phases used and indicates the end of the separation window. Along with Sudan III, it can be used to determine the size of the separation window.

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