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Journal of Chromatography B, 741 (2000) 67–75

JOURNAL OF CHROMATOGRAPHY B

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Capillary electrophoretic separation of vitamins in sodium dodecyl sulfate containing buffers with lower aliphatic alcohols and *n*-hexane as organic modifiers

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Abstract

The effect of lower organic alcohols as co-surfactants (methanol, ethanol, *n*-propanol, isopropanol, propanediol, *n*-butanol and isoamylalcohol) and *n*-hexane as an organic modifier in 12.5 mol/l phosphate buffer with varying SDS concentration was investigated using a set of vitamins and *p*-hydroxybenzoic acid as the test mixture. It was demonstrated that optimum separations can be achieved particularly at high concentrations of the surfactant; the selectivity can be changed by adding a co-surfactant; while propanol and isopropanol show the same properties as co-surfactants, the most efficient alcohols were isoamylalcohol and propanediol. *n*-Butanol was capable of selective separation of *p*-hydroxybenzoic acid in the test mixture. Addition of ethanol appears most effective at higher concentrations (while all the other alcohols are effective already at 5% concentration, the best results with ethanol were obtained when it constituted 20% of the background electrolyte). 5% Concentration of methanol resulted in poor separation of the test mixture, however if 300 μl/10 ml of hexane were added to 20 mmol/l SDS containing phosphate buffer, the resulting separation was practically the same as with 50 mmol/l SDS.

Keywords: Organic modifiers; Vitamins

1. Introduction

The aim of microemulsion electrokinetic chromatography introduced by Watarai [1] has been directed to a further increase of selectivity in capillary electrophoretic separations of non-polar solutes. While micellar electrokinetic chromatography is based on the partition of analysed solutes between the aqueous and micellar phase, the properties of the latter being determined by the chemical nature of the surfactant used, microemulsion based separations offer the possibility to further modify the polarity of the basically apolar pseudophase present in the separation system. This is particularly important if analytes with close hydrophobic properties are to be separated. The microemulsion pseudophase consists usually of a surfactant (typically SDS or TTAB), non-polar modifier (hexane, heptane) and a co-surfactant (typically *n*-butanol). However, as e.g. butanol is considerably soluble in water it remains obscure to what extent it is included in the microemulsion assemblies and to what extent it decreases the polarity of the aqueous phase. In spite of this uncertainty, buffer systems containing a surfactant, apolar modifier and lower aliphatic alcohols can be successfully applied for the separation of non-

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0378-4347/00/$ – see front matter
PII: S0378-4347(99)00382-5

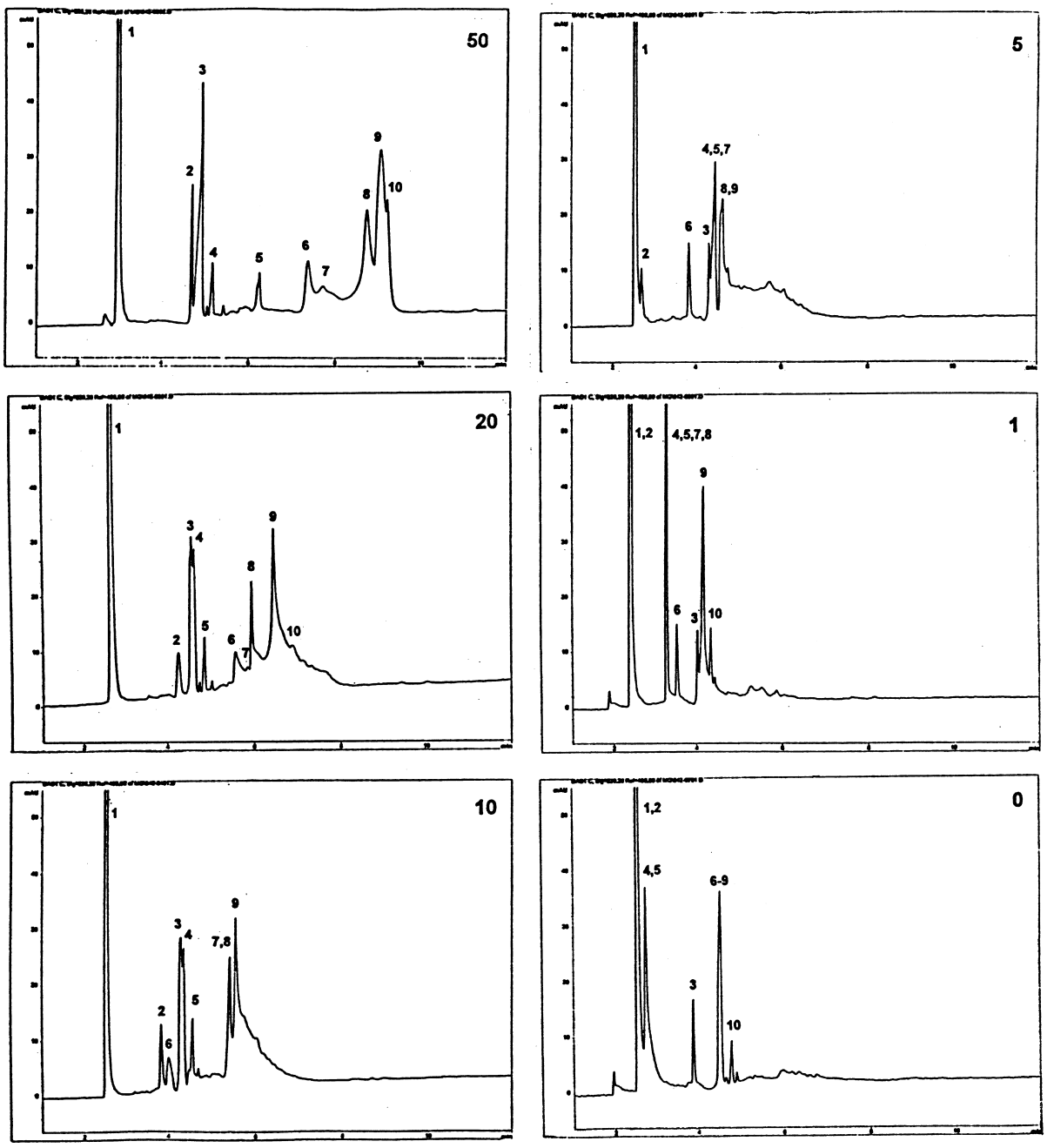

Fig. 1. Separation of the test mixture of vitamins in the presence of varying SDS concentration (numbers of individual plates indicate mmol/l SDS present in the run buffer); 50 cm long capillary 75 μm I.D., 10 kV. 1–Nicotinamide (vitamin PP), 2–*p*-hydroxybenzoic acid, 3–α-tocopherol acetate (vitamin E), 4–riboflavin (vitamin B_2), 5–thiamine (vitamin B_1), 6–ascorbic acid (vitamin C), 7–pyridoxine (vitamin B_6), 8, 9, 10–unidentified, some may represent thiamine degradation products (all these peaks exhibit a distinct UV maximum around 260 nm).

polar analytes. So far separation of this type have been used for model mixtures only in attempts to elucidate the separation mechanisms involved; reports on the separation of vitamins [2], abused drugs [3], proteins [4] and conjugated bile acids [5] can be traced in the literature.

Microemulsions prepared by mixing the organic solvent water (aqueous buffer), surfactant and co-surfactant (such as medium-chain alcohol) are transparent and thermodynamically stable. According to Terabe [5] they consist of an organic solvent containing core surrounded by the surfactant and co-surfactant. Their role is to stabilise the droplet. The higher solubilization power of microemulsions has been claimed also as an advantage offering a wider dynamic range in sample concentration [7]. In the present investigation we have studied the separation of a series of both water and liposoluble vitamins in buffer systems containing various proportions of SDS, lower aliphatic alcohols and non-polar solvents (*n*-hexane).

2. Material and methods

2.1. Chemicals

2.1.1. Test mixture

Idroplurivit (a product of Menarini Ind. Pharm. United, Firenze, Italy) was used as a test mixture. Its composition runs as follows: 1660 UI vitamin E (α-tocopherol acetate), 16 mg thiamine hydrochloride (vitamin B_1), 16 mg riboflavin monophosphate (vitamin B_2), 8 mg pyridoxine chlorhydrate (vitamin B_6), 160 mg nicotinamide (vitamin PP), 500 mg ascorbic acid (vitamin C), 16 mg sodium pantothenate (vitamin B_5), *p*-hydroxybenzoic acid methyl ether served as excipient

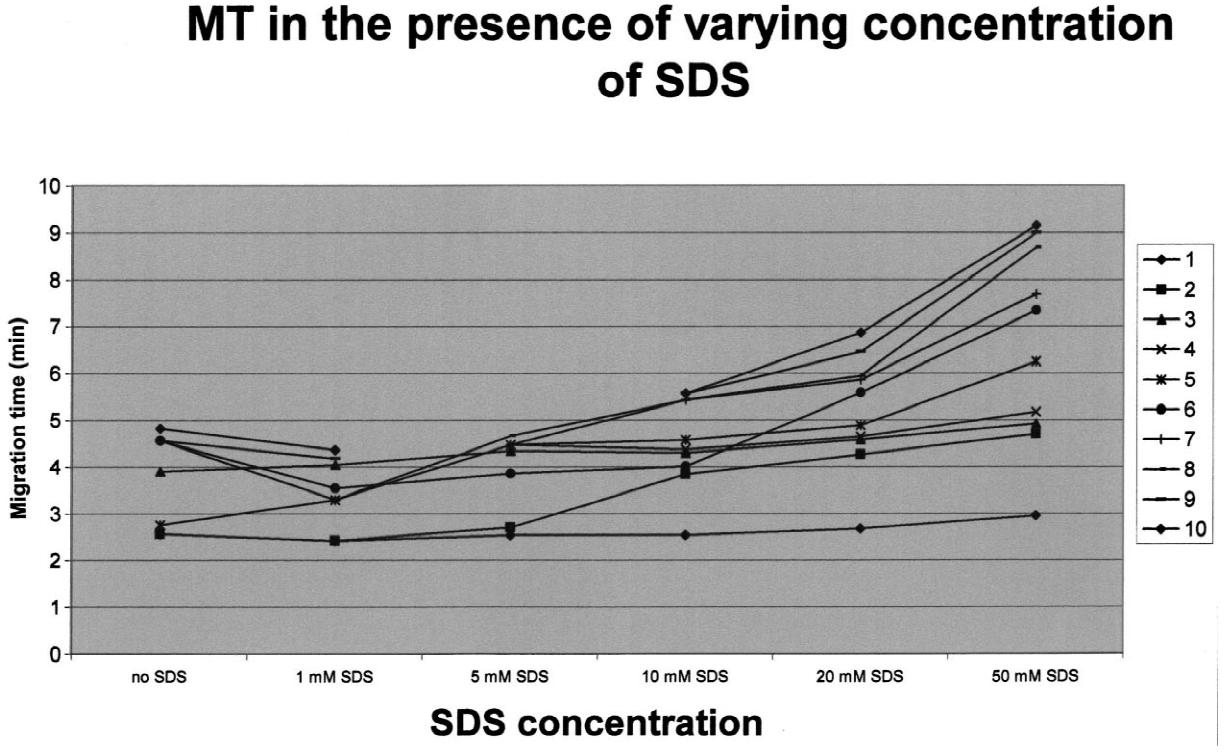

Fig. 2. Diagrammatic representation of migration time changes with increasing concentration of SDS in the background electrolyte (Specification of the analytes by numbers is identical with that in Fig. 1).

The data refer to 20 ml of the test solution; before injection into the capillary electrophoresis system the sample was diluted 1:2000 with Milli Q water.

2.1.2. Run buffer components

All components (sodium phosphate, organic solvents and SDS) were obtained from Carlo Erba, Milano, Italy.

2.2. Procedure

Electromigration separations were done on a Hewlett Packard Capillary Electrophoresis System (Waldbronn, Germany) using a 50 cm×75 μm (I.D.) bare silica capillary (57 cm total length). All runs were done at 10 kV applied to anode. Detection was set to 220 nm. For peak identification diode array detection was used. All runs were done in 12.5 mmol sodium phosphate containing the surfactant, co-surfactant (aliphatic alcohol) and possibly organic modifier (typically *n*-hexane) in amounts specified in Results. Prior to analysis the capillary was washed stepwise with 1 *M* NaOH, 0.1 *M* NaOH, Milli Q water and the run buffer (5 min each). Occasionally the capillary was rinsed with a water – 1 *M* HCl – water wash (5 min each). No post-wash steps were used.

3. Results and discussion

In order to elucidate the role of lower aliphatic alcohol presence in the surfactant (SDS) containing run buffer we started with a set of separation runs using 12.5 mmol/l sodium phosphate with varying concentration of SDS. The results are presented in Figs. 1–3. As emerges from these figures generally better resolution is obtained with higher SDS concentration. It is evident that the separation of ana-

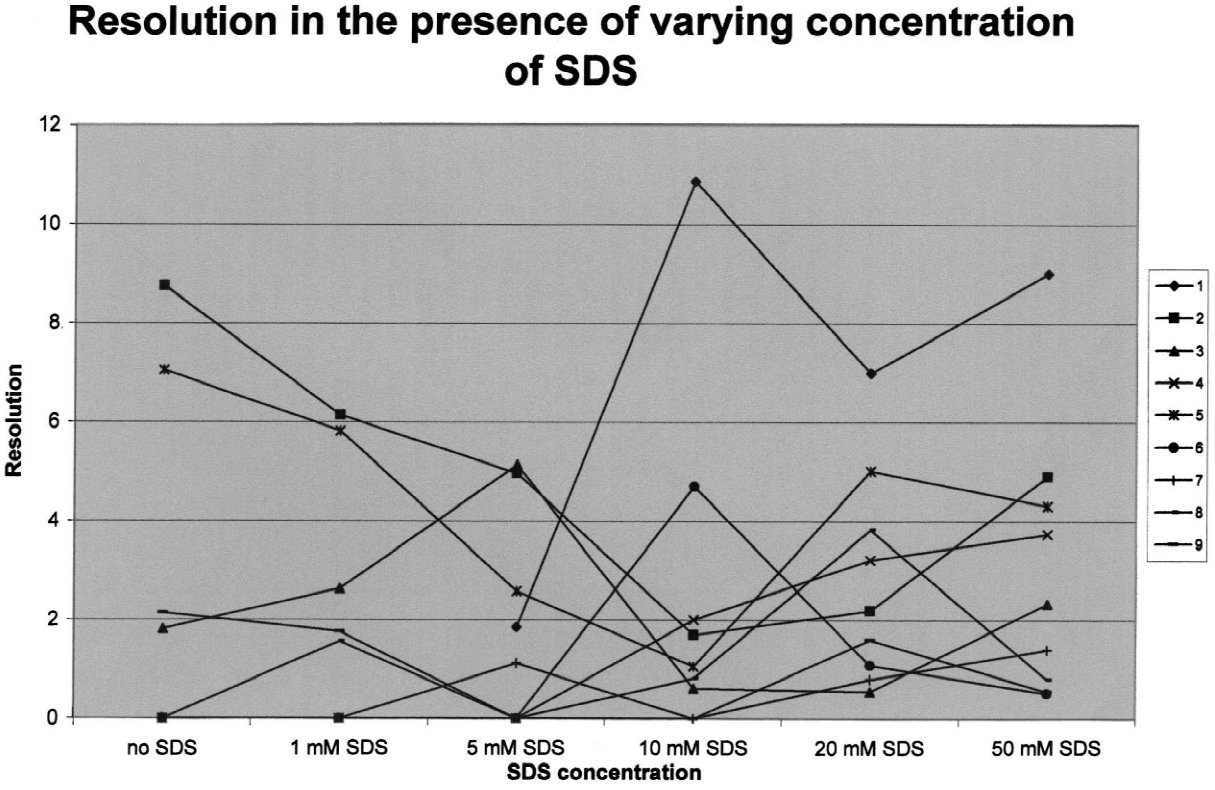

Fig. 3. Resolution of individual members of the test mixture with increasing SDS concentration in the background electrolyte (Specification of the analytes by numbers is identical with that in Fig. 1. Resolution is calculated to the peak that follows the peak in question).

lytes involved is considerably affected by the surfactant concentration both in the sub or supramicellar range; generally in the supramicellar range the separation is the better the higher surfactant concentration is. There is only one member of the test mixture that changes its position relatively to other members of the family, namely ascorbic acid, which moves quite distinctly to higher migration times with higher SDS concentration. Because of this behaviour at 10 m*M* SDS concentration ascorbic acid moves before α-tocopherol acetate, riboflavin and thiamine while in 20 and 50 m*M* SDS it emerges after the peaks of these compounds. This is indicative of an association of this vitamin with SDS micelles particularly if they are present in a high concentration.

Regarding resolution, good results were obtained at 20 and 50 mmol/l of SDS (e.g. at 20 and 50 mmol/l); two components had a resolution less than 1 (ascorbic acid and an unidentified peak, perhaps a thiamine degradation product).

The role of a set of lower aliphatic alcohols is visualised in Figs. 4–6; as shown in Fig. 4, within the set of aliphatic alcohols used no crossing over of migration times occurred. As expected there were practically no differences between propanol and isopropanol. The critical combination of analytes separated that were difficult to separate were *p*-hydroxybenzoic acid, α-tocopherol acetate, riboflavin and thiamine. Propanol and isopropanol if added in the 5% concentration to 50 mmol/l SDS does not separate *p*-hydroxybenzoic acid and α-tocopherol acetate, but the separation of these analytes is easily obtained with the addition of *n*-butanol in the same concentration. Also isoamylalcohol or propanediol are quite effective in separating *p*-hydroxybenzoic acid from the rest of the mixture, however the separation of ascorbic acid from the critical cluster of solutes is worse upon the addition of isoamylalcohol than if any other alcohol or β-cyclodextrin (without the addition of aliphatic alcohol) is present in the

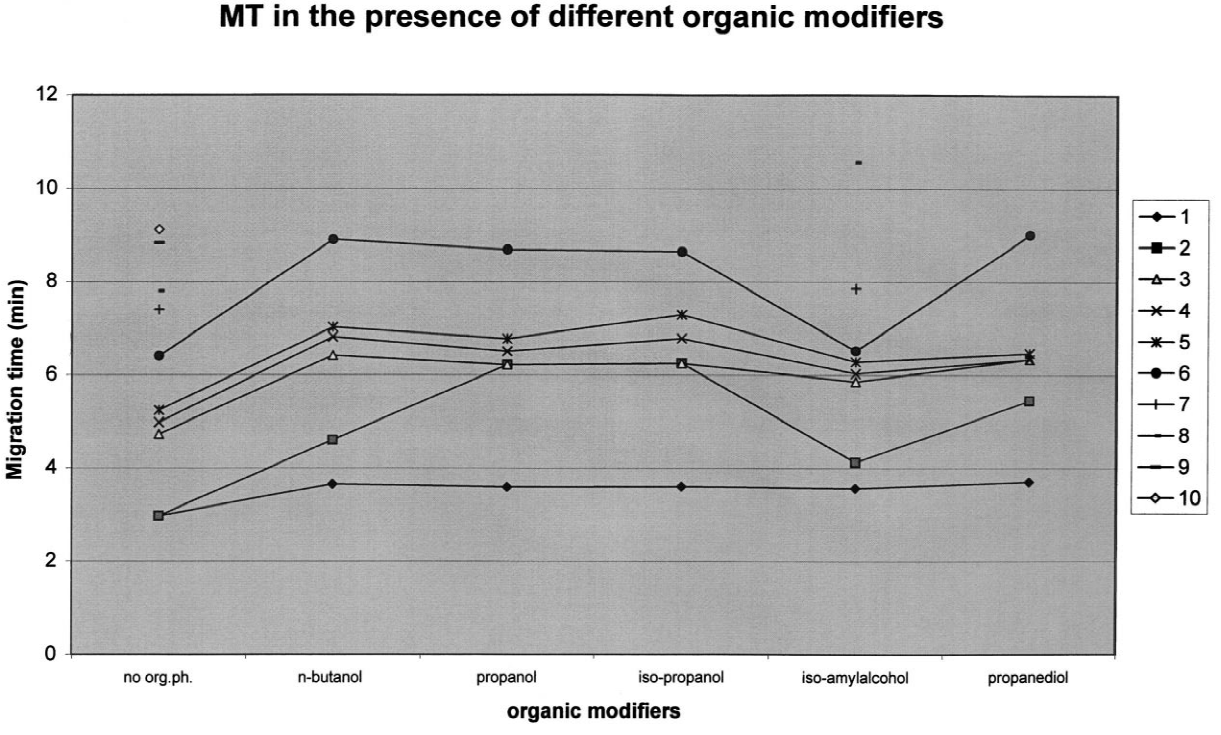

Fig. 4. Diagrammatic representation of migration time changes upon the addition of different lower aliphatic alcohols. All runs in 12.5 mmol/l phosphate buffer, 5% of respective alcohol and 50 mmol/l SDS. Identification numbers of compounds involved are the same as in Fig. 1.

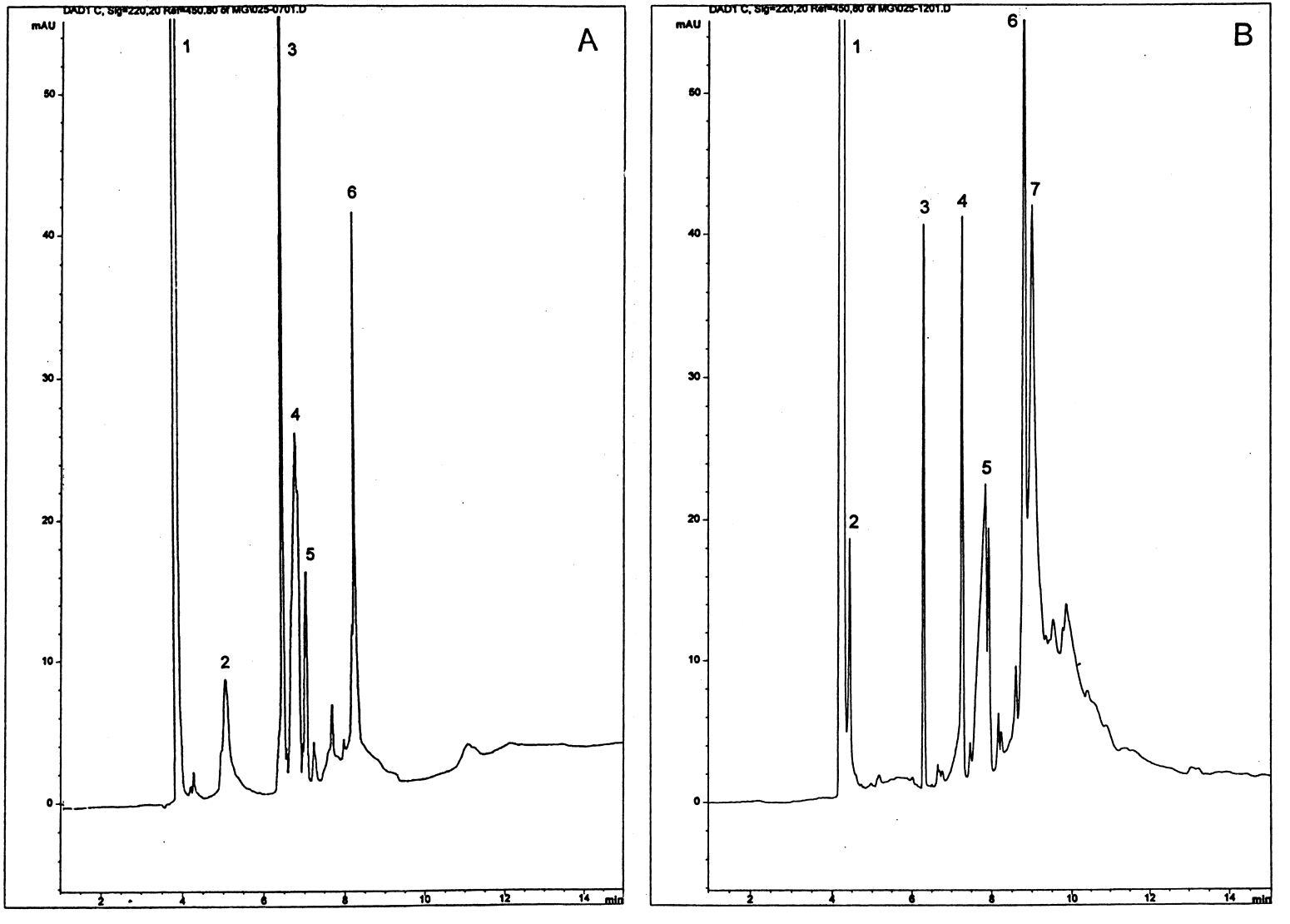

Fig. 5. A representative electropherogram of the test mixture upon the addition of 5% isoamylalcohol (A) and *n*-butanol (B) to the background electrolyte (12.5 mmol/l sodium phosphate, 50 mmol/l SDS).

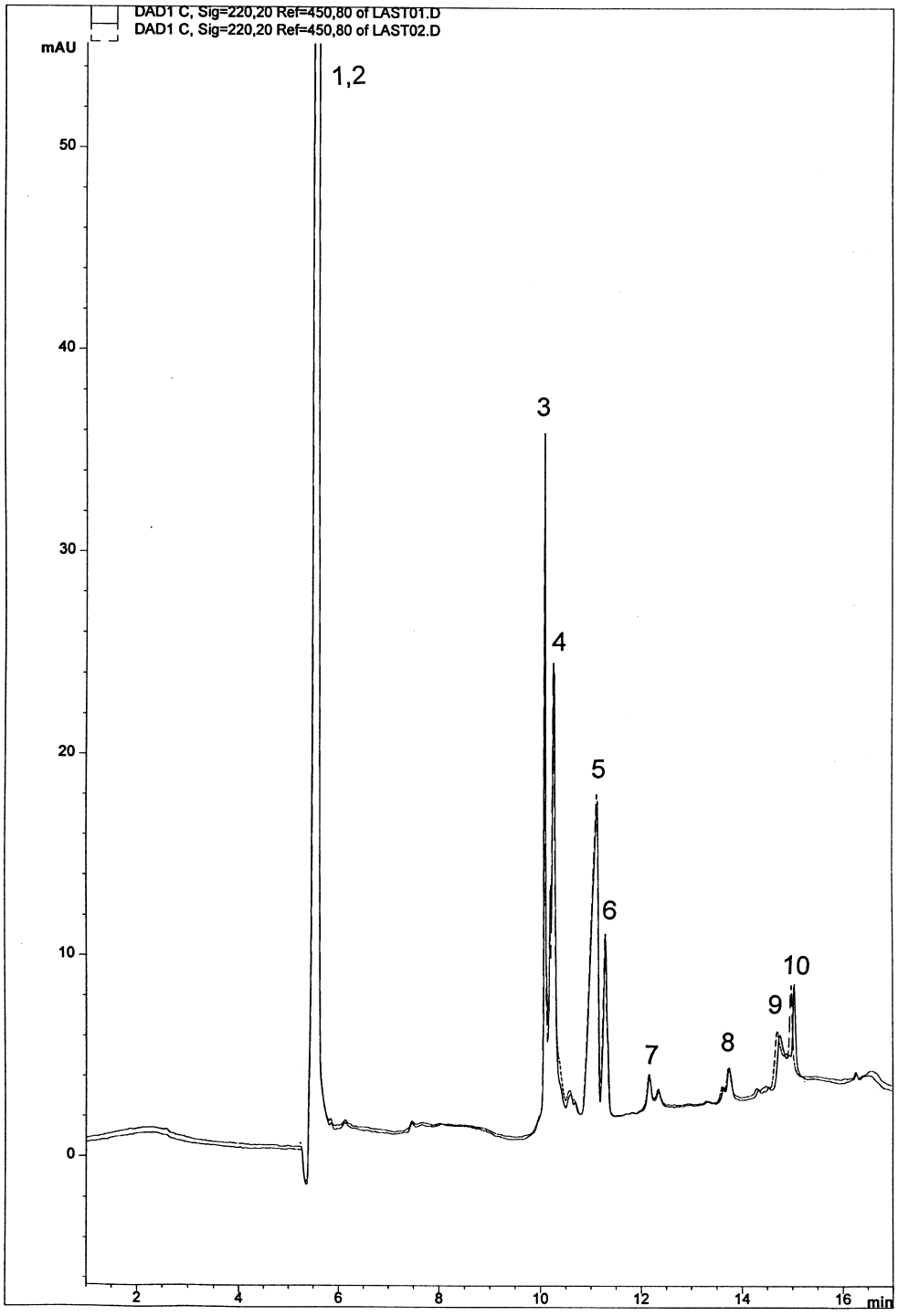

Fig. 6. Separation of the vitamin test mixture in the presence of ethanol (buffer-ethanol mixture 80:20, 50 mmol/l SDS in 12.5 mmol/l phosphate). The figure shows also the repeatibility of the runs (the solid and dashed lines represent the first and last run of a set of five). Peak designation as in Fig. 4.

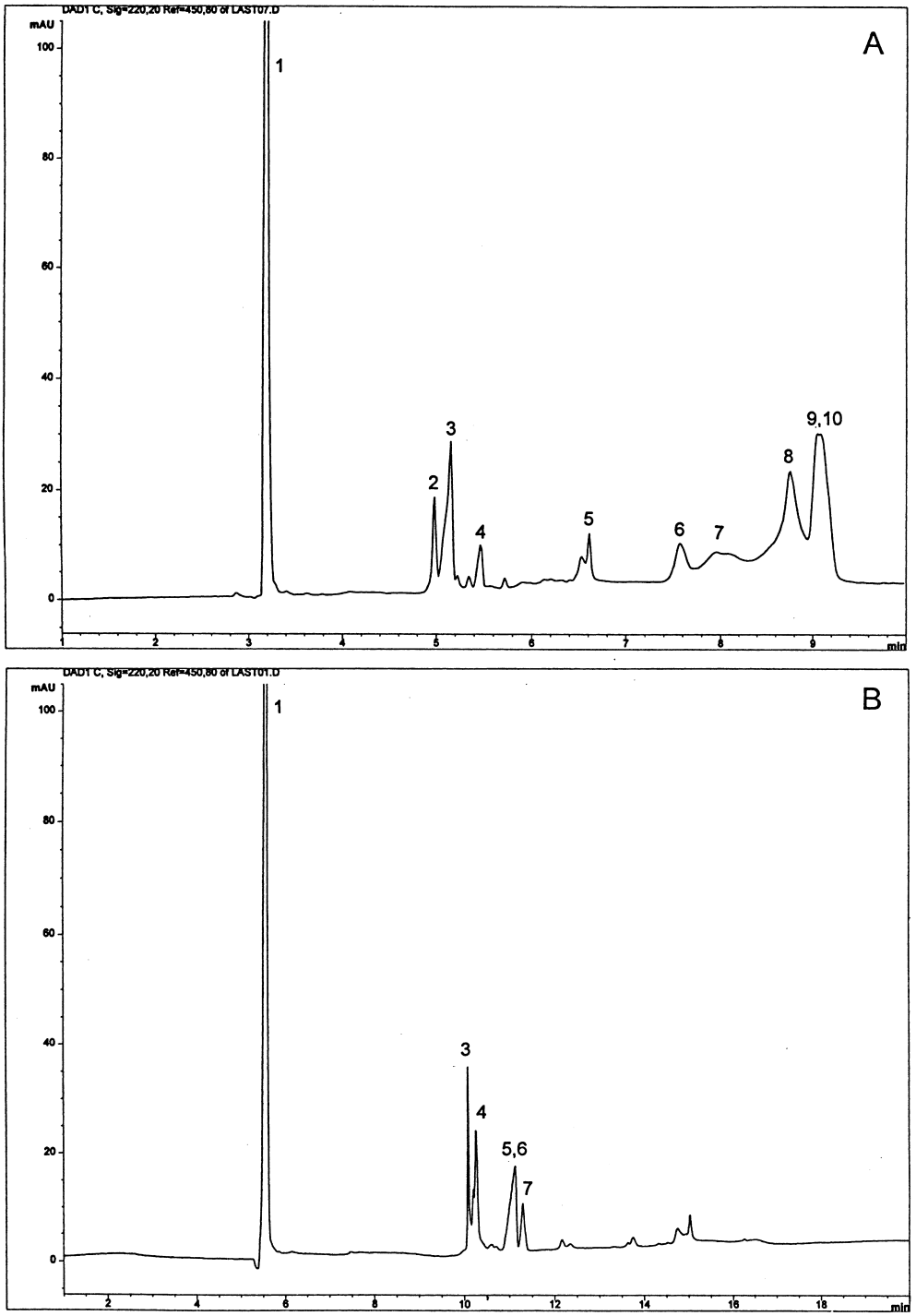

Fig. 7. Separation of the vitamin test mixture in 12.5 mmol/l phosphate containing 20 mmol/l SDS and 5% methanol with 300 μl of *n*-hexane (A) or without *n*-hexane (B). Peak identification as in Fig. 4.

mixture. The addition of γ-cyclodextrin in the concentration of 3 mmol/l was done because according to the literature [6] this addition reportedly improves the resolution. Indeed (see Fig. 4) column ''no organic phase'' γ-cyclodextrin presence apparently improves slightly the differences in migration times (compare Fig. 2, column ''No SDS''), however it makes the peaks of nicotinamide and *p*-hydroxybenzoic acid to fuse. Naturally, it is possible to increase the concentration of the organic modifier in the background electrolyte. Typically if 12.5 mmol/l phosphate buffer-ethanol (80: 20) mixture is used, no separation of nicotinamide and *p*-hydroxybenzoic acid was possible though all the other components were clearly separated.

If the separation is run in 20 mmol/l SDS containing buffer with 5% methanol (selected because it is least likely to form associates (microemulsion)) with the SDS micelles to which 300 μl of *n*-hexane were added, the separation is very similar to that obtained in 50 mmol/l SDS containing buffer (without the presence of any organic modifier (Fig. 7 A,B) The addition of hexane changes the properties considerably as obvious from comparison with Fig. 7 B where the separation run in the absence of *n*-hexane is presented.

4. Conclusions

This experimental work was directed towards elucidating the role of organic co-surfactants and modifiers in the separation of a test mixture the members of which differed considerably in polarity and molecular structure. It was demonstrated that (1) better resolutions are generally achieved with mobile phases of higher surfactant concentrations (50 mmol/l SDS turned the best); (2) addition of lower aliphatic alcohols (5% v/v) changes in some cases the selectivity of the system and changes occasionally also the order of emerging peaks. Isoamyl alcohol and propanediol appeared most efficient in this respect while there was practically no difference between the addition of propanol and isopropanol. *n*-Butanol, the effect of which was generally the same as that of *n*-propanol and isopropanol was capable of separating *p*-hydroxybenzoic acid from tocopherol acetate which upon the addition of propanol or isopropanol was not possible. (3) Further selectivity changes can be obtained by using an increased concentration of alcohol. In our hands 50 mmol/l SDS, 12.5 mmol/l sodium phosphate containing with ethanol 80:20 v/v appeared most suited for the separation of our test mixture. (4) Simultaneous addition of lower alcohol (typically methanol) and an organic modifier (*n*-hexane used) simulated increased concentration of SDS in the background electrolyte. It is concluded that simultaneous addition of an organic modifier, surfactant and co-surfactant to the background electrolyte may change the selectivity of the system in a wide range depending on the chemical nature of the components used.

References

[1] H. Watarai, Chem. Lett (1991) 31.
[2] R.L. Boso, M.S. Bellini, I. Mikšík, Z. Deyl, J. Chromatogr. A 709 (1995) 11.
[3] F. Tagliaro, J. Chromatogr. A, in press
[4] G. Luo, J. Chromatogr. A, in press
[5] Yu-Jer Lin, Jo-Ying Hu, Wei-Chao Lin, Yea-Shiou Ho, Chi-Peng Day, Pei-Lin Wu, Chun-Hsiung Kuei, J. Microcolumn Separ. 10 (1998) 625.
[6] S. Terabe, N. Matsubara, Y. Ishima, Y. Okada, J. Chromatogr. 608 (1992) 23.
[7] S.F.Y. Li, Capillary Electrophoresis, J. Chromatogr. Library, 52, Elsevier, Amsterdam, 1992.