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Micellar electrokinetic chromatography with bis(2-ethylhexyl)sodium sulfosuccinate vesicles Determination of synthetic food antioxidants

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Abstract

Capillary electrokinetic chromatography is suitable for the separation of mixtures of uncharged and charged solutes. In the present work the behavior of six synthetic food antioxidants – 2[3]-*tert*.-butyl-4-hydroxyanisole, 2,6-di-*tert*.-butyl-*p*-cresol, tercbutylhydroquinone, 3,4,5-trihydroxybenzoic acid propyl ester, 3,4,5-trihydroxybenzoic acid octyl ester and 3,4,5-trihydroxybenzoic acid dodecyl ester – was studied in a capillary electrophoresis system using capillary electrokinetic chromatography with vesicles of the surfactant bis(2-ethylhexyl)sodium sulfosuccinate (AOT). Several studies aimed at calculating the critical aggregation concentration of the surfactant were conducted to check that under the conditions used the AOT was in a state of aggregation. Having checked the association shown by the surfactant, we then explored the greater or lesser capacity of the antioxidants to interact with this compound. We followed the evolution of the molecular absorption spectra of each of the antioxidants in the presence of the surfactant at different concentrations and the retention factors were calculated at different pH values. Additionally, in order to determine which species – anionic or neutral – was present at the pH of the buffer used (boric/borate), the pK_a values in acetonitrile–water (20:80) were obtained. Resolution and quantification of the antioxidants demand optimization of the variables involved in the system, such as the percentage of acetonitrile, the concentration of AOT and boric/borate buffer, pH, voltage, etc. When this part of the study had been completed, calibrations were obtained for each of the antioxidants, obtaining good linear correlation coefficients in all cases. Finally, we propose a method that allows the resolution of the six most employed antioxidants in a capillary electrophoretic system in 15 min, using electrokinetic chromatography with AOT as the pseudostationary phase.

Keywords: Pseudostationary phases; Food analysis; Vesicles; Bis(2-ethylhexyl)sodium sulfosuccinate; Surfactants; Antioxidants

1. Introduction

Micellar electrokinetic chromatography (MEKC) is a technique of capillary electrophoresis that was first introduced by Terabe and co-workers [1–3] in 1984. It is based on the use of micellar aggregates of surfactant as a pseudostationary phase in the separation buffer. The technique facilitates the separation of neutral species that could not be resolved using other capillary electrophoretic techniques and also improves the separation of ionic species with similar electrophoretic mobilities, based on their different degrees of hydrophobicity. Sodium dodecyl sulfate

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(SDS) is the micelle-forming surfactant most used as pseudophase. In the present work, instead of micelles we employed bis(2-ethylhexyl)sodium sulfosuccinate (AOT) surfactant vesicles as the pseudostationary phase to accomplish the separation of the synthetic antioxidants.

The term vesicle is used to denote organized structures formed of closed spherical or helicoidal bilayers that may either be simple or have several compartments. There is a considerable body of literature [4] addressing liposomes and vesicles used in different scientific fields as transporters of active substances, with therapeutic actions through tissues, as models of the behavior of cell membranes, etc.

Initially, most authors reported that for a surfactant to form vesicles its structure had to have a polar head and two C_{10}–C_{18} alkyl chains as the lipophilic part owing to the similarity with phospholipid structures. However, later studies carried out by different authors [5,6] indicated that these structures can also be obtained with single-chain amphiphilic molecules with derivatives of diphenylazomethine or biphenyl groups. The surfactant AOT has a two-structure chained like that described above and forms vesicles spontaneously in polar solvents.

AOT has been widely used as a surfactant model to investigate organized systems involving the use of anionic surfactants. One of the reasons why the system is attractive for such studies is that its hydrophilic and lipophilic properties are nearly balanced [7]. Accordingly, microemulsions are formed without the need for the presence of co-surfactants, such as alcohols. Different investigators [8–11] have used AOT in studies on the critical behavior of phase systems. Its surface activity and the effects of electrolytes [12,13] and of co-surfactants [14] on it have also been studied. AOT has also been studied exhaustively in non-polar solvents [15,16].

The solubility of AOT in water is quite low. At 30°C it is about 1.8% (w/w). It is more soluble in water–polar organic solvent solutions and its solubility also increases in water with dissolved salts. In saline solutions, AOT is one of the few systems to exhibit an ultra low oil–water surface tension [17] (with linear alkanes, like oil) without the addition of co-surfactants. Analysis of phase behavior is thus considerably simplified. This may be due to the equilibrium between the lipophilic and hydrophilic characteristics of the surfactant molecule. Shahidzadeh et al. [18] reported that another consequence of this equilibrium is that in saline solutions AOT forms vesicles instead of micellar structures. The presence of relatively large vesicles allowed the authors to follow the redistribution of the surfactant during spontaneous emulsification directly, using phase contrast microscopy. They studied the spontaneous emulsification mechanisms with this surfactant and the relationship with the properties of the compound. In a previous work, other authors [19] studied AOT–apolar solvent–saline solution systems to gain insight into its ability to form microemulsions and liquid crystals of AOT with different water–non-polar solvent compositions.

Recently electrokinetic chromatography with vesicles as pseudophase has been used by Hong et al. [20]. These authors studied thermodynamically stable vesicles and mixed micelles formed from SDS and *n*-dodecyltrimethylammonium bromide (DTAB), two surfactants oppositely charged, as systems of separation. They compared polar group selectivity, retention and efficiency when vesicles, mixed micelles or SDS micelles were used. Some of these authors [21], using immobilized artificial membrane chromatography and lipophospholipid MEKC, studied the differences of retention between these systems of eight β-blockers.

Among the food additives most used today, synthetic antioxidants are outstanding. Antioxidants [22] are used to retard oxidative rancidity, thus reducing the loss of nutritional quality and increasing the shelf life of a wide variety of lipid-containing foods. Antioxidants act by interrupting the chain reaction responsible for producing peroxides, by inactivating the free radicals, ceding a hydrogen, and become stabilized by resonance. Synthetic antioxidants are polyphenolic compounds that are more or less liposoluble and that must not be toxic and should not confer strange tastes to the food they are added to. Among them are the esters of gallic acid (propyl, octyl and dodecyl gallate) and the substituted phenol group, including 2[3]-*tert*.-butyl-4-hydroxyanisole (BHA) and 2,6,-di-*tert*.-butyl-*p*-cresol (BHT). Usually, mixtures of some of them that act synergistically, such as BHA and BHT, or BHA and propyl gallate, are used.

Some authors compared capillary electrophoretic

techniques with high-performance liquid chromatography (HPLC) analysis of four of these synthetic antioxidants [23]. Summanen et al. [24] proposed the use of MEKC to test the purity of phenolic compounds extracted from medicinal plants. More recently, using MEKC, Abrantes et al. [25] separated 11 phenolic antioxidants.

The aim of the present work was to study the behavior of the surfactant AOT as a pseudostationary phase in capillary electrokinetic chromatography. The behaviors of different widely-used synthetic antioxidants – BHA, BHT, tercbutylhydroquinone (TBHQ) and propyl, octyl and dodecyl gallates – were studied in a capillary electrophoresis system using electrokinetic chromatography in the presence of the surfactant AOT with a view to proposing an analytical methodology that would enable the resolution and quantification of these substances in food samples.

2. Experimental

2.1. Apparatus

Experiments were conducted on a P/ACE System 2000 capillary electrophoresis apparatus equipped with a UV detector permitting measurements at 200, 214, 254 and 280 nm (Beckman, Fullerton, CA, USA). The capillary used was 57 cm×75 μm I.D. Detection was accomplished at 50 cm.

Data were recorded on an American CPU computer (with a dtk computer screen and keyboard and an Epson Fx-870 printer, Seiko, Nagano, Japan) that uses the System Gold program to evaluate the electropherograms. The software of the set-up evaluates the corresponding electropherograms, affording migration times and the areas and heights of the electrophoretic peaks. As the quantitative variable, the normalized area was used, whose expression is: $\text{Area}_N = \text{Area} \cdot L_d(\text{cm})/t_m(\text{min})$, where L_d is the distance to the detector and t_m is the migration time.

Conductivity measurements were made on a Crison micro CM 2200 (Barcelona, Spain) conductimeter with two platinized platinum plates of 1 cm^2 separated by 1 cm. Spectrophotometric and nephelometric studies were carried out on Shimadzu UV-160 and Shimadzu R-F 5000 (Shimadzu, Tokyo, Japan) devices, respectively.

2.2. Reagents

BHA, BHT, 3,4,5-trihydroxybenzoic acid propyl ester (PG), 3,4,5-trihydroxybenzoic acid dodecyl ester (DG) were supplied by Sigma. TBHQ and 3,4,5-trihydroxybenzoic acid octyl ester (OG) were from Aldrich and the surfactant AOT and vitamin E (α-tocopherol) were from Fluka. Sigma, Aldrich and Fluka have distributors of their products in Alcobendas (Madrid, Spain). Methanol was from BDH and acetonitrile was from Merck; both were of HPLC grade. The other reagents employed were supplied by Panreac (Barcelona, Spain) and were of analytical-reagent grade.

2.3. Procedure

Solutions of the six antioxidants were prepared in methanol at an approximate concentration of 1 m*M* (except in the case of TBHQ, for which it was 5 m*M*). 200 μl aliquots of these solutions were diluted up to 4 ml with the separation solution. This was injected under pressure into the capillary over 5 s. The capillary contained 20 m*M* boric acid/sodium borate, 20% acetonitrile buffer and 20 m*M* AOT. These are the optimum conditions and the values will vary in the study corresponding to each variable.

Before performing the first determination it was necessary to condition the capillary by washing it over 5 min with water and then for a further 2 min with 0.1 *M* sodium hydroxide. Finally, the capillary was rinsed with the separation solution for 2 min.

Between each analysis, the capillary was rinsed for 1 or 2 min with the buffer solution. After injection, a potential of 24 kV was applied to accomplish separation and detection was performed spectrophotometrically at 280 nm. In all experiments temperature was maintained at 25°C.

3. Results and discussion

3.1. Study of the surfactant

Three experiments were conducted to check that

the surfactant employed (AOT) was in a state of aggregation under the experimental conditions employed.

3.2.1. Conductivity measurements

When performing conductivity measurements on electrolyte solutions, when in some way certain ions become regrouped a change occurs in the conductivity of the solution. The empirical equation of Kohlrausch relates the molar conductivity of the electrolyte with the concentration thus: $\Lambda = \Lambda^0 - bC^{1/2}$.

Therefore, the conductivity of several AOT solutions was measured. The results show a change in slope for values above 1 m*M*, that implies a change in the aggregation state of the surfactant.

3.2.2. Light scattering experiments

If the surfactant undergoes a change in its aggregation state, this change implies a greater dispersal of light because the size of the constituents of the solution increases. Using a spectrofluorimeter, we conducted a nephelometric study in solutions with increasing concentrations of AOT, illuminating the solutions with monochromatic radiation at 300 nm and measuring the intensity of the dispersed light (also at 300 nm) at an angle of 90° with respect to the incident radiation. In this experience, it was observed that for concentrations above 1 m*M* there is a change in the aggregation state.

3.2.3. Current intensity measurements

Recently, some authors [26] have proposed measuring the electric current running through the electrophoretic circuit to assess the critical micellar concentration (cmc) of the surfactant SDS. Since on applying a difference in potential to the capillary of radius r and length L_t an intensity I is produced according to Ohm's law:

$$V = \frac{L_t}{\pi r^2(\sigma_{Na^+} + \sigma_{S^-} + \sigma_{mic})} \cdot I \quad (1)$$

where σ_{Na^+}, σ_{S^-} and σ_{mic} are the specific conductivities of the co-ion, of the surfactant and of the micelle, it is easy to see that for concentrations below the cmc the main contribution to conductivity comes from the sodium ions and from the surfactant in its monomeric form, whereas when the concentration is higher than the cmc the conductivity of the medium is governed by the micelles and some sodium ions. There will therefore be a variation in the intensity of the circuit above and below the cmc.

The values of intensity, generated on applying 24 kV to a capillary with different concentrations of AOT, show a change in the slope for values close to 1 m*M*.

3.2.4. Microscope observations

Microscope photographs were obtained of different solutions in which the concentrations of the surfactant and of the buffer solution were varied. As a precedent to this, reference is made to the 1997 work of Shahidzadeh et al. [18] who studied the spontaneous emulsification of apolar organic media in surfactant solutions and concluded that the presence of salts gives rise to relatively large AOT vesicles. It was observed that on increasing the salt concentration large vesicles were formed. This was expected in view of the characteristics of the surfactant molecule.

3.3. Study of the antioxidants

In order to gain further insight into the nature of the species studied as regards their behavior in an electrophoretic system, several assays were carried out, in particular to know their acid–base characteristics and their greater or lesser degree of interaction with the surfactant.

In relation to acid–base behavior, Fig. 1 shows the results of titration of 25 ml of a solution of each analyte at 0.01 *M* concentration with 0.1 *M* sodium hydroxide in the acetonitrile–water medium used. Study of the titration curves reveals that the three esters of gallic acid have a single K_a value of about 10^{-8}. To determine the corresponding K_a values, the curves were linearized, affording values of $10^{-7.86}$, $10^{-8.12}$ and $10^{-8.47}$ for propyl, octyl and dodecyl gallate, respectively. Since no sharp increase in the titration curve was observed, the other three species can be said to be in neutral form.

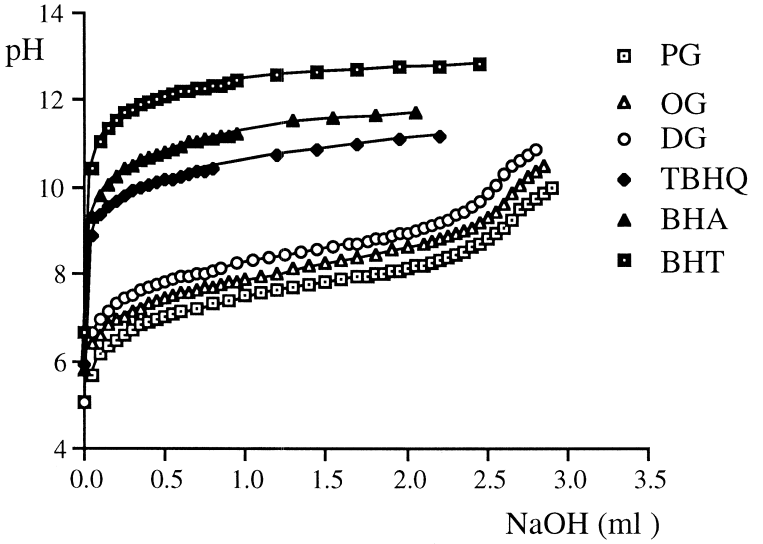

Fig. 1. Titration of the antioxidants with 0.1 *M* NaOH in acetonitrile–water medium.

3.3.1. Interaction of the antioxidants with the surfactant

3.3.1.1. Spectrophotometric study

The evolution of the molecular absorption spectra of each antioxidant in the presence of the surfactant AOT was used to check the effects of the interaction between the antioxidants and the surfactant. These spectrophotometric studies have been used to check the uptake of polar substances into inverse micelles in non-polar solvents [27]. To see whether uptake into vesicles was occurring, the UV spectra of the antioxidants in the presence of AOT at increasing concentrations (from 0 to 40 m*M*) in a medium containing 20% acetonitrile and boric/borate buffer at 20 m*M* were recorded. In the spectra of BHT and DG, a gradual disappearance of a band at 240 nm was observed as the surfactant concentration increased. This is shown in Fig. 2. The other spectra did not show appreciable variations with the rise in AOT concentrations.

From this study it may be inferred that the surfactant aggregates interact strongly with the antioxidants BHT and dodecyl gallate, even though the latter substance has a negative charge, as seen in the study of p*K*. It should be stressed that dodecyl gallate has a 12-atom hydrocarbon chain and hence it would appear that the hydrophobic interactions of the

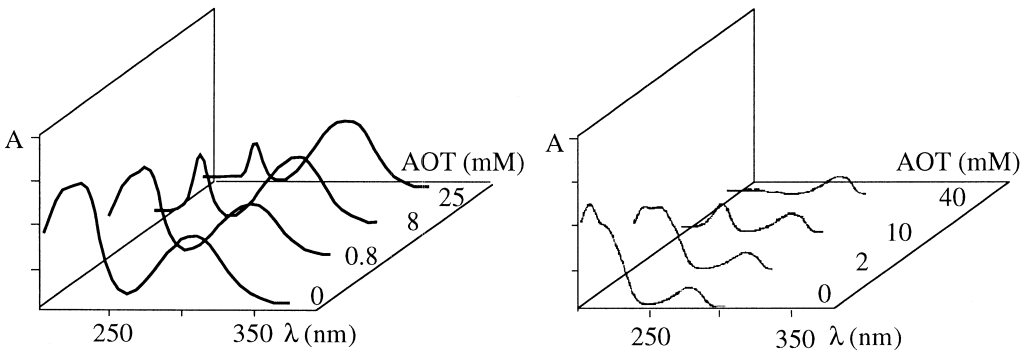

Fig. 2. Effect of surfactant concentration on BHA and DG spectra.

dodecyl chain overcome the electrostatic repulsion between the surfactant aggregates and the antioxidant anion.

3.3.1.2. Calculation of retention factors

The behavior of the antioxidants in the separation system was studied, varying pH in the 5.5–9.5 range without and with AOT. The antioxidants studied are of two types: some are neutral (TBHQ, BHA and BHT) while others were in anionic form (gallates), depending on the pH, as inferred from the pK_a value obtained.

In the absence of surfactant, the mobilities of anionic analytes is defined by:

$$\mu = \mu_{A^-} \frac{\frac{K_a}{[H^+]}}{1 + \frac{K_a}{[H^+]}} \quad (2)$$

where μ is mobility at a given pH, μ_{A^-} is the mobility of the anionic form of the acid, and K_a is the acid dissociation constant. This equation predicts a sigmoidal behavior for the variation in mobility as a function of pH [28].

Using the μ_{A^-} obtained at values far higher than the corresponding pK_a of each anion, and the K_a values obtained, the theoretical curves for the mobility of the different gallates as a function of pH were deduced (Fig. 3).

On plotting the experimental values obtained for the mobilities of propyl, octyl and dodecyl gallate, a sigmoidal behavior similar to that observed in the theoretical curves was seen. In the case of neutral antioxidants in the absence of surfactant, these eluted, as expected, together with the electroosmotic flow.

In the presence of the surfactant AOT, the corresponding retention factors were calculated for the six analytes studied at the above-indicated pH values. According to the equation of Otsuka et al. [2], the retention factor for neutral solutes when micellar electrokinetic chromatography is used, can be expressed as:

$$k' = \frac{t_r - t_{eo}}{t_{eo}\left(1 - \frac{t_r}{t_{mc}}\right)} \quad (3)$$

where t_r, t_{eo} and t_{mc} are the migration time, the electroosmotic flow time, and the micellar time, respectively.

Although the aggregation state of the surfactant employed, AOT, is vesicular instead of micellar, the mechanism of distribution of the analytes between the solution and the pseudophase has the same characteristics. Several substances that, owing to their hydrophobic characteristics may be completely

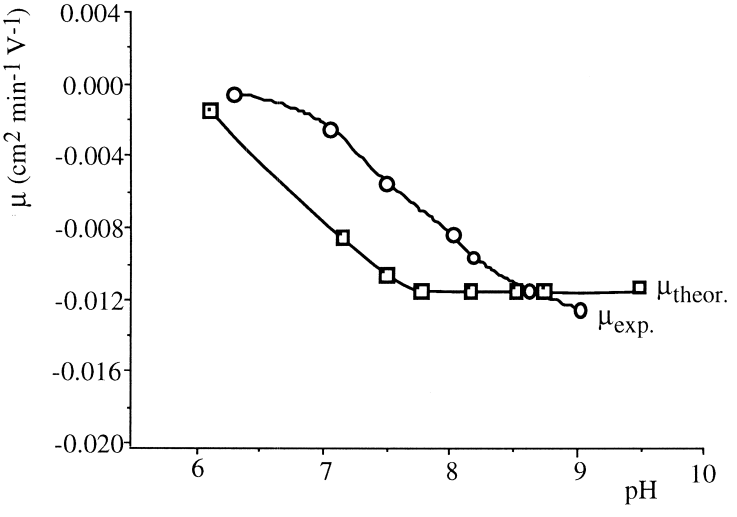

Fig. 3. Dependence of mobility on pH in the absence of surfactant. Theoretical and experimental values for anionic antioxidants.

incorporated into the pseudophase, were studied as vesicular markers. Vitamins K (menaquinone 4) and E (α-tocopherol) were those that best indicated the arrival of the pseudophase at the detector and vitamin K was chosen because it showed the greatest absorbance at the working wavelength.

Otsuka et al.'s expression was applied in the case of the antioxidants TBHQ, BHA and BHT, which are neutral at the pH values studied. As expected, the retention factors did not vary with pH. The antioxidant with the highest k' value was BHT; i.e., it was the analyte that interacted most strongly with the surfactant and that was incorporated into the pseudophase in the highest amounts. This corroborates the previously performed spectrophotometric study that had shown that an important variation occurs in the BHT spectra as the concentration of AOT becomes sufficiently high for vesicular aggregates to be formed.

In the case of charged solutes, the expression for calculating the retention factors using micellar electrokinetic capillary chromatography is similar [29] to Otsuka et al.'s expression for neutral solutes:

$$k' = \frac{\mu - \mu_0}{\mu_{mc} - \mu} \quad (4)$$

where μ is the mobility of the solute in the micellar medium and μ_0 is the electrophoretic mobility – that is, the mobility of the ionic analyte in the absence of surfactant – and μ_{mc} is the mobility corresponding to the micellar phase. Calculation of the corresponding mobilities in the presence and absence of the surfactant and application of the above expression afforded the values of the partition coefficients.

Fig. 4 shows the variation in the retention factors with pH. As may be seen, as pH decreased OG and DG were incorporated in greater amounts to the vesicular aggregates since the electrostatic repulsion due to the charges of the anions had disappeared. This was less pronounced in the case of PG; the value of its retention factor increased very little when pH was lower because its hydrophobic interaction with the surfactant is very reduced owing to the chain of this ester being shorter than those of the octyl and dodecyl esters.

Calculation of the retention factors indicated that for any pH value the analyte that interacted most strongly with the surfactant was DG; this means that the hydrophobic interactions are stronger than the electrostatic repulsions since even if the compound is present in anionic form it is incorporated into the surfactant aggregates. At the working pH values (9.4), BHT was the second analyte that most strongly interacted with the surfactant. Accordingly, the results of this part of the study are consistent with those obtained concerning the evolution of the UV spectra of the different antioxidants in the presence of AOT. In Fig. 5 this interaction between AOT

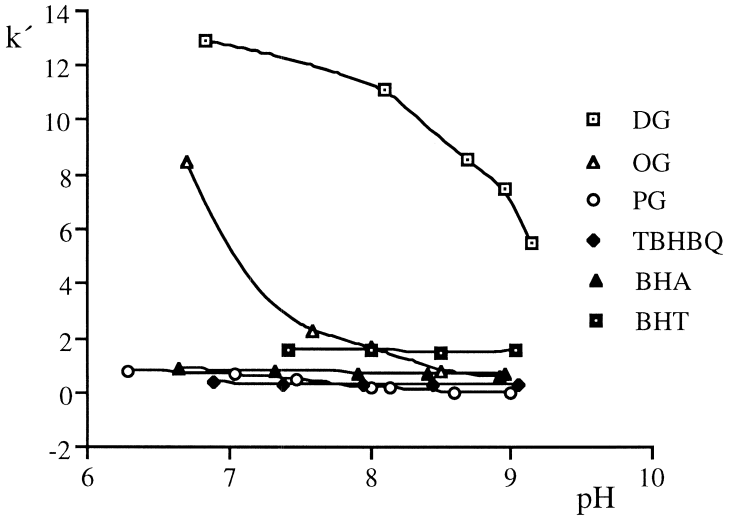

Fig. 4. Retention factors calculated for the different antioxidants using methanol and vitamin K as markers.

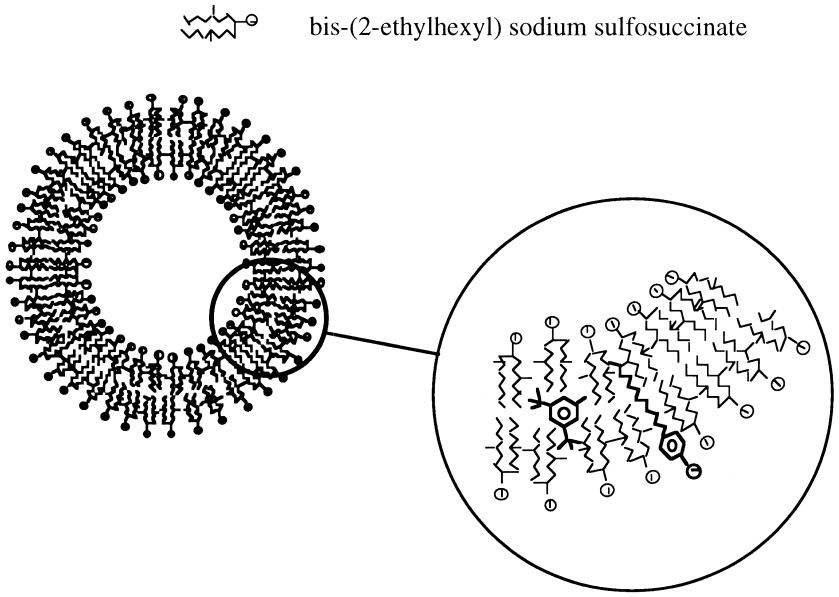

Fig. 5. Schematic representation of the interaction of BHT and DG with AOT vesicles.

vesicles and the antioxidants BHT and DG is represented.

4. Separation and quantification

4.1. Optimization of separation

To achieve total resolution of the analytes it is necessary to optimize the variables affecting the electrokinetic capillary chromatography separation step. To do this, the procedure explained in Experimental was implemented with different buffer solutions in which the different conditions were varied.

First, the effect of the percentage of acetonitrile was studied, varying this in solution from 5 to 40% (Fig. 6). The presence of an organic solvent in the separation buffer had a fairly strong effect on migration and hence on the resolution of the analytes since the distribution of these between the buffer solution and the pseudophase may be modified. As expected, as the percentage of acetonitrile was increased the time corresponding to the electroosmotic flow gradually increased since organic solvents may interact with the wall of the capillary. Concentrations of acetonitrile higher than 40% did not give good results owing to the poorer dissolution of the surfactant, an impoverishment in the stability of the current intensity, and a poorer resolution of the first peaks (TBHQ, BHA) which eluted next to the electroosmotic flow.

When working with the electrokinetic chromatography technique, since the analytes are distributed between the buffer and surfactant vesicles the concentration of the latter is a variable that must be taken into account. To study its effect, the six antioxidants were separated using different concentrations of surfactant solutions (from 5 to 100 m*M*). As with values above 50 m*M* AOT, the resolution of the electrophoretic peaks became progressively worse and, in particular, disappeared after 60 m*M* (Fig. 7). The reason for this phenomenon, is a possible increase in vesicle size as the surfactant concentration is increased. From the experimental point of view, this can be corroborated since when the solution at 60 m*M* was placed in a sonication bath, the electropherogram was partially recovered; the disturbance produced reduced vesicle size and separation was improved.

From this study, it may be deduced that when applying electrokinetic capillary chromatography in

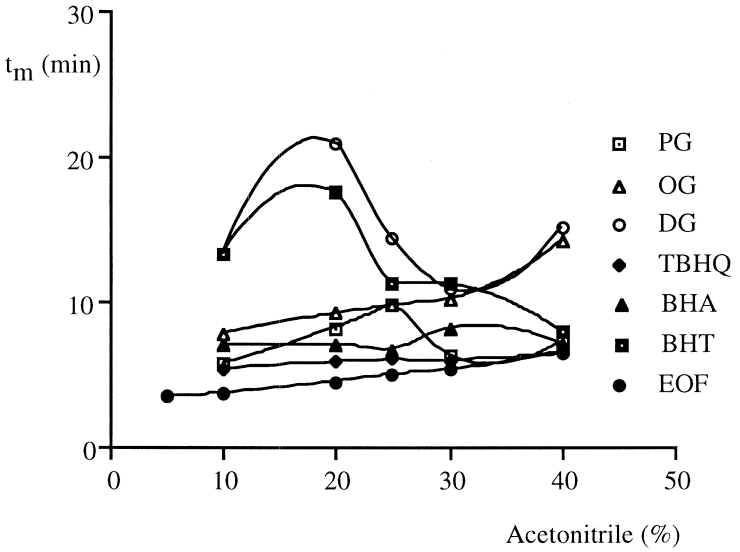

Fig. 6. Dependence of mobility on the percentage of acetonitrile in the separation buffer.

the presence of vesicles these must be of reproducible size and shape and the experimental results suggest that this can be achieved more readily when they are small. It is therefore recommended to work at surfactant concentrations below 40 m*M*. A concentration of 20 m*M* was selected as optimum for resolution of the analytes.

Since ionic strength exerts important effects on solute mobility, the buffer concentration should be considered as an important variable in separation efficiency. We therefore used solutions with boric/borate concentrations in the 10–60 m*M* range. The plots of mobility versus the inverse of the square root of the concentration were straight and with a slight slope corresponding to the expression: $\mu_{ep} = e/(3 \cdot 10^7 ZhC^{1/2})$. Another factor to be taken into consideration when the concentration of ions in the medium is raised is the increase in the electric current passing through the circuit and hence an increase in the Joule heat generated. So that this heat can be dissipated it is appropriate to work at low intensities; thus a boric/borate concentration of 20 m*M* fulfils the conditions for good resolution and a low electric current.

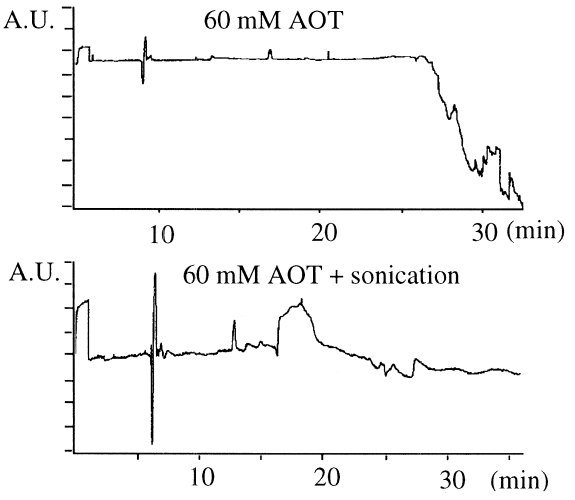

Fig. 7. Effect of AOT concentration on electrokinetic capillary chromatographic separation of phenolic food antioxidants.

Voltage is another variable that affects the separation process when electrophoretic techniques are used. High voltages produce a greater number of theoretical plates and at the same time, on reducing analysis times, diffusion is minimized. However, application of high voltages is limited owing to the generation of an elevated current intensity which produces more heat through the Joule effect. It was observed that for values above 27 kV deviations in Ohm's law occurred. Fig. 8 shows the migration times of the six antioxidants using different voltages for their separation. As seen, for voltages below 24 kV good resolution was achieved. Higher voltages,

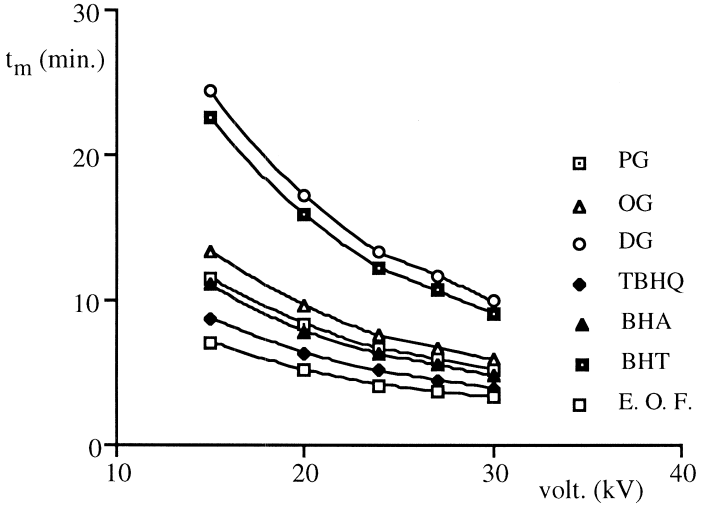

Fig. 8. Influence of applied voltage on the migration time of phenolic food antioxidants.

although still permitting analyte separation, generated a high current intensity as well as the elution of TBHQ next to the peak of the electroosmotic flow, hindering its quantification. A potential of 24 kV simultaneously afforded good resolution and quite short analysis times and was therefore chosen as the working potential.

Finally, the effect of variations in the injection volume was studied. This volume depends on the injection time since the sample is introduced in the capillary under pressure over a certain time. To calculate the viscosity of the solution [30] the following expression was used:

$$\frac{L_d \eta_2}{2L_t} + \left(1 - \frac{L_d}{2L_t}\right) \cdot \eta_1 = \frac{\Delta t \Delta P D^2}{32 L_t L_d} \quad (5)$$

where η_1 is the viscosity of the reference solution (distilled water) and η_2 is the viscosity of the medium; L_d is the length of the capillary up to the detection window. Experimentally, the time taken by the boundary between the reference and separation solutions in travelling is measured. Once the viscosity of the medium was known, and applying the Poiseuille equation:

$$\mathrm{Vol}_t = \frac{\Delta P D^4 \pi}{128 \eta L_t} \quad (6)$$

where ΔP is the applied pressure, D is the inner diameter of the capillary, L_t is the length of the capillary and η is viscosity, the volumes injected were calculated. A value of 3.67 nl/s was obtained for the injection flow-rate. The quantitative response, that is the corrected area, was related linearly to the amount injected. However, on injecting volumes higher than 29.4 nl, a poorer peak resolution was obtained and quantification was hindered. Therefore, an injection volume of 18.3 nl was chosen, equivalent to an injection time of 5 s.

4.2. Analytical characteristics

The electropherograms corresponding to different solutions containing the six antioxidants, except for TBHQ, at increasing concentrations in the $2 \cdot 10^{-6}$ M–$2 \cdot 10^{-4}$ M range were recorded. In the case of TBHQ, owing to its lower sensitivity, a concentration one-order of magnitude higher was used. Injection was carried out over 5 s and the analytes were separated applying a potential of 24 kV, using a separation solution containing 20% acetonitrile, 20 mM of the surfactant AOT and 20 mM boric/borate buffer at pH 9.4. In Fig. 9 a electropherogram is showed employing these conditions. Once the analytes had been quantified using the normalized area, this was plotted against the concentration, obtaining the corresponding calibration straight lines whose equations are shown in Table 1. It may be seen that

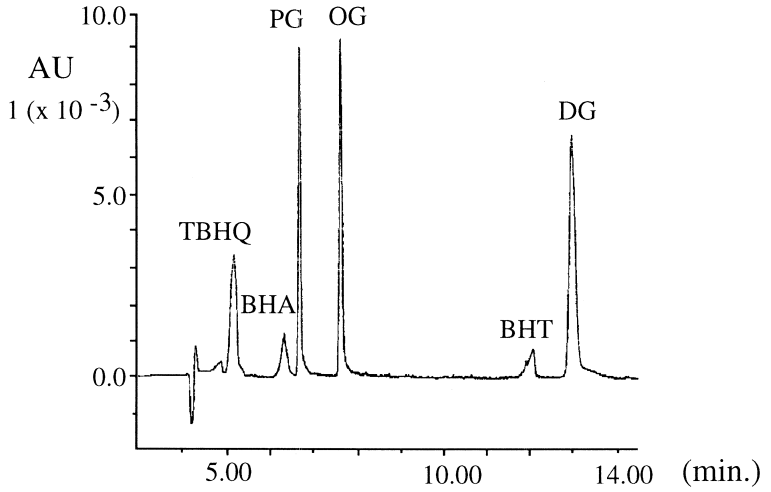

Fig. 9. Electrokinetic chromatographic separation of phenolic food antioxidants. Conditions: hydrodynamic injection, 18 nl; applied voltage 24 kV; separation buffer, 20 m*M* borax (pH adjusted to 9.4), 20% acetonitrile, 20 m*M* AOT.

good linear correlation coefficients were obtained for all the antioxidants.

Among all the analytical characteristics it is of interest to know the reproducibility of the method on the same or on different days. For this, the electropherograms corresponding to 10 different injections on one day and on 10 different days were quantified, obtaining the results shown in Table 1.

5. Conclusions

Using different techniques, it is seen that the surfactant AOT has a ''critical aggregation concentration'' of about 1 m*M* in the medium used and that the size of the aggregates is strongly affected by the salt concentration and by the concentration of the surfactant itself. The separation and quantification of three neutral analytes and three anionic ones – three present in neutral form in the presence of the surfactant – were also studied. Finally, the variables of the electrophoretic system were optimized, permitting the separation of the six antioxidants using electrokinetic chromatography. The calibration straight lines of the antioxidants were obtained and their retention factors were calculated.

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Table 1
Analytical characteristics of electrokinetic chromatographic separation of antioxidants using the optima conditions

Antioxidants	Intercept	Slope (area units/*M*)	r^2	D.L.[a]	RSD (%)	
					Intra-day ($n=10$)	Inter-day ($n=10$)
TBHQ	-0.02 ± 0.04	$(4.9\pm0.1)\cdot10^3$	0.996	$3.43\cdot10^{-6}$	7.23	9.05
BHA	0.005 ± 0.005	$(6.69\pm0.06)\cdot10^3$	0.999	$2.51\cdot10^{-6}$	4.81	8.07
PG	-0.09 ± 0.02	$(20.7\pm0.3)\cdot10^3$	0.998	$0.81\cdot10^{-6}$	11.0	13.2
OG	-0.04 ± 0.04	$(22.8\pm0.5)\cdot10^3$	0.996	$0.74\cdot10^{-6}$	11.3	13.8
BHT	0.022 ± 0.006	$(2.5\pm0.07)\cdot10^3$	0.997	$6.72\cdot10^{-6}$	10.0	7.38
DG	0.004 ± 0.03	$(20.9\pm0.4)\cdot10^3$	0.997	$0.80\cdot10^{-6}$	10.4	13.1

[a] D.L. = Detection limit for a signal-to-noise ratio of 3.

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