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Potential of microemulsion electrokinetic chromatography for the separation of priority endocrine disrupting compounds

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Abstract

This work examines the potential of microemulsion electrokinetic chromatography for the separation of several priority endocrine disrupting compounds (EDCs). The optimised microemulsion system comprised 25 m*M* phosphate buffer pH 2, 80 m*M* octane, 900 m*M* butanol, 200 m*M* sodium dodecyl sulphate and was further modified with 20% propanol. The use of a low pH buffer resulted in the suppression of electroosmotic flow within the capillary. Reversal of the conventional electrode polarity resulted in faster migration of hydrophobic compounds. Test analytes included the octylphenol, nonylphenol and nonylphenol diethoxylate, which are breakdown products of the alkylphenolic detergents. The synthetic oestrogens diethylstilbestrol and ethynyloestradiol were also included in the separation along with the plastic monomer bisphenol-A. Test analytes were selected due to their reported presence in environmental samples namely industrial and domestic wastewater treatment effluents and sludges. Using the optimised method a separation of six EDCs was achieved within 15 min. The optimised method was then applied to the analysis of a spiked wastewater influent sample with UV detection of all six compounds at 214 nm.

Keywords: Microemulsion electrokinetic chromatography; Endocrine disruptors; Alkylphenol ethoxylates; Surfactants; Steroids; Phenols

1. Introduction

Recent years have seen the investigation of a number of compounds that interfere with the normal action of the endocrine system collectively known as endocrine disrupting compounds (EDCs). An EDC has been defined as ‘‘an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function’’ [1]. Compounds that interfere with the action of endogenous oestrogen hormones are also known as environmental oestrogens, oestrogen mimics, oestrogenic chemicals or xenoestrogens [2].

The significance of oestrogen-mimicking EDCs lies in the adverse biological effects that have been observed following elevated exposure in wildlife populations with concern now focused on the possible implications for humans [3]. Compounds identified as priority EDCs include various members of distinct chemical groups such as the bioflavanoids, synthetic oestrogens, phenols, alkylphenols, poly-

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chlorinated biphenyls, organochlorine pesticides, dioxans and furans. EDCs including bisphenol-A, ethynyloestradiol and nonylphenol have been detected in wastewaters [4–6] and are known to persist in ''treated'' effluents due to incomplete removal by conventional treatment facilities [7–9]. Many of these compounds have been detected in the wider aquatic environment following subsequent discharge of these effluents to surface waters. Recycling of water resources i.e. water abstraction downstream of wastewater treatment facilities and infiltration of groundwaters has also resulted in the detection of some EDCs in drinking water supplies [4,8–11].

Characterisation of wastewaters and effluents is therefore important to determine the efficiency of current treatment technologies for the removal of EDCs prior to discharge. As a result, there is an increasing need for the development of sensitive analytical techniques to facilitate the routine analysis of often complex mixtures of EDCs. To this end, our research focuses on the development of novel capillary electrophoresis (CE)-based separations of mixtures of priority EDCs due to the ability of the technique to resolve complex mixtures of structurally diverse charged and neutral compounds. Particular focus is given to the development of novel separations of oestrogen mimicking EDCs previously detected in the environmental samples. Target analytes for this study included the synthetic oestrogens; ethynyloestradiol used in the contraceptive pill and diethylstilbestrol previously used as an illegal growth promoter in cattle. Breakdown products of the alkylphenol polyethoxylated (APE) surfactants under investigation included nonylphenol diethoxylate, octylphenol and nonylphenol. While parent APEs are not oestrogenic, partial breakdown during wastewater treatment can result in the formation of intermediate degradation products exhibiting increased toxicity and biological potency [12]. Structures of analytes under investigation are illustrated in Fig. 1.

The hydrophobic nature of these compounds means that for CE-based separations they must be solubilised using high concentrations of organic modifiers, surfactants [13–17] and/or cyclodextrins [18–23]. This can lead to both excessive current generation and longer analysis times when using additives whose mobility opposes the direction of

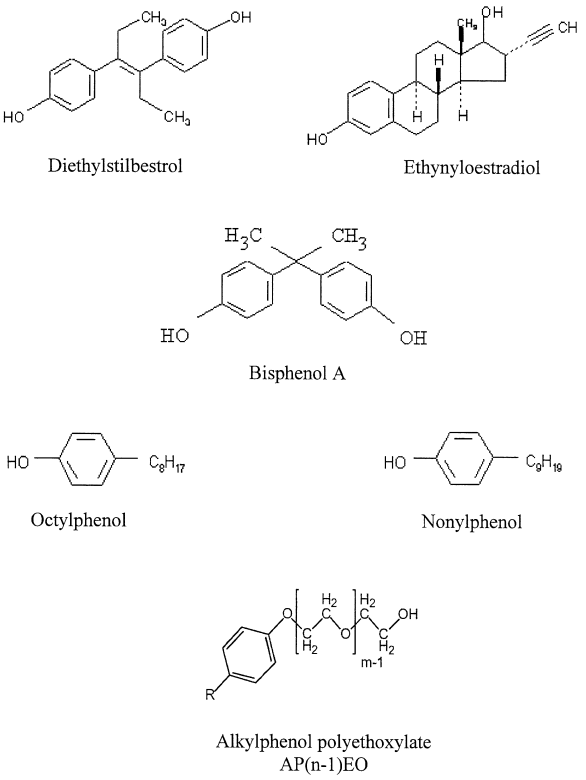

Fig. 1. Structures of target analytes.

electroosmotic flow (EOF) in conventional high-pH separations (15–20 min). This is often an issue for hydrophobic compounds where strong analyte partition and/or inclusion often occurs.

Our group has previously investigated micellar electrokinetic chromatography (MEKC) [24] and cyclodextrin (CD)-modified MEKC [25] for the simultaneous separation of a diverse selection of EDCs. This study investigates microemulsion electrokinetic chromatography (MEEKC) for the separation of alkylphenols and oestrogens. Microemulsions are microheterogeneous liquids, with characteristics including optical transparency, thermodynamic stability and high solubilisation power [26]. This separation mode was investigated due to the high solubilising power of microemulsions. MEEKC has been applied to the separation of a number of different compound groups including water- and lipid soluble vitamins [26], steroids [27], proteins [28] as well as a range of pharmaceutical compounds [29].

The majority of publications in the area so far have used a high pH for the analysis in order to generate high EOF velocities. Separation in MEEKC is achieved due to the differential partitioning of analytes between the oil droplet and surrounding aqueous buffer zone, as well as their individual mobilites determined by their mass-to-charge ratios. For further background theory on the technique the reader is referred to some recent reviews [30,31].

An advantage of MEEKC over conventional MEKC systems includes easier analyte penetration and partition into microemulsion droplet, which has been described as being less rigid than a surfactant micelle [32]. However, to create and maintain a stable microemulsion system, the concentrations of the oil, water and surfactant must be maintained within a narrow range. As a result, many of the published MEEKC methods are similar, as the opportunity for variation of the concentration of system components is somewhat limited. Analytes that are highly soluble in the droplet may also exhibit long analysis times due to the high surfactant concentrations used in order to maintain dispersion of the oil in the aqueous buffer.

In previous studies it was found that suppression of EOF through the use of low pH buffer and electrode polarity reversal proved useful for the separation of a selection of endocrine disruptors which had long migration times in CD-MEKC [25]. At low pH values, phenolic compounds are uncharged and should interact with the surfactant-coated oil droplets on the basis of their hydrophobicity. Suppression of EOF also minimises possible analyte repulsion from negatively charged micelles, which could occur at high pH where both phenols and micelles (anionic surfactants) are negatively charged. When conventional electrode polarity is reversed (to allow detection at the anode), the use of an anionic surfactant such as sodium dodecyl sulphate (SDS) can lead to a faster migration of associated analytes. While the developed EOF-suppressed CD-MEKC method achieved a faster analysis of alkylphenols in a CE system than reported previously, resolution of octylphenol (OP) and nonylphenol (NP) needed further improvement.

As far as we are aware, MEEKC has not been investigated for the separation of EDCs, therefore previously reported separations of structurally similar compounds were investigated [35]. The analysis of fatty acids with a broad spectrum of alkylchain lengths (C_2–C_{20}) has been achieved using a microemulsion system [36] and this suggests that the technique could be applicable to the extended chains of alkylphenol detergents and their breakdown products, which has not been attempted previously. MEEKC has been used in the analysis of 10 corticosteroids at pH 10 [27]. A similar system is investigated further for the separation of several priority EDCs including oestrogens and alkylphenols. The potential of EOF suppression will also be investigated in an attempt to reduce analysis times for hydrophobic alkylphenols.

2. Experimental

2.1. Instrumentation

Separations were performed using a Beckman P/ACE 5500 system (Beckman Coulter, 4300 N. Harbor Boulevard, P.O. Box 3100, Fullerton, CA 92834-3100, USA), equipped with a photo-diode array detection (DAD) system. The DAD detector range was 190 nm to 300 nm. The CE instrument was operated using Windows P/ACE Station Software version 1.21. Integration data was calculated by P/ACE Station, using the USP (United States Pharmacopoeia) method. Peak efficiency (N) was defined as $N = 16(t/w)^2$ where t is peak migration time and w is the width of the base of the component peak. Peak resolution (defined as the resolution between the peak of interest and the preceding peak) was calculated using $R = 2(t_2 - t_1)/w_2 + w_1$. The fused-silica capillaries (Beckman) used were 57 cm long with an I.D. of 50 μm. Bare fused-silica capillaries with an I.D. of 50 μm (Beckman) and effective lengths of 30, 40 and 50 cm were used.

2.2. Reagents

Sodium phosphate buffer, propanol, butanol, octane, heptane, acetonitrile, HCL, NaOH, SDS, nonylphenol (NP), octylphenol (OP), bisphenol-A (BPA), ethynyloestradiol (EO) and diethylstilbestrol (DES) were all purchased from Sigma Aldrich, Dublin, Ireland and used without further purification. Nonyl-

phenol monoethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO) and nonylphenoxy acetic acid (NPAA) were purchased from Dr Ehrenstorfer, Augsburg, Germany. The pH of the phosphate buffer was adjusted using 5 *M* HCl and 0.1 *M* NaOH.

2.3. CE separation conditions

Buffers were prepared by weighing the appropriate mass of surfactant (SDS), the co-surfactant and the core phase together, followed by the organic modifier (propanol), buffered phosphate solution and were made up to the mark with deionised water. All run buffers were sonicated for half an hour prior to use and were either prepared fresh each day or used within 3 to 4 days of preparation. Buffers were also filtered through a 0.45 μm syringe (Gelman) before use.

The daily start-up procedure involved a 5 min pre-rinse with deionised water followed by a 3 min 0.1 *M* HCl rinse, and a 10 min buffer rinse to equilibrate the capillary. The standard method involved a 3 min pre-rinse with buffer followed by a 5 s hydrodynamic injection of standard/sample. Separations were performed at 20 kV constant voltage with detection at 214 nm UV (unless otherwise stated). For optimisation of standard separation, a capillary of 30 cm (effective length) and 50 μm I.D. was used. After all method development was completed, the optimised buffer conditions were determined to be 25 m*M* phosphate pH 2, 200 m*M* SDS, 900 m*M* butanol, 80 m*M* heptane, with 20% propanol.

2.4. Standard preparation

Stock solutions of analytes were prepared in 100% propanol and diluted with run buffer prior to analysis. They were stored at 4°C in the dark and were covered with tinfoil to prevent photodegradation of analytes. Nonylphenol monoethoxylate, nonylphenol diethoxylate and nonylphenoxyacetic acid were prepared in 100% MeCN and diluted with run buffer for injection. All optimisation separations were performed in triplicate.

2.5. Sample preparation

A sewage influent sample was obtained and spiked with two target analytes in order to investigate possible sample matrix effects. Sample collection and solid-phase extraction procedures were carried out, using a developed method [25]. Samples were then diluted with buffer prior to analysis as indicated in relevant figure legends.

3. Results and discussion

3.1. High-pH MEEKC

The majority of MEEKC separations to-date have used high pH buffers (pH 9 or greater) due to the greater magnitude of EOF generated resulting in faster analysis times. Initial work focused on the investigation of an existing MEEKC method, and assessed its suitability for the separation of several priority endocrine disruptors. The method had been developed for the separation of corticosteroids at high pH, whose chemical structure is similar to that of the synthetic oestrogens of interest to this work [27]. Successive injections of the synthetic oestrogen ethynyloestradiol were performed using a similar system. Differences in this study included heptane (used as the core phase in place of hexanol) as this has been used in a majority of applications and the injection mode was hydrodynamic as opposed to electrokinetic. While the baseline remained relatively constant the migration time of the oestrogen increased with each successive run (data not shown). This suggested problems with either the equilibration of the capillary surface as a new capillary was used, and/or a build-up of surfactant due to the high concentrations present in the run buffer (120 m*M*).

The lengthy migration times were attributed to the preferential partitioning of the analyte into the oil droplet as opposed to the surrounding aqueous buffer. The negatively charged SDS micelles migrate against the direction of the EOF, resulting in increased migration times for associated analytes. In order to remove a build up of SDS, various pre-rinses were investigated including 0.1 *M* NaOH and also methanol. These did not reduce the time needed for analysis. A reduction in the concentration of the SDS would have reduced analysis times but was not considered, as the surfactant concentration used is generally 3% or greater in order to maintain the stability of the microemulsion solution [38]. As the

migration time of ethynyloestradiol eventually exceeded 35 min, the run conditions were deemed unsuitable for the analysis of synthetic oestrogens. The method had been optimised for corticosteroids, which have greater aqueous solubility than that of target EDCs. The long migration time of ethynyloestradiol illustrated a strong affinity for the negatively charged micelles. As anionic micelles migrate against the direction of the EOF at high pH values under the influence of electrophoresis, shorter migration times are expected using the EOF-suppressed system with reversal of conventional electrode polarity.

3.2. EOF-suppressed MEEKC

EOF-suppressed MEEKC was investigated to overcome the issues of lengthy migration times of hydrophobic analytes. A low pH buffer was used to eliminate EOF in the capillary with reversal of conventional electrode polarity allowing detection at anode. This approach had been investigated previously by Pedersen-Bjergaard et al. [33] for the separation of fat-soluble vitamins, who used 66.6% (w/w) 25 m*M* phosphate buffer pH 2.5, 20% 2-propanol, 6.6% 1-butanol, 6% SDS and 0.8% *n*-octane as the separation medium.

A similar separation system using 20 m*M* phosphate pH 2, 120 m*M* SDS, 900 m*M* butanol, 80 m*M* heptane was investigated further for the analysis of octylphenol and nonylphenol with and without organic modifier. Some of the solvents investigated previously for the modification of microemulsion systems include methanol, ethanol, tetrahydrofuran, propanol and acetonitrile although the latter has resulted in instable baselines [33]. As with MEKC analysis, the organic modifier can only be added up to a certain concentration (%) after which peak efficiencies are compromised due to the disruption of the microemulsion.

An organic concentration of 20% was needed to achieve partial separation of the two components through the alteration of the affinity for the core phase (data not shown). This improved resolution was, however, at the cost of increased analysis times. Improved separation of hydrophobic analytes after propanol addition has been observed previously [37]. This is attributed to a higher viscosity than other organic solvents (MeCN and MeOH) leading to EOF reduction and improved separation. After the addition of 20% propanol in this study the migration time for both peaks more than doubled. The degree of partitioning of the analytes into the core phase was altered through the addition of an organic modifier to the system. The addition of an organic solvent also reduces the associated system current generated, which can help to minimise the effects of Joule heating.

3.3. Optimisation of standard separation

The compounds chosen for the optimisation of the standard separation were octylphenol, nonylphenol, nonylphenol diethoxylate, diethylstilbestrol, ethynyloestradiol and bisphenol-A (Fig. 2). The conditions did not resolve the isomers of nonylphenol present even though a technical mixture of isomers was used. Resolution of isomers was not a priority of the study.

3.3.1. Surfactant concentration

The concentration and nature of the surfactant and the co-surfactant used to form the microemulsion are thought to be the most important factors for optimis-

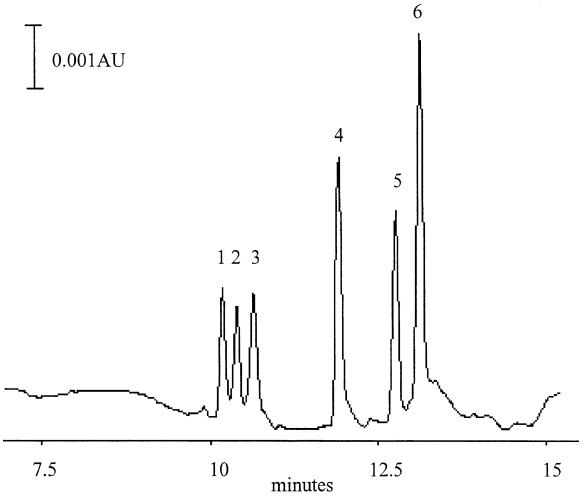

Fig. 2. Separation of a mixture of endocrine disruptors using standard conditions. Separation conditions: 25 m*M* phosphate pH 2, 120 m*M* SDS, 900 m*M* butanol, 80 m*M* heptane, with 20% propanol. Analytes: 50 mg/l in 70% buffer and 30% propanol). Peak identification: 1 = octylphenol; 2 = nonylphenol; 3 = nonylphenol diethoxylate; 4 = diethylstilbestrol; 5 = rthynyloestradiol, 6 = bisphenol-A.

ing the selectivity of MEEKC analysis [38]. A neutral surfactant was not investigated as it would have the same mobility as the neutral oil droplet and would not allow separation. The anionic surfactant SDS has previously proven useful in developed MEKC and CD-MEKC applications for the separation of EDCs [24,25], and was selected for further investigation. SDS (80 m*M*) was the lowest concentration of surfactant investigated in order to maintain the stability of the microemulsion and 200 m*M* the highest to minimise current generation. Gabel-Jensen et al. [38] reported that the use of SDS as a surfactant at pH 9.2 led to relatively long analysis times for the more hydrophobic compounds; however, in an EOF-suppressed environment the reverse should be true.

As illustrated in Fig. 3, higher concentrations of SDS resulted in shorter analysis times coupled with a stable and reproducible separation system, as illustrated by the low RSD of analyte migration times. Due to the high concentrations of surfactant used in the microemulsion, it was essential to rinse the capillary with water for 5 min before start-up each day and before instrument shutdown at the end of the day. Without this rinse step surfactant precipitation on the ends of the capillary and inside the capillary led to blockages and current problems. SDS (200 m*M*) was selected as the optimum surfactant concentration, as it resulted in the shortest migration times coupled with acceptable peak efficiencies for all six analytes.

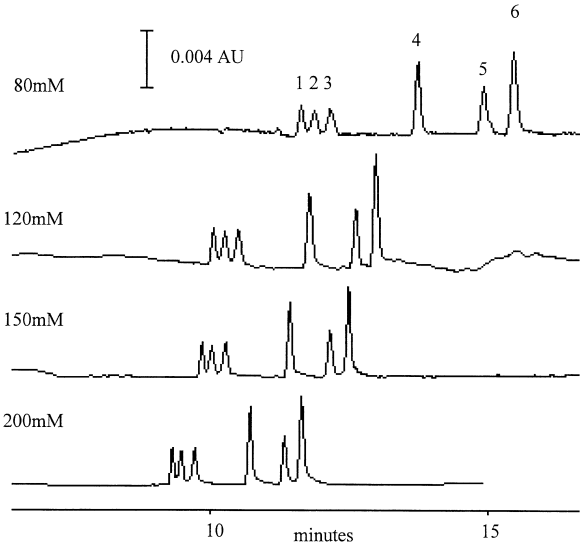

Fig. 3. Variation of surfactant concentration. Separation conditions: 25 m*M* phosphate pH 2, various SDS, 900 m*M* butanol, 80 m*M* heptane, with 20% propanol. Analytes: 50 mg/l in 70% buffer and 30% propanol. Peak identification: 1 = octylphenol; 2 = nonylphenol; 3 = nonylphenol diethoxylate; 4 = diethylstilbestrol; 5 = ethynyloestradiol; 6 = bisphenol-A.

3.3.2. Organic modifier concentration

The % concentration of organic modifier (propanol) in the run buffer was varied (Fig. 4). Propanol was selected as a recent study [33] found that modification with acetonitrile led to baseline instability. As expected, increasing the concentration of the organic modifier led to an increase in the migration times of the analytes. A concurrent improvement in the resolution of octylphenol and nonylphenol (peaks 1 and 2) was observed at higher concentrations of propanol from co-migration (resolution 0) at 10% propanol to a resolution of 1.21 at 20%. Higher concentrations of propanol (<20%) were not investigated as excessive amounts of organic could destabilise the microemulsion. In MEKC applications the limit of addition is around 30% organic solvent before micelle disruption occurs

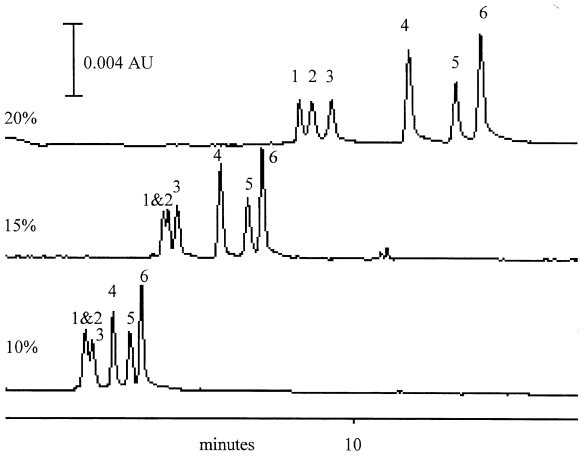

Fig. 4. Variation of organic modifier concentration. Separation conditions: 25 m*M* phosphate pH 2, 200 m*M* SDS, 900 m*M* butanol, 80 m*M* heptane, with various propanol concentrations. Analytes: 50 mg/l in 70% buffer and 30% propanol. Peak identification: 1 = octylphenol; 2 = nonylphenol; 3 = nonylphenol diethoxylate; 4 = diethylstilbestrol; 5 = ethynyloestradiol; 6 = bisphenol-A.

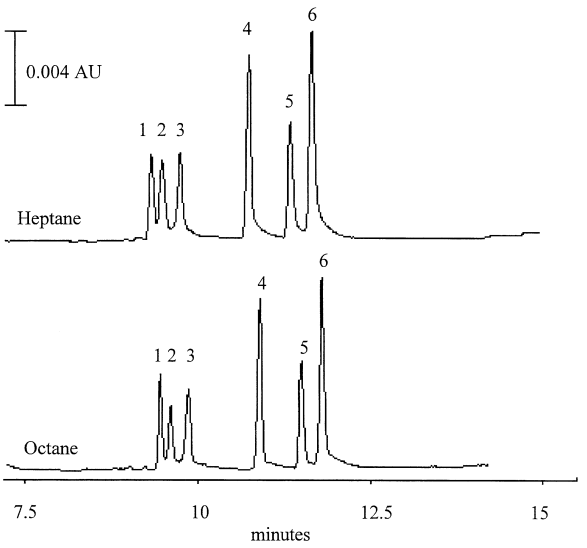

Fig. 5. Comparison of octane and heptane as core phases. Separation conditions: 25 m*M* phosphate pH 2, 200 m*M* SDS, 900 m*M* butanol, 80 m*M* core phase, with 20% propanol. Analytes: 50 mg/l (70% buffer 30% propanol). Peak identification: 1 = octylphenol; 2 = nonylphenol; 3 = nonylphenol diethoxylate; 4 = diethylstilbestrol; 5 = ethynyloestradiol; 6 = bisphenol-A.

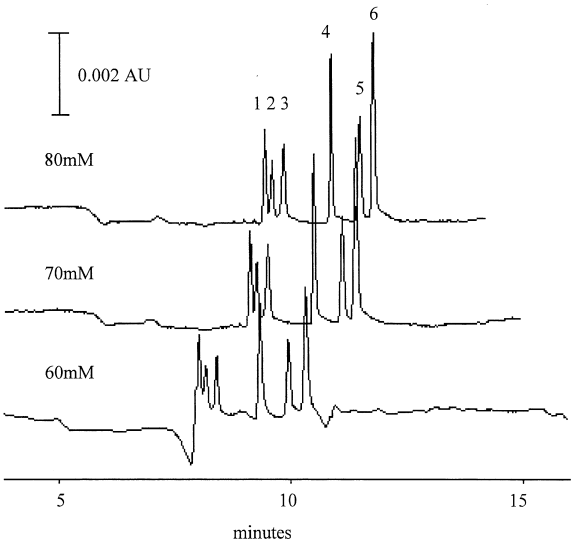

Fig. 6. Variation of concentration of octane core phase. Separation conditions: 25 m*M* phosphate pH 2, 200 m*M* SDS, 900 m*M* butanol, various concentration of core phase, with 20% propanol. Analytes: 50 mg/l in 70% buffer and 30% propanol. Peak identification: 1 = octylphenol; 2 = nonylphenol; 3 = nonylphenol diethoxylate; 4 = diethylstilbestrol; 5 = ethynyloestradiol; 6 = bisphenol-A.

[37]. Propanol addition was optimal at 20% for the separation of the six target EDCs.

3.3.3. Core phase selection and concentration

The core phase investigated initially was heptane as this has been used in the majority of MEEKC applications. It has been reported that better resolution has been obtained when alcohols with higher carbon numbers in the molecule were used [27]. Analysis times increased when alcohols with increasing carbon numbers were used. A slight increase in migration time was observed when octane was investigated as a comparison to heptane (Fig. 5). Table 1 compares the integration data for each analyte when octane and heptane were used as core phases and it can be seen that superior peak resolution, peak efficiencies (*N*) and peak area precision for each analyte was achieved when octane was employed. The concentration of the core phase was then optimised from 60 m*M* to 90 m*M* (Fig. 6). At the highest concentration of octane investigated (90

Table 1
Comparison of integration data for octane and heptane as core phases

Peak	Resolution $N=3$		Peak area (RSD) $n=3$, %		Peak efficiency (N) $n=3$		Migration (RSD) $n=3$, %	
	Octane	Heptane	Octane	Heptane	Octane	Heptane	Octane	Heptane
Octylphenol	0	0	3.67	6.04	151681	107894	0.96	0.23
Nonylphenol	1.37	1.24	5.56	7.41	93868	70648	0.93	0.21
Nonylphenol diethoxylate	2.05	1.82	6.96	11.51	103570	78745	0.92	0.21
Diethylstilbestrol	8.82	7.50	3.17	7.42	149390	115772	0.93	0.25
Ethynyloestradiol	5.28	4.68	5.20	5.38	144987	114749	0.89	0.24
Bisphenol-A	2.46	2.24	7.84	5.73	135120	100645	0.81	0.21

Conditions as in Fig. 5.

m*M*) a stable microemulsion could not be formed, attributed to the inability of the SDS to maintain core-phase dispersion in the aqueous buffer. Efficiency and % RSD data for peak areas were optimal when 80 m*M* octane was used.

3.3.4. Co-surfactant concentration

The co-surfactant aids the stability of the microemulsion system by lowering the surface tension to zero [32]. The co-surfactant also influences the extent of partition of the analyte between the oil and the aqueous buffer, thereby influencing the selectivity of the analysis [37]. Butanol concentration was varied for the separation (Fig. 7). The addition of 1000 m*M* butanol disrupted the formation of the microemulsion and could not be used. A reduction in the concentration of the co-surfactant led to a reduction in resolution of the analytes e.g. resolution of OP and NP at 900 m*M* was 1.26 and at 700 m*M* was 0.87. This can be attributed to the increase in the size of the oil droplet as a result of increased co-surfactant concentration [37]. The larger droplet has a different charge density and therefore this also affects the partition and migration of analytes. Nine hundred millimolar was selected as the optimum concentration as the additional time required for analysis was offset by the corresponding improvements in resolution and peak efficiency.

3.3.5. Optimised separation

The method described has been optimised specifically for the target analytes. This method has not been shown previously for EDC determination (Fig. 8). A standard curve was plotted using the optimised method and the linear regression was calculated for each analyte showing a linear response. Integration data for the optimised separation are listed in Table 2.

3.4. Separation of nonylphenol ethoxylates and other EDCs

In order to investigate if additional components that could be analysed using this system, a number of different combinations of nonylphenol ethoxylates were injected and identified. Unfortunately, this system was unable to resolve octylphenol and nonyl-

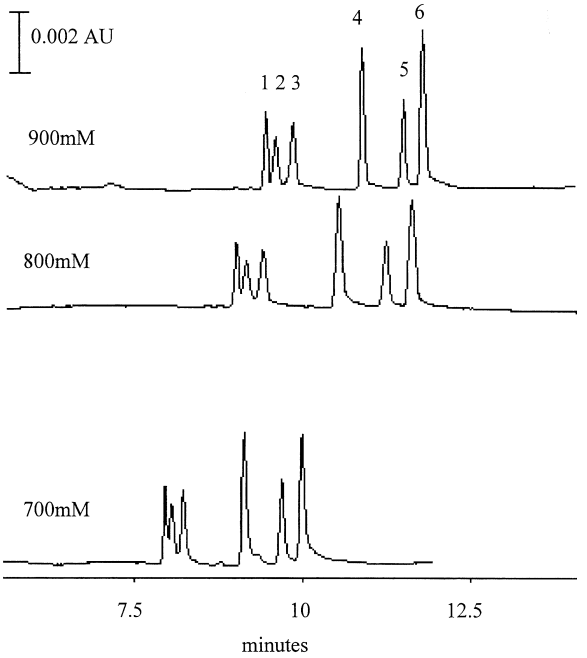

Fig. 7. Variation of co-surfactant concentration. Separation conditions: 25 m*M* phosphate pH 2, 200 m*M* SDS, various butanol concentrations, 80 m*M* octane, with 20% propanol. Analytes: 50 mg/l in 70% buffer and 30% propanol. Peak identification: 1 = octylphenol; 2 = nonylphenol; 3 = nonylphenol diethoxylate; 4 = diethylstilbestrol; 5 = ethynyloestradiol; 6 = bisphenol-A.

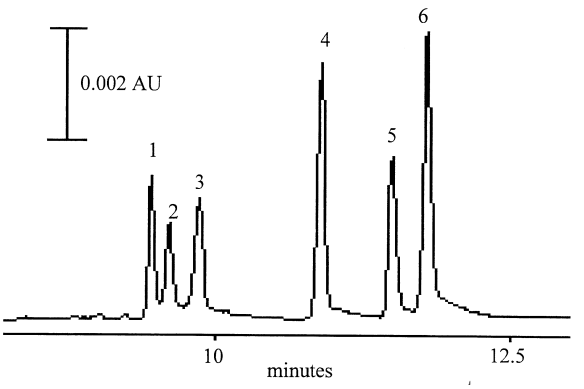

Fig. 8. Optimised separation of six EDCs. Separation conditions: 25 m*M* phosphate pH 2, 200 m*M* SDS, 900 m*M* butanol, 80 m*M* heptane, with 20% propanol. Peak identification: 1 = octylphenol; 2 = nonylphenol; 3 = nonylphenol diethoxylate; 4 = diethylstilbestrol; 5 = ethynyloestradiol; 6 = bisphenol-A. Analytes: 50 mg/l in 70% buffer and 30% propanol.

Table 2
Integration data for optimised separation ($n=3$)

Analyte $n=3$	Resolution	Area (RSD, %)	Plates (N)	Migration (RSD, %)
Octylphenol	0	3.7	151681	0.96
Nonylphenol	1.37	5.6	93868	0.93
Nonylphenol diethoxylate	2.05	7.0	103570	0.92
Diethylstilbestrol	8.83	3.2	149390	0.93
Ethynyloestradiol	5.28	5.2	144987	0.89
Bisphenol-A	2.46	7.8	135120	0.81

Conditions as in Fig. 8.

phenol from all the nonylphenol ethoxylates, as illustrated in Fig. 9. Nonylphenol was seen to co-migrate with nonylphenol monoethoxylate (b), while nonylphenol diethoxylate co-migrates with nonylphenoxy acetic acid (e). One possible solution to the co-migration would have been to increase the length of the capillary, which may have aided the resolution of these compounds but this would have been at the cost of increased analysis times coupled with increased current generation.

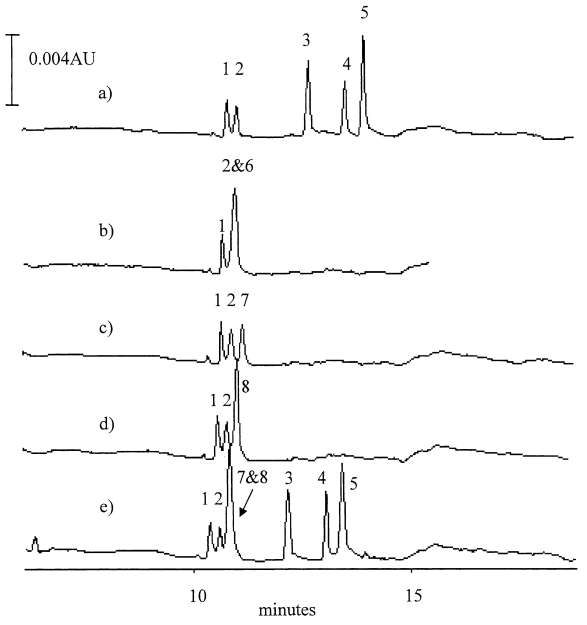

Fig. 9. Separation of mixtures (a–e) of nonylphenol ethoxylates. Separation conditions: 25 mM phosphate pH 2, 120 mM SDS, 900 mM butanol, 80 mM heptane, with 20% propanol. Analytes: 25 mg/l in 83% buffer and 17% MeCN. Peak identification: 1 = octylphenol; 2 = nonylphenol; 3 = diethylstilbestrol; 4 = ethynyloestradiol; 5 = bisphenol-A; 6 = nonylphenol monoethoxylate; 7 = nonylphenol diethoxylate; 8 = nonylphenol acetic acid.

3.5. Sample analysis

A wastewater influent solid-phase extract was obtained and investigated to show the potential of the developed method. Fig. 10 shows a comparison of the standard mixture of six compounds, a spiked wastewater sample (500 mg/l) subsequently extracted using solid-phae extraction (SPE), a standard mixture of octylphenol and nonylphenol in acetonitrile, and an unspiked wastewater extract. As the SPE

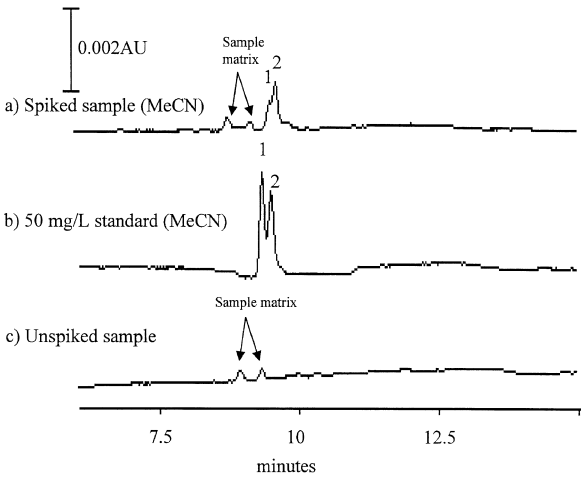

Fig. 10. Analysis of samples and standards using the optimised method. Separation conditions: 25 mM phosphate pH 2, 200 mM SDS, 900 mM butanol, 80 mM core phase, with 20% propanol. Peak identification: 1 = octylphenol; 2 = nonylphenol.

method had been optimised for these two compounds no additional analytes were investigated. Both the spiked sample and the unspiked sample were diluted 1 in 10 with run buffer prior to analysis. This meant that the spiked sample injected should have contained the equivalent of 50 mg/l OP and NP. Using the SPE method, the analytes were extracted into acetonitrile as opposed to propanol. In order to ensure compatibility between standards and samples, an alternative standard of OP and NP was prepared.

The standard was prepared in acetonitrile and treated as the real sample and sample blank by 1 in 10 dilution with the run buffer prior to analysis (final injection concentration of spiked sample and standard 50 mg/l). Fig. 10 illustrates that the migration times of OP and NP were similar in both systems but with a reduction in the resolution of OP and NP from 1.37 (Table 2) to 0.41 (Table 3). This was attributed to the presence of MeCN in the standard as opposed to the use of propanol, which was employed in the optimised method. The SPE method used had been developed previously for the extraction of OP and NP into MeCN [25], thus the sample was spiked with these two components.

Recoveries of 25.6%, and 50.8% were calculated for OP and NP, respectively (calculated using the 50 mg/l standard). The relatively low recoveries of the analytes are attributed to their hydrophobic nature. Due to their low aqueous solubilities they are expected to associate with particulate fraction of the influent after spiking, which was not accounted for in the extraction procedure. Neither analyte was detected in the unspiked sample illustrating that they were either not present or that the detection limits of the technique are as yet not sensitive enough to allow their detection in real samples, even with a preconcentration step. The literature would suggest that these compounds are present in the ng/l to μg/l range in such samples. A preconcentration factor of 100 would therefore not be expected to bring the compounds into the mg/l detection range of the developed method.

4. Conclusions

The first application of MEEKC to separation of a selection of EDCs has illustrated the potential of this technique. EOF-suppression allows rapid analysis of alkylphenols, which tend to have long migration times in conventional MEKC systems (>20 min). The developed method has also achieved resolution of intact alkylphenol peaks in a comparable and/or shorter time than previously reported using alternative CE methods [20,22,39,40].

Further investigation of the method could potentially allow complete resolution of additional alkylphenolic detergent breakdown products and oestrogens. Alternative options for the resolution analytes currently co-migrating would be addition of a further solubilising agent to the run buffer system e.g. cyclodextrins.

The majority of MEEKC applications to-date have employed UV detection but in order for the technique to be useful for the analysis of real environmental samples, a more sensitive detection method is required. The optical transparency of the microemulsion means that MEEKC analysis is also compatible with fluorescence detection [32]. Detection limits in CE may also be improved through investigation of stacking and sweeping techniques [34].

The ultimate application of developed methods could also include analysis of the various sources of EDC exposure, which include certain foods, cosmetic and medical formulations, industrial products and environmental samples such as wastewaters and sludges.

Table 3
Integration data for spiked sample ($n=6$)

Analyte $n=6$	Resolution $n=6$	Area (RSD, %) $n=6$	Plates (N) $n=6$	Migration (RSD, %) $n=6$
Octylphenol	0.00	8.37	6153	1.81
Nonylphenol	0.41	8.17	34357	1.70

Conditions as in Fig. 10.

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