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Selectivity control in the separation of aromatic amino acid enantiomers with sulphated β-cyclodextrin

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Abstract

Control of selectivity in the enantiomeric separation of three aromatic amino acids (phenylalanine, tyrosine and tryptophan) is demonstrated by electrokinetic capillary chromatography utilising temperature variations coupled with the use of sulphated-β-cyclodextrin (s-β-CD) as a pseudostationary phase. The concentration of s-β-CD and temperature were used as experimental variables to control the observed selectivity. A double-coated capillary was used and proved very robust with reproducibility of migration times being <2.0% R.S.D. between runs and <2.6% on using a new capillary. The system was modelled successfully using an artificial neural network (ANN) comprising one input layer, two hidden layers and one output layer. The model accurately described the observed separations with a correlation coefficient of 0.999 being observed between predicted and observed migration times. Selectivity optimisation was achieved using the normalised resolution product and minimum resolution criteria, with both providing optima at different experimental conditions. The selectivity changes observed also allowed the estimation of electrolyte temperatures within the capillary at high operating currents (>100 μA). Using a 50 μm i.d. capillary and an electrolyte comprising 20 mM phosphate and 15 mM s-β-CD, a temperature of 52 °C was calculated within the capillary at an applied voltage of +30 kV.

Keywords: Neural networks, artificial; Enantiomer separation; Electrokinetic chromatography; Amino acids; Cyclodextrins

1. Introduction

Capillary electrophoresis (CE) separates species based on differences in the electrophoretic mobility of the analytes of interest [1]. It is a powerful technique and has been used to separate a large variety of charged species [2]. CE shows many advantages over more traditional chromatographic methods for ionic analytes, such as ion chromatography (IC) and high-performance liquid chromatography (HPLC), including high efficiency, reduced analysis time and smaller sample volumes.

However, one disadvantage of CE is the lack of a convenient means to vary the separation selectivity of the system. This drawback can be overcome by the use of pseudostationary phases (p-SPs) in the background electrolyte (BGE) that can interact with the analytes of interest and greatly increases the selectivity control. CE methods in which p-SPs are used are termed electrokinetic chromatography (EKC). EKC has been particularly well suited to chiral separations [3] due the high efficiency and low sample consumption, and in the case of chiral separations, charged cyclodextrin derivatives have been the most widely utilised p-SPs and these have been applied to a large variety of different analytes [4].

The analysis of amino acids is an important area of research in chemistry and biochemistry. EKC is particularly useful for both enantiomeric and non-enantiomeric separations of amino acids and the separation of free and derivatized amino acids has been achieved using micelles, cyclodextrins, crown-ethers and proteins as p-SPs [3]. Cyclodextrins are particularly useful for chiral separations and have the advantage that substituents can be added in order to alter their selectivity. The separation of enantiomers is generally attributed to inclusion of a hydrophobic portion of the analyte into the cavity of the cyclodextrin and further hydrogen bonding interactions with the hydroxyl groups on the cyclodextrin opening [5]. Sulphated-cyclodextrins have been widely applied to the analysis of pharmaceutical compounds [6–9], di- and tripeptides [10–12] and amino acids [13,14].

Although the use of p-SPs such as cyclodextrins can facilitate the separation of a wide variety of enantiomeric

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analytes, the full separation of all components in a complex mixture can still be problematic. It is therefore often necessary to utilise further experimental parameters such as pH, temperature, applied voltage, use of further additives, etc. in order to achieve a desired separation [15–17]. However, to successfully develop a separation, the effect of these additional experimental parameters should be predictable so that the correct separation selectivity can be attained. The use of artificial neural networks (ANNs) to model such systems has been reported previously [18–23], but these systems are very specific and the outputs of the ANNs often involve the number of peaks or peak height. Furthermore, the application of ANNs to chiral separations is limited, especially when describing the separation of more than a single component. The aim of the present work was to demonstrate that the selectivity of enantiomeric separations (using three UV-absorbing amino acids as test analytes) could be modelled and optimised using an ANN when an anionic cyclodextrin was used as a chiral p-SP (with temperature used as a further variable). The outputs of the model were to be the observed mobilities of the analytes, such that the proposed system could be easily applied to other chiral separations of amino acids or even other chiral compounds.

2. Experimental

2.1. Instrumentation

The CE instrument used was a Hewlett-Packard ^{3D}CE (Walbron, Germany). Separations were carried out using Polymicro (Phoenix, AZ, USA) fused silica capillaries of 25 and 50 μm i.d. with a length of 55 cm (46.5 cm to detector). Injection was performed by applying a 50 mbar pressure for 3 or 5 s (depending on capillary i.d.) to the injection side of the capillary. Separations were performed in the co-electroosmotic flow (EOF) mode, i.e. with migration of both the free analytes and the EOF being towards the negative electrode, using detection by direct spectrophotometry at 210 nm.

2.2. Capillary coating procedures

A capillary showing pH-independent EOF based on work by Katayama et al. [24] was used for this study and the procedure for its preparation has been reported previously [25]. Briefly, this involved coating a fused silica capillary with the cationic polymer poly(diallyldimethylammmonium) chloride (PDDAC), followed by coating with the anionic polymer dextran sulphate. This resulted in a stable, positive EOF of $\sim 45 \times 10^{-9}$ m^2 s^{-1} V^{-1} at 25 °C.

2.3. Reagents

Phosphoric acid was obtained from BDH Analar (Kilsyth, Australia). All further chemicals were obtained from Sigma–Aldrich (Milwaukee, WI, USA). Sulphated-β-cyclodextrin was obtained with an average degree of substitution of 7–11. PDDAC, with a molecular mass of 400 000–500 000, was obtained as a 20% (w/v) solution. All chemicals were used without further purification.

Amino acid standards of 10 mM concentration (phenylalanine, tyrosine and tryptophan) were prepared in 10 mM NaOH. Electrolytes were prepared by diluting 100 mM phosphoric acid to 20 mM and titrating to pH 2 with NaOH. Sulphated-β-cyclodextrin (s-β-CD) was dissolved in each electrolyte to give the desired concentration prior to use. Individual enantiomers were identified by injection of the optically pure component and comparing migration times. All electrolytes were degassed using an ultrasonic bath for 2 min and prior to use. EOF values were determined by injecting acetone.

2.4. Artificial neural networks

Calculations involving ANNs were performed using the Trajan Neural Network Simulator, Release 3.0 (Trajan Software, Durham, UK).

3. Results and discussion

3.1. Factors influencing separation selectivity

Both β- [26] and γ- [27] sulphated cyclodextrins have been used as chiral separating agents. Sulphated-β-CD has also been used in the separation of metal ions [28] where the sulphate groups on the cyclodextrin acted as cation-exchange sites, analogous to sulfonated cation-exchangers. From this, it can be expected that interactions between the s-β-CD and the protonated amino acid test analytes will be a combination of host–guest complexation, most likely between the aromatic group on the amino acids and the hydrophobic core of the cyclodextrin, and ion-pair (IP) interactions between the positive charge on the amino acids and the negative sulphate groups on the cyclodextrin.

Fig. 1 shows the effect of addition of s-β-CD to the electrolyte on the separation of the three amino acids. Full separation of all six enantiomers was not obtained even at 20 mM s-β-CD. Resolution of D-phenylalanine, L-tyrosine and D-tryptophan was problematic and under some conditions the EOF peak co-migrated with L-phenylalanine. It should be noted that the varying peak heights observed for L-tryptophan were due to decomposition in the sample vial. From previous work in this laboratory [29] and the work by Muzikar et al. [28] it can be expected that a major component of the interactions occurring between the amino acids and s-β-CD will be IP in nature. The observed separation should therefore be influenced by changes in the concentration of a competing ion in the electrolyte (Na^+ for the work presented here). Fig. 2 shows the effect of increasing the concentration of sodium chloride in the presence of

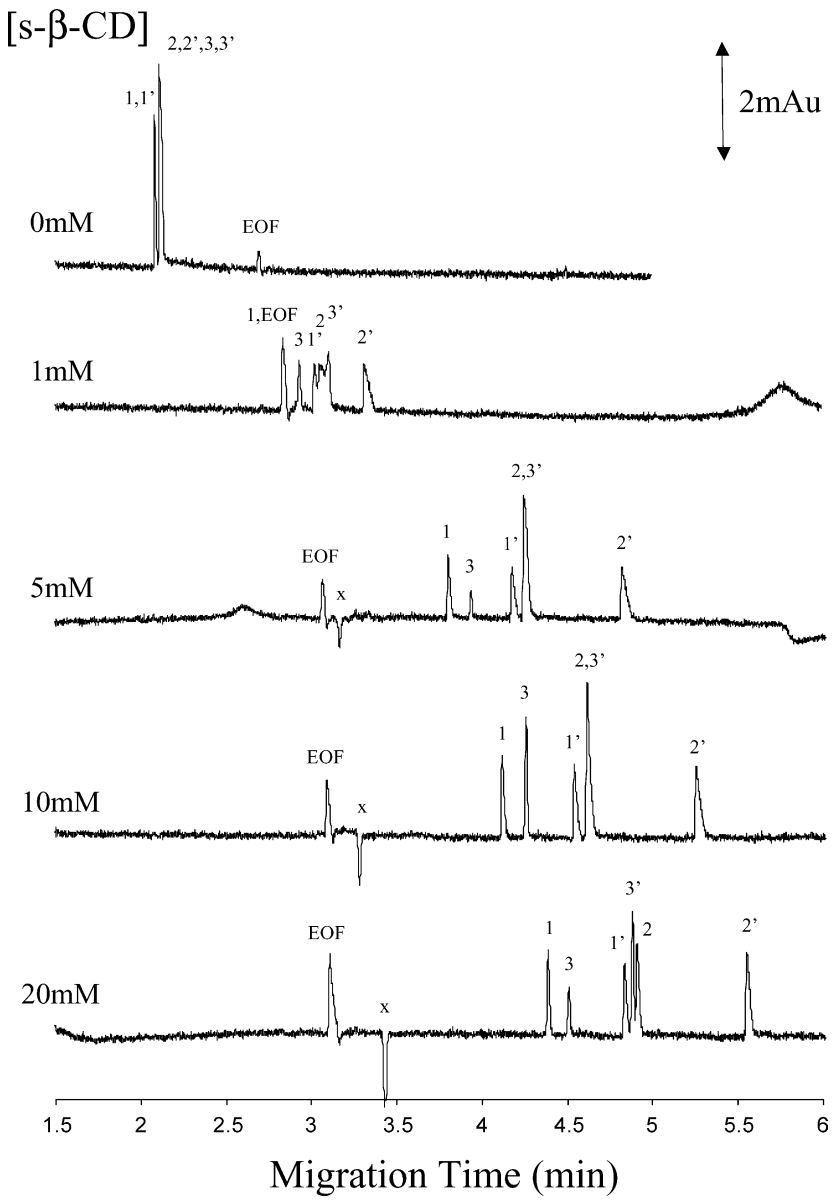

Fig. 1. Effect of [s-β-CD] on the separation of the three amino acids. Peaks are 1 = L-phenylalanine, 1′ = D-phenylalanine, 2 = L-tyrosine, 2′ = D-tyrosine, 3 = L-tryptophan, 3′ = D-tryptophan and x = system peak associated with the addition of s-β-CD. Conditions: 20 mM phosphate electrolyte at pH 2.0. Separation at +30 kV and 25 °C with detection at 210 nm. Capillary: 55 cm (46.5 cm to detector) × 25 μm.

s-β-CD. It can be seen that as the concentration of competing ion increased, the interaction between the analytes and the s-β-CD decreased, most likely due to competition for IP sites on the s-β-CD by sodium ions.

Temperature is known to have a large effect on enantiomeric separations by EKC with cyclodextrins due to its influence on the analyte-cyclodextrin complexation process. It has been shown that better enantiomeric separations are achieved at lower temperatures [5,30] due to increased migration times and an increase in the stability of the inclusion complex, although this can depend on many factors and may vary from case to case. Fig. 3 shows the effect of varying temperature on the separation of the three amino acids in the presence of 15 mM s-β-CD. Increasing the temperature from 15 to 60 °C led to the expected increase in mobilities for all analytes. However, it can be seen that increasing the temperature also altered the selectivity of the system, with different migration orders being observed at 15 and 60 °C. It should also be noted that baseline resolution of all six enantiomers could be achieved at 60 °C.

3.2. Optimisation of the separation using an artificial neural network

From Figs. 1 and 3, it can be seen that it should be possible to optimise the separation using a strategy in which two influential experimental parameters (two factors) are varied, these being [s-β-CD] and temperature. However, since the

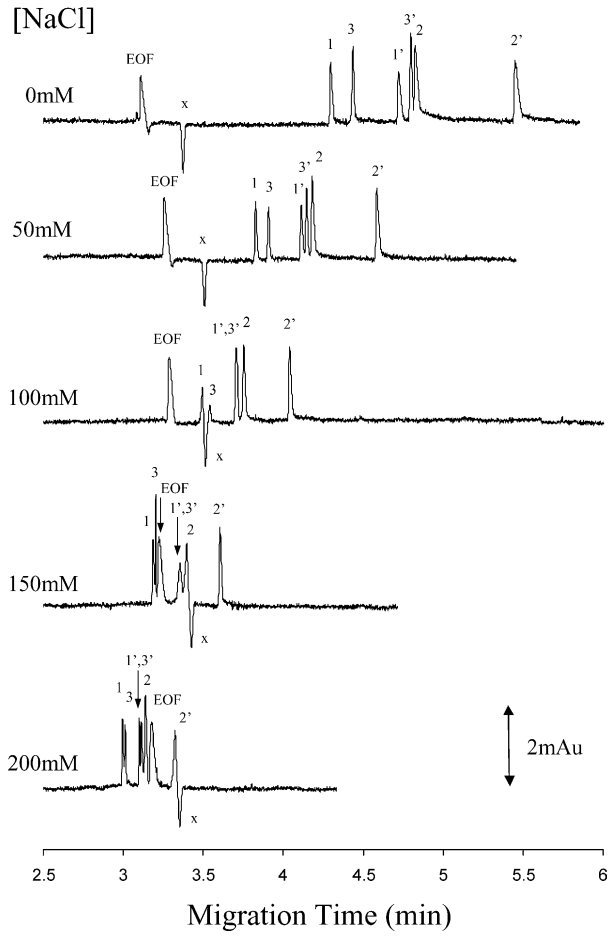

Fig. 2. Effect of competing ion concentration in a electrolyte containing 15 mM s-β-CD. Peaks are 1 = L-phenylalanine, 1′ = D-phenylalanine, 2 = L-tyrosine, 2′ = D-tyrosine, 3 = L-tryptophan, 3′ = D-tryptophan and *x* = system peak associated with the addition of s-β-CD. Conditions: 20 mM phosphate +15 mM s-β-CD at pH 2.0. Separation at +30 kV and 25 °C with detection at 210 nm. Capillary: 55 cm (46.5 cm to detector) × 25 μm.

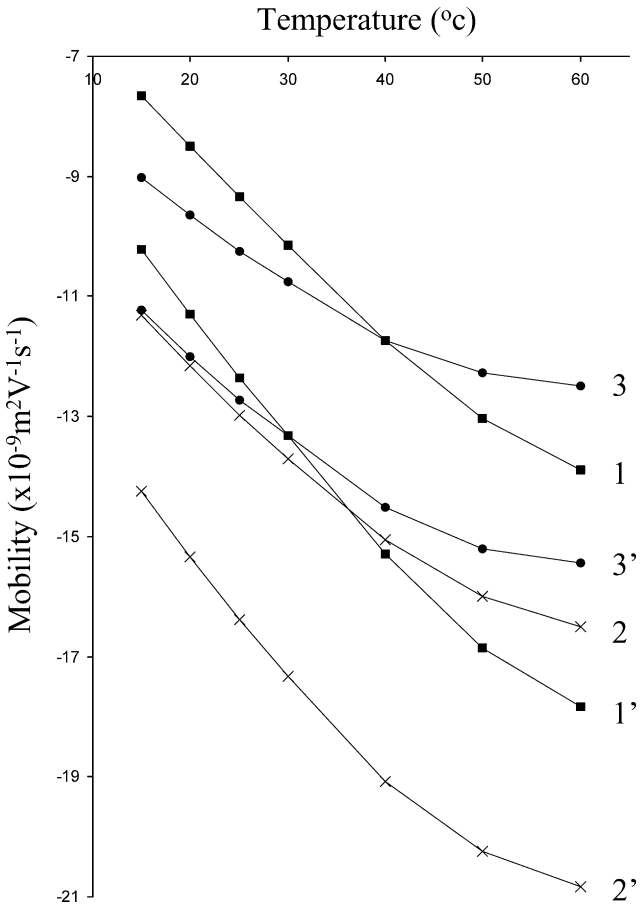

Fig. 3. Effect of temperature on mobilities of the amino acids in an electrolyte containing 15 mM s-β-CD. Peaks are 1 = L-phenylalanine, 1′ = D-phenylalanine, 2 = L-tyrosine, 2′ = D-tyrosine, 3 = L-tryptophan and 3′ = D-tryptophan. Conditions: 20 mM phosphate +15 mM s-β-CD at pH 2.0. Capillary: 55 cm (46.5 cm to detector) × 25 μm.

temperature effect is difficult to model accurately using a physical model, the use of an ANN was investigated. An ANN is a soft modelling approach that does not require any explicit knowledge of the system being modelled. This is an advantage for complex systems where several parameters can simultaneously affect the observed separation. ANNs have been used to model several CE [31], MEKC [19] and enantiomeric [21,32] separations.

The two-dimensional parameter space defined by 0–20 mM s-β-CD (0, 0.5, 1.0, 2.5, 5.0, 10, 15 and 20 mM) and 15–60 °C (15, 20, 25, 30, 40, and 60 °C) was used and migration times for each of the analytes were measured at 56 points within this parameter space. Different ANN architectures were tested and the number of data points used to train the ANN was varied. An ANN having a 2-5-5-6 architecture, i.e. consisting of four layers (an input layer, two hidden layers, and an output layer) with two nodes in the input layer ([s-β-CD] and temperature), five nodes in both the hidden layers and six in the output layer (corresponding to the mobilities of the six enantiomers), was found to be optimal. Best results were also obtained when 28 data points were used to train the ANN with the further 28 points being used to verify the predictive capabilities of the model. Table 1 shows the errors associated with differing numbers of training iterations. It can be seen that 20 000 iterations led to the lowest errors in the verification set. Further training iterations, while lowering the error in the training set, increased the error associated with the verifications set. Thus, 20 000 iterations were used to train the current model.

Fig. 4 shows the correlation between observed mobilities and those predicted mobilities using the ANN. It can be seen that very good correlation was achieved with an R^2 value of 0.9993 being obtained. This allowed the model to accurately predict analyte mobilities anywhere within the two-dimensional parameter space and therefore enabled it to be used to determine optimal separation conditions. The two optimisation criteria used for this purpose were the normalised resolution product (NRP) criterion and the minimum resolution (MR) criterion. The NRP criterion, Eq. (1), is maximised when all peaks are evenly spaced throughout

Table 1
Errors vs. training iterations using a 2-5-5-6 ANN

Number of training iterations	RMS error in training set	RMS error in verification set	R^2 on correlation graph[a]
1000	1.6940	1.3800	0.9819
3000	0.7023	0.5754	0.9976
7000	0.5144	0.5657	0.9985
10000	0.3205	0.4805	0.9987
15000	0.2680	0.3764	0.9992
20000	0.2030	0.3636	0.9993
30000	0.1777	0.3911	0.9992
40000	0.1680	0.4086	0.9992

[a] Using all 56 points within the experimental space.

the separation window. Values range between 0 and 1, with 1 corresponding to the separation where all peaks are evenly spaced.

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

The MR criterion, Eq. (2), reports the value of resolution (R_s) for the two adjacent peaks having the worst resolution.

$$r_{\text{min}} = \min \left(\sum_{i=1}^{n-1} R_{s(i,i+1)} \right) \quad (2)$$

Figs. 5 and 6 show the observed and predicted separations using conditions calculated with the NRP (Fig. 5) and MR (Fig. 6) criteria. It can be seen that both criteria yielded separations with baseline resolution of all six enantiomers, but the separation derived using the MR criterion was superior. Both criteria predict very different electrolyte compositions with the NRP criterion predicting a low concentration of s-β-CD and low temperature while the MR criterion predicted a high concentration of s-β-CD and a high temperature. Agreement between observed and predicted migration times was also very good for both optima, with deviations being less than 1% in both cases. It should be noted that

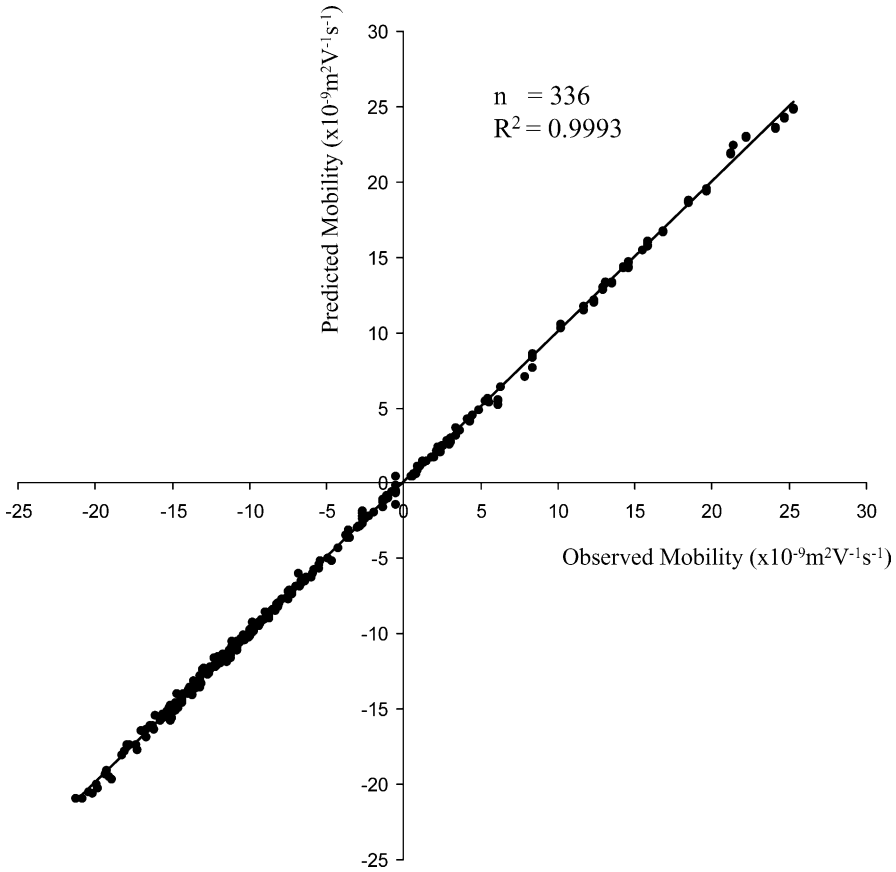

Fig. 4. Correlation between observed mobilities and those predicted using the 2-5-5-6 ANN. All 56 experimental conditions included in the correlation graph, i.e. both those used to train and verify the model.

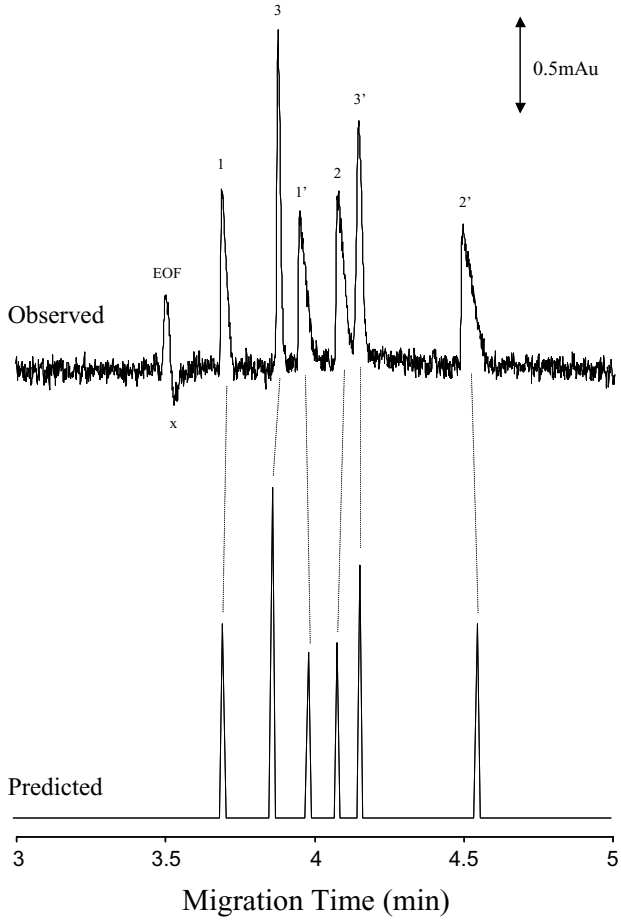

Fig. 5. Optimised separation at 1.5 mM s-β-CD and 15 °C. Conditions calculated using the normalised resolution product criterion. Peaks are 1 = L-phenylalanine, 1′ = D-phenylalanine, 2 = L-tyrosine, 2′ = D-tyrosine, 3 = L-tryptophan, 3′ = D-tryptophan and *x* = system peak associated with the addition of s-β-CD. General conditions: 20 mM phosphate electrolyte at pH 2.0. Separation at +30 kV with detection at 210 nm. Capillary: 55 cm (46.5 cm to detector) × 25 μm.

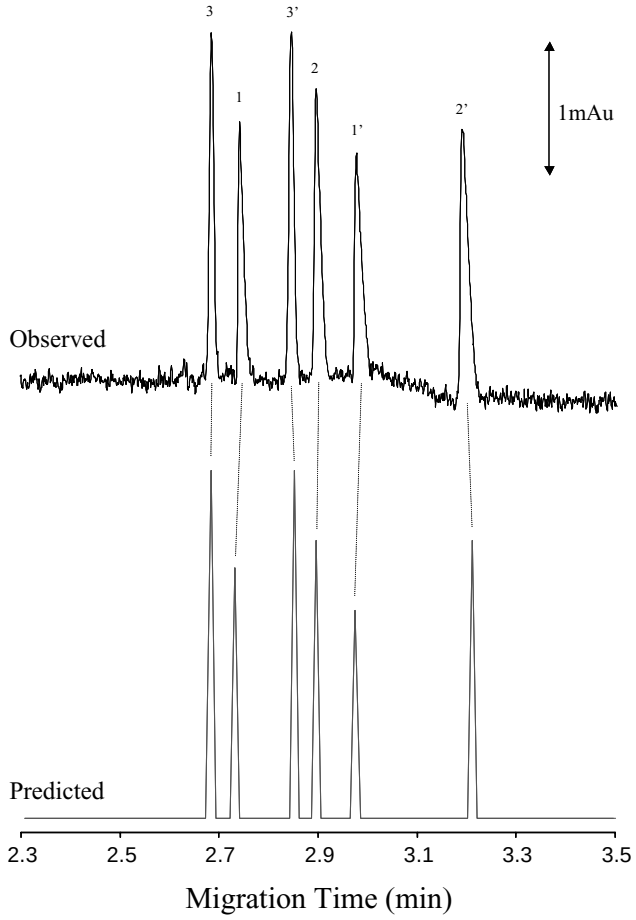

Fig. 6. Optimised separation at 13 mM s-β-CD and 60 °C. Conditions calculated using the minimum resolution criterion. Peaks are 1 = L-phenylalanine, 1′ = D-phenylalanine, 2 = L-tyrosine, 2′ = D-tyrosine, 3 = L-tryptophan and 3′ = D-tryptophan. General conditions: 20 mM phosphate electrolyte at pH 2.0. Separation at +30 kV with detection at 210 nm. Capillary: 55 cm (46.5 cm to detector) × 25 μm.

the ANN only predicts mobilities (migration times), and the peak heights and widths shown in the predicted electropherograms have been inserted manually. The precision of the system is also very good, even at higher temperatures, with reproducibility of migration times being less than 1% over 13 successive runs at 60 °C.

The model was also used to individually optimise the separation of each amino acid. Fig. 7 shows the optimised separations obtained when only individual pairs of amino acid enantiomers were considered. It can be seen that very similar conditions were calculated for each amino acid. It is also interesting to note that a high temperature was predicted for each of the separations, in contrast to previous work which has suggested that lower temperatures were best for enantiomeric separations [5,30]. The use of higher temperature also facilitated rapid separations (all under 3.3 min). The best enantiomeric separation was not achieved at the highest concentration of s-β-CD in the parameter space, but at values around 12 mM. This agrees with previous work using cyclodextrins where it was found that enantiomeric resolution increased initially with increasing concentration of cyclodextrin up to some optimum value, after which the resolution tended to decrease [33,34].

3.3. Temperature calculations using selectivity effects

Fig. 8 shows the effect on selectivity arising from variation in the applied voltage. In conventional CE, the applied voltage affects the observed migration times of all analytes, with lower voltages corresponding to longer migration times (for co-EOF separations). This generally means that variations in voltage do not lead to selectivity changes. However, it can be seen in Fig. 8 that the migration order of the amino acids is dependent on the applied voltage. It can also be seen that the selectivity changes observed were very similar to those observed when changing the temperature at which the separations were performed. The separations shown in Fig. 8 were performed in a 50 μm i.d. capillary, whereas previous separations have used 25 μm i.d.

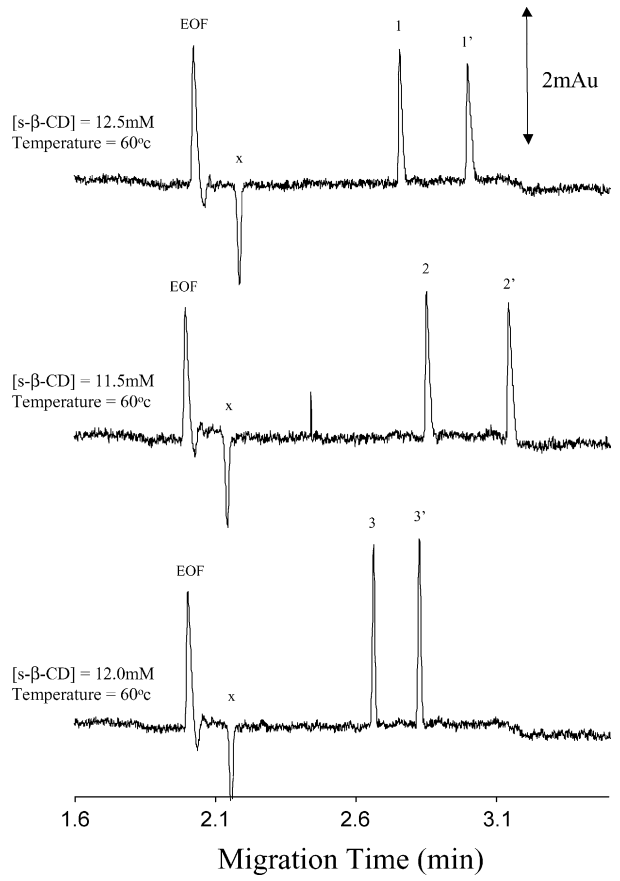

Fig. 7. Optimised enantiomeric separations for individual pairs amino acid enantiomers. Peaks are 1 = L-phenylalanine, 1′ = D-phenylalanine, 2 = L-tyrosine, 2′ = D-tyrosine, 3 = L-tryptophan, 3′ = D-tryptophan and *x* = system peak associated with the addition of s-β-CD. Conditions: 20 mM phosphate electrolyte at pH 2.0. Separation at +30 kV with detection at 210 nm. Capillary: 55 cm (46.5 cm to detector) × 25 μm.

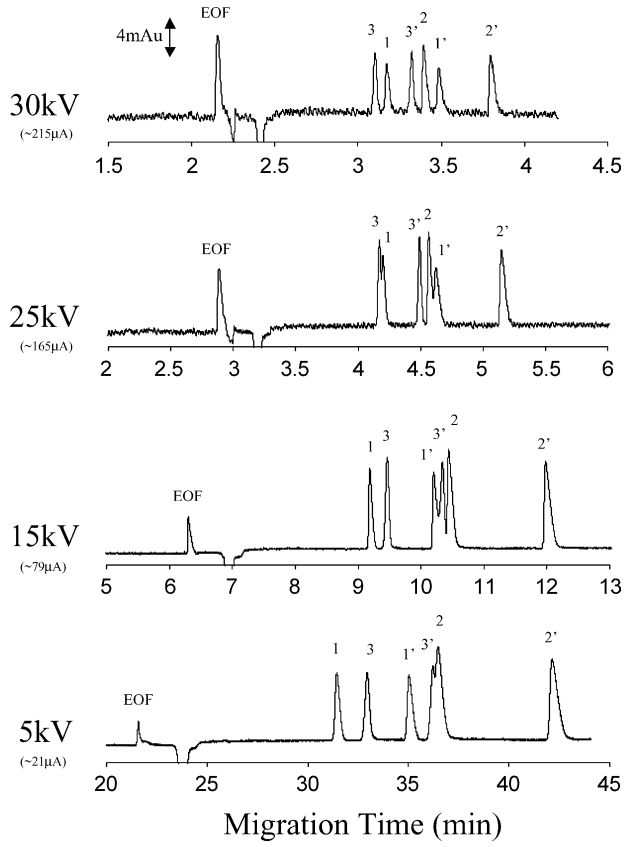

Fig. 8. Effect of voltage on the enantiomeric separation of the three amino acids. Peaks are 1 = L-phenylalanine, 1′ = D-phenylalanine, 2 = L-tyrosine, 2′ = D-tyrosine, 3 = L-tryptophan and 3′ = D-tryptophan. Conditions: 20 mM phosphate electrolyte + 15 mM s-β-CD at pH 2.0. Capillary: 55 cm (46.5 cm to detector) × 50 μm, kept at 25 °C.

capillaries. This increase in capillary diameter led to large increases in the operating current for applied voltages larger than 15 kV. The selectivity changes observed on increasing voltage were therefore most likely due to a corresponding increase in the temperature of the electrolyte within the capillary. The effect of temperature on the separation selectivity for L-phenylalanine and L-tryptophan is shown in Fig. 9. It can be seen that a linear relationship between selectivity and temperature was observed between 25 and 60 °C and this provides a means for estimating the temperature within the capillary, based solely on the relative migration positions of the two amino acids. It should be noted that L-phenylalanine and L-tryptophan were chosen here because they exhibited a relatively large selectivity change over the range of temperatures examined. Using this method, it was possible to predict the temperature within the capillary for the separations shown in Fig. 8, especially those performed at high operating currents (>100 μA) by comparing the observed selectivity coefficients with Fig. 9. Using this method the temperatures calculated for operating voltages of 20, 25 and 30 kV (corresponding to currents of approximately 117, 165

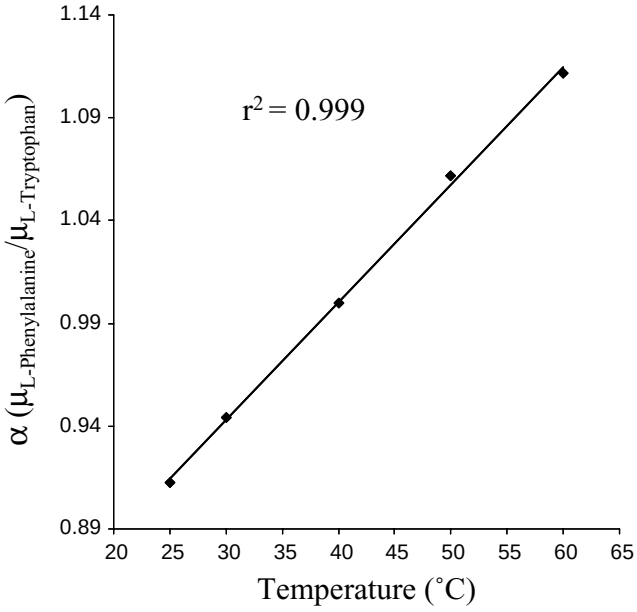

Fig. 9. Separation selectivity (α) vs. temperature for L-phenylalanine and L-tryptophan. Conditions: 20 mM phosphate electrolyte + 15 mM s-β-CD at pH 2.0. Separations undertaken at +30 kV while currents were ~41, 44, 50, 56 and 62 μA at 25, 30, 40, 50 and 60 °C, respectively. Capillary: 55 cm (46.5 cm to detector) × 25 μm.

and 215 μA) were 32.9, 43.7 and 51.2 °C, respectively. This shows that excessive operating currents can dramatically increase the internal temperatures within the capillary and can have dramatic effects on the observed selectivity. Temperatures were also calculated using the method outlined by Burgi et al. [35] where measured EOF values were related to changes in the electrolyte viscosity (which in turn were related to the temperatures within the capillary). Temperatures calculated using this method were 34.7, 41.5 and 52.2 °C and were within 2.2 °C of the temperatures calculated using the selectivity method.

4. Conclusions

The selectivity of enantiomeric separations for three aromatic amino acids (phenylalanine, tyrosine and tryptophan) can be varied predictably through the use of a sulphated-β-cyclodextrin combined with temperature control. The observed selectivity changes can also be successfully modelled using an artificial neural network, enabling optimisation of the separation. Since observed selectivity changes are related to temperature, this provided a means with which to estimate temperatures within the capillary at high operating currents and showed that when performing such separations, the effect of the increased temperature on the observed selectivity can be quite significant. It should be noted that performing separations at high operating currents may not be optimal for many application and hence the technique is best used as a diagnostic tool only rather than a method to control selectivity. The described system is potentially applicable to any cationic analytes, such as many pharmaceutically important compounds, that show appreciable interaction with sulphated-β-cyclodextrin. The technique could also be extended to other cyclodextrins which may show more interaction with target analytes.

References

[1] R. Kuhn, S. Hoffstetter-Kuhn, Capillary Electrophoresis: Principles and Practice, Springer, Berlin, 1993.
[2] S.F.Y. Li, Capillary Electrophoresis: Principles, Practice and Applications, Elsevier, Amsterdam, 1992.
[3] A. Rizzi, Electrophoresis 22 (2001) 3079.
[4] T. de Boer, R.A. de Zeeuw, G.J. de Jong, K. Ensing, Electrophoresis 21 (2000) 3220.
[5] W. Lindner, B. Bohs, V. Seidel, J. Chromatogr. A 697 (1995) 549.
[6] A.M. Stalcup, K. Gahm, Anal. Chem. 68 (1996) 1360.
[7] A. Gomez-Gomar, E. Ortega, C. Calvet, B. Andaluz, R. Merce, J. Frigola, J. Chromatogr. A 990 (2003) 91.
[8] M.D. Egger, Y. Liu, J. Sevcik, E. Tesarova, R. Rozhkov, R.C. Larock, D.W. Armstrong, Electrophoresis 24 (2003) 2650.
[9] C. Perrin, H. Fabre, M. Maftouh, D.L. Massart, Y. Vander Heyden, J. Chromatogr. A 1007 (2003) 165.
[10] K. Verleysen, S. Sabah, G. Scriba, A. Chen, P. Sandra, J. Chromatogr. A 824 (1998) 91.
[11] F. Suss, C.E. Sanger-van de Griend, G. Scriba, Electrophoresis 24 (2003) 1069.
[12] F. Suss, W. Poppitz, C.E. Sanger-van de Griend, G. Scriba, Electrophoresis 22 (2003) 2416.
[13] C. Perrin, Y. Vander Heyden, M. Maftouh, D.L. Massart, Electrophoresis 22 (2001) 3203.
[14] S. La, S. Ahn, J. Kim, J. Goto, O. Choi, K. Kim, Electrophoresis 23 (2002) 4123.
[15] P. Dzygiel, P. Wieczorek, J.A. Jonsson, J. Chromatogr. A 793 (1998) 414.
[16] S. Chinaka, S. Tanaka, N. Takayama, K. Komai, T. Ohshima, K. Ueda, J. Chromatogr. B 749 (2000) 111.
[17] S. Fanali, J. Chromatogr. A 792 (1997) 227.
[18] J. Havel, E.M. Pena, A. Rojas-Hernandez, J.-P. Doucet, A. Panaye, J. Chromatogr. A 793 (1998) 317.
[19] J. Havel, M.C. Breadmore, M. Macka, P.R. Haddad, J. Chromatogr. A 850 (1999) 345.
[20] L. Pokorna, A. Revilla, J. Havel, J. Patocka, Electrophoresis 20 (1999) 1993.
[21] V. Dohnal, M. Farkova, J. Havel, Chirality 11 (1999) 616.
[22] M. Jalali-Heravi, Z. Garkani-Nejad, J. Chromatogr. A 927 (2001) 211.
[23] M. Pacheco, J. Havel, Electrophoresis 23 (2002) 268.
[24] H. Katayama, Y. Ishihama, N. Asakawa, Anal. Chem. 70 (1998) 2254.
[25] P. Zakaria, M. Macka, P.R. Haddad, Electrophoresis 23 (2002) 1844.
[26] W. Jianjun, Z. Guojun, Y. Liu, S. Wanru, Analyst 126 (2001) 438.
[27] Y.T. Iwata, A. Garcia, T. Kanamori, H. Inoue, T. Kishi, I.S. Lurie, Electrophoresis 23 (2002) 1328.
[28] M. Muzikar, J. Havel, M. Macka, Electrophoresis 23 (2002) 1796.
[29] P. Zakaria, M. Macka, P.R. Haddad, J. Chromatogr. A 985 (2003) 493.
[30] A. Guttman, N. Cooke, J. Chromatogr. A 680 (1994) 157.
[31] M. Farkova, E.M. Pena-Mendez, J. Havel, J. Chromatogr. A 848 (1999) 365.
[32] V. Dohnal, H. Li, M. Farkova, J. Havel, Chirality 14 (2002) 509.
[33] S.A.C. Wren, R.C. Rowe, J. Chromatogr. 603 (1992) 235.
[34] S.A.C. Wren, J. Chromatogr. 636 (1993) 57.
[35] D.S. Burgi, K. Saloman, R. Chien, J. Liq. Chromatogr. 14 (1991) 847.