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Determination of micelle/water partition coefficients of cosmetic preservatives Optimisation of the capillary electrophoretic method

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Abstract

The hydrophobicity of a group of molecules used as preservatives for aqueous cosmetics, containing surfactants in the form of micelles, was evaluated by means of micellar electrokinetic capillary chromatography (MECC). Five different preservatives mixtures were examined, with a composition analogous to that employed in the preservation of cosmetic formulations. Sodium dodecyl sulphate and sodium dodecyl(polyoxyethylene)$_{1–4}$ sulphate were used as model surfactant and surfactant present in real cosmetic samples, respectively. The appropriate choice of the micellar velocity marker turned out to be a crucial point for the application of the MECC approach. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Parabens; MECC; Surfactants; Partition coefficients

1. Introduction

The aim of preservation of aqueous based cosmetics is to ensure that they are microbiologically safe and stable [1]. Adequately preserved products help to prevent microbial contamination during manufacturing and use, which minimises the potential health hazards to consumers. Up to now the choice of optimal preservative molecules or preservatives mixture is based on empirical testing and is often viewed as an art rather than a science. As a consequence, no correlation has ever been established between structure and properties of the molecules normally employed as preservatives.

In this research the attention has been focused on the preservation of water-based products such as shampoos, which mainly consist of micellar surfactant solutions. The partition between aqueous and micellar phase of a group of molecules that exhibit a preservative action against a wide spectrum of moulds, yeast and bacteria was examined. It cannot be excluded that their activity could be related to the extent of their presence in water, almost the only phase where the micro-organisms can develop. Five different preservatives mixtures were characterised; all of them contained four parabens (esters of 4-hydroxybenzoic acid) and different combinations of other four antibacterial agents. This research can be considered as an analytical preliminary study concerning the assessment of a possible relationship between antimicrobial activity and micelle/water partitioning of aromatic preservatives. The micelle/water partition

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coefficients of the preservatives were determined by means of micellar electrokinetic capillary chromatography (MECC) according to the model proposed by Terabe [2,3]. Such a method was applied to determine the micelle–solute interaction for a wide number of compounds [4], with an extension to cationic and anionic solutes [5,6]. It was also employed to study the effect of physico-chemical properties and molecular structure of the micelle–water partitioning [7]. Moreover, MECC presents an interesting alternative to micellar liquid chromatography (MLC) to estimate *n*-octanol/water partition coefficients [8,9]. Compared to MLC, MECC has the advantages related to the absence of a stationary phase: faster pre-conditioning and cleaning of the system, higher reproducibility and decreased error caused by intercolumn variability [8].

MECC is also a useful method for the separation and analysis of the parabens in real samples, in addition to MLC [10,11]; it was also employed to determine the imidazolidinyl urea, one of the mostly used preservatives, in cosmetic preparations containing the parabens [12,13].

In the present study sodium dodecyl sulphate (SDS) was chosen as model surfactant system because it has been widely used in the detergency field and it has been extensively studied and characterised; moreover the analytical method here proposed has been developed using SDS micelles.

Successively two selected mixtures were analysed also in a micellar solution of sodium dodecyl(polyoxyethylene)$_{1–4}$ sulphate, which is actually used in detergency products as a valid alternative to SDS because it is less irritating.

2. Experimental

2.1. Reagents

All reagents were of analytical-reagent grade. Methylparaben, ethylparaben, propylparaben, butylparaben, benzyl alcohol, 2-phenylethanol, 2-phenoxyethanol, 4-chloro-3-methylphenol, 4-chloro-3,5-dimethylphenol, *o*-phenylphenol were purchased from Sigma Aldrich (St. Louis, MO); 2,4,4′-trichloro-2′-hydroxy-diphenylether (Triclosan®) was supplied by Res Pharma (Italy). Their molecular structures are reported in Fig. 1. SDS was supplied by Sigma (St. Louis, MO), sodium dodecyl(polyoxyethylene)$_{1–4}$ sulphate, Zetesol 1070® (SDES, aqueous solution of 70% w/w SDES) by Zschimmer & Schwarz S.p.A. (Italy). Sodium dihydrogen phosphate and sodium tetraborate were purchased from Fluka (St. Louis, MO); methanol and Sudan III from Sigma (St. Louis, MO); 1–(4-*n*-butyl-2-pyridylazo)-2-naphthol (PAN-C_4) and 1-(4-*n*-octyl-2-pyridylazo)-2-naphthol (PAN-C_8) were synthesised in our lab.

Bidistilled water from a Milli-Q, water purification system (Millipore Corp., Bedford, MA) was used throughout the work.

2.2. Apparatus

The electrophoretic analysis was carried out on an electrophoresis apparatus Eureka 2100 model (Kontron) equipped with Diode Array (190–500 nm) detector. A fused silica capillary with 62.5 cm of length (50 cm to the detection window) and 0.050 mm of internal diameter was used (Supelco Celect).

A Metrohm 654 pH-meter, equipped with a combined glass–calomel electrode was employed for pH measurements. All buffers and samples were filtered (0.22 μm cellulose acetate filters, Millipore) and degassed by an ultrasonic bath (BRANSON 2200). Critical micellar concentration (c.m.c.) measurements were performed with a digital-tensiometer (K10, KRÜSS).

2.3. Electrolyte solutions

The proper amount of surfactant was dissolved in a buffer solution of sodium dihydrogen phosphate 3.30×10^{-2} M and sodium tetraborate 8.3×10^{-3} M (pH=6.90). The employed surfactant concentrations ranged from 20 to 100 mM for both SDS and SDES.

2.4. Sample preparation

A stock solution 1.0×10^{-3} M of each analyte in buffered SDS or SDES 20.0 mM was prepared; the same surfactant solutions were used for the dilution of the sample. The mixture compositions were chosen on the basis of preservative products employed in the cosmetic field. An effort was made to supply the widest antimicrobial activity spectrum. The four

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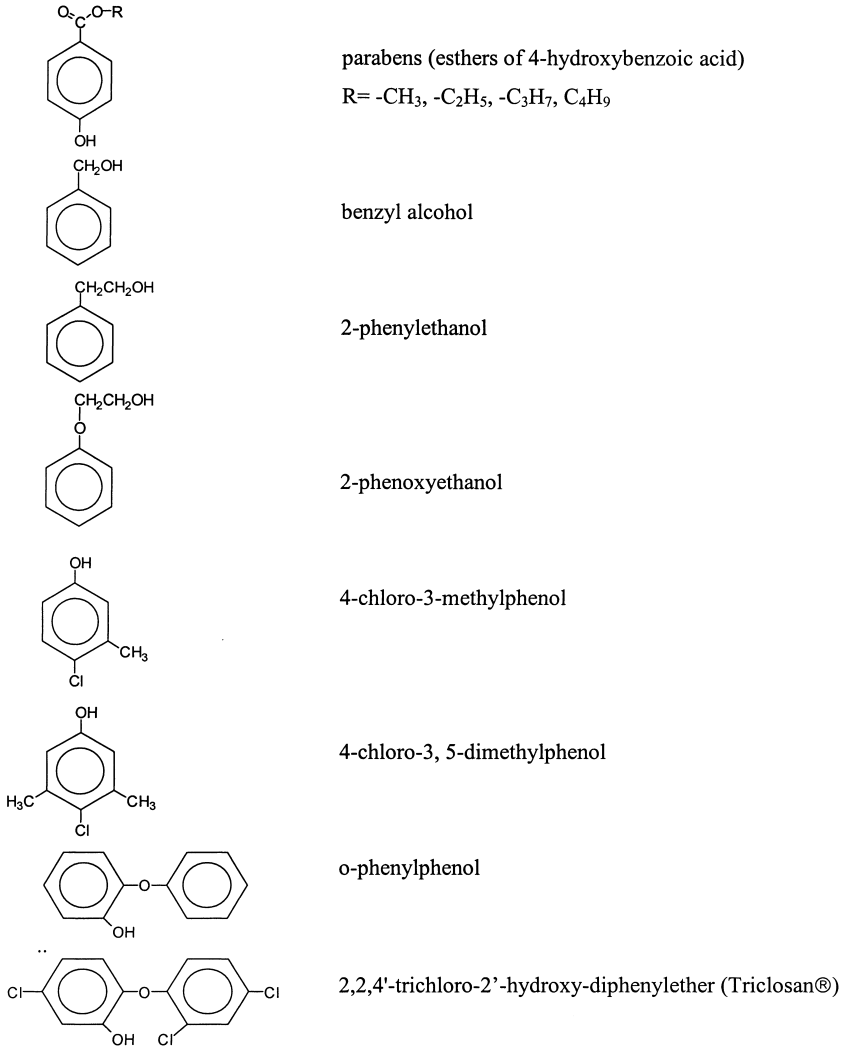

Fig. 1. Examined cosmetic preservatives: molecular structure and formula.

parabens present in all the mixtures were chosen because of their activity against moulds, yeast and Gram (+) bacteria. The other preservatives were then added to broaden the spectrum of action also against Gram (−) bacteria. The preservatives concentration in the analysed mixtures was chosen taking into account the ratio preservative/surfactant generally encountered in detergency cosmetic products, keeping as reference the parabens concentration (2.0×10^{-5} M each). About 1% V/V of methanol and 1×10^{-5} M of Sudan III (or PAN-C_n) were added to each sample solution as electroosmotic (v_{eo}) and micellar (v_{MC}) velocity marker, respectively. The overall concentrations of both preservatives and surfactant were considerably lower than in real samples (i.e. preservatives 0.1–0.8% w/w, surfactant 10–20% w/w) to ensure the appropriate experimental conditions for MECC.

Tables 1 and 2 report the five examined mixtures and the analytes concentration.

2.5. Electrophoretic procedure

When a new capillary was used, it was washed with water for 10 min, activated with 0.1 M NaOH

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Table 1
Molecules present in the examined mixtures

Mixture	Composition
1	Parabens+2-phenoxyethanol+Triclosan®
2	Parabens+2-phenylethanol+Triclosan®
3	Parabens+benzyl alcohol+2-phenoxyethanol
4	Parabens+benzyl alcohol+4-chloro-3-methylphenol
5	Parabens+*o*-phenylphenol+4-chloro-3,5-dimethylphenol

Table 2
Analytes concentration in the examined mixtures

Preservative	Concentration (M)
R-paraben	2.0×10^{-5}
Benzyl alcohol	3.2×10^{-5}
2-Phenoxyethanol	2.5×10^{-5}
2-Phenylethanol	2.8×10^{-5}
o-Phenylphenol	4.1×10^{-6}
4-Chloro-3-methylphenol	4.9×10^{-6}
4-Chloro-3,5-dimethylphenol	1.1×10^{-5}
Triclosan®	3.6×10^{-6}

for 10 min and rinsed with water for 10 min. Then it was equilibrated with the running buffer for 15 min at 20 kV. The same treatment was applied daily before starting the analysis. Each time the buffer composition was changed, the capillary was washed with water for 3 min, washed with methanol for 3 min, rinsed with water for 3 min, activated with 0.1 M NaOH for 3 min, rinsed with water for 3 min. Then it was equilibrated flushing the running buffer for 5 min and applying a constant potential of 30 kV for 20 min. The capillary was rinsed for 3 min with the buffer between runs.

The sample was introduced by applying a negative pressure at the cathodic capillary end for 4 s. The separations were run at 20, 22, 25, 27 and 30 kV keeping the capillary temperature constant at 35.0±0.1°C. The diode-array detection was performed in the 200–350 nm wavelength range.

3. Results and discussion

3.1. Preliminary analytes separation

Before starting with the determination of the partition coefficient, an electrophoretic analysis of a mixture containing all the examined compounds together with methanol and Sudan III was run. This was in order to establish their migration order and their resolution. All the compounds were identified by comparing the spectrum and the migration time of each peak with those recorded for the solutions of the pure standards. A 60 mM SDS solution buffered at pH 6.90 was chosen as the model surfactant system; the operating voltage was 30 kV; methanol and Sudan III were used as electroosmotic velocity marker and micellar velocity marker, respectively. Since at this pH all the examined molecules are in their neutral form their migration times differ only because they have different micelle/water partition coefficients. All the analytes were separated (see Fig. 2), falling within the elution window with the exception of Triclosan®, which migrates slower than Sudan III and partially coeluted with it.

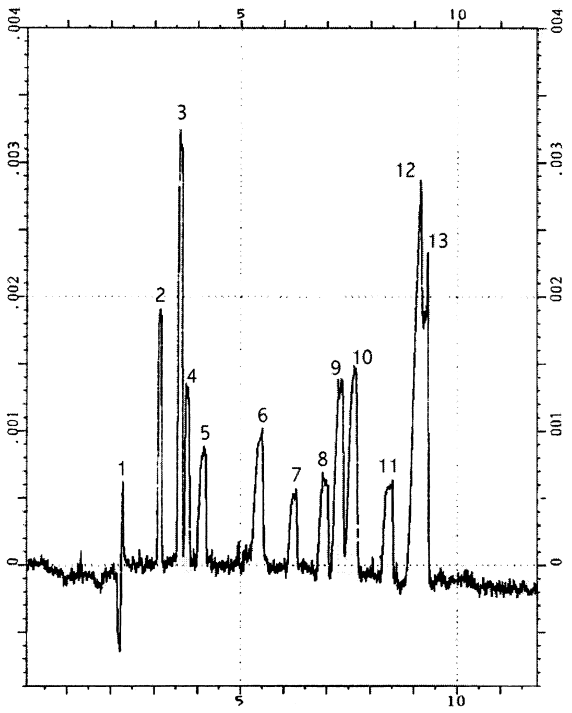

Fig. 2. Pherogram of the examined preservatives. SDS 60 mM; pH=6.90; applied voltage=30 kV; UV detection at 220 nm. Migration order: (1) methanol, (2) benzyl alcohol, (3) 2-fenoxyethanol, (4) 2-fenylethanol, (5) methylparaben, (6) ethylparaben, (7) 4-chloro-3-methylphenol, (8) propylparaben, (9) 4-chloro-3, 5-dimethylphenol, (10) *o*-phenylphenol, (11) butylparaben, (12) Sudan III and (13) Triclosan®.

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3.2. Examination of the proposed analytical method

The applicability of the electrophoretic model was checked by running preliminary measurements on a mixture containing only the four parabens (all present in every mixture further considered), dissolved in SDS together with methanol and Sudan III. Prior to the determination of the solute capacity factors (k') at different surfactant concentrations, the existence of possible temperature variations was verified by examining the capacity factor behaviour as a function of both the applied voltage and the electroosmotic velocity.

Fig. 3 reports the results obtained in the analysis run with 0.050 M of SDS at five different applied voltages. A linear correlation between k' values and electroosmotic velocity can be observed, with the slope increasing with the hydrophobicity of the molecule; an analogous behaviour was observed when plotting k' versus applied voltage. In order to eliminate the effect of v_{eo} on k', each line of the plot was extrapolated to v_{eo}=0, yielding the corresponding k'_0 values [3]. The same procedure was followed for the other examined SDS concentrations.

Plots of k'_0 as a function of the surfactant concentration are linear according to the following equation:

$$k'_0 = K_{MW}\,\bar{v}(C_{sf} - \text{c.m.c.}) \quad (1)$$

where K_{MW} is the micelle/water partition coefficient, $\bar{v}$ is the surfactant partial molar volume and C_{sf} is the total surfactant concentration. Fig. 4 shows that a good linear correlation exists although no common intersection is observed. Moreover the values of the intersection on the x-axis are negative resulting in meaningless negative values of the c.m.c.

Considering the Terabe's equation [3] adopted to calculate k'_0, it clearly demonstrates the importance of an accurate evaluation of both electroosmotic and micellar velocity; in particular, a slight variation of t_{MC} exerts a strong effect on the k'_0 evaluation. Acetone was used instead of methanol as v_{eo} marker, but no changes were observed. On the other hand, PAN-C_4 was chosen as v_{MC} marker; this molecule possesses an amphiphilic structure, which allows a suitable strong interaction with the micelles. In absence of added surfactant, PAN-C_4 and Sudan III migrated with the same velocity as acetone and methanol, thus evidencing that their migration was only due to the electroosmotic effect. In the presence of surfactant micelles PAN-C_4 migrates slightly slower than Sudan III. Due to its very low concentration (about 1.0×10^{-5} M) it cannot significantly modify the aggregate. On the contrary, it was observed that more concentrated organic modifiers, when added to the buffer to extend the elution window [14], can alter the micellar structure. In this

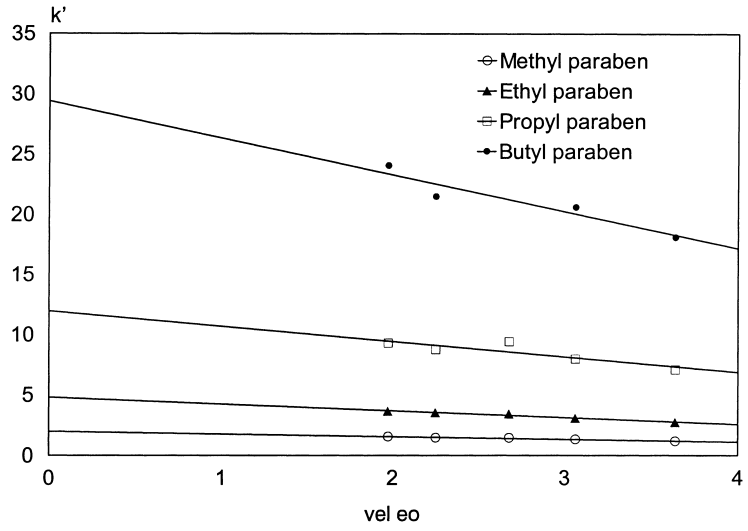

Fig. 3. Capacity factor behaviour in function of the electroosmotic velocity for the four parabens. SDS 50 mM; pH=6.90; applied voltages: 20, 22, 25, 27, 30 kV.

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