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Separation of two groups of oestrogen mimicking compounds using micellar electrokinetic chromatography

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Abstract

Two groups of compounds are being investigated due to their reported oestrogen mimicking characteristics in the environment. Separation of phenolic compounds and synthetic oestrogens using micellar electrokinetic chromatography is reported. Photodiode array detection is used for both separations. A standard separation buffer can be used for both groups of compounds including zwitterionic buffer cyclohexylamino-1-propanesulfonic acid, 20 m*M* at pH 11.5. It was found necessary to include 15% acetonitrile and 25 m*M* sodium dodecyl sulfate to aid separation and maintain analytes in solution. Optimum separations are achieved using 20 kV with hydrodynamic injection for 5 s. The relative standard deviation (RSD) for reproducibility was investigated for a mixture of phenols and synthetic oestrogens. For these compounds RSD was found to be $<0.6\%$ in all cases. Peak efficiencies ranged from 76 000 to 150 000 theoretical plates for different analytes. Application to environmental samples is discussed.

Keywords: Water analysis; Environmental analysis; Estrogens; Alkylphenols; Phenols

1. Introduction

Considerable scientific evidence indicates that a multitude of environmental contaminants can modulate or mimic the actions of steroid hormones and, in some cases, produce biological responses qualitatively similar to those produced by endogenous hormones [1].

Compounds identified as oestrogen mimics, are members of distinct groups of chemicals including pesticides, alkylphenols, plasticisers, and also synthetic oestrogens. Phenolic oestrogen mimics such as bisphenol A and pentachlorophenol have been detected in both domestic sewage and industrial wastewaters [2].

Wastewater and sewage contains the vast proportion of the environmental load of oestrogen mimics and is a major source of a mixture of oestrogen mimicking chemicals as a result of direct effluent discharge. Conventional wastewater treatment processes have proved ineffective in completing the removal of these compounds from wastewaters due to the stable nature of many oestrogen mimicking compounds and their resulting poor biodegradability [3].

Advances in civilisation and rising population levels have resulted in an increasing need to treat and recycle available water resources. It is estimated that 30% of all UK water is of a recycled nature [1]. Following re-use the water is returned to the aquatic environment, usually via sewage treatment works of

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varying processes and performance, which improves its quality, but it may be abstracted again further downstream. In the UK and other European countries with a high population density, the volume of effluent discharged from sewage treatment works can be considerable, sometimes contributing up to 50% of the flow of a river, a figure that can rise to 90% in periods of low rainfall.

The group of steroid hormones including oestrone, oestriol and 17-β-oestradiol, and the synthetic oestrogen ethinyloestradiol, have all been reported to be present in various effluents. The synthetic oestrogen ethinyloestradiol used in the contraceptive pill is excreted into the wastewater system from where it has been seen to make its way back to drinking waters [4].

Phenols are common water pollutants because they are the products of many industrial processes. Alkylphenol polyethoxylates (APEOs) are widely used in cleaning products and as industrial cleaning aids. The majority of APEOs are used in aqueous solutions; therefore, they are discharged into municipal and industrial wastewaters which enter sewage treatment plants. During different steps of treatment biodegradation processes take place which lead to the formation of biorefractory metabolites [1]. Nonyl- and octylphenol are two metabolite compounds of high toxicity, which are released following sewage treatment, to the aquatic environment. These highly branched species are undesirable in the environment because of their poor biodegradability [5]. Alkylphenolic compounds are used in a variety of applications and are found in both domestic and industrial products. Some of the applications include shampoos, some detergent-containing petrols and pesticides formulations [6]. Alkylphenols which have exhibited oestrogenic behaviour have been identified as components of wastewater [7]. State-of-the-art techniques for the analysis of oestrogen mimicking compounds include biological assay [8], high-performance liquid chromatography (HPLC) [9], liquid chromatography–mass spectrometry (LC–MS) [10], gas chromatography (GC) [11], and GC–MS [12].

Capillary zone electrophoresis (CZE) has been used for the analysis of synthetic oestrogens [13] and phenolic compounds [14] but is not suited to analysis of alkylphenolic compounds.

The hydrophobic nature of this group of compounds due to long carbon chain extensions demands the increased selectivity and efficiency of micellar mode separation techniques. The hydrophobic portion of the micelle aids the solubility of hydrophobic compounds which can also be improved through the addition of organic modifiers to the run buffer system. Cyclodextrin modification of a micellar electrokinetic chromatographic separation can further improve selectivity due to the differential partitioning of solutes between the micelles and cyclodextrins. An organic modifier can be employed to influence the extent of partition of a solute between the micellar and chiral phases. The technique of cyclodextrin-modified micellar electrokinetic chromatography (MEKC) has been employed for the individual separation of structural homologues of natural and synthetic oestrogens [15,16], phenolics [17] and alkylphenols [18].

This work examines the potential of MEKC for the simultaneous determination of a selection of environmentally relevant oestrogen mimics from two environmentally significant groups. These two classes of compounds have been chosen due to their reported simultaneous occurrence in wastewaters and drinking waters [1,4]. The individual compounds selected for analysis within each class are those which have been reportedly found in environmental waters.

Existing chromatographic techniques are widely used for these compounds of interest. However, no attempt has been shown to analyse the compounds simultaneously using state of the art techniques. This is due perhaps to the limitations of the techniques, notably, varying analyte volatility of GC, need for gradient elution in HPLC, large solvent demands and variable molecule charges and masses.

The advantage of MEKC for analysis of phenols, alkylphenols and synthetic oestrogens lies in its ability to effect simultaneous separation of a wide variety of long- and short-chain neutral analytes and charged analytes with very high efficiency.

The separation is optimised to enable analysis of both phenolic compounds and synthetic oestrogens using similar conditions. Diode array detection (DAD) is used. The structures of compounds being investigated are shown in Fig. 1.

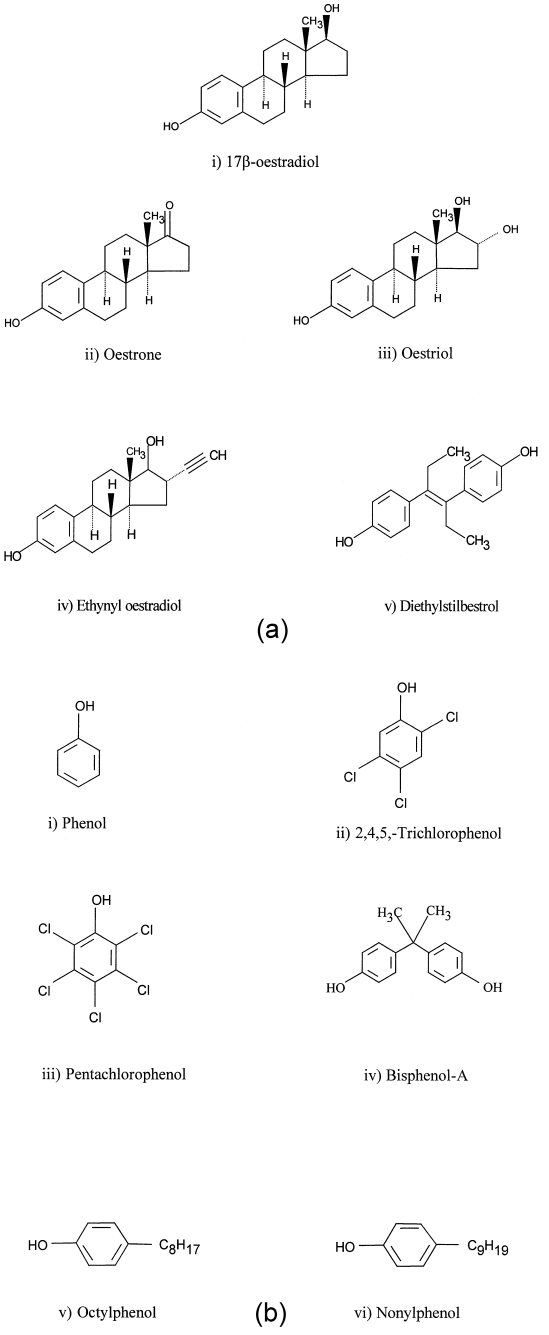

Fig. 1. Structures of (a) synthetic oestrogens and (b) phenolic compounds.

2. Experimental

2.1. Instrumentation

Separations were performed using a Beckman P/ACE 5500 system which is equipped with a DAD system. The DAD range was 190 nm to 300 nm. The capillary electrophoresis (CE) instrument was operated using Windows P/ACE Station software version 1.21. The fused-silica capillaries (Beckman) used were 57 cm×50 μm I.D. and 87 cm×75 μm I.D.

2.2. Reagents

Cyclohexylamino-1-propanesulfonic acid (CAPS) was purchased from Sigma–Aldrich, Dublin, Ireland. HCl, NaOH, methanol (HPLC grade), acetonitrile (ACN, HPLC grade), sodium dodecyl sulfate (SDS) were purchased from Sigma–Aldrich. Phenols and synthetic oestrogens (Sigma–Aldrich) were used without further purification. CAPS buffer pH was adjusted using 0.1 *M* HCl and 0.1 *M* NaOH.

2.3. CE separation conditions

The separation buffer contained CAPS, SDS, acetonitrile and methanol were added as shown in the figure legends. The optimum separation system for both groups of compounds was found to be 20 m*M* CAPS, 25 m*M* SDS, 15% ACN at pH 11.5. All buffers were prepared with deionised water and filtered through a 0.2-μm filter (Gelman) prior to use. Buffers were prepared fresh prior to use as SDS was found to precipitate out of solution if stored.

A standard method involved: a routine pre-rinse of 3 min each; 0.1 *M* NaOH, deionised water and buffer. Separations were carried out at 20 kV constant voltage. Injections were hydrodynamic for 5 s. Detection was carried out using DAD scanned from 190 to 300 nm.

2.4. Standard preparation

Stock solutions of both the phenolic compounds and the synthetic oestrogen diethylstilbestrol (DES), β-oestradiol and ethinyloestradiol were made up in 100% acetonitrile. Oestriol and oestrone were pre-

pared in 100% methanol. Aliquots of these stocks were taken in acetonitrile–buffer (50:50).

2.5. Sample preparation

As the ultimate application of this technique would be in the analysis of environmental samples a river water sample (River Shannon, Limerick, Ireland) was spiked with the five synthetic oestrogens and six phenols/alkylphenols, in order to investigate possible sample matrix effects. The stock oestrogens were dissolved in ACN and then added to the river water sample. This meant that the injection sample contained river water–ACN–100 m*M* SDS (5:4.35:0.65).

3. Results and discussion

3.1. Preparation of standard mixture of compounds

It was found that preparation of analytes in methanol provided limited solubility for some compounds, primarily the alkylphenols. However, as a separation system was required for both groups of compounds it was desirable to use similar conditions throughout. A buffer containing acetonitrile was investigated as an alternative. It was found that acetonitrile improved the solubility of the more hydrophobic analytes. It was found that injecting a sample in 100% acetonitrile caused increased resistance at the inlet end of the capillary on application of voltage. This resulted in repeated capillary breakages. To overcome these problems of sample plug resistance and solubility of analytes, electrophoretic buffer was added to the injection sample. Consequently standards and samples were injected in solutions as similar in composition to the electrophoretic run buffer as possible.

3.2. Separation of synthetic oestrogens

The most commonly encountered oestrogen is ethinyloestradiol, a semi-synthetic oestrogen, present in very low dosage in oral contraceptive pills. DES is a synthetic oestrogen which was administered to women in the 1940s to prevent miscarriages, but was banned due to reproductive abnormalities that resulted. The metabolism of oestrogen hormones occurs when circulating oestradiol is converted to oestrone in the liver and further metabolism of oestrone leads to oestriol formation. 17β-Oestradiol is the most biologically active oestrogen produced by the ovary and it is interconvertable to a less potent compound oestrone and metabolises further to oestriol [19].

These oestrogenic compounds are nonpolar compounds consisting of a phenanthrene ring with hydroxy groups (Fig. 1a).

As a result of their nonpolar nature they are difficult to separate using CZE. This has been achieved by Potter et al. [13] using a zwitterionic buffer at a very high pH where the oestrogens are negatively charged. Fig. 2 shows the application of CZE to the analysis of four synthetic oestrogens (β-oestradiol, oestriol, oestrone and ethinyloestriol) using an organic-modified buffer system. Using a methanol modified buffer it was found that two analytes, β-oestradiol and ethinyloestradiol co-migrated due to similarities in their charge and mass.

As a result of analyte comigration and poor solubility MEKC was investigated. The separation conditions including, pH, buffer concentration, SDS concentration and percentage organic modifier were varied in order to achieve the optimum separation

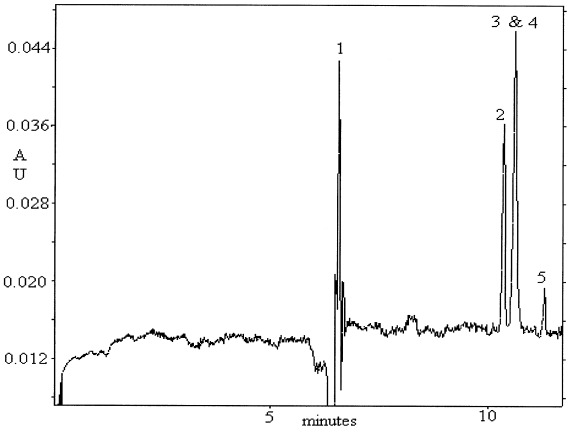

Fig. 2. CZE separation of four oestrogens. 100 m*M* CAPS buffer, pH 11.5; 20% MeOH; separation voltage 30 kV; detection at 200 nm; capillary 57 cm×50 μm I.D.; hydrodynamic injection 5 s; injection in 100% MeOH. 1=MeOH, 2=oestriol, 3&4=β-oestradiol and ethinyloestradiol, 5=oestrone.

conditions for five synthetic oestrogens. The optimum conditions chosen for this separation were 20 m*M* CAPS buffer, pH 11.5, 25 m*M* SDS and 15% ACN at 25 kV (Fig. 3).

3.2.1. Buffer pH

The effect of buffer pH was investigated. It was found that varying the buffer pH from 11.5 to 9, deteriorated the separation of synthetic oestrogens. The buffer contained 25 m*M* SDS and 10% ACN. Fig. 4 illustrates the effect of varying pH for this separation. At pH values lower than 11.5, migration times were seen to decrease for all compounds down to pH 10, and thereafter the migration times increased dramatically. It was found that lowering the pH caused peak splitting. This may be due to the breakdown of the analytes, due to lower analyte solubility as a result of protonation. It is likely also, that analyte interaction with the micelle is increased slightly as the compounds become less polar.

Optimisation of pH was repeated with an increased SDS concentration of 50 m*M* in order to compensate for the protonation of the negatively charged oestrogens at pH values below 11.5.

The increased SDS concentration improved the solubility of the compounds and thus the efficiencies

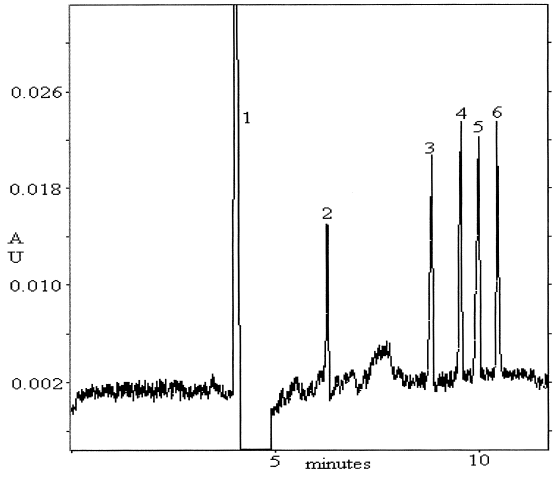

Fig. 3. MEKC separation of five synthetic oestrogens. 20 m*M* CAPS buffer, pH 11.5; 25 m*M* SDS; 15% ACN; separation voltage 25 kV; detection at 200 nm; capillary 57 cm×50 μm I.D.; hydrodynamic injection 5 s; injection in ACN–electrophoretic buffer (50:50). 1=ACN, 2=oestriol, 3=oestrone, 4=β-oestradiol, 5=DES, 6=ethinyloestradiol.

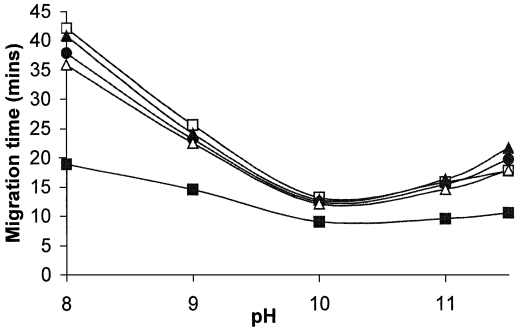

Fig. 4. Graph of migration times of five synthetic oestrogens as a function of buffer pH. 35 m*M* CAPS buffer; 50 m*M* SDS; 10% ACN; separation voltage 20 kV; detection at 200 nm; capillary 57 cm×50 μm I.D.; hydrodynamic injection 5 s; injection in ACN–electrophoretic buffer (50:50). □=DES; ▲ethinyloestradiol; ●=β-oestradiol; △=oestrone; ■=oestriol.

of the analyte peaks at lower pH values. The overall migration times of the compounds were greatly increased by the addition of SDS without a significant improvement in resolution of the compounds. Table 1) shows the effect of varying buffer pH on analyte efficiency with 50 m*M* SDS. As expected efficiency increased with increasing pH. pH 11.5 was determined to be the optimum for the separation.

Efficiencies in the region of 40 000 were obtained for oestriol and 60 000 for ethinyloestradiol at pH 11.5. At pH 10 these values were halved due to the splitting effect. In addition it was observed that the migration order of ethinyloestradiol and DES was reversed at pH 11.0. This effect is due to the reversal of polarity of these species and greater interaction of DES with the micelle, leading to its increased migration time.

3.2.2. Variation of buffer concentration

The effect of varying the concentration of the run buffer from 10 m*M* to 50 m*M* was investigated. It was found that increasing the concentration of the run buffer lead to an increase in the migration times of all of the oestrogens which in turn impacted upon the resolution of the analytes (Fig. 5). Table 1 shows the efficiencies of the analyte peaks as a function of buffer concentration. The optimum concentration of CAPS buffer was determined to be 20 m*M*. This concentration was found to counteract the effect of

Table 1
Efficiencies of five synthetic oestrogens as a function of buffer pH, CAPS concentration and SDS concentration

	Efficiency (plates)				
	Oestriol	Oestrone	DES	β-Oestradiol	Ethinyloestradiol
pH[a]					
11.5	40 439	–	–	34 094	39 984
11	12 915	21 467	19 384	–	10 006
10	6778	7963	12 502	4083	16 193
9	8030	13 238	16 744	24 883	10 838
8	29 960	22 420	16 235	31 309.5	20 808
CAPS (m*M*)[b]					
10	–	70 468	72 865	42 572	38 492
20	25 657	68 985	39 368	66 052	49 354
30	48 880	54 919	56 929	56 168	70 086
40	30 031	29 105	–	–	31 964
50	68 759	39 789	–	–	46 664
SDS (m*M*)[c]					
10	173 121	93 780	116 043	64 410	91 801
20	62 417	54 597	–	–	37 270
25	25 657	68 985	39 368	66 052	49 353
30	42 347	26 660	59 551	52 899	37 580

[a] 35 m*M* CAPS buffer; 50 m*M* SDS; 10% ACN; separation voltage 20 kV; detection at 200 nm; capillary 57 cm×50 μm I.D.; hydrodynamic injection 5 s; injection in ACN–electrophoretic buffer (50:50).
[b] CAPS buffer pH 11.5; 25 m*M* SDS; 10% ACN; separation voltage 20 kV; detection at 200 nm; capillary 57 cm×50 μm I.D.; hydrodynamic injection 5 s; injection in ACN–electrophoretic buffer (50:50).
[c] 20 m*M* CAPS buffer, pH 11.5; 25 m*M* SDS; 10% ACN; separation voltage 20 kV; detection at 200 nm; capillary 57 cm×50 μm I.D.; hydrodynamic injection 5 s; injection in ACN–electrophoretic buffer (50:50).

increased migration times due to the SDS concentration of 25 m*M* in the electrophoretic buffer.

3.2.3. SDS concentration

It was necessary to add SDS to the electrophoretic buffer to achieve resolution of synthetic oestrogens with similar chemical nature. The variation of the concentration of SDS in the run buffer was seen to have a significant impact upon the separation of synthetic oestrogens.

The migration times of the compounds were seen to increase with increasing concentration of SDS as shown in Fig. 6. This was due to a greater degree of analyte interaction with the micelle at higher SDS concentrations. The concentration of SDS was varied from 10 m*M* to 40 m*M*.

The difference in polarity of the synthetic oestrogens analysed was evident during the variation of the buffer SDS concentration as the most polar oestrogen oestriol had improved peak efficiency at lower concentrations of SDS. The optimum SDS concentration was found to be 25 m*M*. This concentration allowed baseline resolution of all compounds with acceptable peak efficiencies.

3.2.4. Organic modifier composition

To achieve separation of synthetic oestrogens organic modifier (acetonitrile) was added to the run buffer to maintain the on-capillary solubility of the analytes. The presence of the organic modifier in the buffer also aided in the resolution of the compounds and reduced the associated system current generated. The % organic added to the buffer was varied from 5% to 20%. Increasing the % organic composition of the run buffer lead to increased migration times of the compounds and also increased resolution between the compounds (Fig. 7). 15% ACN was found to provide the optimal separation.

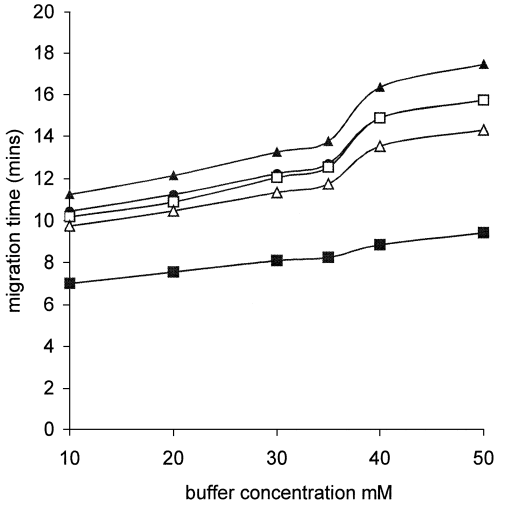

Fig. 5. Graph of migration times of five synthetic oestrogens as a function of buffer concentration. CAPS buffer, pH 11.5; 25 m*M* SDS; 10% ACN; separation voltage 20 kV; detection at 200 nm; capillary 57 cm×50 μm I.D.; hydrodynamic injection 5 s; injection in ACN–electrophoretic buffer (50:50). Symbols as in Fig. 4.

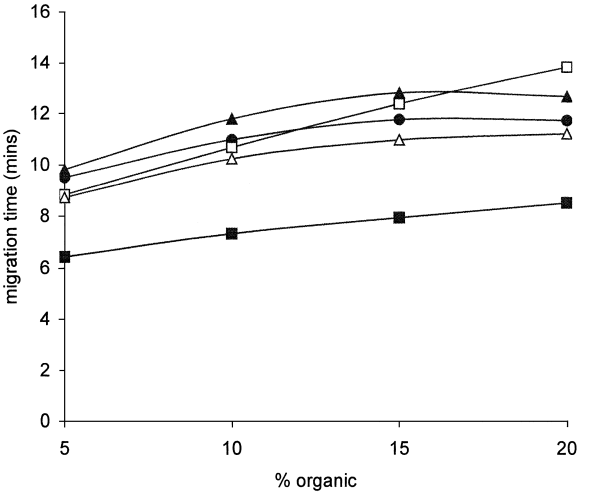

Fig. 7. Graph of migration times of five synthetic oestrogens as a function of organic composition. 20 m*M* CAPS buffer, pH 11.5; 25 m*M* SDS; separation voltage 20 kV; detection at 200 nm; capillary 57 cm×50 μm I.D.; hydrodynamic injection 5 s; injection in ACN–electrophoretic buffer (50:50). Symbols as in Fig. 4.

3.2.5. Separation voltage

As expected it was found that variation of voltage resulted in a significant variation in migration times of analytes. Increasing voltage from 20 kV to 30 kV reduced the analysis time for synthetic oestrogens from 15 min to 10 min at 30 kV. The optimum voltage applied was 25 kV.

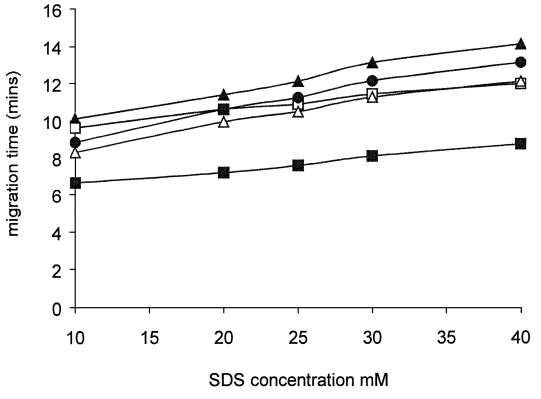

Fig. 6. Graph of migration times of five synthetic oestrogens as a function of SDS concentration. 20 m*M* CAPS buffer, pH 11.5; 10% ACN; separation voltage 20 kV; detection at 200 nm; capillary 57 cm×50 μm I.D.; hydrodynamic injection 5 s; injection in ACN–electrophoretic buffer (50:50). Symbols as in Fig. 4.

3.3. Separation of phenols

Phenols (Fig. 1b) are common water pollutants because they are products of many industrial processes [6]. Presence of the alkylphenol nonylphenol and bisphenol A in effluents indicates contamination and these have been found to be oestrogenic in aquatic animals [4]. Fig. 8 illustrates the separation of both phenols and synthetic oestrogens. The separation includes the six phenolic compounds phenol, pentachlorophenol, trichlorophenol, bisphenol A, 2-*sec*.-butylphenol and nonylphenol. These compounds have been identified in the literature [1–4] as having oestrogen mimicking capabilities. Phenolic compounds such as pentyl-, hexyl- and heptylphenols have not been included in this study. The latter compounds are not prioritised in the literature as being present in environmental samples to the same extent as those presented here.

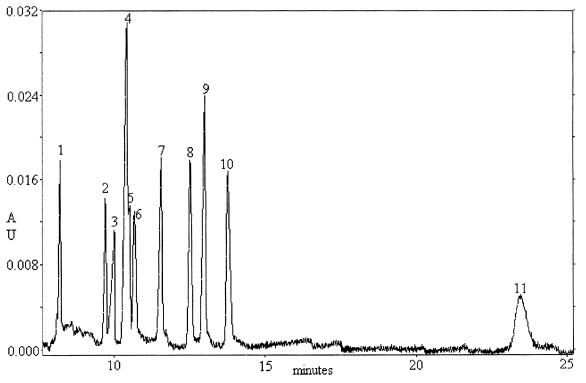

Fig. 8. Separation of six phenols and five oestrogens. 20 m*M* CAPS buffer, pH 11.5; 25 m*M* SDS; 15% SDS; separation voltage 20 kV; detection at 200 nm; capillary 57 cm×50 μm I.D.; hydrodynamic injection 5 s; injection in ACN–electrophoretic buffer (50:50). 1=Oestriol, 2=phenol, 3=trichlorophenol, 4=bisphenol A, 5=pentachlorophenol, 6=butylphenol, 7=oestrone, 8=β-oestradiol, 9=DES, 10=ethinylestradiol and 11=nonylphenol (concentrations from 20 to 70 mg l^{-1}).

Table 2
Reproducibility of MEKC separation of oestrogen and phenol mixture[a]

Synthetic oestrogen	Resolution	Average t_m (min)	RSD (n=5) (%)
Oestriol	0	8.3	0.6
Phenol	12.5	9.7	0.5
Trichlorophenol	1.8	10.0	0.6
Bisphenol A	2.1	10.5	0.4
Pentachlorophenol	0.9	10.6	0.4
Butylphenol	1.6	10.7	0.5
Oestrone	7.7	11.6	0.2
β-Oestradiol	5.8	12.5	0.4
DES	2.9	13.0	0.3
Ethinyloestradiol	4.2	13.8	0.5
Nonylphenol	36.5	23.4	0.4

[a] 20 m*M* CAPS buffer, 25 m*M* SDS; 15% ACN; separation voltage 20 kV; detection at 200 nm; capillary 57 cm×50 μm I.D.; hydrodynamic injection 5 s; injection in ACN–electrophoretic buffer (50:50). t_m=Migration time.

The separation conditions for this mixture including the phenols are identical to those used for the separation of synthetic oestrogens. Fig. 8 indicates that the two classes of oestrogen mimicking compounds can be separated in the same analysis. This is useful, as many of these compounds will be simultaneously present in wastewaters and effluents arising from industrial and domestic systems. Reproducibility (RSD) of analysis of the phenols was found to be <0.6% for each analyte (n=5) (Table 2). The efficiency of analyte separation in terms of theoretical plates (N) ranged from 104 000 for phenol to 76 000 for nonylphenol.

3.3.1. Effect of buffer pH

The effect of buffer pH on migration time for bisphenol A is illustrated in Fig. 9. The analysis was found to take longer between pH 4 and pH 6 and above pH 10. The optimum range for separation of the phenols is between pH 7.5 and pH 9.5. However, separation of these species is possible using MEKC at pH 11.5. This pH was used as it has been optimised for the synthetic oestrogen separation.

3.3.2. Effect of buffer concentration

The buffer concentration was varied from 10 m*M* to 30 m*M*. It was found that 20 m*M* gave the best resolution of analyte peaks as for the synthetic oestrogens (Table 3). The buffer conditions optimised for the oestrogen separation were then applied to the analysis of several environmentally relevant phenols.

3.3.3. Effect of SDS concentration

The concentration of SDS in the run buffer was varied from 10 m*M* to 25 m*M* for the separation of six phenols. 25 m*M* SDS was chosen for this separation. The more polar phenols namely phenol and trichlorophenol were baseline resolved at lower concentrations of SDS while the peak efficiency of

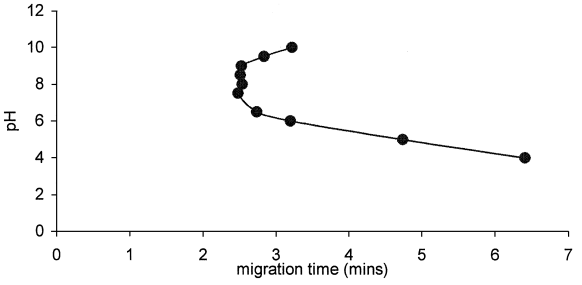

Fig. 9. Graph of migration time of bisphenol A as a function of pH. 10 m*M* buffers; separation voltage 20 kV; detection at 200 nm; capillary 57 cm×50 μm I.D.; hydrodynamic injection 5 s.

Table 3
Limits of detection for 11 oestrogen mimics (separation conditions as for Table 2) ($S/N=2$)

Compound	LOD (mg l^{-1})
Oestriol	7.2
Phenol	2.0
Trichlorophenol	4.9
Bisphenol A	4.0
Pentachlorophenol	6.7
Butylphenol	3.8
Oestrone	6.8
β-Oestradiol	6.8
DES	7.2
Ethinyloestradiol	7.4
Nonylphenol	5.5

the hydrophobic compound nonylphenol was reduced. MEKC was found to be particularly necessary for separation of nonyl- and octylphenols which co-migrate without SDS in the run buffer. Separation of these components can be seen in Fig. 10 using an 87 cm capillary and similar buffer conditions. Increasing the concentration of SDS in the run buffer was found to increase the solubility of the alkylphenols and increase the peak efficiency but at the cost of lengthy migration times.

To overcome the separation problem with alkylphenols, future work will involve the use of a cyclodextrin-modified MEKC. This principle has been previously shown to be successful [18].

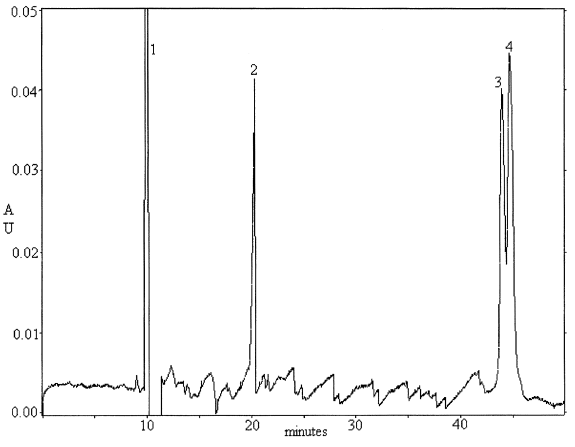

Fig. 10. MEKC separation of three phenols. 37.5 m*M* CAPS, pH 11.5; 25 m*M* SDS; 10% ACN; separation voltage 20 kV; detection at 200 nm; capillary 87 cm×75 μm I.D.; hydrodynamic injection 5 s. 1=ACN, 2=trichlorophenol, 3=octylphenol, 4=nonylphenol.

3.4. Reproducibility of analysis

Table 2 illustrates reproducibility of this separation including 11 compounds in a mixture. Using MEKC, reproducibilities of <0.6% RSD were obtained for phenolic and synthetic oestrogen analytes. This result is based on five repeated separation runs of the analyte mixture.

3.5. Limits of detection (LODs)

By employing the optimised separation as illustrated in Fig. 8, the LOD of each compound was determined for this preliminary study. Table 3 shows the LOD values of 11 analytes, which range from 2 mg l^{-1} for phenol and 7.4 mg l^{-1} for ethinyloestradiol. The LOD values are based on a S/N ratio of 2. These concentrations are orders of magnitude greater than required for environmental sample analysis. However, follow-on work will incorporate sample preconcentration and laser-induced fluorescence detection to achieve limits within the desired range.

3.6. Environmental sample analysis

A spiked river water sample was used to assess the MEKC separation technique. Genuine environmental samples were not considered as the detection limits (Table 3) for the test analytes are not sensitive enough at this stage to allow their determination in environmental samples.

Different sample injection techniques were investigated. The first injection procedure involved passing the sample through a 0.2-μm filter prior to injection. The optimised separation procedure (Section 3.2) was used. The only oestrogen identified was the most polar of the synthetic oestrogens oestriol. The polarity of the compound aided its solubility in the water sample. The other four oestrogens and phenols which are more hydrophobic in nature were not detected.

The second procedure involved filtration of the sample followed by 50:50 dilution with ACN. As before the only compound identified was the most polar of the synthetic oestrogens oestriol. The hydro-

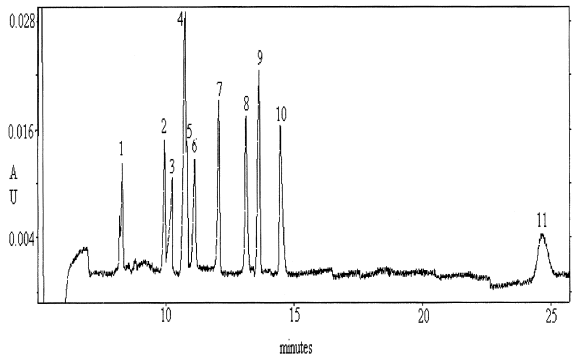

Fig. 11. Spiked river water sample. 20 m*M* CAPS buffer, pH 11.5; 25 m*M* SDS; 15% ACN; separation voltage 20 kV; detection at 200 nm; capillary 57 cm×50 μm I.D.; hydrodynamic injection 5 s; injection sample river water with 12.5% ACN. 1=Oestriol, 2=phenol, 3=trichlorophenol, 4=bisphenol A, 5=pentachlorophenol, 6=butylphenol, 7=oestrone, 8=β-oestradiol, 9=DES, 10=ethinylestradiol and 11=nonylphenol (spike concentrations of 20–70 mg l^{-1}).

phobic nature of the other compounds may have lead to their association with particulate matter in the sample which was then removed during the filtration process or they may have come out of solution.

The third procedure involved the injection of a sample with the following composition: river water–ACN–100 m*M* SDS (5:4.35:0.65). This spiked sample (20–70 mg l^{-1} analyte) was not filtered.

The addition of SDS and acetonitrile solubilised the 11 test analytes (some of which are quite hydrophobic in nature) and maintained them in solution. When this was not carried out samples were found to precipitate out of solution and required filtration.

Fig. 11 shows the analysis of a river water sample (River Shannon) spiked with 20–70 mg l^{-1} of each analyte. The response is comparable to that obtained from the separation of standards.

Further investigation into sample preparation is required for effluent samples.

4. Conclusions

Preliminary investigations have shown the potential of these separation conditions for analysis of the two environmentally important classes of oestrogen mimicking compounds, synthetic oestrogens and phenols. We have identified the conditions for separation using UV detection at 200 nm. The power of the MEKC technique in providing a highly efficient separation of complex analytes has been demonstrated. The potential for application to environmental samples has been shown. While further work is required for this to be useful for application to sewage effluent and waste waters, this work identifies the potential utility of CE for this purpose.

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References

[1] E.J. Routledge, D. Sheahan, C. Desbrow, G. Brighty, M. Waldock, J.P. Sumpter, Environ. Sci. Technol. 32 (1998) 1559.
[2] R.A. Rudel, S.J. Melly, P.W. Geno, G. Sun, J.G. Brody, Environ. Sci. Technol. 32 (1998) 861.
[3] H.H. Tabak, R. Bunch, Dev. Ind. Microbiol. 11 (1970) 367.
[4] G.W. Aherne, R.J. Briggs, Pharm. Pharmacol. 41 (1989) 735.
[5] C. Desbrow, E. Routledge, G.C. Brighty, J. Sumpter, M. Waldock, Environ. Sci. Technol. 32 (1998) 1549.
[6] M. Warhurst, website location: http://easyweb.easynet-.co.uk/mwarhurst/ape.html
[7] M.A. Blackburn, M.J. Waldock, Water Res. 29 (1995) 1623.
[8] D. Feldman, A. Krishnan, Environ. Health Perspect. 103 (1995) 129.
[9] G. Ghosh, S.K. Roy, M.K. Majumdar, Indian-Drugs 30 (1993) 301.
[10] L.G. Mackay, M.Y. Croft, D.S. Selby, R.J. Wells, J. Assoc. Off. Anal. Chem. 80 (1997) 401.
[11] O.H. Masi, W.M. Gulick Jr., J. High Resolut. Chromatogr., Chromatogr. Commun. 10 (1987) 647.
[12] W.N. Sawaya, K.P. Lone, A. Husain, B. Dashti, S. Al-Zenki, Food Chem. 63 (1998) 563.
[13] K.J. Potter, R.J.B. Allington, J. Algaier, J. Chromatogr. A 652 (1993) 427.
[14] Y.C. Chao, C.W. Whang, J. Chromatogr. A 663 (1994) 229.
[15] Y.J. Deng, J.X. Zhou, M.D. Perkins, S.M. Lunte, Anal. Commun. 34 (1997) 129.
[16] J.J. Berzas, J. Roderiguez, G. Castaneda, M.J. Pinilla, Chromatographia 49 (1999) 65.
[17] L.H. Leenhers, Beckman Applications Brief, 1991.
[18] Y. He, H.K. Lee, J. Chromatogr. A 749 (1996) 227.
[19] A.J. Ji, N.F. Nunez, D. Machacek, J.E. Ferguson, M.F. Iossi, P.C. Kao, J.P. Launders, J. Chromatogr. B 669 (1995) 15.