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Comparison of micellar and microemulsion electrokinetic chromatography for the analysis of water- and fat-soluble vitamins

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Abstract

Separation and determination of water- and fat-soluble vitamins by micellar (MEKC) and microemulsion electrokinetic chromatography (MEEKC) are compared. MEKC is only useful in the quantitative analysis of water-soluble vitamins when sodium dodecylsulfate (SDS) is used as the surfactant. However, the separation of mixtures containing water- and fat-soluble vitamins is only achieved by MEEKC using a microemulsion prepared by mixing SDS as the surfactant, butanol as the co-surfactant, octane as the non-polar modifier and propanol as the second co-surfactant. The injection time and the solvent used for the dilution of samples have a significant effect on the analysis of lypophilic compounds. The most reproducible results in the analysis of fat-soluble vitamins are obtained by using the same microemulsion electrolyte as the solvent for samples and an injection time of 10 s.

Keywords: Micellar electrokinetic chromatography; Microemulsion electrokinetic chromatography; Background electrolyte composition; Vitamins

1. Introduction

Capillary electrophoresis (CE) has become increasingly popular in the analysis of pharmaceutical preparations since Terabe et al. [1], in 1984, introduced micellar electrokinetic chromatography (MEKC) [2]. This mode of electrophoresis permits the separation of neutral components, which migrate in the separation window in order of increasing hydrophobicity. MEKC is a useful technique in the analysis of water-soluble compounds. The separation and analysis of lypophilic analytes, however, may be difficult in MEKC due to the strong affinity of lypophilic compounds to the micelle resulting in long separation times and poor resolution [3].

The analysis of water- and fat-soluble vitamins in one single run cannot be achieved by typical HPLC methods [4], but some studies suggest that this analysis may be performed by CE [3,5–12]. The separation of fat-soluble vitamins by MEKC is extremely difficult because these compounds have an extreme partition to the micelles that results in high retention and poor resolution [3,6,8,9] making it necessary to add organic solvents to the buffered electrolytes for the analysis of highly lypophilic compounds [8,9,13]. Addition of organic solvents, usually acetonitrile or short chain aliphatic alcohols, to micellar solutions has been applied in the separation of vitamin mixtures [3,5–8,12,14,15]. The addition of organic solvents increases the solubility

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of lypophilic analytes in the separation electrolyte with respect to typical MEKC but in some cases there are problems of solvent evaporation [16].

Microemulsion electrokinetic chromatography (MEEKC) [17,18] is another CE method that presents some advantages with respect to the use of organic solvent additives in MEKC. In fact, MEEKC is a variation of MEKC where a microemulsion droplet, a non-polar pseudophase, is formed by mixing an immiscible organic solvent, such as octane, with a buffered aqueous solution. A surfactant and a co-surfactant, such as butanol, are added to stabilise the emulsion by coating the outside of the droplet. The aim of MEEKC is to increase selectivity in the separation of non-polar solutes. The use of MEEKC has the advantage over MEKC that it dissolves highly lypophilic compounds more effectively, reduces the loss of solvent through evaporation, and results in a greater separation of the lypophilic compounds [18]. Despite the great potential of this technique, few studies into the use of microemulsion systems in the analysis of fat-soluble vitamins have been conducted [5,9–11].

The main disadvantage of MEEKC is the relatively long times needed for the separation of lypophilic compounds. However, as a CE method it has the advantage that in principle an increase in the analysis time should not affect the efficiency and the separation resolution should also be maintained.

In the present study we have studied and compared MEKC and MEEKC in the analysis of water- and fat-soluble vitamins in order to evaluate the advantages and disadvantages of each mode of CE and to determine the best CE mode for the analysis of water- and fat-soluble vitamin mixtures. The main interest of this study is focused on the separation of a wide group of fat- (A palmitate, E acetate, D_3 and K_3) and water-soluble (B_1, B_2, B_3, B_6, B_{12} and C) vitamins. The study also compares two different types of surfactants; one anionic, sodium dodecylsulfate (SDS); and the other cationic, cetyltrimethylammonium chloride (CTAC).

2. Experimental

2.1. Chemicals

Vitamins A palmitate, E acetate, K_3, D_3, and B_2 were supplied by Sigma (Germany). Vitamins B_1 and B_3 were from Roche (Switzerland), vitamin B_{12} from Roussel-Uclaf (France), and vitamins B_6 and C from Basf Española (Spain).

SDS (>99%) was supplied by Sigma, and Cetyltrimethylammonium Chloride (CTAC) was obtained from Fluka (Switzerland). Boric acid (analytical grade) was from Riedel-de Haen (Germany) and sodium borate (analytical grade) from Panreac (Spain).

All solvents were HPLC grade and were employed as supplied by manufacturers. Methanol, ethanol, propanol, and butanol were obtained from Carlo Erba (Italy). Pentane, hexane, heptane, and octane were from Fluka.

Fort Dodge kindly supplied multi-vitamin formulation ''Duphafral Multi'' which has been used as a commercial preparation. The composition of this pharmaceutical formulation is: vitamin B_1 (10 mg ml^{-1}), Vitamin B_2 phosphate (5 mg ml^{-1}), Vitamin B_6 (3 mg ml^{-1}), vitamin PP (35 mg ml^{-1}), dexpanthenol (25 mg ml^{-1}), vitamin B_{12} (20 μg ml^{-1}), vitamin A palmitate (9 mg ml^{-1}), vitamin E acetate (20 mg ml^{-1}) and vitamin D_3 (0.2 mg ml^{-1}). Excipients and antioxidants: citric acid, tioglycolic acid, phenol, benzylic alcohol, EDTA, 2[3]-*tert*.-butyl-4-hydroxyanisole (BHA) and 2,6-di-*tert*.-butyl-*p*-cresol (BHT).

2.2. Preparation of solutions

Individual stock solutions containing approximately 200 mg L^{-1} of the vitamins were prepared and stored in a light protected environment. As vitamin C suffers degradation in solution, its stock solution was prepared daily. Stock solutions of the fat-soluble vitamins were prepared by dissolving them in butanol and storing them at 0 °C. Water-soluble vitamins were dissolved in water or microemulsion solutions and stored at 4 °C. Working solutions were freshly prepared each day by dilution of the stock solutions.

A 40 m*M* buffer of sodium borate–boric acid at pH 8.5±0.1 was selected for the preparation of all the background electrolytes (BGEs). Fotsing et al. [19] found that this buffer is more efficient and generates a lower current than other buffers in the analysis of commercial pharmaceutical vitamin prep-

arations by capillary electrophoresis. All the BGEs were prepared daily and, prior to use, passed through a 0.22 μm filter and degassed in an ultrasonic bath.

Microemulsions were prepared by mixing appropriate volumes of the organic solvent and the co-surfactant with weighted amounts of the surfactant, boric acid and sodium borate to a fixed volume. The mixed solution was then sonicated for 30 min until the surfactant was dissolved, and a clear and stable microemulsion was obtained. In these conditions the microemulsion is optically transparent and stable for several months.

2.3. Apparatus and electrophoretic conditions

Analyses were performed using a Waters Capillary Ion Analyzer (Waters, MA, USA) with an unmodified fused-silica capillary (Waters) of 63 cm (57 cm to the detector)×75 μm I.D. Waters Millenium 32 software was employed for integration and data handling. All the electrophoretic measurements were made at a constant temperature of 25 °C. UV detection was undertaken at 254 nm.

Each new capillary column was conditioned by first rinsing it with 1 *M* NaOH for 10 min, then 0.1 *M* NaOH for 10 min, followed by pure water for 10 min and finally with BGE solution for 10 min. Prior to use, the capillary was washed daily for 5 min with 0.1 *M* NaOH, water for 5 min, and the BGE solution for 10 min. The capillary was flushed between runs with 0.1 *M* NaOH, water and the BGE at predetermined times, which depended on the BGE employed.

The evaluation of the current generated in the analysis of vitamins by the different CE modes of analysis indicates that a voltage of 15 kV can be applied without generation of Joule heating. Reproducible migration times are obtained at this voltage in all the CE modes evaluated.

3. Results and discussion

3.1. Micellar electrokinetic chromatography (MEKC)

Conventional MEKC is only useful in the separation of mixtures containing water-soluble vitamins if surfactants are added. We have tested two different surfactants, SDS and CTAC. The use of SDS as an anionic surfactant allows the separation of water-soluble vitamins as was reported [3,20–22]. On the other hand, CTAC does not function satisfactorily as a surfactant as it fails to separate nicotinamide, pyrodoxine and cobalamine. Fat-soluble vitamins cannot be separated in the aqueous MEKC buffers studied given that their high affinity to the micelles make them appear too close to the micelle peak. Moreover, these vitamins precipitate during electrophoresis due to their low solubility in aqueous electrolytes and may block the capillary, as was also found by Pedersen-Bjergaard et al. [8]. These problems can be solved by the addition of an organic modifier to aqueous MEKC buffers. Moreover, the increase in the lypophilicity of the electrolyte improves the separation of the fat-soluble vitamins [23,24]. Our results show that the greater the lypophilicity of the modifier, the greater the separation of vitamins A, E, D_3, and K_3 (Fig. 1), but only vitamin K_3 can be baseline separated from the other fat-soluble vitamins when butanol, the most lypophilic solvent evaluated, is added to the SDS-based electrolyte and the samples are dissolved in a ethanol/chloroform mixture. CTAC gives similar separation results for vitamins A, D_3, and K_3 but with lower migration times and in the case of vitamin E its precipitation blocks the capillary. The resolution of water-soluble vitamins increased when organic modifiers were added to the BGE due to the changes in its viscosity. With the cationic surfactant (CTAC), the use of methanol, ethanol, propanol, and

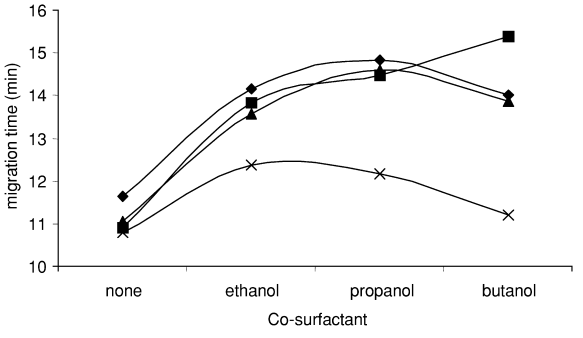

Fig. 1. Electrophoretic behaviour of fat-soluble vitamins in MEKC with the addition of organic modifiers. (♦ A, ■ E, ▲ D, × K) Experimental: all solutions contain 40 m*M* SDS as the micellar solution and 5% of organic modifier, 10 s injection time, samples dissolved in a chloroform–ethanol mixture.

butanol modifiers was also tested. The best results in terms of vitamin separation are obtained when butanol is added to the BGE although nicotinamide and cobalamine are still not separated. Despite the improvement in the separation of the vitamins, the addition of organic solvent additives in MEKC has two problems. Firstly, organic modifiers may be incorporated into the micelles causing them to swell and decreasing the migration time of those solutes with an opposite charge to the surface charge of the micelles i.e. vitamin B_1 with SDS and vitamin B_2 phosphate with CTAC, when high content of the most hydrophobic alcohols are added to the BGE (Fig. 2). Secondly, the use of an excessive amount of additive, 15% or more in the case of methanol and over 10% for other alcohols, results in solvent evaporation in the BGE whose composition changes and gives irreproducible migration times for the vitamins. The first of these problems is easily solved by increasing the surfactant concentration, but the second cannot be avoided.

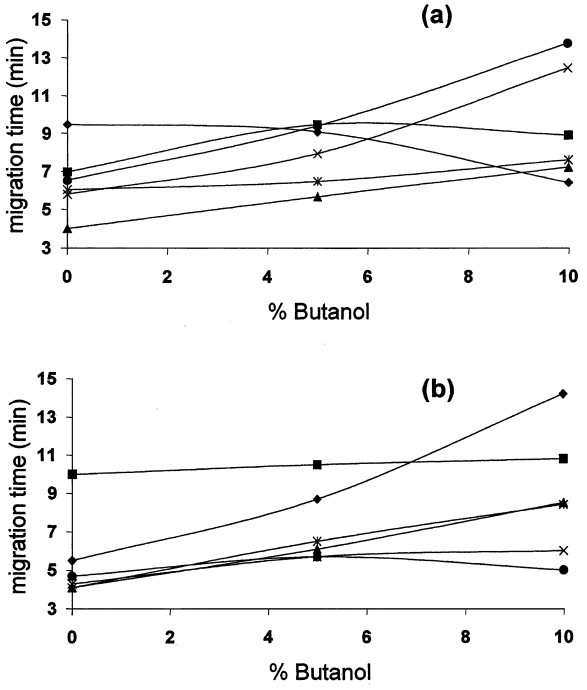

Fig. 2. Electrophoretic behaviour of water-soluble vitamins in MEKC with the addition of butanol as organic modifier. (a) anionic surfactant, SDS; (b) cationic surfactant, CTAC. (♦ B_1, ■ B_2, ▲ B_3, × B_6, * B_{12}, • C) Experimental: BGE Borate–Boric Acid 40 m*M* at pH 8.5±0.1, 40 m*M* surfactant, 20 s injection time.

3.2. Microemulsion electrokinetic chromatography

The main advantage of MEEKC over MEKC in the analysis of lypophilic compounds is that microemulsions are relatively non-volatile and may be prepared with a higher proportion of organic additives without loss of solvent through evaporation. The solvating properties of microemulsions also enable the resolution of solutes of different hydrophobicities.

The choice of butanol as the co-surfactant was determined using the results obtained in the analysis of fat-soluble vitamins by MEKC. Different organic solvents (pentane, hexane, heptane and octane) have been evaluated as internal organic phase in the microemulsions. The concentration of the surfactant has to be optimised in order to obtain the solubilisation of the organic solvents in the microemulsion. SDS electrolyte (40 m*M*) can solubilise 1% (v/v) pentane or hexane, but a higher concentration is needed to form microemulsions with the same amount of heptane or octane. SDS, at a concentration of 80 m*M*, can form microemulsions with all the organic solvents evaluated and it permits a stable baseline to be obtained. Results show small changes in the electrophoretic behaviour of fat-soluble vitamins with the use of different internal organic phases. Gabel-Jensen et al. also found the same effect in the separation of different neutral organic compounds by MEEKC [25]. However, the highest separation efficiency was achieved with the most lypophilic solvent, octane and hence we selected this solvent as the internal organic phase.

The analysis of commercial multi-vitamin preparations requires the use of organic solvents to ensure the solubility of fat-soluble vitamins and organic additives added in the preparation of drugs. Determination of the best solvent is required in order to obtain the best resolution for vitamin separation by MEKC [26] and MEEKC [9]. The results obtained in the analysis indicate that higher sample solvent lypophilicity results in greater migration times and increased resolution. The use of an emulsion containing 80 m*M* SDS, 1% (v/v) octane, and 5% (v/v) butanol allows the separation of vitamin E from

vitamins A and D_3 when samples are dissolved in ethanol/chloroform mixtures, but it is not possible to separate the same vitamin mixture when the sample is dissolved in the microemulsion. Although the efficiency of separation when this microemulsion electrolyte is used as the solvent is lesser than that obtained with ethanol/chloroform mixtures, microemulsions give the most reproducible results given their lesser volatility and the fact that sample composition is not changed by solvent vaporization. In a series of five consecutive analysis of the same vial, reproducibility (RSD%) of the migration time was >5% for a sample dissolved in ethanol–chloroform and <3% when the same sample was dissolved in the microemulsion electrolyte.

In order to obtain the separation of fat-soluble vitamins through the use of microemulsion, it is necessary to increase its lypophilicity. Pedersen-Bjergaard et al. [10] found that the addition of a second co-surfactant, propanol, to the microemulsion allows the complete separation of three fat-soluble vitamins (A palmitate, E acetate and D_3) in a suppressed electro-osmotic flow (EOF) environment. We studied the effect of adding different proportions of propanol to microemulsions containing butanol as the co-surfactant and octane as the non-polar solvent in the presence of EOF. The increased lypophilicity of the electrolyte resulting from the addition of propanol leads to greater resolution in the separation of fat-soluble vitamins, and baseline separation of the fat-soluble vitamins evaluated is achieved through the addition of 15% propanol (Fig. 3). Greater proportions of propanol result in greater resolution but also in longer migration times and an unstable baseline.

The injection time also has an important effect on the separation efficiency of lypophilic compounds in MEKC and MEEKC systems. Altria [9] found that longer injection times resulted in poor separation in terms of peak shape, reduced times and lower resolution. We observed the same effects in the analysis of fat-soluble vitamins. In the analysis of

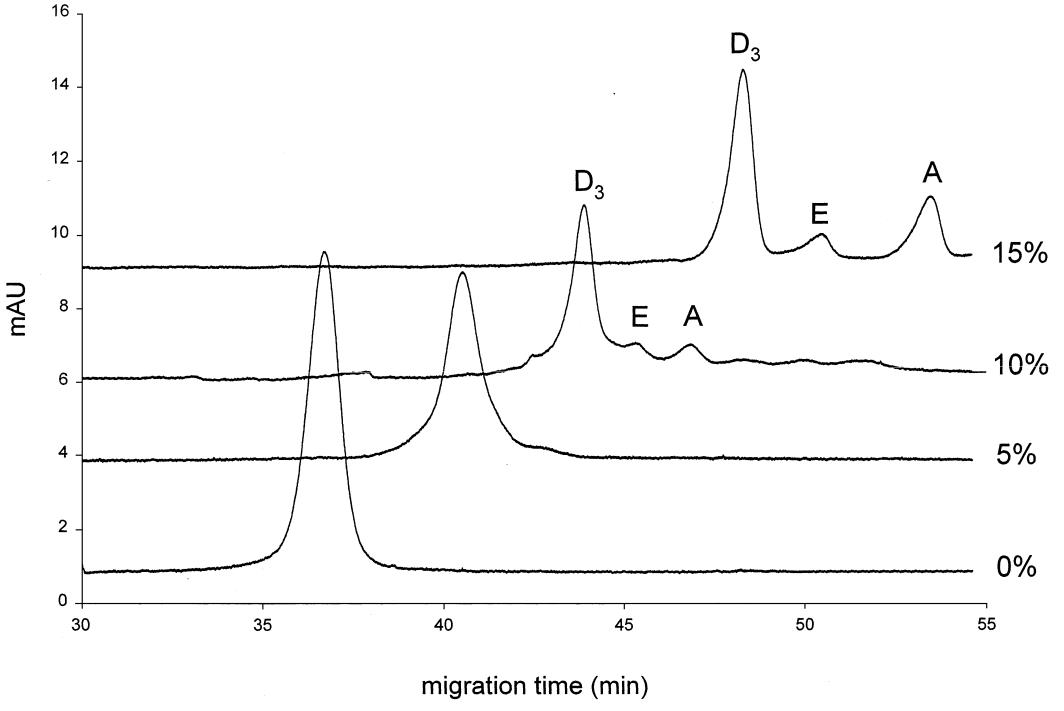

Fig. 3. Separation of fat-soluble vitamins by MEEKC by adding 1-propanol to the electrolyte as a second co-surfactant at different proportions. Experimental: microemulsion containing 40 m*M* borate buffer, 80 m*M* SDS, 5% butanol, 0.8% octane, 0–15% propanol; 10 s injection time; samples dissolved in the microemulsion.

real samples, the use of an injection time of 10 s gives the best results in terms of sensitivity, efficiency and resolution for the fat-soluble vitamins without significant changes occurring in the results for water-soluble vitamins.

4. Conclusions

The present study evaluates and compares MEKC and MEEKC capillary electrophoresis systems in the separation of water- and fat-soluble vitamins with the use of two surfactants, SDS and CTAC.

MEKC is only useful in the quantitative analysis of water-soluble vitamins, and SDS–micellar solutions are superior to CTAC systems in terms of separation efficiency. Fat-soluble vitamins cannot be separated by MEKC and their individual analysis at low concentrations is only possible in this CE mode.

The addition of organic solvent additives to the electrolyte is needed to analyse lypophilic analytes. In MEKC, it has the advantage that lower surfactant concentrations are required in order to separate water-soluble vitamins. Moreover, this mode of CE also allows the separation of vitamin K_3 from other fat-soluble vitamins. However, complex mixtures of fat-soluble vitamins cannot be separated by this technique.

MEEKC proved to be the most efficient technique in the separation of vitamins. It gives better resolution than MEKC in the separation of fat-soluble vitamins due to the greater solubility, stability and separation efficiency of microemulsions. The separation of a broad group of water- and fat-soluble vitamins (B_1, B_2, B_3, B_6, B_{12}, C, A palmitate, D_3, E acetate, and K_3) is obtained when the microemulsion is prepared with SDS as the surfactant, octane as the non-polar modifier, butanol as the co-surfactant, and propanol as the second co-surfactant. In these conditions, the lypophilic compounds are completely solubilised and there is no problem of precipitation during electrophoretic measurement. Baseline separation of the fat-soluble vitamins is achieved maintaining the same efficiency in the separation of

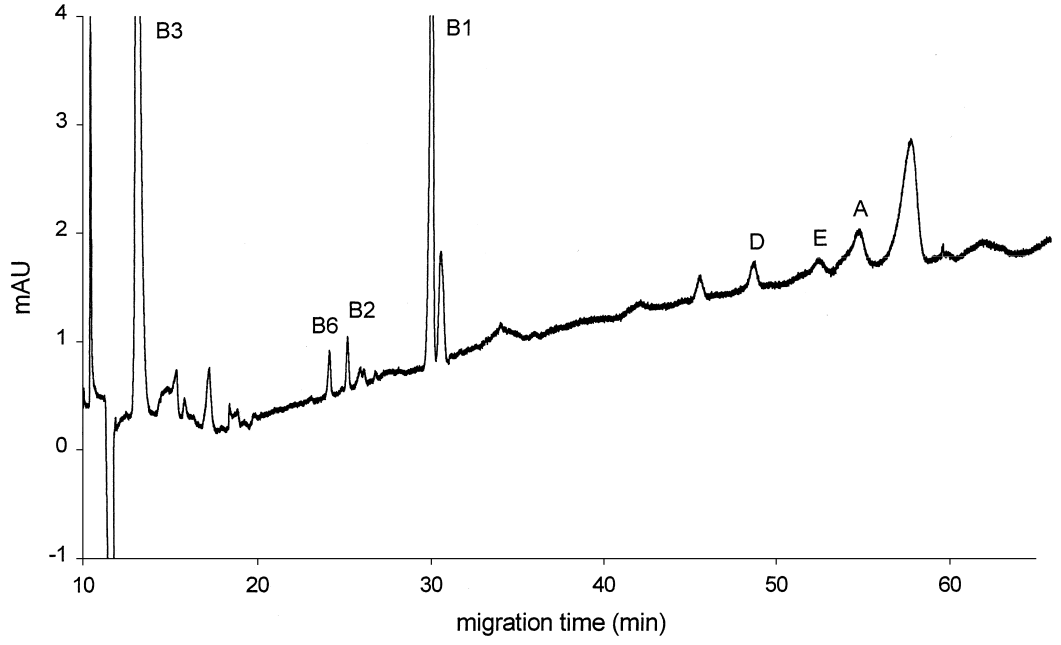

Fig. 4. Electropherogram of a commercial multi-vitamin drug (Duphafral Multi). Unmarked peaks correspond to non-analysed vitamins in this study and organic additives present in the commercial drug (see Experimental section for composition). Experimental: microemulsion containing 40 m*M* borate buffer, 80 m*M* SDS, 5% butanol, 0.8% octane, 15% propanol; 10 s injection time; sample dissolved in a microemulsion.

water-soluble vitamins as in other CE modes. An electropherogram with the complete separation of water- and fat-soluble vitamins in a commercial multivitamin drug is shown in Fig. 4.

The solvent used in sample dilution and injection time has a significant effect on the results obtained. The use of the microemulsion electrolyte as the diluting solution and an injection time of 10 s give the best results.

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