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Rapid determination of water- and fat-soluble vitamins with microemulsion electrokinetic chromatography

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ABSTRACT

A rapid, reliable and reproducible method based on microemulsion electrokinetic chromatography (MEEKC) for simultaneous determination of 13 kinds of water- and fat-soluble vitamins has been developed in this work. A novel microemulsion system consisting of 1.2% (w/w) sodium lauryl sulphate (SDS), 21% (v/v) 1-butanol, 18% (v/v) acetonitrile, 0.8% (w/w) *n*-hexane, 20 mM borax buffer (pH 8.7) was applied to improve selectivity and efficiency, as well as shorten analysis time. The composition of microemulsion used as the MEEKC running buffer was investigated thoroughly to obtain stable separation medium, as well as the optimum determination conditions. Acetonitrile as the organic solvent modifier, pH of the running buffer and 1-butanol as the co-surfactant played the most important roles for the separation of the fat-soluble vitamins, water-soluble vitamins and stabilization of system, respectively. The 13 water- and fat-soluble vitamins were baseline separated within 30 min. The system was applied to determine water- and fat-soluble vitamins in commercial multivitamin pharmaceutical formulation, good accuracy and precision were obtained with recoveries between 97% and 105%, relative standard derivations (RSDs) less than 1.8% except vitamin C, and acceptable quantitative results corresponding to label claim.

1. Introduction

As is well known, vitamins are a broad group of organic compounds that are minor, but essential, constituents of food required for the normal growth, self-maintenance and functioning of human and animal bodies. These compounds can be classified in two main groups: water- and fat-soluble vitamins. Absence of vitamins causes serious physiological problems [1].

Individual vitamin, as well as certain combinations of two or three vitamins could be chromatographed isocratically by typical high-performance liquid chromatography (HPLC) [2,3]. However, the analysis of water- and fat-soluble vitamins in one single run cannot be achieved by this method [4]. Some studies suggested that water- and fat-soluble vitamins may be performed by capillary electrophoresis (CE) [5–8]. Water-soluble vitamins such as B or C group possess an acidic function and those can be separated using capillary zone electrophoresis (CZE) [9]. In fact, most of simultaneous separations of water-soluble vitamins had been performed by micellar electrokinetic chromatography (MEKC), because the selectivity was much improved by this mode [10–12]. Whereas, fat-soluble vitamins such as vitamins A, E and D_3 are neutral, poor water-solubility, it should be required to use a chromatography-based method. In MEKC or microemulsion electrokinetic chromatography (MEEKC) modes, micelle/microemulsion droplet was taken as the pseudostationary phase to separate the neutral compounds. Ong et al. [11] separated a mixture of seven water- and two fat-soluble vitamins simultaneously using MEKC by the addition of cyclodextrin. Gong et al. [12] separated hydrophobic solutes with MEKC by the addition of an organic solvent. But separation of fat-soluble analytes is difficult in MEKC mode, because these compounds have an extreme affinity to the micelles, which results in long migration time and poor resolution [11–13]. Further work done by Hansen et al. [10] found that 1-butanol modified MEKC delivered similar separation efficiency to MEEKC for the analysis of neutral aromatic compounds.

MEEKC is an electrodriven separation technique, it uses microemulsion buffer to separate charged or neutral analytes based on both their hydrophobicities and electrophoretic mobility. MEEKC has the advantage over MEKC due to enhanced solubilization capacity for highly lipophilic compounds, enlarged migration window; it could increase selectivity in the separation of non-polar solutes [6,8,14–19]. Sanchez and Salvado [17] compared MEKC and MEEKC for the determination of water- and fat-soluble vitamins, it was found that MEEKC accomplished separation of mixtures containing water- and fat-soluble vitamins; however MEKC only

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solved the task of water-soluble vitamins analysis. The main disadvantage of MEEKC was the relatively long times (about an hour) needed for the separation of fat-soluble vitamins. Several techniques have been developed in the various electrophoretic modes to improve the disadvantage [18,19]. Pedersen-Bjergaard [18] used hydrophobic interaction electrokinetic chromatography to separate vitamins A palmitate, E acetate, and D_3 in the medium consisted of 80 mM tetradecylammonium in acetonitrile–water (80:20, v/v), three fat-soluble vitamins were baseline separated within 11 min. The reversed electrode polarity stacking mode [14,19] with pH 2.5 buffer to suppress the electroosmotic flow (EOF) had been also used for the rapid determination of fat-soluble vitamins. However, both rapid methods could not separate water- and fat-soluble vitamins simultaneously. Using bis(2-ethylhexyl) sodium sulfosuccinate (AOT) as pseudostationary phases, Delgado-Zamarreno et al. [5] attempted to separate four water- and three fat-soluble vitamins simultaneously with MEKC in short time, unluckily neutral water-soluble vitamin such as vitamin B_2 could not be separated from system peak. Though a complex multivitamin pharmaceutical formulation was analyzed by MEEKC using a sodium lauryl sulphate (SDS)–octane–1-butanol microemulsion [16] within 10 min, only seven kinds of vitamins were determined, and it was easily achieved to separate vitamin A from E.

The target of the present work was to separate water- and fat-soluble vitamins simultaneously by MEEKC within a short time. Therefore, it was required to seek for a novel microemulsion to stabilize separation medium, as well as to improve selectivity and efficiency. A mixture of 3 fat-soluble vitamins [retinol (vitamin A), tocopherol (vitamin E), cholecalciferol (vitamin D_3)] and 10 water-soluble vitamins [nicotinamide (vitamin PP), cyanocobalamine (vitamin B_{12}), riboflavin phosphate (vitamin B_2), pyridoxine hydrochloride (vitamin B_6), thiamine hydrochloride (vitamin B_1), pantothenic acid (vitamin B_3), folic acid (vitamin M), biotin (vitamin H), ascorbic acid (vitamin C), calcium pantothenate (vitamin B_5)] was selected as the test mixture. Moreover, the commercial multivitamin formulations sample was also assayed to evaluate the accuracy and precision of the method.

2. Experimental

2.1. Chemicals and reagents

Vitamin A was purchased from Sigma (St. Louis, MO, USA), vitamins E, D_3, B_1, B_2, B_6, B_{12}, B_3, PP, C, M, H, B_5 and C were all purchased from the Chinese Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

SDS, 1-butanol, *n*-octane, *n*-hexane and ethyl acetate were obtained from Sinopharm Chemical Reagent Co. (Shanghai, China), and all of them were of an analytical grade. Methanol and acetonitrile (HPLC grade) were from Hanbon Sci.&Tech. Co. (Jiangsu, China). All chemicals and solvents were used without further purification. High purity water purchased from Huajing MicroElectronics Co. (Wuxi, Jiangsu, China) was used in all procedures.

Centrum multivitamin sample was obtained from Wyeth (Jiangsu, China).

2.2. Preparation of microemulsions

The final microemulsion as MEEKC running buffer was prepared in the following way: 1.2 g SDS, 0.8 g *n*-hexane, 21 ml 1-butanol and 18 ml acetonitrile was mixed in a 100-ml volumetric flask, then 20 mM borate buffer (pH 8.7) was added to 100 ml in volume. The mixture was sonicated for 30 min to obtain a clear and highly stable microemulsion. The other microemulsions used during the optimization studies were prepared in a similar manner. All solutions were filtered through a 0.45-μm filter and degassed in an ultrasonic bath prior to use.

2.3. Preparation of standard solutions and sample solution

100 mg of each vitamin standard was accurately weighed, fat-soluble vitamin was dissolved with 1-butanol, vitamins B_2, M and H were dissolved in microemulsion buffer, and the other water-soluble vitamins were dissolved in water to prepare 10 mg/ml of the stock solutions for each analyte. Individual stock solutions were stored in a dark environment at −4 °C. As vitamin C suffers degradation in solution and vitamins B_2, M and H suffer degradation in alkaline solution, these stock solutions were prepared daily. Working solutions were prepared each day by dilution of the stock solutions with the microemulsion that was used as the running buffer.

One tablet of commercial multivitamin was pulverized and extracted with 50 ml microemulsion for 15 min in an ultrasonic bath. Subsequently it was filtrated through 0.45 μm nylon filter. The filtrate was directly injected to the CE instrument automatically.

2.4. Instrumentation and conditions

The CE-system was a PACE/MDQ from Beckman (Palo Alto, CA, USA) equipped with a diode-array detector and a temperature control device. An uncoated fused-silica capillary (Yongnian Photoconductive Fibre Factory, Hebei Province, China) (66 cm × 75 μm i.d.) with an effective length of 60 cm was used. All separation was carried out with a voltage of 25 kV at 25 °C. Sample injections were performed in hydrodynamic way with 0.4 psi pressure for 4 s. The detection wavelength was 205 nm.

Prior the first use, the capillary was conditioned by flushing 1 M NaOH, 0.1 M NaOH and finally water for 10 min, respectively. The capillary was equilibrated at the beginning of each day with the running buffer for 10 min. The rinsing procedure was found to be very important to obtain reproducible migration times. The best reproducibility was achieved by flushing the capillary between the runs as follows: 3 min with water and 3 min with the microemulsion buffer. Vials of running buffer were replaced every injection to avoid the electrolysis of the solutions.

3. Results and discussion

3.1. Optimization of the microemulsion composition

Microemulsions are microheterogeneous liquids which have characteristic properties such as optical transparency, thermodynamic stability and high solubilization capacity [20]. Some work [14–23] was performed to assess previously reported MEEKC conditions for the separation of acidic, neutral and basic analyses. Most of these experiments were carried out in a common microemulsion of 0.81% (w/w) heptane, 6.61% (w/w) 1-butanol, 3.31% (w/w) SDS and 89.27% (w/w) 10 mM sodium tetraborate buffer [23]. Analysis of highly hydrophobic analytes in this buffer is extremely difficult because these compounds have an extreme partition to the microemulsion droplet that results in long migration time or poor resolution. In order to achieve stable microemulsion and rapid separation, microemulsion system in the present work was modified by decreasing SDS concentration, in reverse, addition of lots of amount of co-surfactant (1-butanol) to stabilize the microemulsion. Demulsification was not observed during storage of the buffer for 6 months.