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Short communication

Comparative effects of sodium dodecyl sulfate and sulfobutyl ether-β-cyclodextrin as pseudostationary phases in the electrokinetic chromatographic separation of hydrophobic compounds

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Abstract

An alternative approach to the separation of hydrophobic compounds by electrokinetic chromatography using a negatively charged cyclodextrin, sulfobutyl ether-β-cyclodextrin (SBE-β-CD), as a pseudostationary phase is described. While the separation power of SBE-β-CD is comparable with sodium dodecyl sulfate for compounds with relatively low retention factors, a considerable improvement in resolution and small differences in selectivity were observed with this pseudostationary phase for highly hydrophobic compounds exemplified by C_5–C_{11} phenones. Moreover this approach allows electrokinetic chromatography to be performed in reversed flow mode where the pseudostationary phase elutes in the opposite direction to the electroosmotic flow, even at neutral pH and without the need for treated capillaries or addition of organic modifiers, thereby expanding the elution range.

Keywords: Pseudostationary phases; Phenones; Cyclodextrins

1. Introduction

Micellar electrokinetic chromatography (MEKC) with an anionic micellar pseudostationary phase migrating with a different velocity to that of the aqueous mobile phase, is one of the more popular modes of electrokinetic separation for neutral compounds. However, separation of highly hydrophobic compounds by this method often represents a considerable challenge as these compounds will migrate together with the micellar phase, leading to inadequate resolution. Reduction of retention factors towards the optimal values ($\tilde{k}'_{opt} = \sqrt{t_{MC}/t_0}$), where t_{MC} corresponds to migration time of micellar phase and t_0 corresponds to migration time of electroosmotic flow [1]) is required to obtain resolution. This can be realised in several ways.

Organic modifiers like methanol [2–4], isopropanol [5], acetonitrile [2,6] or urea [7] can be used to increase the solubility of hydrophobic compounds in an aqueous mobile phase.

Bile salts have lower solubilizing power than sodium dodecyl sulfate (SDS) and can therefore prove useful for separation of hydrophobic compounds [8–10]. Separation of highly hydrophobic phospholipids was recently demonstrated using the mixed effect of organic modifiers and bile salts [11,12].

Cyclodextrins can be used to reduce the polar character of the aqueous mobile phase and the distribution of hydrophobic compounds between aqueous and micellar phase can be shifted by inclusion into the cyclodextrin cavity [13,14].

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Separations of highly hydrophobic compounds by MEKC using an ionic polymer in the presence of high concentrations of organic modifiers [15] and improvement of separation of hydrophobic compounds in $^{2}H_{2}O$ based buffer solutions [16] have also been reported.

In this study we assess the potential of sulfobutyl ether-β-cyclodextrin (SBE-β-CD) as a pseudostationary phase in electrokinetic chromatography (EKC), focusing on the separation of hydrophobic compounds.

2. Experimental

2.1. Chemicals and reagents

All standards were obtained from Aldrich (Poole, UK). SBE-β-CD was obtained from Pfizer Central Research (Sandwich, UK). It has an average degree of substitution of 6.5 as determined by NMR and CE. All other chemicals used were of analytical grade purity. All aqueous mobile phases and separation buffers were filtered using a 0.45 μm filter (Anachem, Bedfordshire, UK) and degassed by sonication under vacuum.

2.2. Electrokinetic chromatography

The EKC was performed on a HP^{3D}CE capillary electrophoresis system (Hewlett-Packard, Waldbronn, Germany) equipped with a diode array detection (DAD) system operating at 220 nm. The capillary compartment temperature was maintained at 25°C. Fused-silica capillaries (Composite Metal Services, Hallow, UK) were 64.5 cm (56 cm to detector)×375 μm O.D.×50 μm I.D.. A CE capillary burner EK 1.2 from Capital HPLC (Broxburn, Edinburgh, UK) was used to prepare the detector window. Hydrodynamic injection (35 mbar over 10 s) was used to introduce samples. The new capillary was rinsed with 0.1 *M* NaOH (1000 mbar×30 min), followed by deionised water (1000 mbar×30 min) and separation buffer (1000 mbar×30 min). The capillary was rinsed between injections with 0.1 *M* NaOH (1000 mbar×1 min) followed by deionised water (1000 mbar×1 min) and separation buffer (1000 mbar×2 min). All data were collected and analysed using HP^{3D}CE CHEMSTATION software (Hewlett-Packard).

2.3. Results and discussion

SBE-β-CD is a derivative of β-cyclodextrin which on average has 6.5 of the hydroxyl groups in position 6 substituted through an ether bond by $-C_4-SO_3Na$ groups. In aqueous solutions SBE-β-CD is present in its anionic form over a wide pH interval [17].

In fused-silica capillaries filled with borate buffer at pH 9.2 strong cathodic electroosmotic flow is formed. Upon the addition of negatively charged SBE-β-CD to this separation buffer a two-phase system is formed. Aqueous mobile phase moves with the velocity of electroosmotic flow (μ_{eo}) towards the cathode. SBE-β-CD pseudostationary phase moves with the velocity and direction determined by the ratio between the electroosmotic mobility and the electrophoretic mobility of SBE-β-CD ($\mu_{SBE\text{-}\beta\text{-}CD}$) as schematically shown in Fig. 1. Separation of analytes between these two phases is based on differential

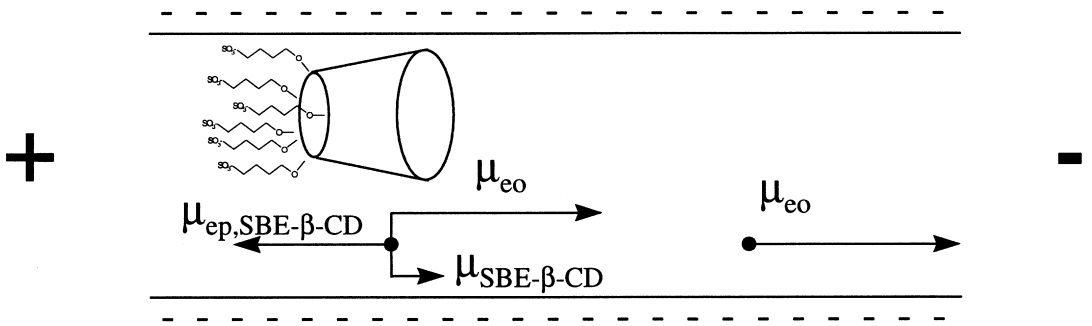

Fig. 1. Schematic representation of EKC with SBE-β-CD pseudostationary phase at pH 9.2. μ_{eo} = Electroosmotic mobility, $\mu_{ep,SBE\text{-}\beta\text{-}CD}$ = electrophoretic mobility of SBE-β-CD, $\mu_{SBE\text{-}\beta\text{-}CD}$ = apparent mobility of SBE-β-CD.

interaction with the SBE-β-CD phase. Depending on the nature of the analyte this may involve either polar/ionic interactions with the negatively charged sulfonic acid groups and/or inclusion complexation within the cyclodextrin cavity.

The separation of a test mixture using SBE-β-CD compared to that obtained using a more established pseudostationary phase formed by SDS is shown in Fig. 2. It can be concluded from this comparison that when SBE-β-CD is used as a pseudostationary phase, a similar separation to that obtained using SDS is observed. However in some instances e.g.

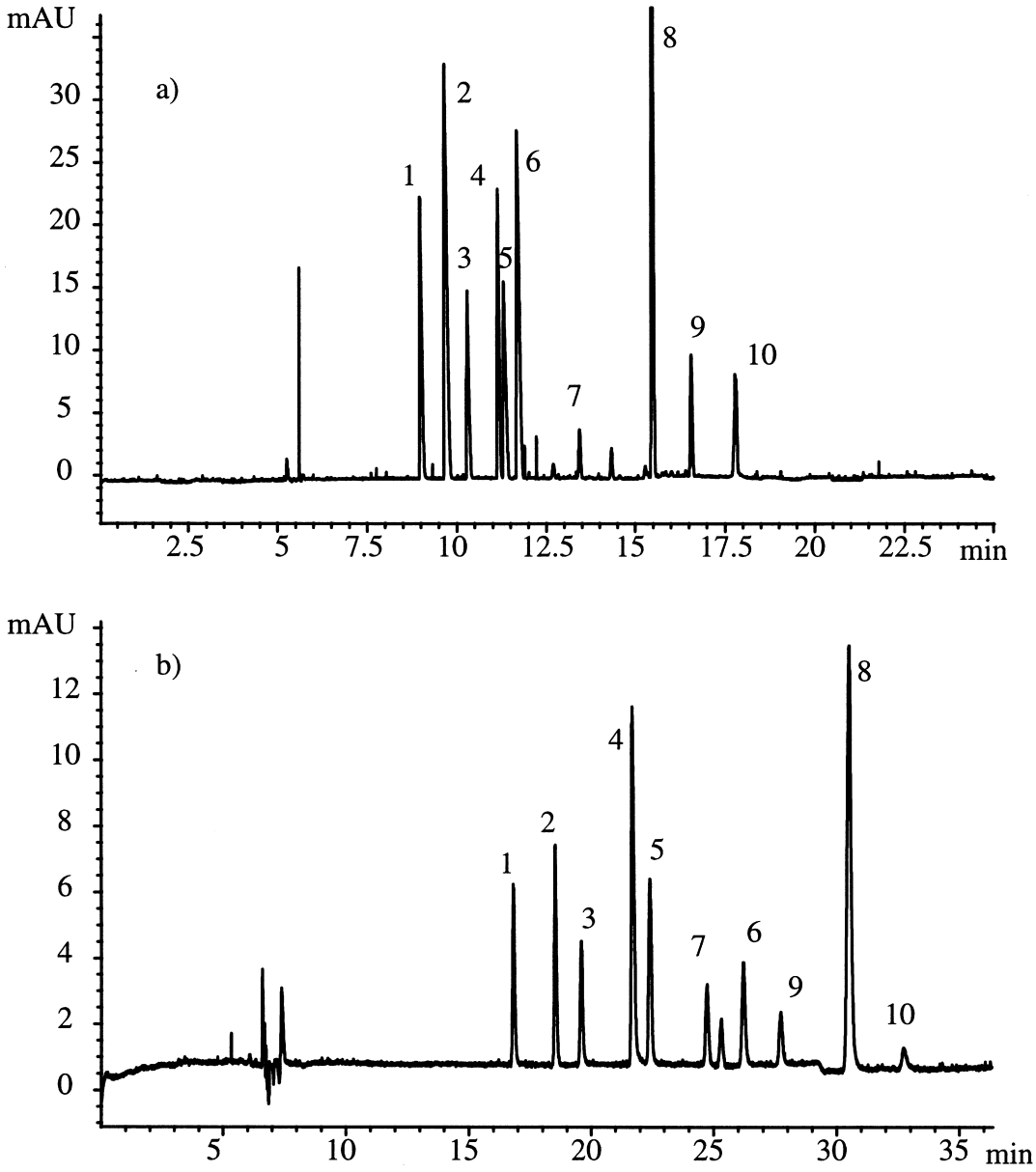

Fig. 2. EKC of aromatic compounds. Buffer: (a) 25 m*M* borate, pH 9.2, 100 m*M* SDS, (b) 25 m*M* borate, pH 9.2, 50 m*M* SBE-β-CD. See Section 2 for other conditions. 1=Aniline, 2=benzyl alcohol, 3=benzaldehyde, 4=nitrobenzene, 5=acetophenone, 6=phenylacetone, 7=toluene, 8=chlorobenzene, 9=ethylbenzene, 10=naphthalene.

phenylacetone and chlorobenzene (Fig. 2.) stronger retention and changes in elution order were observed. These differences may be rationalised by considering very different retention mechanisms involved: SDS – hydrophobic interactions within SDS micelles vs. SBE-β-CD – inclusion complexation within the cyclodextrin cavity.

Currently there are no reliable markers for determination of the elution time of the electroosmotic flow (t_{eo}) when SBE-β-CD is used as pseudostationary phase. This is because methanol, which is commonly used as an electroosmotic flow marker in MEKC with SDS as a pseudostationary phase, may be retained by SBE-β-CD. Similarly we are not aware of any reliable marker for the determination of the elution time of SBE-β-CD ($t_{SBE\text{-}\beta\text{-}CD}$). Consequently the elution range ($t_{SBE\text{-}\beta\text{-}CD}/t_{eo}$) cannot be reliably determined for this system.

However, the ratio between the retention time of last eluting compound (naphthalene) and retention time of baseline disturbance due to methanol is larger for SBE-β-CD (5.07) than for SDS (3.18) indicating a larger elution range for the SBE-β-CD system.

When a test mixture consisting of four hydrophobic compounds (hexanophenone, octanophenone, decanophenone and dodecanophenone) was analysed by EKC with pseudostationary phase formed by SDS

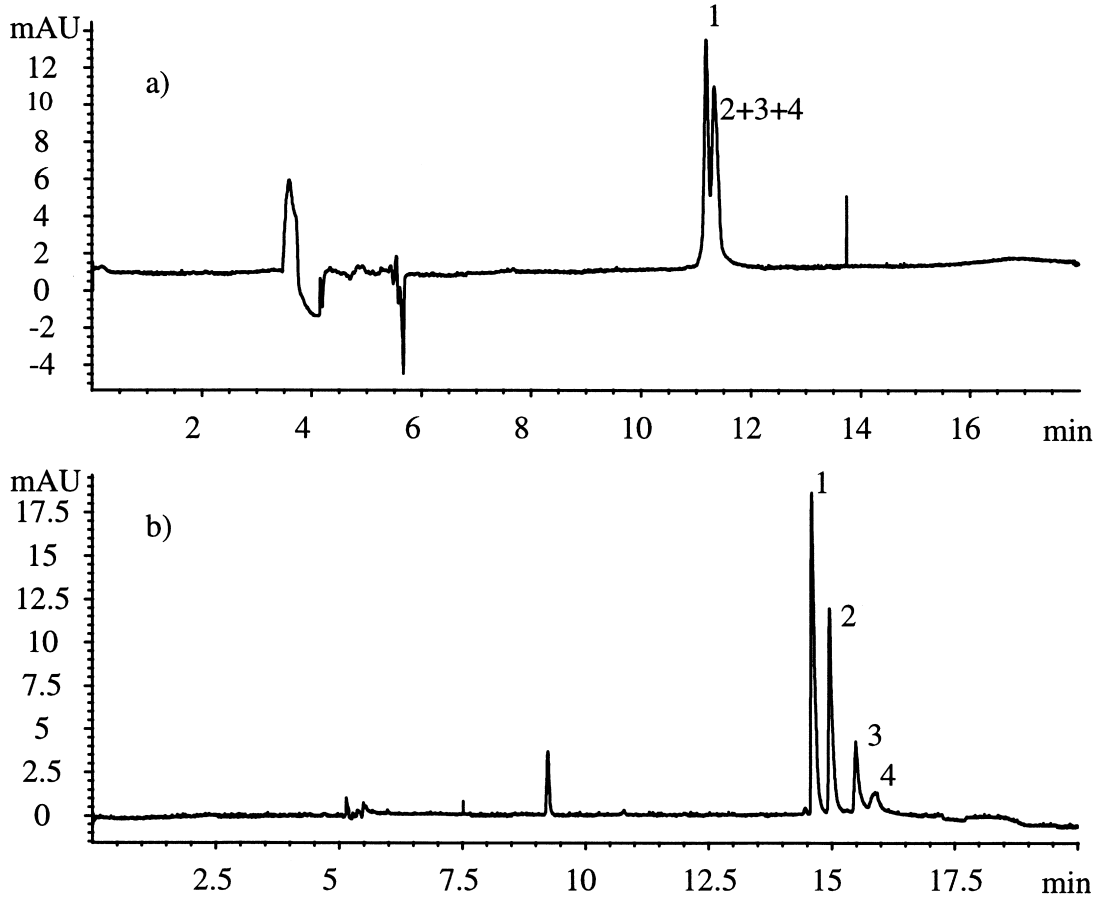

Fig. 3. EKC of hydrophobic compounds. Buffer: (a) 25 m*M* borate, pH 9.2, 100 m*M* SDS. (b) 25 m*M* borate pH 9.2, 50 m*M* SBE-β-CD. Applied voltage: +30 kV. See Section 2 for other conditions. 1=hexanophenone, 2=octanopheno ne, 3=decanophenonee, 4=dodecanophenone.

at pH 9.2 only a partial separation was obtained (Fig. 3a). When the same separation was attempted using the pseudostationary phase formed by SBE-β-CD, baseline separation of all four hydrophobic compounds was obtained (Fig. 3b).

The fact that all the highly hydrophobic compounds were detected in order of their hydrophobicity at the cathodic end indicates that electroosmotic flow dominates the net migration and that the SBE-β-CD pseudostationary phase migrates in the same cathodic direction. When an identical experiment was performed but with the pH of separation buffer reduced to 7.0, EKC with SDS again provided only a partial separation of the hydrophobic test mixture (Fig. 4a). Use of SBE-β-CD led to a complete resolution of the four hydrophobic compounds (Fig. 4b). In this case all hydrophobic compounds were detected at anodic side in reversed elution order compared to EKC under normal conditions.

This is because the electrophoretic mobility of SBE-β-CD is in absolute value higher than electroosmotic flow at pH 7.0, resulting in anodic movement of the pseudostationary phase, as schematically indicated in Fig. 5. This separation mechanism in which the pseudostationary phase migrates in an opposite direction to electroosmotic movement, is described in the literature as reversed-flow EKC (RF-EKC) [18,19]. However to the best of our knowledge, this is the first report of RF-EKC performed at neutral pH in untreated capillaries and without organic modifiers.

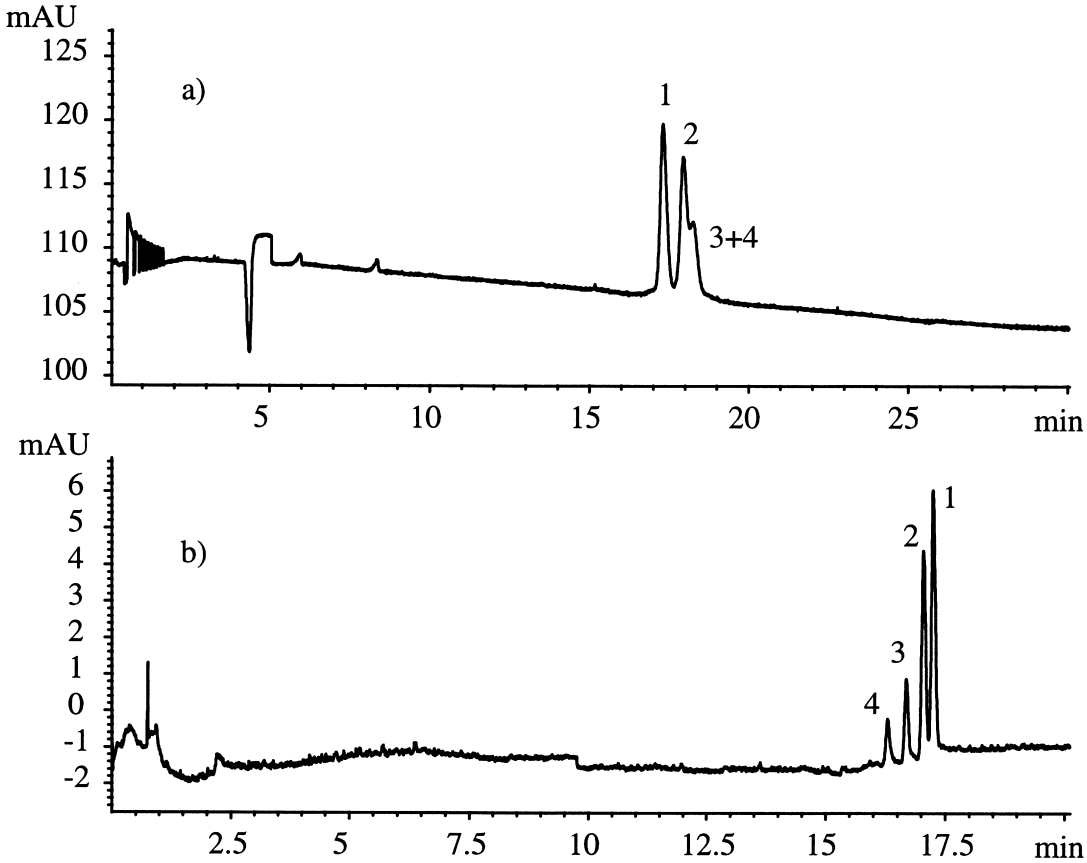

Fig. 4. EKC of hydrophobic compounds. Buffer: (a) 25 m*M* phosphate, pH 7.0, 100 m*M* SDS. (b) 25 m*M* phosphate, pH 7.0, 50 m*M* SBE-β-CD. Applied voltage: (a) +30 kV, (b) −30 kV. See Section 2 for other conditions and Fig. 3 for peak identification.

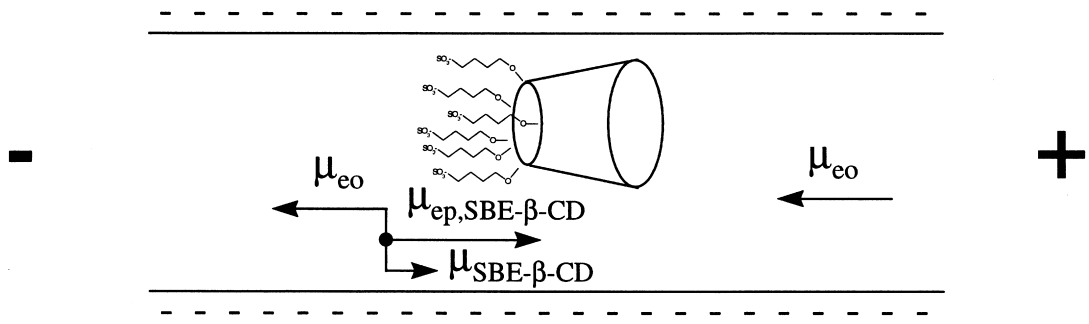

Fig. 5. Schematic representation of EKC with SBE-β-CD pseudostationary phase at pH 7.0. μ_{eo} = electroosmotic mobility, $\mu_{ep,SBE\text{-}\beta\text{-}CD}$ = electrophoretic mobility of SBE-β-CD, $\mu_{SBE\text{-}\beta\text{-}CD}$ = apparent mobility of SBE-β-CD.

3. Conclusions

The main features of EKC with an SBE-β-CD pseudostationary phase are an extended elution window, subtle differences in selectivity and an improved separation of hydrophobic compounds. This separation system also gives reversed-flow EKC at neutral pH, providing a normal-phase HPLC type of separation based on polarity.

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