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Short communication

Microemulsion electrokinetic chromatography for the separation of retinol, cholecalciferol, δ-tocopherol and α-tocopherol

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Abstract

A microemulsion electrokinetic chromatographic method was used to separate fat-soluble vitamins. The separation of retinol, cholecalciferol, and δ- and α-tocopherol was performed using a microemulsion containing 0.75% (v/v) *n*-heptane, 30 mM bis(2-ethylhexyl)sodium sulfosuccinate (AOT), 5% (v/v) 1-butanol, 15% (v/v) 1-propanol and 15% (v/v) methanol in 20 mM boric acid–sodium borate buffer. The effect of the different microemulsion constituents was studied, including the type and concentration of surfactant, buffer, oil and co-surfactants. The presence of methanol in the microemulsion was found to be necessary to achieve the separation of the tocopherols. Detection was carried out at 200, 265 and 325 nm for the tocopherols, cholecalciferol and retinol, respectively. Calibration curves and precision data were obtained for each analyte. Good linear relationships were found between the analytical signal and the analytes concentration in the 25–500 mg L^{-1} for retinol and cholecalciferol, and 25–300 mg L^{-1} for tocopherols ranges. The precision of the method afforded relative standard deviations in the 4.0–10% range.

Keywords: Microemulsion electrokinetic chromatography; Retinol; Cholecalciferol; Tocopherols; Bis(2-ethylhexyl)sodium sulfosuccinate

1. Introduction

Microemulsions are nanometre-sized droplets of oil-in-water or water-in-oil, stabilized by a surfactant and often a short-chain alcohol as co-surfactant. The possibility of using microemulsions as separation media for capillary electrophoresis was demonstrated for the first time by Watarai [1], and the technique was termed microemulsion electrokinetic chromatography (MEEKC). MEEKC is a useful technique for the separation of both charged and neutral solutes, covering a wide range of solubility. Separation is based on both hydrophobic interactions and the electrophoretic mobility of solutes. Thus, substances that are not resolved by micellar electrokinetic chromatography (MEKC) can be separated using MEEKC [2]. Several authors have studied selectivity in MEEKC [3–5].

Sodium dodecyl sulfate (SDS) is the most commonly used surfactant in microemulsions; the immiscible oil is typically octane or heptane, and the most frequently employed co-surfactant is 1-butanol [6]; the microemulsion also contains an aqueous buffer. In the present work, the surfactant used was bis(2-ethylhexyl)sodium sulfosuccinate (AOT). This surfactant was used in previous works to separate synthetic food antioxidants [7] and water and fat-soluble vitamins [8] using MEKC. In this work, retinol and α-tocopherol have been separated, but no cholecalciferol.

MEEKC has been used in the separation analgesics [9], neutral steroids [10], cardiac glycosides [11] and numerous drugs and pharmaceutical products [12–15]. Few papers have described the separation of vitamins using MEEKC. Boso et al. [16] studied the separation of water- and fat-soluble vitamins (E and A) using anionic (SDS) and cationic (trimethyltetradecylammonium bromide, TTAB) surfactants. Altria [12] applied a MEEKC method to analyze a wide range of both water-soluble and insoluble pharmaceutical products, vitamins and excipients, but only with vitamin A and D_3 as the fat-soluble vitamins. Pedersen-Bjergaard et al. [17] used MEEKC for the separation and determination of vitamin A palmitate, vitamin D_3, and vitamin E acetate in pharmaceutical products. Sánchez and Salvadó [18] separated mixtures containing water- and fat-soluble vitamins using SDS as the surfactant, butanol as the co-surfactant,

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octane as the non-polar solvent, and propanol as a second co-surfactant. Aurora-Prado et al. [19] determined a water-soluble vitamin – folic acid – in tablets using MEEKC. Recently, Chang et al. [20] have separated α-, γ-, δ-tocopherol and α-tocopherol acetate using cyclodextrin-modified microemulsion chromatography.

The aim of this work was to develop a rapid and simple MEEKC method for the separation of vitamin A, D_3 and δ- and α-tocopherol, in reasonably short times, using the surfactant AOT for the first time in MEEKC; these analytes were not resolved using MEKC. Different oils and co-surfactants were studied in order to achieve the separation of analytes.

2. Experimental

2.1. Apparatus

MEEKC was performed with a Hewlett-Packard ^{3D}Capillary Electrophoresis system (Waldbronn, Germany) equipped with fused-silica capillaries of 58.5 cm (50 cm to the detector) ×75 μm I.D. ×375 μm O.D. from Composite Metal Services (Ilkley, UK). The capillary was maintained at 25 °C. Detection was performed at 200, 265 and 325 nm, for vitamins E, D_3 and A, respectively.

2.2. Reagents

α-Tocopherol (>98%) and δ-tocopherol (90%) were supplied by Sigma (Madrid, Spain). Vitamin D_3 (>99.0%), vitamin A alcohol (>99.0%), sodium dodecyl sulfate (SDS), *n*-octane (≥99.5%), *n*-nonane (>99.8%), 1-penthanol and 1-hexanol were from Fluka (Madrid, Spain). Bis(2-ethylhexyl)sodium sulfosuccinate was from Aldrich (Madrid, Spain). Acetonitrile, methanol, hexane and 1-octanol were from Merck (Darmstadt, Germany) all were of special LC quality. *N*-hexane (96%), disodium tetraborate decahydrate and sodium hydroxide were supplied by Scharlau (Barcelona, Spain); 1-butanol and 1-propanol were from Panreac (Barcelona, Spain). All chemicals used for the preparation of the buffer electrolytes were of analytical reagent grade. Ultra-high-quality water was obtained with an Elgastat UHQ (High Wycombe, UK) water purification system.

2.3. Procedure

Uncoated fused-silica capillaries were conditioned before use. All new capillaries were pre-treated by sequentially flushing 1.0 M NaOH, water, 20 mM borate buffer, and microemulsion solution (10 min each). This procedure was also applied at the beginning of the day, reducing the time length of the steps by a half. Between runs, the capillaries were rinsed with 0.1 M NaOH and water (3 min each) prior to the passage of the microemulsion. Temperature was maintained at 25 °C.

Solutions of the vitamins were prepared in methanol at concentrations close to 1000 mg L^{-1}. 100 μL aliquots of this solution were diluted up to 500 μL with the microemulsion and then injected electrokinetically into the capillary at 24 kV for a fixed period of 5 s. The same potential, 24 kV, was applied to achieve separation. Detection was performed spectrophotometrically at 325 nm for vitamin A; at 265 nm for vitamin D_3, and at 200 nm for tocopherols.

2.4. Preparation of microemulsions

The final microemulsion was prepared by mixing 5% (v/v) 1-butanol, 0.75% (v/v) *n*-heptane, 30 mM AOT, 20 mM boric acid–sodium borate buffer, 15% (v/v) 1-propanol and 15% (v/v) methanol. The resulting solution was sonicated for at least 30 min until a clear and stable microemulsion was obtained. In these conditions all vitamins were resolved in 25 min.

3. Results and discussion

3.1. Optimization of the microemulsion medium

Variation of the microemulsion components can affect the separation selectivity [5]; thus, we carried out a preliminary study to find the best conditions for analyte separation. In order to achieve the separation of all vitamins, the microemulsion should contain a surfactant, an organic solvent, and several co-surfactants: methanol, propanol and butanol.

To choose the surfactant, SDS, AOT, and mixtures of both surfactants were tested; AOT was chosen because it allowed the separation of the vitamins in a shorter time. AOT has not been used as surfactant in MEEKC; some authors [21] have reported that microemulsions containing 3.3% AOT are not stable. However, the microemulsions prepared under the conditions described in this work (between ∼0.7 and ∼2.7%) were stable even over months.

The surfactant concentration was studied using microemulsions prepared as in Section 2.3 varying the AOT concentration between 15 and 60 mM. Small amounts of the surfactant produced a less stable microemulsion, and the separation of vitamins D_3 and E was not possible. Higher concentrations increased the retention of analytes, ionic strength, and the background current (from 18 μA for 15 mM AOT to 52 μA for 60 mM AOT). Thus, on increasing the amount of surfactant the separation of vitamins improved, but the analysis time also increased (Fig. 1). Furthermore, sensitivity decreased when the amount of AOT increased. Owing to this, 30 mM AOT was chosen as optimum because it allowed the separation of analytes in a shorter time with good sensitivity.

The effect of the type of oil on the separation process was evaluated using *n*-hexane, *n*-heptane, *n*-octane, *n*-nonane and *n*-decane to generate the oil droplets; longer alkanes did not produce stable microemulsions. When the chain length of the alkane was varied, no significant differences in separation selectivity were observed. However, the migration times increased slightly with the length of the chain, because the most lipophilic solvents provided the strongest retention in the micelles. This is in agreement with the conclusions of some authors that the nature of the oil phase plays a minor role in the selectivity of the separation [22]. Because odd-numbered alkanes have lower toxicity than even-numbered alkanes [23], *n*-heptane was chosen as the oil. The percentage of *n*-heptane in the oil droplet was also studied