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Cyclodextrin-modified microemulsion electrokinetic chromatography for separation of α-, γ-, δ-tocopherol and α-tocopherol acetate

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Abstract

Different forms of tocopherols, together with tocotrienols, are collectively named as vitamin E, and each possesses different degree of medical, biological and physiochemical significance. The main difficulty of separating different forms of tocopherols lay in their highly structural similarities and hydrophobicities. Microemulsion electrokinetic chromatography (MEEKC), claimed to attain high peak efficiency with great solubilization power, has not previously been applied to the separation of tocopherols. The effects that various parameters, such as buffer system, type and concentration of cyclodextrins, temperature, and sample matrix, have on the separation of tocopherols by MEEKC have been investigated. By using a buffer mixture of 4% (w/w) sodium dodecyl sulfate (SDS), 6.6% (w/w) 1-butanol, 0.8% (w/w) *n*-octane, 20% (w/w) 2-propanol, 68.6% (w/w) phosphate (25 mM, pH 2.5), and 25 mM heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD), the separation of α-, γ-, and δ-tocopherol, α-tocopherol acetate, as well as the antioxidant butylated hydroxytoluene (BHT) at −26 kV, 25 °C was completed within 35 min. The practical potential of the present approach has been further validated by the determination of tocopherols in a vitamin E preparation, with the result of 132.63 (RSD 1.25%), 176.51 (RSD 0.29%), and 64.32 mg (RSD 3.34%) per 500 mg capsule for α-, γ- and δ-tocopherol, respectively.

Keywords: Microemulsion electrokinetic chromatography; Tocopherol; Cyclodextrin

1. Introduction

Tocopherols (structures shown in Fig. 1) and tocotrienols share common structures with a chromanol head and a phytyl tail. They are collectively named as vitamin E that has been shown to be the most effective lipid-soluble antioxidant in nature, interfering with one or more propagation steps of the lipid peroxidation process [1]. Due to their medical, biological, and physiochemical significance, tocopherols have been extensively studied. Among four tocopherols (α-, β-, γ- and δ-tocopherol), α-tocopherol has been reported to possess the highest biological activity [2,3], whereas γ-tocopherol has been found to be able to trap mutagenic electrophiles and to complement α-tocopherol [4]. In addition to these naturally occurring forms, α-tocopherol acetate was also included in many studies, since it is frequently used in the industrial application due to its great stability [5].

A number of methods such as normal phase high-performance liquid chromatography (HPLC), reversed-phase high performance thin-layer chromatography (C_{18}RP-HPTLC), reversed-phase high performance liquid chromatography (C_{18}RP-HPLC), gas chromatography (GC), and nano-liquid chromatography have been developed for the separation of distinct forms of tocopherols [6-12]. Owing to a difficulty of separating β- and γ-tocopherol, they were sometimes measured as a combined fraction [6,7]. Since β-tocopherol is usually present at trace levels in vegetable oils, it was not included in some studies [6,8,9]. In recent years, capillary electrophoresis (CE) has become increasingly popular in the pharmaceutical analysis owing to its high resolution and low sample and solvent consumption. The analyses of different forms of tocopherols using electrokinetic separation techniques have been demonstrated, including capillary electrochromatography (CEC) [8,9,13] and micellar electrokinetic chromatography (MEKC) [14].

Microemulsions [15] are solutions containing nanometer-sized droplets dispersed throughout another immiscible liquid [16,17]. Microemulsion electrokinetic chromatography

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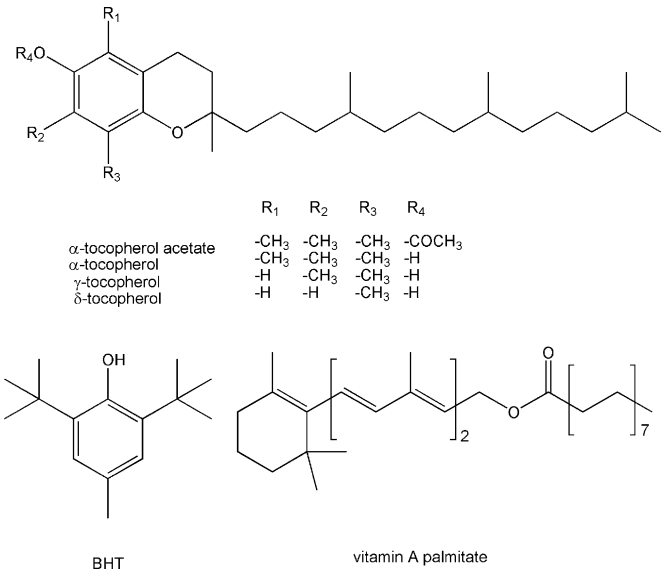

Fig. 1. Structures of different forms of tocopherols used, vitamin A palmitate, and BHT.

(MEEKC) is an electro-driven separation technique which applies microemulsion buffers to separate both charged and neutral solutes covering a wide range of water solubilities [16,18]. Oil-in-water microemulsions are typically used in MEEKC separations, which combine specific proportions of surfactant, oil, and water. A short-chain alcohol as cosurfactant is often added to further lower the surface tension of the droplets and to stabilize the microemulsion system [16,17]. The high solubilization power of microemulsions offers a great advantage on analyzing high lipophilic compounds. It has been demonstrated successfully for the separation of lipid-soluble vitamins [19–21].

Great solubilization power of microemulsions together with its high separation efficiencies [21,22] makes MEEKC a possible alternative for the separation of tocopherols. To the best of our knowledge, MEEKC has not yet been studied on the separation of tocopherols. Therefore, the aim of the present paper was to develop an MEEKC system for the separation of α-, γ-, δ-tocopherol, α-tocopherol acetate and the antioxidant butylated hydroxytoluene (BHT) which was added to prevent oxidation loss of tocopherols during analysis. The impacts of several parameters such as the species and concentrations of cyclodextrins, phosphate concentration, species of cosurfactants and temperature that have on the separation of tocopherols by MEEKC were carefully investigated.

2. Experimental

2.1. Chemicals

α-, γ-, δ-Tocopherol, and α-tocopherol acetate were purchased from Supelco (Bellefonte, PA, USA). Vitamin A palmitate was purchased from ICN Biochemicals Inc. (Aurora, OH, USA). Sodium dihydrogen phosphate, *n*-octane, 2-propanol, 1-butanol, γ-cyclodextrin (γ-CD) and hydrochloric acid were purchased from Merck (Darmstadt, Germany). Methanol was purchased from J.T. Baker (Phillipsburg, NJ, USA). Butylated hydroxytoluene (BHT), heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD), heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CD) and sodium dodecyl sulfate (SDS) were purchased from Sigma (St. Louis, MO, USA). Hydroxypropyl-β-cyclodextrin (HP-β-CD) and decanophenone were purchased from Aldrich (Milwaukee, WI, USA). Sudan III and pyrene were purchased from Janssen Chimica (Geel, Belgium). Carboxymethyl-β-cyclodextrin (CM-β-CD; degree of substitution, 2) and 6-amino-β-cyclodextrin (A-β-CD) were purchased from Advanced Separation Technologies (Whippany, NJ, USA). β-Cyclodextrin sulfobutyl ether, 4 sodium salt (SBE-β-CD; degree of substitution, 5.5) was purchased from CyDex (Overland Park, KS, USA). Ethanol was purchased from Cheng-Xin-Tang (Taipei, Taiwan). Hexane and acetone were purchased from ALPS (Taipei, Taiwan). Sodium hydroxide, naphthalene and citric acid monohydrate were purchased from Wako (Osaka, Japan). Vitamin E supplement capsule named Gamma E was purchased from Enerex Botanicals Ltd. (Port Moody, Canada).

2.2. Apparatus

The experiments of capillary electrophoresis were carried out with Beckman P/ACE MDQ capillary electrophoresis system (Beckman Coulter, Fullerton, CA, USA) equipped with photodiode array detector. Separations were performed using a 59 cm (49 cm effective length) × 50 μm ID fused-silica capillary from Polymicro Technologies (Phoenix, AZ, USA). New capillaries were pre-conditioned before use by flushing successively with 1.0 M sodium hydroxide for 20 min, 0.2 M sodium hydroxide for 20 min, de-ionized water for 20 min, methanol for 20 min, de-ionized water for 5 min and running buffer for 20 min. At the beginning of the experiment each day, the capillary was flushed by methanol for 10 min, de-ionized water for 5 min, and running buffer for 10 min. Between CE runs, the capillary was washed by methanol for 5 min and de-ionized water for 5 min, and then equilibrated with running buffer for 5 min. All the solutions were filtered through a 0.45 μm filter (Millipore, Bedford, MA, USA) before use. Operation conditions were as follows: voltage, −26 kV; temperature, 25 °C; injection time, 5.0 s (hydrodynamic, 0.5 psi); detection wavelength, 205 nm. The voltage of −26 kV was selected in this study because optimum resolution and speed were achieved.

2.3. Preparation of optimized microemulsion buffer

To prepare the microemulsion, 4% (w/w) SDS with 6.6% (w/w) 1-butanol were mixed firstly. Then 0.8% (w/w) *n*-octane, 20% (w/w) 2-propanol, 68.6% (w/w) 25 mM sodium dihydrogen phosphate buffer pH 2.5 were added to the SDS solution in sequence. The mixture was subjected to sonication for at least 30 min to ensure the formation of a transparent, stable microemulsion. Finally, 25 mM of DM-β-CD was added prior to being subjected to sonication for at least 10 min until it was fully dissolved.

2.4. Preparation of standard solutions

Standard stock solutions of vitamin A palmitate, α-, δ-tocopherol, and α-tocopherol acetate were prepared in 2-propanol at a concentration of around 20,000 μg/mL, while γ-tocopherol and BHT were prepared in 2-propanol at concentrations of around 5000 and 10,000 μg/mL, respectively. Tocopherol stock solutions were stored and protected from light at −20 °C. The samples were prepared by diluting with a sample matrix to obtain a standard mixture containing 100 μg/mL of α-, δ-tocopherol, α-tocopherol acetate, BHT, and γ-tocopherol unless otherwise stated. The optimized sample matrix contained 4% (w/w) SDS, 6.6% (w/w) 1-butanol, 0.8% (w/w) *n*-octane, 20% (w/w) 2-propanol, 68.6% (w/w) water, and 25 mM DM-β-CD, which was prepared as stated in the previous section except that the phosphate buffer was replaced by pure water. For obtaining the droplet mobility, 200 μg/mL of vitamin A palmitate was added into the running sample.

2.5. Preparation of the test solution

Known amount of the inner material of a soft gel capsule of vitamin E supplement was transferred to a volumetric flask and diluted with 2-propanol to 5 mL. The sample was either subjected to CE analysis or mixed with α-tocopherol acetate and BHT prior to CE analysis.

2.6. Calculations

The peak efficiency was represented by number of theoretical plates (N) which was calculated based on the following equation:

$$N = 5.54\left(\frac{t_m}{W_{50}}\right)^2 \quad (1)$$

where t_m is the peak migration time (min) and W_{50} is the peak width at half-height (min).

The resolution (R_s) was calculated based on the following equation:

$$R_s = 1.18\frac{t_{m(2)} - t_{m(1)}}{W_{50(1)} + W_{50(2)}}$$

2.7. Calibration and validation

Linearity of the signal against concentration was evaluated by measuring the relative responses of the analytes at the concentration range 20–160 μg/mL for α- and δ-tocopherol and 20.8–166.4 μg/mL for γ-tocopherol with respect to α-tocopherol acetate that was used as internal standard. Calibration curves obtained were used to calculate the concentrations of the analytes in the test solutions. Standard mixtures were prepared at three concentration levels of α-, γ- and δ-tocopherol, and analyzed three times a day for 3 different days to assess the intra-assay precision, intermediate precision, and accuracy.

3. Results and discussion

3.1. Method development

Several MEEKC compositions (without containing cyclodextrins) were tested in preliminary experiments for the separation of α-, β-, γ-, δ-tocopherol, α-tocopherol acetate, and the antioxidant BHT. The peaks for the tocopherols completely overlapped and only one broad peak appeared. The main difficulty of separating the analytes lay in their highly structural similarity and the hydrophobicity. Since β-tocopherol is usually present at trace levels [6,8,9], the present study focused on the separation of the remaining forms of tocopherols. The MEEKC conditions that was used by Pedersen-Bjergaard et al. [20] for the separation of fat-soluble vitamins A palmitate, E acetate, and D_3 was tested for the separation of the analytes (without BHT) in this study, with a result of three, but not well-separated, peaks. Although the separation was not quite successful, the electropherogram indicates that the separation order is related to their hydrophobicity (hydrophobic analytes migrate faster). Because the separation medium contained 66.6% (w/w) phosphate buffer (25 mM, pH 2.5), 20.0% (w/w) 2-propanol, 6.6% (w/w) 1-butanol, 6.0% (w/w) SDS, and 0.8% (w/w) *n*-octane, electroosmotic flow (EOF) of the system was suppressed.

Cyclodextrins (CDs), commercially available in numerous types and sizes, offer a highly hydrophobic cavity which possesses complexation properties as a result of host/guest interactions based on hydrophobicity and steric hindrance [23,24]. Together with its low UV absorption leading to a sensitive detection [25], cyclodextrins were thought to be promising for further separation of the tocopherols. List of cyclodextrins tested and their influences on the separation of tocopherols by MEEKC were summarized in Table 1. Of the tested CDs, 20 mM γ-CD and 20 mM 6-amino-β-cyclodextrin (A-β-CD) were not soluble in the buffer electrolytes. The results suggested that the addition of heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD) and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CD) into the aforementioned MEEKC buffer enabled the separation of α-, γ-, δ-tocopherol, α-tocopherol acetate, and BHT. However, the addition of TM-β-CD prolonged the analysis time (about 1.5 min longer) without providing a greater resolving power than that of using DM-β-CD. Therefore, DM-β-CD was chosen for the rest of studies.

Next we tested the impact of the buffer compositions on the separation of tocopherols by MEEKC. By changing 25 mM phosphate buffer (pH 2.5) to 25 mM citrate solutions (pH 2.5), similar separation results were obtained. Thus, the phosphate buffer was kept unchanged. When 50, 75, 100 mM phosphate solutions (pH 2.5) were tested, baseline disturbance occurred very often as a result of high Joule heats generated, which impeded the use of high concentrations of phosphate buffers. Herein, we also tested the separation of tocopherols in the presence of different cosurfactants, including methanol, ethanol, 1-propanol, and 1-butanol. Cosurfactant is a very important part of the MEEKC composition and has been found to be influential on the separation [17,22]. The total analysis time was

Table 1
Effect of cyclodextrins on the separation of a sample mixture containing four tocopherols and BHT[a]

Cyclodextrin	Concentration (mM)	Separation performance
HP-β-CD	20	3 peaks for the four tocopherols 1 peak for BHT
DM-β-CD	20	4 peaks for the four tocopherols 1 peak of BHT
TM-β-CD	20	4 peaks for the four tocopherols 1 peak of BHT
γ-CD	20	N.D.[b]
A-β-CD	20 8.4[c]	N.D.[b] 2 broad peaks for the four tocopherols 1 broad peak for BHT
SBE-β-CD	25[c]	3 small peaks for the four tocopherols 1 small peak of BHT
CM-β-CD	25[c]	3 peaks for the four tocopherols 1 peak of BHT

[a] Microemulsion composition, 66.6% (w/w) 25 mM phosphate buffer pH 2.5, 20.0% (w/w) 2-propanol, 6.6% (w/w) 1-butanol, 6.0% (w/w) SDS, and 0.8% (w/w) *n*-octane; applied voltage, −26 kV; temperature, 25 °C.
[b] The two CDs were not soluble in the buffer electrolytes.
[c] Experimental conditions as above, except for 68.6% (w/w) 25 mM phosphate buffer pH 2.5 and 4.0% (w/w) SDS.

shorter when alcohols of higher polarity were used, and the separation order was the same as reported by Huang et al. [26]. Although the total analysis time was shorter, the resolution decreased (peaks overlapped) when using methanol and ethanol. Therefore, 1-butanol, which was demonstrated to be the most suitable cosurfactant [20], was left unchanged. Besides, we also tested different amount of 2-propanol added. Although the electrolyte containing 25% (w/w) 2-propanol provided acceptable resolution, baseline disturbance was a problem. These findings were concordant with the report of Pedersen-Bjergaard et al. [20].

3.2. Method optimization

3.2.1. Influence of the concentration of DM-β-CD

Based on the results addressed above, the addition of cyclodextrins appeared to be the most influential factor. Thus, the concentration of DM-β-CD was varied to investigate the separation performance of tocopherols with respect to speed, resolving power, and reproducibility. The baseline became irregular and the electric current easily dropped to zero when the buffer electrolytes contained more than 20 mM DM-β-CD. In order to have a wider concentration range of DM-β-CD (0–25 mM), we changed the concentration of SDS in the buffer electrolytes from 6% (w/w) to 4% (w/w). We note that addition of DM-β-CD above 25 mM encountered the problems of unstable electric current and baseline. It was found that the migration time prolonged and the resolution improved as the concentration of DM-β-CD increased (as shown in Fig. 2), which implies that DM-β-CD increased the distribution equilibria between the analytes and the microemulsions. In other words, guest/host interactions of

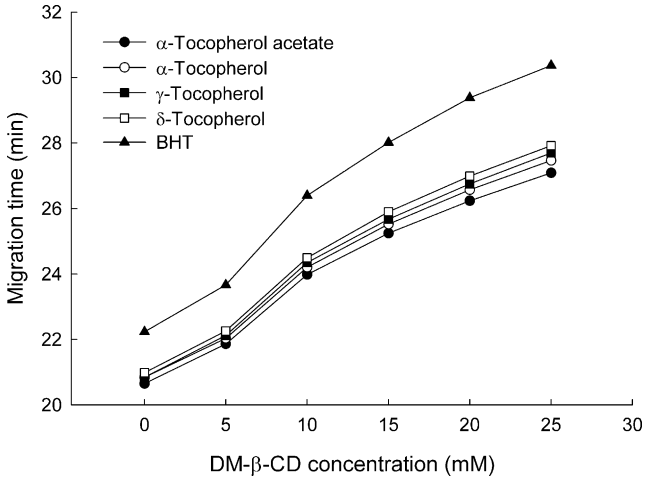

Fig. 2. Effect of DM-β-CD on migration time. Running buffer composition, 68.6% (w/w) 25 mM phosphate buffer pH 2.5, 20.0% (w/w) 2-propanol, 6.6% (w/w) 1-butanol, 4.0% (w/w) SDS, and 0.8% (w/w) *n*-octane with 0–25 mM DM-β-CD; applied voltage, −26 kV; temperature, 25 °C.

the analytes with DM-β-CD occurred. In addition, the changes in the viscosity and zeta potential of the system should also play minor roles in changing the migration time. The migration times for BHT increased form 22.23 to 30.37 min when the DM-β-CD concentration was changed from 0 to 25 mM. With increasing the DM-β-CD concentration from 0 to 25 mM, the resolution values for α-/γ- and γ-/δ-tocopherols improved from 0 to 1.83 and from 0.81 to 1.59, respectively.

3.2.2. Influence of temperature

A range of temperature from 15 to 45 °C was tested and the results are displayed in Fig. 3. Shorter analysis times were obtained using higher temperature. However, as the temperature increased to 45 °C, instability of the electric current encountered. Most of the time, the currents dropped to zero within 2 min. The separation at 15 °C was good, but the analysis took about 40 min

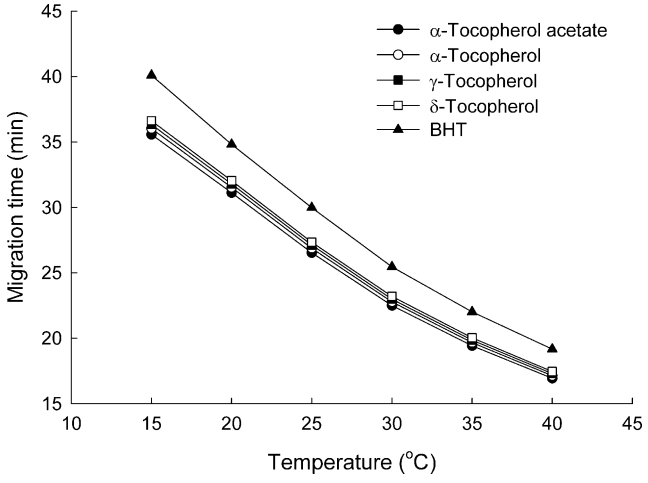

Fig. 3. Effect of temperature on migration time. Running buffer composition, 68.6% (w/w) 25 mM phosphate buffer pH 2.5, 20.0% (w/w) 2-propanol, 6.6% (w/w) 1-butanol, 4.0% (w/w) SDS, and 0.8% (w/w) *n*-octane with 25 mM DM-β-CD; applied voltage, −26 kV; temperature, 15–40 °C.

to be completed. In terms of speed, the results obtained at 35 °C were somehow superior to those conducted at 25 °C. However, the performance at 35 °C was less reproducible, mainly because poor temperature control of the system and an unstable baseline. Thus, the temperature was set at 25 °C.

3.2.3. Influence of sample matrix

In MEEKC, sample matrix is an important factor. Thus, the sample matrix should be prepared as close as possible to the composition of the running buffer in order to avoid disturbing the microemulsion adjacent to the sample injection plug inside the capillary [18,23]. The mixtures of standard stock solutions were diluted with four different sample matrixes, while the amount of 2-propanol in the injected samples was kept at 10%. The results of using the four sample matrixes, which are 2-propanol, microemulsion running buffer (unstacked) with DM-β-CD, microemulsion running buffer (stacked) without DM-β-CD, microemulsion running buffer (stacked) with DM-β-CD, are listed in Table 2. The difference between stacking and unstacking sample matrixes was water used instead of phosphate buffer. Among the tested composition, 2-propanol provided the lowest peak efficiencies. The other three matrixes provided comparable efficiencies, microemulsion running buffer (stacked) with DM-β-CD was more stable. Therefore, it was chosen as the sample matrix.

3.3. Separation mechanism

According to the above results, the optimized separation condition was found to be achieved using a microemulsion running buffer with 4% (w/w) SDS, 6.6% (w/w) 1-butanol, 0.8% (w/w) *n*-octane, 20% (w/w) 2-propanol, 68.6% (w/w) 25 mM phosphate buffer pH 2.5, and 25 mM DM-β-CD at −26 kV, 25 °C. Under the above-mentioned condition, α-, γ-, δ-tocopherol, α-tocopherol acetate and the antioxidant BHT were separated as presented in the electropherogram exhibited in Fig. 4. The aqueous phase was composed of the phosphate buffer, together with 20% (w/w) 2-propanol and 25 mM DM-β-CD. While 0.8% (w/w) *n*-octane served as the oil droplet, the surfactant, 4% (w/w) SDS, and the cosurfactant, 6.6% (w/w) 1-butanol, were used to stabilize the droplet formed. Under reversed polarity, the negatively charged oil droplets migrated towards the detector. Compounds were separated in order of decreasing hydrophobicity.

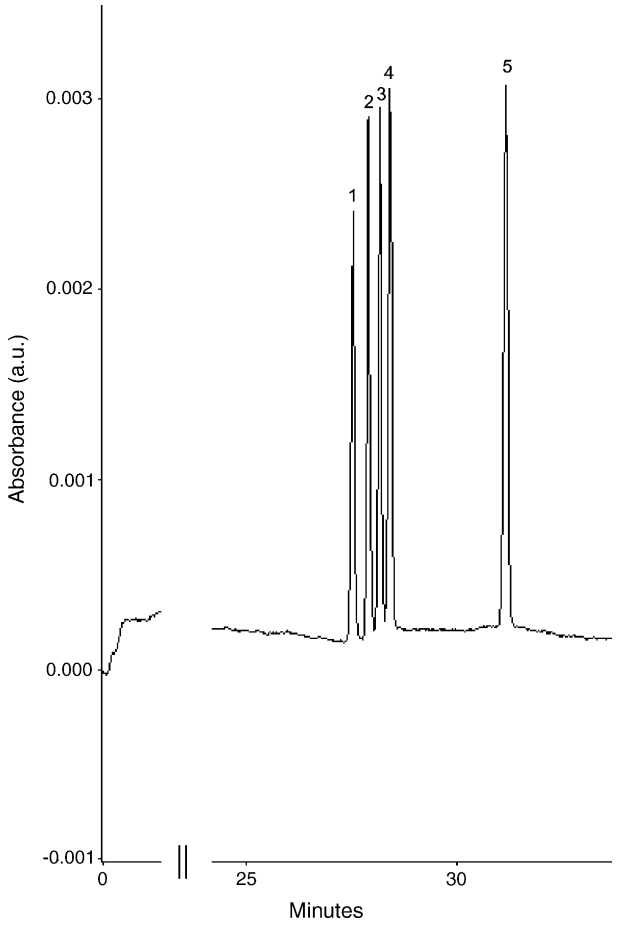

Fig. 4. Electropherogram of four tocopherols studied and BHT under the optimized condition. Running buffer composition, 68.6% (w/w) 25 mM phosphate buffer pH 2.5, 20.0% (w/w) 2-propanol, 6.6% (w/w) 1-butanol, 4.0% (w/w) SDS, and 0.8% (w/w) *n*-octane with 25 mM DM-β-CD; applied voltage, −26 kV; temperature, 25 °C. Peaks: 1, α-tocopherol acetate (100 μg/mL); 2, α-tocopherol (120 μg/mL); 3, γ-tocopherol (124.8 μg/mL); 4, δ-tocopherol (120 μg/mL); 5, BHT (100 μg/mL).

Table 2
Effect of sample matrixes (A–D) on peak efficiency of four tocopherols and BHT

Compound	Efficiency ($\times 10^5$)			
	A[a]	B[b]	C[c]	D[d]
α-Tocopherol acetate	2.1	4.1	4.5	4.8
α-Tocopherol	2.3	4.6	4.7	5.3
γ-Tocopherol	1.9	4.3	4.6	5.2
δ-Tocopherol	2.2	4.5	4.3	4.9
BHT	3.6	4.0	4.3	4.6

Values are from means of duplicates; other experimental conditions as Fig. 4.
[a] 2-Propanol.
[b] Microemulsion running buffer (unstacked) with DM-β-CD.
[c] Microemulsion running buffer (stacked) without DM-β-CD.
[d] Microemulsion running buffer (stacked) with DM-β-CD.

Under the separation conditions, the EOF mobility of the system is negligible. No peak for acetone (EOF marker) was detected within 60 min in 25 mM phosphate buffer (pH 2.5). In order to assess the mobility of the droplets, several markers including decanophenone, naphthalene, pyrene, and Sudan III were tested. None of them migrated faster than tocopherols, indicating that they interacted more weakly with the emulsion droplets than did the tocopherols. Thus, vitamin A palmitate that is neutral, highly hydrophobic and was chosen as the marker. The separations of vitamin A palmitate, tocopherols, and BHT by MEEKC in the presence and absence of DM-β-CD are depicted in Fig. 5. In the presence of 25 mM DM-β-CD, the mobilities of vitamin A palmitate, α-, γ-, δ-tocopherol, α-tocopherol acetate and BHT were calculated to be 7.05×10^{-5} with 0.32% relative standard deviation (RSD), 6.67×10^{-5} (0.31% RSD), 6.61×10^{-5} (0.31% RSD), 6.55×10^{-5} (0.31% RSD), 6.76×10^{-5} (0.30%

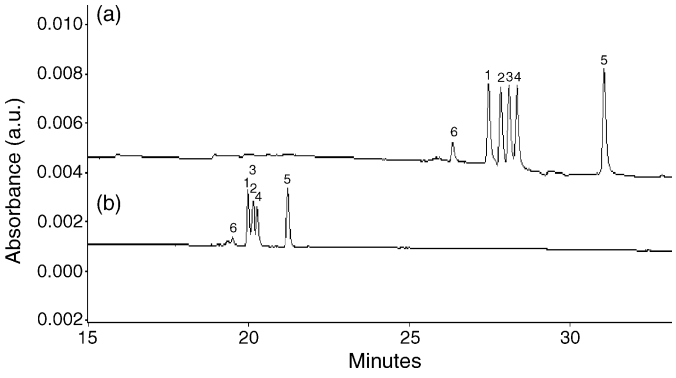

Fig. 5. Electropherograms of vitamin A palmitate, four tocopherols, and BHT under optimized conditions in the (a) presence and (b) absence of DM-β-CD. Peaks: 1, α-tocopherol acetate; 2, α-tocopherol; 3, γ-tocopherol; 4, δ-tocopherol; 5, BHT; 6, vitamin A palmitate. Other conditions are the same as in Fig. 4.

Table 3
Linear relationships between peak-area-ratios and concentrations (μg/mL)

Compound	Linear range (μg/mL)	Intercept	Slope	r^a
α-Tocopherol	20–160	−0.0552	0.0099	0.9988
γ-Tocopherol	20.8–166.4	−0.0472	0.0099	0.9993
δ-Tocopherol	20–160	−0.0630	0.0115	0.9988

[a] Correlation coefficient.

RSD), 5.98×10^{-5} (0.32% RSD) $cm^2\ V^{-1}\ s^{-1}$, respectively. In the absence of DM-β-CD, their mobilities were 9.49×10^{-5} (0.13% RSD), 9.18×10^{-5} (0.14% RSD), 9.18×10^{-5} (0.14% RSD), 9.12×10^{-5} (0.17% RSD), 9.26×10^{-5} (0.17% RSD), 8.70×10^{-5} (0.60% RSD) $cm^2\ V^{-1}\ s^{-1}$, respectively. The result supports the interactions of DM-β-CD with the analytes.

3.4. System performance

The linearity ranges and the characteristics of the regression lines of each tocopherol using α-tocopherol acetate as the internal standard are listed in Table 3. The limits of detection (LOD) and limits of quantitation (LOQ) at the signal-to-noise ratios of 3 and 10, respectively, for the analytes are listed in Table 4. For the four tocopherols (α-, γ-, δ-tocopherol, and α-tocopherol acetate), intra-assay precision ($n = 3$) and intermediate precision ($n = 9$) in terms of migration times of the method at three concentration levels (50, 100, 150 μg/mL for α-, δ-tocopherol, and α-tocopherol acetate; 52, 104 and 156 μg/mL for γ-tocopherol) were less than RSD 0.70% and 1.89%, respectively. Intra-assay precision ($n = 3$) and intermediate precision ($n = 9$) of the peak-area-ratios (with respect to α-tocopherol acetate) of the method at the three concentration levels were less than 2.86 and 2.86% (RSD), respectively. Accuracy at the three concentration levels was in the range of 94.80–104.77%. These data are summarized in Table 5.

Table 4
Limits of detection (LOD) and limits of quantitation (LOQ)

Compound	LOD (μg/mL)	LOQ (μg/mL)
α-Tocopherol acetate	1.41	4.71
α-Tocopherol	1.88	6.27
γ-Tocopherol	1.93	6.42
δ-Tocopherol	1.57	5.19
BHT	1.44	4.81

Values are from means of three replicates; other experimental conditions as Fig. 4.

3.5. Analysis of the test solution

A sample of Gamma E capsule preparation which is claimed to contain α-, β-, γ- and δ-tocopherol was tested using the optimized experimental condition. The electropherograms of the sample with and without addition of α-tocopherol and BHT are depicted in Fig. 6. Using α-tocopherol acetate as internal standard, the amounts of α-, γ- and δ-tocopherol were measured and the results as compared to the label claim are listed in Table 6. Since β- and γ-tocopherol could not be separated using the present method, the amount of γ-tocopherol could be overestimated.

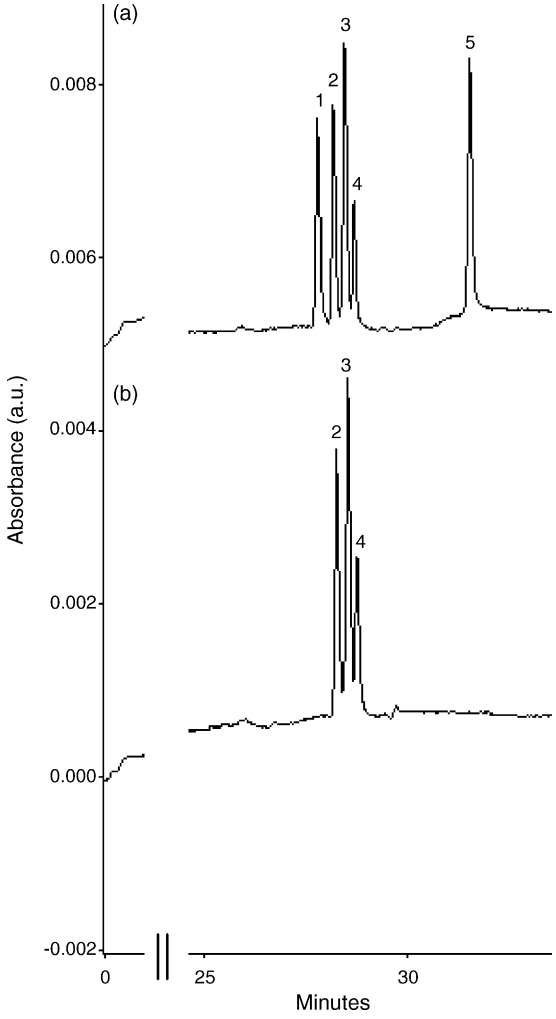

Fig. 6. Electropherograms of vitamin E supplement (a) with and (b) without the addition of α-tocopherol acetate and BHT under optimized conditions. Peaks: 1, α-tocopherol acetate; 2, α-tocopherol; 3, γ-tocopherol; 4, δ-tocopherol; 5, BHT. Other conditions are the same as in Fig. 4.

Table 5
Intra-assay precision ($n=3$) and intermediate precision ($n=9$) of migration times (t_m) and peak-area-ratios, and accuracy ($n=9$) data at three concentration levels

Compound concentration (μg/mL)	Intra-day precision (RSD %)		Intermediate precision (RSD %)		Accuracy (RSD %)
	t_m	Peak-area-ratios	t_m	Peak-area-ratios	
α-Tocopherol acetate					
50	0.34	–	1.84	–	–
100	0.33	–	1.60	–	–
150	0.64	–	0.89	–	–
α-Tocopherol					
50	0.34	0.58	1.86	1.09	97.81
100	0.33	2.37	1.62	2.37	98.54
150	0.68	1.48	0.93	1.88	94.80
γ-Tocopherol					
52	0.32	0.80	1.87	1.34	99.91
104	0.31	1.85	1.62	2.19	98.74
156	0.69	0.85	0.95	2.41	97.20
δ-Tocopherol					
50	0.32	1.15	1.89	1.15	104.77
100	0.31	2.86	1.64	2.86	103.27
150	0.70	1.12	0.98	1.13	104.56

Experimental conditions as Fig. 4.

Table 6
Comparison of label claim with the estimation of the amount of each tocopherol determined

Compound	Detected amount (mg/500 mg) mean[a] (RSD %)	Label claim (mg/500 mg)
α-Tocopherol	132.63 (1.25)	136
β-Tocopherol	NA	3
γ-Tocopherol	176.51 (0.29)	168
δ-Tocopherol	64.32 (3.34)	62

[a] $n=3$.

4. Conclusion

MEEKC is a promising analytical technique for the determination of highly hydrophobic analytes because it combines the strengths of CE and the great solubilization ability of microemulsion. In this study, we have shown that MEEKC is a powerful separation technique for the separation of tocopherols. We have found that addition of cyclodextrins such as DM-β-CD, to the buffer electrolyte notably improved the separation of different forms of tocopherols by MEEKC. Under the optimized conditions, α-, γ-, δ-tocopherol, α-tocopherol acetate and the antioxidant BHT were successfully separated by MEEKC for the first time. The practical potential of this present method for the determination of tocopherols has been validated. Based on the results presented in this study, the potential of MEEKC for the separation of other hydrophobic compounds is worth further investigation.

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