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Separation of opiate alkaloids by electrokinetic chromatography with sulfated-cyclodextrin as a pseudo-stationary phase

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Abstract

The separation of six related opiate alkaloids (morphine, thebaine, 10-hydroxythebaine, codeine, oripavine and laudanine) was studied using sulfated-cyclodextrin (s-CD) as a cation-exchange pseudo-stationary phase. Cation-exchange interactions between the cationic analytes and the anionic s-CD (7–11 mol of sulfate groups per mole CD) were found to be the predominant mechanism, allowing the separations to be performed at low pH where the opiates are protonated and exhibit very similar mobilities. The concentrations of the s-CD and the competing ion (Na^+ or Mg^{2+}) in the electrolyte were used to govern the extent of the ion-exchange interactions. Interactions with the sulfated-cyclodextrin differed for each analyte, with oripavine exhibiting the strongest interaction and 10-thebaine and laudanine showing the weakest interactions. Despite the very similar structures of the analytes, these differences resulted in significant changes in separation selectivity. The separation was modelled using a migration equation derived from first principles and based on ion-exchange interactions between the s-CD and the opiates. Constants within the model were obtained by non-linear regression using a small subset of experimentally determined migration times. These constants related to the ion-exchange affinities of the s-CD for the various opiates. When the model was used to predict migration times under other experimental conditions, a very good correlation was obtained between observed and predicted mobilities ($r^2=0.996$). Optimisation of the system was performed using the normalised resolution product and minimum resolution criteria and this process provided two optimised separations, each exhibiting a different separation selectivity.

Keywords: Background electrolyte composition; Electrokinetic chromatography; Pseudo-stationary phases; Alkaloids; Cyclodextrins; Opiates

1. Introduction

Opiate compounds have long been used either therapeutically or illicitly and as such the determination of such compounds is important in the pharmaceutical industry or forensic analysis. Traditionally, capillary gas chromatography (cGC) and high-performance liquid chromatography (HPLC) have been the most popular techniques for the determination of opiate compounds. However, these techniques have some limitations and problems can arise for compounds that are thermally degradable, polar or non-volatile [1]. Recently the use of capillary electrophoresis (CE) has proven to be a viable alternative which overcomes many of the problems encountered when using cGC or HPLC. Generally CE separations of opiates are either performed in acidic or basic background electrolytes (BGEs)

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where the opiates are either fully or partially protonated, respectively. Unger et al. [2] separated various alkaloid classes, including the opiates thebaine, codeine and morphine, using a 1:1 ammonium acetate acetonitrile BGE at pH 3.1, while Tagliaro et al. [3] used CE to analyse hair samples for cocaine and morphine using borate BGEs at pH 9.2.

Although straight CE has been applied successfully to the determination of opiates, this approach is limited due to lack of a convenient method to vary the separation selectivity and thereby to enable the method to be used with samples of widely differing compositions. Separation in CE is generally based on differences in the charge/size ratio of the analytes, so that analytes with very similar charge/size ratios, such as many of the opiates, are often hard to separate. Selectivity changes can be brought about by varying the pH, ionic strength or using an organic modifier in the BGE, but these approaches generally lead to only small selectivity variations or are simply inapplicable to the particular analytes being separated, e.g. varying pH for analytes with very similar pK_a values generally leads to only small selectivity changes.

The use of additives (often referred to as ''pseudo-stationary phases'' or pseudo-SP) in CE to interact with the analytes and improve separation has become a popular approach to the analysis of many pharmaceutical compounds, including opiates. This approach has been termed electrokinetic chromatography (EKC) since it combines the electrophoretic separation of CE with a chromatographic component resulting from the addition of a pseudo-SP. Generally the pseudo-SP is simply added to the BGE and differences in the partitioning of the analytes between the aqueous phase and the pseudo-SP are established, leading to improved separation. The pseudo-SP can comprise cationic, anionic, zwitterionic or neutral polymers, large molecules or surfactant micelles, with the last approach being termed micellar electrokinetic chromatography (MEKC). The most common surfactant used in MEKC has been sodium dodecyl sulfate (SDS) and several papers have been published in which various opiate related compounds have been separated in acidic or basic BGEs [4–6]. As well as SDS, cationic surfactants such as cetyltrimethylammonium bromide (CTAB) [7] and zwitterionic surfactants such as 3-*N*,*N*-dimethylmyristylammoniopropanesulfonate [8] have also been used.

Cyclodextrins (CDs) have proven popular in the analysis of many pharmaceutical compounds, especially for enantiomeric separations. As with the use of micelles, separation is attributed to differences in partitioning of the analyte between the aqueous BGE and the hydrophobic core of the CD. Many cyclodextrins have been used including neutral, anionic or cationic CDs, as well as CDs functionalised with a variety of substituents. Bjornsdottir and Hansen [5] successfully separated six opiates including morphine, thebaine and codeine using heptakis(2,6-di-*O*-methyl)-β-cyclodextrin and then applied this to real samples of opium and drug preparations.

Although previous reports on the separation of opiate compounds using EKC have been published, generally these have not included a detailed study of the mechanism of separation and have employed a qualitative approach to optimisation. Furthermore, these separations have generally relied on partitioning of the opiate between the aqueous BGE phase and a hydrophobic pseudo-SP. The use of an anionic CD to vary the selectivity of opiate-related compounds has been investigated briefly by Jelinek et al. [9] who used 2-*O*-carboxymethy-β-cyclodextrin (CM-CD) to separate morphine, codeine, thebaine and papaverine. It was found that the addition of CM-CD to an acidic BGE at pH 3.7 reduced the mobility of all analytes and this was attributed to formation of complexes between the opiates and the oppositely migrating CM-CD.

To our knowledge no EKC system based on ion-exchange interactions has been demonstrated, especially for the analysis of opiate-related compounds. The aim of the present study was to investigate an EKC system based on an anionic sulfated-cyclodextrin for the separation of opiate-related compounds. The possibility of modelling and optimising the system based on ion-exchange interactions was also investigated.

2. Experimental

2.1. Instrumentation

The CE instrument used was an Agilent ^{3D}CE

(Walbron, Germany). Separations were carried out using Polymicro (Phoenix, AZ, USA) fused-silica capillaries (50 cm (41.5 cm to detector)× 50 μm I.D.). Injection was performed by applying a 50-mbar pressure for 5 s to the anodic side of the capillary. Separations were performed in the co-electroosmotic flow (co-EOF) mode, i.e. with migration of both the opiates and the EOF being towards the negative electrode. Detection was by direct spectrophotometry at 200–210 nm. All separations were performed at +15 kV.

2.2. Capillary coating procedures

A dual-coated capillary which gave a pH-independent EOF was used for all separations. For this purpose a coated capillary similar to that described by Katayama et al. [10] was used, where the capillary surface was first coated with polybrene, a cationic polymer, resulting in a reversed EOF, and then coated with dextran sulfate, an anionic polymer, resulting in a pH-independent cathodic EOF. For the work presented here polybrene was replaced by poly(diallydimethylammonium chloride) (PDDAC) which resulted in very similar pH-independent EOF in the final capillary. The capillary was coated by flushing for 30 min with NaOH, 15 min with water, allowing to stand for 30 min, flushing for 15 min with 1% PDDAC, standing for 15 min, flushing for 2 min with water, 15 min with dextran sulfate, standing for 30 min and finally flushing for 5 min with water. Capillaries prepared in this manner were very robust and produced stable EOF values for the entire range of BGEs used. Capillaries were equilibrated by flushing with each new BGE for 15 min prior to use. This produced stable, reproducible migration times for all BGEs tested. EOF values were determined by an injection of acetone as a neutral marker.

2.3. Reagents

Analytical-grade citric acid and sodium citrate were obtained from Sigma–Aldrich (Milwaukee, WI, USA). Sulfated-cyclodextrin (s-CD) with a typical substitution of 7–11 mol of sulfate/mol CD was obtained from Aldrich and used without further purification.

The opiates were generously donated by Tasmanian Alkaloids, and were obtained as codeine phosphate, morphine, thebaine barbiturate, an ~1:1 mixture of 10-β-hydroxythebaine and thebaine, oripavine and laudanine. These were diluted to 500 mg/l stock solutions and further diluted to prepare the mixed 50-mg/l mixed standards.

Citrate BGEs were prepared by mixing the appropriate amount of citric acid with sodium citrate to produce a 20-m*M* BGE. The appropriate mass of s-CD was then dissolved in the BGE and the pH adjusted to 3.50 with 1 *M* HCl. All BGEs were degassed and filtered through 0.45-μm filters (Activon, Thornleigh, Australia) immediately prior to use.

3. Results and discussion

3.1. Selectivity

It can be expected that two possible interactions will occur between s-CD and the opiates, namely partitioning into the hydrophobic cavity of the CD and, since the opiates are protonated at the pH of the BGE, electrostatic interaction with the sulfate groups on s-CD. Fig. 1 shows the effect on mobility of varying both the concentration of s-CD and NaCl in the BGE. It can be seen that increasing the concentration of s-CD decreased the mobility of the three opiates shown (this effect was also observed for the other opiates but these have been omitted from the figure for clarity). This resulted from the opposing migration directions of the opiates and the s-CD towards the detector and injection end of the capillaries, respectively. This trend did not clarify the exact nature of the interaction between the opiates and the s-CD since both hydrophobic partitioning and electrostatic interactions would result in decreased analyte mobility. However, if the interaction with the s-CD was predominantly ion exchange in nature, then varying the nature and concentration of a competing cation in the BGE should increase the observed mobility by decreasing the interaction between the opiates and the s-CD. This is indeed observed in Fig. 1 as $[Na^+]$ was increased and also in Fig. 2 where increasing concentrations of the competing cation (Na^+ or Mg^{2+}) increased the

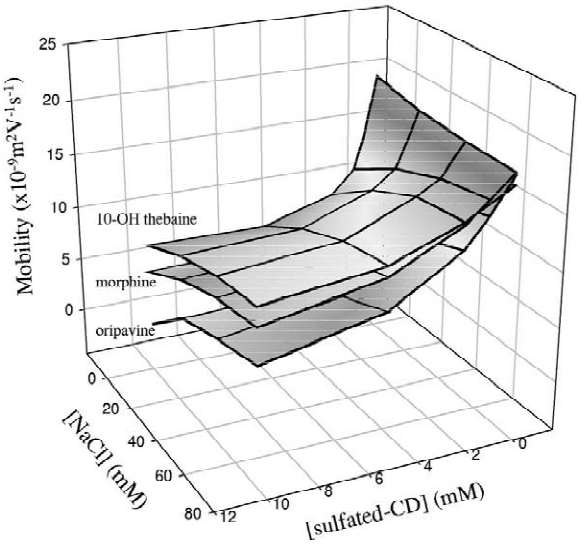

Fig. 1. Plot of mobility versus [sulfated-CD] and [NaCl] for morphine, 10-hydroxythebaine and oripavine. Conditions: 20 m*M* citrate BGE at pH 3.50, 50 cm (41.5 cm to detector)×50 μm capillary.

observed mobility of all the opiates. Fig. 2 also shows the effect of changing the competing cation from Na^+ to Mg^{2+}. It can be seen that Mg^{2+} exerted a much stronger effect than Na^+, as would be expected due to higher charge and stronger ion exchange interaction with the sulfate groups on the CD. However, when using Mg^{2+} as the competing ion, there was also a large increase in the observed EOF (t_m for EOF of 6.7 min ($\mu_{EOF}=34\cdot10^{-9}$ m^2 V^{-1} s^{-1}) at 0 m*M* $MgCl_2$, compared to t_m for EOF of 14 min ($\mu_{EOF}=16\cdot10^{-9}$ m^2 V^{-1} s^{-1}) at 50 m*M* $MgCl_2$) so that the migration times of the opiates remained approximately the same. The change in EOF was far less pronounced when Na^+ was used as the competing ion with the migration time of the EOF going from 6.5 min ($\mu_{EOF}=35\cdot10^{-9}$ m^2 V^{-1} s^{-1}) at 0 m*M* NaCl to 7.5 min ($\mu_{EOF}=31\cdot10^{-9}$ m^2 V^{-1} s^{-1}) at 100 m*M* NaCl. Baselines at >2 m*M* $MgCl_2$ were also less stable than when NaCl was used. The most probable reason for these observations was the stronger interaction of Mg^{2+} with the negative wall of the capillary (due to the final layer of dextran sulfate), leading to decreased EOF. Due to these phenomena NaCl was used as the competing ion in all subsequent work.

From these results it can reasonably be concluded

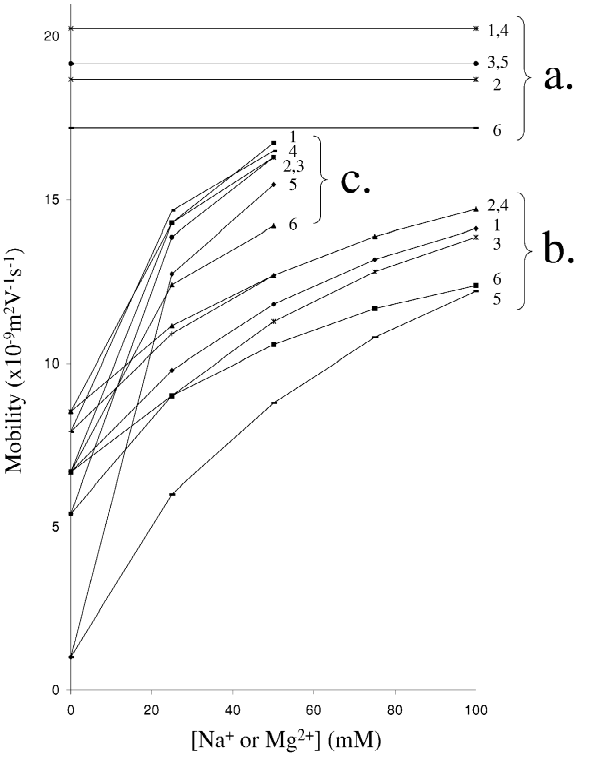

Fig. 2. Effect of competing ion on the separation of opiates in a BGE containing 3.0 m*M* s-CD. (a) Observed electrophoretic mobility with no ion exchange interaction (i.e. no additives in the BGE); (b) effect on mobility of increasing Na^+ concentration; and (c) effect on mobility of increasing Mg^{2+} concentration. Analytes: 1=morphine, 2=10-hydroxythebaine, 3=thebaine, 4=codeine, 5=oripavine and 6=laudanine. Conditions: 20 m*M* citrate BGE at pH 3.50, 50 cm (41.5 cm to detector)×50 μm capillary.

that the predominant interaction between the opiates and the s-CD is ion exchange in nature rather than hydrophobic partitioning. It can also be seen that at high competing ion concentrations the observed migration order approached that of the standard CE separation, which also indicated that ion exchange was the main interaction. Finally, it was also found that the use of neutral β-cyclodextrin as a pseudo-SP exerted very little effect on the separation of the opiates, again suggesting that the interaction between the opiates and the β-cyclodextrin ring is rather small, especially at acidic pH values.

3.2. Modelling the system

Since the interaction between the opiates and the

s-CD involved ion exchange interactions it was possible to model the system using a similar ion exchange model to that used by Breadmore et al. [11] for the separation of inorganic anions using the soluble polymer PDDAC.

If ion exchange interactions are the predominant mechanism of separation, then the interaction of the analytes with the s-CD can be represented by the following equilibrium:

$$Op^+_{bge} + Na^+_{cd} \overset{K_{ie}}{\rightleftharpoons} Op^+_{cd} + Na^+_{bge} \quad (1)$$

where Op^+ represents the opiate, Na^+ the competing ion and the subscripts bge and cd refer to the species in the BGE and bound to the cyclodextrin, respectively. The equilibrium constant for Eq. (1) is:

$$K_{ie} = \frac{[Op^+]_{cd}[Na^+]_{bge}}{[Op^+]_{bge}[Na^+]_{cd}} \quad (2)$$

An expression for the analyte retention factor can be derived from first principles [11]:

$$k' = w_{\%} K_{ie} Q [E^+]^{-1} \quad (3)$$

where $w_{\%}$ is the mass percent of the pseudo-SP, Q is the ion-exchange capacity of the pseudo-stationary phase and $[E^+]$ is the concentration of the competing ion in the BGE (Na^+ for the work presented here).

The observed mobility of an analyte can be expressed in terms of this retention factor [11]:

$$\mu_{ob} = \frac{1}{1+k'} \cdot \mu_{bge} + \frac{k'}{1+k'} \cdot \mu_{cd} \quad (4)$$

where μ_{ob} is the observed mobility and μ_{bge} and μ_{cd} are the mobilities of the free opiate in the BGE and bound to the cyclodextrin, respectively.

Combining Eqs. (3) and (4) leads to a final equation describing the separation of the opiates in the presence of the s-CD:

$$\mu_{ob} = \frac{1}{1 + w_{\%} K_{ie} Q [Na^+]^{-1}} \cdot \mu_{bge} + \frac{w_{\%} K_{ie} Q [Na^+]^{-1}}{1 + w_{\%} K_{ie} Q [Na^+]^{-1}} \cdot \mu_{cd} \quad (5)$$

In Eq. (5), $w_{\%}$ and $[Na^+]$ are known parameters. Q can be estimated from the average degree of substitution of the s-CD or can be included as one of the unknown parameters, along with K_{ie}, μ_{bge} and μ_{cd}, and determined by non-linear regression. Non-linear regression was performed using the solver function in Microsoft Excel 97 for all analytes by a minimisation of least squares.

Initial application of Eq. (5) to the separation of the opiates showed that the $[Na^+]^{-1}$ relationship did not accurately represent the observed trends in μ_{ob}. Replacing this with an $[Na^+]^{-x}$ term (Eq. (6)) dramatically improved the results allowing the model to accurately predict the observed separations of the opiates. This outcome is most probably due to the fact that the displacement of Na^+ ions by the opiates is not a 1:1 process, as assumed in Eq. (1). Since the exact displacement ratio will depend on a range of parameters and will be difficult to predict, its value was determined by non-linear regression, together with other unknown parameters in Eq. (6):

$$\mu_{ob} = \frac{1}{1 + w_{\%} K_{ie} Q [Na^+]^{-x}} \cdot \mu_{bge} = \frac{w_{\%} K_{ie} Q [Na^+]^{-x}}{1 + w_{\%} K_{ie} Q [Na^+]^{-x}} \cdot \mu_{cd} \quad (6)$$

3.3. Application of the migration model

Eq. (6) has five unknown parameters, K_{ie}, μ_{bge}, μ_{cd}, Q and x, with two variables, $w_{\%}$ and $[Na^+]$. This implied that a minimum of six experimental data points was required to determine all of the unknowns. A two-dimensional data set of observed mobilities was obtained over the parameter space determined by 0, 1, 2, 5, 10 m*M* s-CD and 0, 25, 50, 75 m*M* NaCl was chosen, comprising of 20 data points. From these data non-linear regression was performed on the four corner points of the parameter space and two points taken close to the centre of the parameter space (a total of six points, termed the primary dataset) to determine the five unknowns in Eq. (6). The total dataset comprising all 20 points was then used to evaluate the predictive power of the model.

Table 1 shows the constants obtained by non-linear regression of the primary dataset. Two of the five constants are related to the analytes themselves, i.e. K_{ie} and μ_{bge}, while the other three, μ_{cd}, number of ion exchange sites (Q) and x, are related to the BGE system and would vary only when a new BGE

Table 1
Parameters derived from non-linear regression applied to Eq. (6)

Analyte	K_{ie}	μ_{bge} [a]
Analyte specific constants		
Oripavine	29.7	19.2
Morphine	22.5	20.2
Thebaine	22.1	19.2
Codeine	19.8	20.2
Laudanine	18.1	17.2
10-Hydroxythebaine	15.7	18.7
Electrolyte specific constants		
μ_{cd}	−78.9	
No. of ion exchange sites	4.7	
x	2.1	

[a] $\times 10^{-9}$ m^2 V^{-1} s^{-1}.

or pseudo-SP was used. The constants obtained agreed well with expected and observed trends. The analytes are listed in Table 1 in order of decreasing affinity for the s-CD and this order supports the observed trends seen in Fig. 1 with oripavine showing the strongest affinity for the s-CD, 10-hydroxythebaine the weakest and morphine having an intermediate value. μ_{bge} values also agreed with observed trends, with migration times predicted for a pure CE separation (i.e. in the absence of s-CD and NaCl) varying by less than 0.66% compared to the observed migration times. Table 1 shows a μ_{cd} value (relating to the mobility of the opiate–cyclodextrin complex) of $-78.9 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}, showing that the negative charge on the s-CD was not fully neutralised on complexing with the opiates. Q is related to the number of ion exchange sites contained on each of the s-CD molecules and Table 1 shows a value 4.7, which was reasonable since the average degree of substitution quoted by the manufacturer is 7–11 mol sulfate/mol of s-CD and it can be assumed that not every sulfate group will be available for interaction with the bulky opiate ions. The final constant shown in Table 1 is x and the calculated value of 2.1 implies that there was an inverse square relationship between the analyte retention factor and the competing ion concentration.

Fig. 3 shows the correlation between observed mobilities and those calculated using Eq. (6) and the constants shown in Table 1. It can be seen that excellent correlation is obtained with an r^2 value of 0.996 obtained.

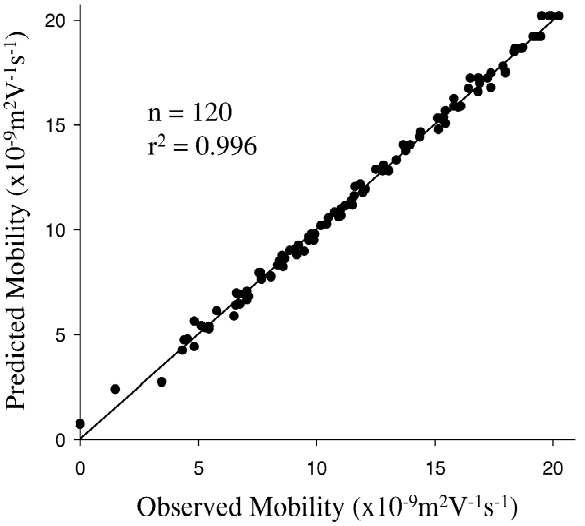

Fig. 3. Correlation between observed and predicted mobilities using Eq. (6) and the constants shown in Table 1. A total of 120 data points was used for the correlation including 36 points used to derive the constants.

3.4. Optimisation of separation conditions

The availability of a model to describe the migration behaviour of the opiates introduces the possibility of optimising the separation using a suitable algorithm. Optimisation was performed using the normalised resolution product (r_{norm}) and minimum resolution product (r_{min}) criteria. The normalised resolution product is designed to reach a maximum value when all the analytes are evenly separated and is given by [11]:

$$r_{norm} = \prod_{t=1}^{n-1} \left(\frac{R_{s(i,i+1)}}{\frac{1}{n-1} \sum_{t=1}^{n-1} R_{s(i,i+1)}} \right) \quad (7)$$

where $R_{s(i,i+1)}$ is the resolution between adjacent peaks and n is the number of peaks in the separation. r_{norm} ranges from 0 to 1, with 0 implying complete overlap of at least one pair of peaks, with a value of 1 being reached when all the analytes are evenly spaced over the electropherogram.

Optimisation was also carried out using the minimum resolution criterion. This criterion takes into account only the peak pair having the worst resolution and is calculated as follows [11]:

$$r_{\min} = \min\left(\sum_{i=1}^{n-1} R_{s(i,i=1)}\right) \quad (8)$$

The process followed in the optimisation involved calculation of r using a defined set of experimental data covering the parameter space and then performing a separation under the predicted optimal conditions. Observed mobilities were then compared with predicted values and if the discrepancies exceeded a predefined limit of 1%, the migration data for the selected optimum were added to the optimisation data set and the process repeated.

Fig. 4 shows the resolution surface obtained using the normalised resolution product criterion. It can be seen that the optimum separation, i.e. that producing the highest resolution, was calculated to be at 1 m*M* s-CD and 45 m*M* NaCl. This should result in a separation where the peaks are as evenly spread over the entire separation as possible. The optimum separation is shown in Fig. 5a with the predicted separation shown in Fig. 5b. Although it can be seen that full separation is not obtained using these conditions, the agreement obtained between predicted and observed migration times was <0.83%. However, since all of the opiates migrated in a very small time window (less than 30 s), even this small deviation between observed and predicted mobilities led to co-migration. A further complicating factor was the slightly tailed nature of the peaks which also contributed to the co-migration of 10-hydroxythebaine with thebaine and oripavine with laudanine. This was especially evident for oripavine which interacted strongly with the s-CD, leading to broadened peaks. Laudanine also produced a relatively small peak at 210 nm, which further contributed to the lack of resolution between oripavine and laudanine.

The system was also modelled using the minimum

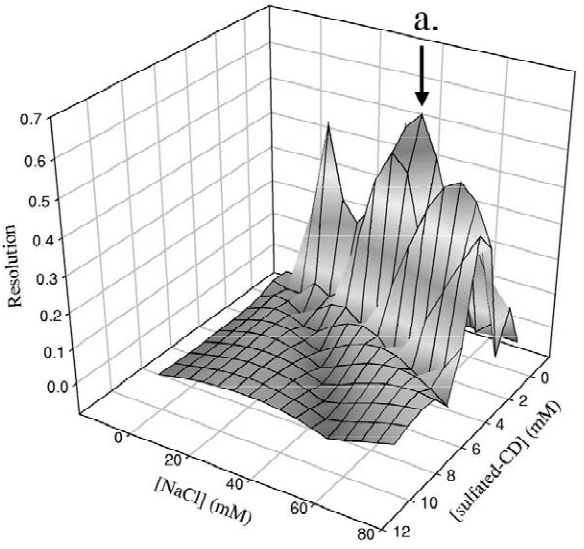

Fig. 4. Resolution surface obtained using the normalised resolution product criterion (Eq. (7)). (a) Optimum at 1 m*M* s-CD and 45 m*M* NaCl.

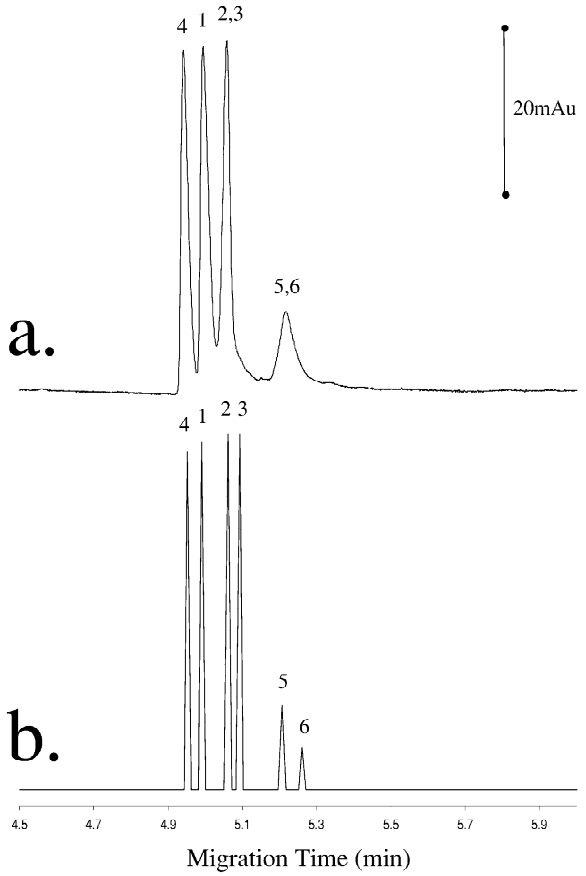

Fig. 5. Optimal separation calculated using the normalised resolution product criterion. (a) Observed separation, (b) predicted separation. Conditions: 1 m*M* s-CD and 45 m*M* NaCl. Peaks are: 1 = morphine, 2 = 10-hydroxythebaine, 3 = thebaine, 4 = codeine, 5 = oripavine and 6 = laudanine. BGE: 20 m*M* citrate at pH 3.50, 50 cm (41.5 cm to detector) × 50 μm capillary. Detection at 210 nm.

resolution criterion, shown in Eq. (8). This criterion considers only the two adjacent peaks having the worst resolution and as such could possibly give a better optimum than that calculated using the normalised resolution product criterion. Fig. 6 shows the resolution surface obtained using the minimum resolution criterion. It can be seen that the optimum separation was calculated to be at 5 m*M* s-CD and 10 m*M* NaCl, and Fig. 7 shows the separation obtained at these conditions. Full resolution was again not obtained but the separation was superior to that obtained using the normalised resolution product criterion. The difference between observed and predicted migration times was $<1\%$ as can be seen when comparing Fig. 7a and b. The cause for the co-migration of morphine and laudanine was again the small error in prediction and the fact that the peak for laudanine was very much smaller than morphine at 210 nm and it migrated within the tail of the morphine peak. Lowering the wavelength of detection did not alleviate the problem. A further complication with the separation was the system peak seen after oripavine (Fig. 7a). This occurred in a reproducible manner, particularly at higher s-CD concentrations, and caused problems with quantification of oripavine which has the strongest interaction with s-CD. This problem could be overcome by including the system peak in the optimisation process [12].

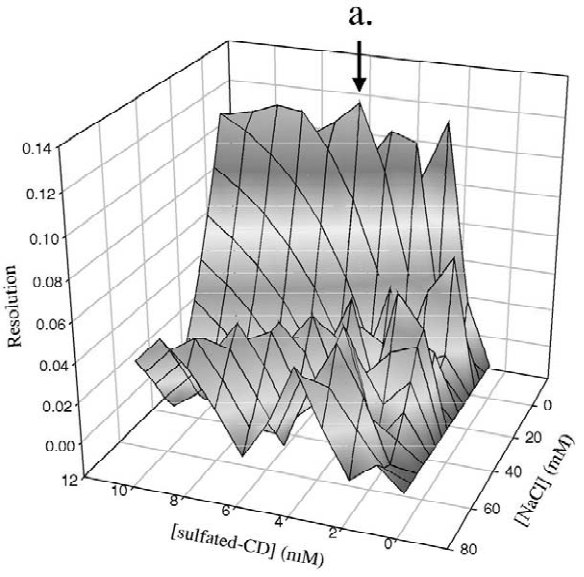

Fig. 6. Resolution surface obtained using the minimum resolution criterion (Eq. (8)). (a) Optimum at 5 m*M* s-CD and 10 m*M* NaCl.

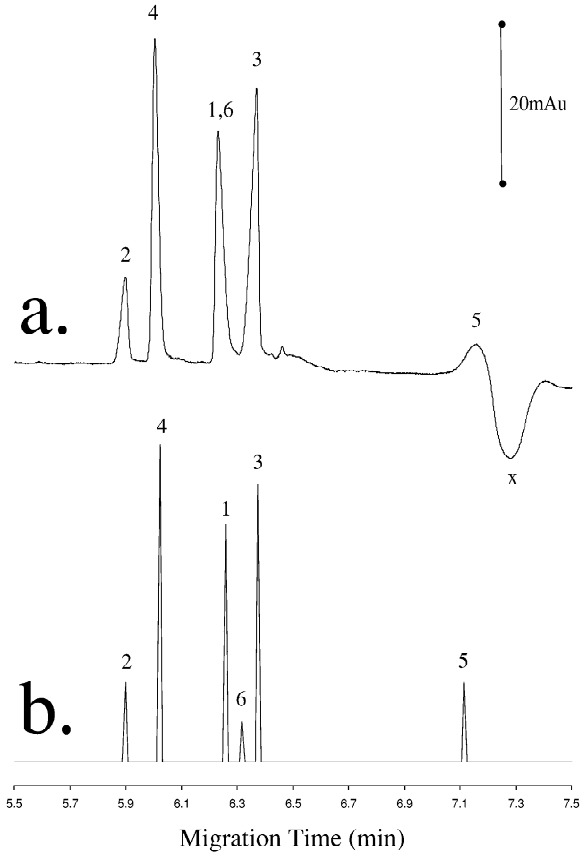

Fig. 7. Optimal separation calculated using the minimum resolution criterion. (a) Observed separation; and (b) predicted separation. Conditions: 5 m*M* s-CD and 10 m*M* NaCl. Peaks are: 1 = morphine, 2 = 10-hydroxythebaine, 3 = thebaine, 4 = codeine, 5 = oripavine 6 = laudanine and *x* = system peak occurring prior to the EOF. BGE: 20 m*M* citrate at pH 3.50, 50 cm (41.5 cm to detector) × 50 μm capillary. Detection at 210 nm.

4. Conclusions

A sulfated CD has been demonstrated to be a suitable cation-exchange pseudo-stationary phase additive for separation of opiate alkaloids. The extent of the ion exchange interactions between the s-CD and the protonated opiates can be easily controlled by varying either the concentration of s-CD in the

BGE or the nature and concentration of the competing ion, such as Na^+ or Mg^{2+}. A mathematical model derived from first principles in terms of the ion exchange equilibrium taking place can be used to accurately describe observed separations at any concentration within the experimental space using an initial subset comprising only six individual experiments. The system is not only useful for the opiates studied but potentially can be applied to any organic cation that exhibits an ion exchange interaction with s-CD, possibly including many classes of pharmaceutically important compounds. The possibility also arises to include further cyclodextrins in the system to permit simultaneous enantiomeric separations.

References

[1] I.S. Lurie, J. Chromatogr. A 780 (1997) 265.
[2] M. Unger, D. Stockigt, D. Belder, J. Stockigt, J. Chromatogr. A 767 (1997) 263.
[3] F. Tagliaro, C. Poiesi, R. Aiello, R. Dorizzi, S. Ghielmi, M. Marigo, J. Chromatogr. 638 (1993) 303.
[4] C. Zhang, W. Thormann, J. Chromatogr. A 764 (1997) 157.
[5] I. Bjornsdottir, S.H. Hansen, J. Pharm. Biomed. Anal. 13 (1995) 687.
[6] M. Krogh, S. Brekke, F. Tonnesen, K.E. Rasmussen, J. Chromatogr. A 674 (1994) 235.
[7] V.C. Trenerry, R.J. Wells, J. Robertson, J. Chromatogr. A 718 (1995) 217.
[8] O. Naess, K.E. Rasmussen, J. Chromatogr. A 760 (1997) 245.
[9] I. Jelinek, B. Gas, I. Zuskova, E. Niklickova, I. Buben, Chem. Listy 91 (1997) 487.
[10] H. Katayama, Y. Ishihama, N. Asakawa, Anal. Chem. 70 (1998) 2254.
[11] M.C. Breadmore, P.R. Haddad, J.S. Fritz, Electrophoresis 21 (2000) 3181.
[12] P. Zakaria, M. Macka, P.R. Haddad, Electrophoresis 23 (2002) 1844.