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Brush-type chiral stationary phase for enantioseparation of acidic compounds
Optimization of chiral capillary electrochromatographic parameters

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Abstract

The capillary electrochromatographic separations of three acidic enantiomers (carprofen, coumachlor and warfarin) were studied on a capillary column packed with 5 μm (3*R*,4*S*)-Whelk-O 1 chiral stationary phase. The influence of several experimental parameters (mobile phase pH, type of background electrolyte, acetonitrile ratio, temperature, applied voltage and ionic strength) on electroosmotic flow velocity, retention factor, selectivity factor, efficiency, resolution and effectiveness of chiral separation was evaluated. It was notable that the optimum resolution of the acidic enantiomers was achieved at pH 3.0 phosphate buffer, suggesting that capillary electrochromatography in the ion-suppressed mode can be applied for chiral separations of a range of acidic compounds.

Keywords: Chiral stationary phases, electrochromatography; Enantiomer separation; Electrochromatography; Carprofen; Coumachlor; Warfarin; Profens

1. Introduction

Capillary electrochromatography (CEC), an emerging analytical separation technique, is a hybrid of both high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) [1]. In CEC, the electroosmotic flow (EOF) is used to propel the mobile phase through the capillary column in one of three different formats: open tubular column [2], packed column [3], and monolithic column [4]. Due to the plug-like flow profile in the column, CEC combines the high efficiency of CE with high selectivity and high sample loading capacity of HPLC. Since Mayer et al. [5] first separated 1,1′ binaphthyl-2,2′-diylhydrogenphosphate and 1-phenylethanol enantiomers with open tubular CEC columns in 1992, chiral separation by CEC has attracted a growing interest in recent years [6].

A wide range of chiral stationary phases (CSPs), based on proteins [7], cyclodextrins [8], macrocyclic antibiotics [9,10], imprinted polymers [11], chiral acrylamides [12], polysaccharide derivatives [13], anion exchangers [14] and brush-type CSPs [15,16] have been used to pack CEC columns to address a greater range of chiral separation problems. Although in CEC, the use of (3*R*,4*S*)-Whelk-O 1 CSP

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has already demonstrated its high selectivity for a wide range of neutral enantiomers [15,16], there are no reports dealing with CEC enantioseparations of acidic compounds using this CSP. In particular, separation of acidic enantiomers in the ion-suppressed mode of CEC is seldom considered [17].

In this paper, the 5 μm (3*R*,4*S*)-Whelk-O 1 CSP was evaluated in ''ion-suppressed'' mode where low pH electrolytes were used for enantioseparation of acidic compounds in neutral forms. The experimental parameters such as type of background electrolyte (BGE), pH, organic solvent ratio, temperature, separation voltage and ionic strength were examined to deduce their effects on EOF, peak shape, retention factor, resolution, efficiency and selectivity as well as effectiveness of chiral separation.

2. Experimental

2.1. Reagents and chemicals

The 5 μm (3*R*,4*S*)-Whelk-O 1 CSP (pore size 100 Å) was a gift from Regis Technologies, Inc. (Morton Grove, IL, USA). Racemic carprofen, coumachlor and warfarin (Fig. 1), and thiourea (EOF marker) were purchased from Aldrich (Milwaukee, WI, USA). Acetonitrile (ACN) was of HPLC grade and purchased from Burdick and Jackson (Muskegon, MI, USA). Hydrochloric acid (HCl) was supplied by Fisher Scientific (Springfield, NJ, USA). Phosphoric acid (H_3PO_4) was purchased from EM Science (Gibbstown, NJ, USA). Sodium chloride (NaCl), 2-(4-morpholino)ethanesulfonic acid (MES), sodiumhydrogenphosphate (Na_2HPO_4) and sodiumdihydrogenphosphate (NaH_2PO_4), were of analytical grade and obtained from Sigma (St. Louis, MO, USA). Water used in all of the experiments was purified by a Barnstead Nanopure II Water System (Barnstead International, Dubuque, IA, USA).

2.2. Instrumentation and materials

All CEC enantioseparations were performed using an Agilent capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany) equipped with an autosampler, a diode-array detector and a Chemstation software. Throughout the CEC studies an

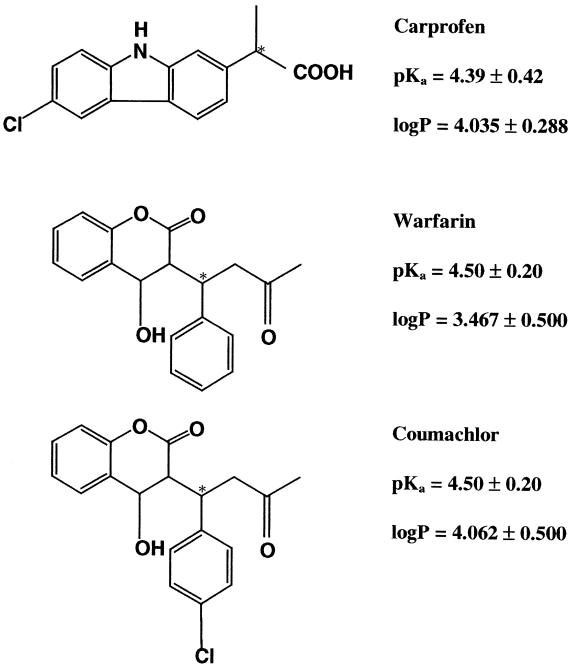

Fig. 1. The chemical structures, pK_a and log *P* values of carprofen, coumachlor and warfarin.

external pressure of 11 bar was applied to both inlet and outlet buffer vials. The samples were injected electrokinetically at 6.0 kV for 8.0 s. A Knauer pneumatic pump K-1900 (Wissenschaftliche Gerätebau, Knauer, Berlin, Germany) capable of operating up to 700 bar, was used to pack capillary columns. Production of retaining frits was carried out using a homemade frit burner.

2.3. Column preparation

Fused silica capillary (363 μm O.D., 75 μm I.D., obtained from Polymicro Technologies, Phoenix, AZ, USA) was slurry packed with (3*R*,4*S*)-Whelk-O 1 CSP [18]. The slurry was prepared by dispersing about 15 mg CSP in 0.3 ml ACN followed by sonication for 10 min. Subsequently, the slurry was transferred into a stainless steel packing reservoir. After fitting the capillary outlet with a 0.5-μm metal screening as a temporary frit, the capillary inlet was connected to the outlet of the reservoir. Using the pneumatic pump, the packing solvent, ACN, was delivered through the reservoir and capillary at 300

bar for a period of 12 h. During this entire period, the capillary was immersed in a sonicator to achieve homogenous packing. Prior to the frits fabrication, the capillary was flushed with 10 m*M* NaCl for 3 h at 300 bar. Using the same pressure, the inlet and outlet frits were prepared by applying heat on a small segment of the packed capillary with the frit burner for 14 s. After pumping ACN through the column, the residual particles behind the frits were flushed out. A detection window was fabricated by burning a 1–2 mm segment of polyimide coating of the unpacked segment just next to the outlet frit. The column with a packed bed length of 25.0 cm, unpacked length of 2.0 cm on the inlet side and 8.3 cm from detection end to the outlet end (total capillary length of 35.3 cm) was aligned on the cartridge. The column was preconditioned with the desired mobile phase for 60 min by applying 11 bar pressure from the inlet vial. Further conditioning was done with 11 bar pressure at both side of the column, slowly increasing the voltage by 5.0 kV each 20 min until reaching the maximum operating voltage of 30.0 kV and stable baseline.

2.4. Mobile phase and samples preparation

All mobile phases were obtained by first preparing neat aqueous buffers of desired pH as a 12.5 m*M* stock solution. The BGE buffer, MES buffer of desired pH was prepared with HCl; phosphate buffer of desired pH was prepared using either NaH_2PO_4 or Na_2HPO_4 solution with 12.5 m*M* H_3PO_4. The pH of the aqueous buffer was checked and adjusted on an Orion 420A pH meter (Beverly, MA, USA) before addition of an appropriate volume ratio of ACN. The final mobile phase was degassed for 30 min before use. Racemic solutions of each analyte were dissolved in ACN–water (60:40, v/v) at a concentration of about 2 mg/ml.

2.5. Calculations

The resolution (R_s) of enantiomers was calculated by the Chemstation software using the peak width at half the peak height method and given by:

$$R_s = \frac{2.35/2(t_{R2} - t_{R1})}{W_{50(1)} + W_{50(2)}} \quad (1)$$

where t_{R2} and t_{R1} are the retention times for the late and early eluting enantiomers, respectively; $W_{50(1)}$ and $W_{50(2)}$ are the peak width at half height for peak 1 and peak 2, respectively.

Chiral selectivity (α) was calculated using the equation:

$$\alpha = \frac{t_{R2}}{t_{R1}} \quad (2)$$

where t_{R2} and t_{R1} are the retention times for the late and early eluting enantiomers, respectively.

The separation efficiency and capacity factor of the late eluting enantiomer were calculated using the equations:

$$N_2 = 5.54\left(\frac{t_{R2}}{W_{50(2)}}\right)^2 \quad (3)$$

$$k'_2 = \frac{t_{R2} - t_o}{t_o} \quad (4)$$

where t_o is the retention time for thiourea.

The EOF velocity, u_{eof}, was calculated using the equation:

$$u_{eof} = \frac{L_{eff} + L_{inlet}}{t_o} \quad (5)$$

where L_{eff} represents the packed bed length of the capillary column; L_{inlet} is the unpacked length on the inlet side; t_o is the retention time of thiourea.

The effectiveness (E) of CEC enantiomeric separations, originally introduced by Berthod et al. for HPLC [19], was calculated using the equation:

$$E_2 = \frac{\alpha R_s}{k'_2} \quad (6)$$

where E_2 represents the E-factor of late eluting enantiomer. It should be noted that ''E'' factor was basically designed for just a quick screening of chromatographic quality criterion and has no thermodynamic or physical relevance.

3. Results and discussion

Most CEC applications are focused on separations of neutral and hydrophobic compounds; in order to broaden the CEC applications, it is necessary to study CEC separations for charged analytes [4]. In

contrast to CEC separation of neutral compounds, the CEC separation of acidic compounds using standard stationary phase is difficult [20]. This is because the acidic analytes are less retained at high pH by the stationary phase due to electrostatic repulsion with the negatively charged silanol groups of the packing material [17]. Moreover, the negatively charged analytes migrate opposite to the EOF and become undetectable if they have mobility greater than the EOF [17,21–23]. For these analytes, low buffer pH can suppress the analytes ionization and increase their interactions with the stationary phase, thus better separations can be achieved. However, it should be noted that ion-suppression mode is operational only when the pH of the mobile phase is well below the pK_a of the acidic compounds [17].

After an initial chiral screening of 10 acidic compounds using MES buffer at pH 6.0 and phosphate buffer at pH 4.4 [16], three acidic chiral drugs, carprofen, coumachlor and warfarin showed some degree of enantioselectivity using (3*R*,4*S*)-Whelk-O 1 CSP. Therefore, the three aforementioned compounds were chosen as model test analytes to study the effect of several experimental parameters (mobile phase pH, ACN content, temperature, applied voltage and ionic strength) on the EOF velocity, k_2', N_2, α, R_s and E_2 value.

3.1. Effects of pH and type of BGE of mobile phase

In order to study the effects of buffer pH on the enantiomeric separation, CEC of all acidic enantiomers were performed in the mobile phases containing 80% (v/v) ACN along with 2.5 m*M* MES or phosphate buffer, each in pH range of 3.0–7.0. Although MES is not ideal BGE to study at lower pH range of 3–4 (MES pK_a = 6.1), it was chosen because a previous study by Wolf et al. [16] indicated that MES buffer provides higher enantioselectivity of neutral compounds compared to acetate or phosphate buffer.

From the EOF velocity vs. pH plot (Fig. 2 inset), it is clear that as the pH of the mobile phase buffer increased from 3.0 to 7.0, the EOF velocity showed a general increasing trend, which is caused by the expected dissociation of silanol groups (pK_a 2–4) and hence zeta potential increased at higher pH values [24,25]. It should be noted that at pH 3.0, (3*R*,4*S*)-Whelk-O 1 CSP can still provide reasonable EOF velocity [45 mm/min for MES and 55 mm/min for phosphate buffer, respectively (Fig. 2 inset)] which was sufficient for CEC separations. Furthermore, due to different ion mobility of BGE, the phosphate buffer and MES buffer yielded much different EOF velocities at the same pH. Additionally, the pK_a value of BGEs and ionic strength seemed to play a role in the profile of EOF velocity vs. pH plot. For example, when the mobile phase containing monobasic phosphate (natural pH of 4.3) was titrated with H_3PO_4, a significant decrease of EOF velocity was observed as buffer pH decreased from 4.3 (natural pH of NaH_2PO_4) to 3.0, which is probably related to a synergistic effect of increase in ionic strength and a decrease in mobility of phosphate ion. Similarly, using MES (pK_a = 6.1 for basic amine functional group) as BGE and by adding HCl to decrease the pH value from 7.0 to 6.0, a significant reduction in EOF was observed.

The carprofen chromatograms shown in Fig. 2a–c using MES buffer indicated that pH 3.0 yielded the best R_s, while pH 7.0 provided the lowest R_s value. Compared to MES buffer, the R_s of carprofen enantiomers with phosphate buffer was always higher under the same experimental pH values of 3.0 and 5.0 (Fig. 2d–e). However, the data for the two BGE cannot be compared at pH 7.0 since peak splitting was observed with a phosphate buffer at this pH value (Fig. 2f). Using MES buffer, the retention time of carprofen enantiomers were longer at pH 5.0 than at pH 3.0, which can be explained by an increase in ionization of acidic carprofen enantiomers (pK_a = 4.39 ± 0.42). In this respect, increased ionization increased the electrophoretic mobility of negatively charged carprofen towards the anodic end (injection end in normal polarity CEC), which in turn delayed its elution towards the cathodic end (detection end in normal polarity CEC). It is interesting to note that using phosphate buffer over the same pH range caused only a very slight increase in the retention time (Fig. 2d,e). With pH 7.0 MES buffer (Fig. 2c), the high EOF velocity was probably contributing to the decrease of retention time and R_s of carprofen; while using phosphate buffer at the same pH (Fig.

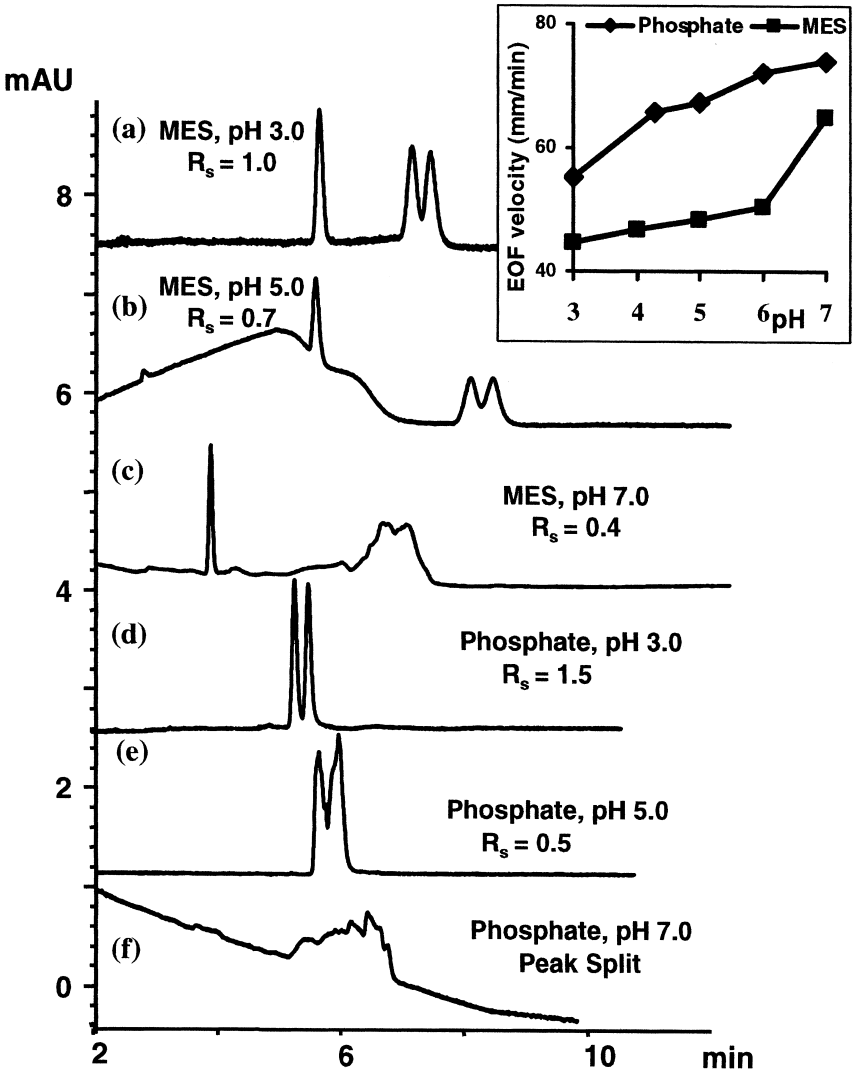

Fig. 2. Electropherograms (a–f) showing the effect of pH and type of background electrolyte on separation of carprofen enantiomers. Inset: EOF velocity at various pH values. Conditions: mobile phase, ACN–water (80:20, v/v) containing 2.5 m*M* BGE, 25 °C, separation voltage 30 kV.

2f), retention time and R_s were difficult to measure due to peak splitting.

Similar to carprofen, the other two acidic analytes, coumachlor and warfarin, also provided the maximum R_s value with a mobile phase containing phosphate buffer pH 3.0 (Table 1). The improved R_s values of carprofen, coumachlor and warfarin enantiomers at low pH values using either phosphate buffer or MES can be explained with the ion-suppressed mode [17]. In this mode, when the buffer pH was decreased below the pK_a of the acidic analytes (Fig. 1), the effective charge of acidic analytes was practically zero due to suppressed ionization. Therefore, the uncharged analytes enhanced the analyte–chiral selector interaction [26]. Additionally, the electrostatic repulsion effects due to the negatively charged residual silanol groups on this CSP were also suppressed. Thus, the neutral enantiomers of carprofen, coumachlor and warfarin interacted strongly with the CSP under such low pH conditions.

Table 1
Effect of pH and type of BGE on the chromatographic data for enantioseparation of coumachlor and warfarin[a]

Analyte	Buffer	pH	R_s	α	N_2
Coumachlor	Phosphate	3.0	3.8	1.11	22 000
		5.0	1.8	1.11	4000
		7.0	–[b]		
	MES	3.0	1.1	1.08	4000
		5.0	0.6	1.07	2000
		7.0	–[b]		
Warfarin	Phosphate	3.0	1.8	1.05	22 000
		5.0	1.3	1.05	10 000
		7.0	–[b]		
	MES	3.0	1.0	1.04	10 000
		5.0	0.4	1.03	7000
		7.0	–[b]		

[a] Conditions: mobile phase, ACN–water (80:20, v/v) containing 2.5 m*M* BGE, 25.0 °C, 30 kV.
[b] No data available due to split peaks.

Consequently, improved resolution with higher efficiency was achieved at pH 3.0 and the results shown in Table 1 confirm this point. It is important to note that although MES worked well in the system at pH 3.0 (Fig. 2a), MES is not normally used at this pH because pH 3.0 is outside the normal range (pH 5.1–7.1) of MES buffering capacity.

3.2. Effects of ACN concentration

In this set of experiments, the ionic strength and pH of the mobile phase (2.5 m*M* phosphate buffer, pH 3.0) were fixed and the volume fraction of ACN was varied from 40 to 80% (v/v). Fig. 3 illustrates the effects of ACN content of the mobile phase on the EOF velocity, k_2', N_2, α, R_s and E_2 values. As shown in Fig. 3a, EOF velocity first decreased slightly from ~48 to ~47 mm/min when %ACN increased from 40 to 60% (v/v). Further increase in %ACN higher than 60% (v/v) caused a dramatic increase in the EOF velocity due to the increase ratio of permittivity to viscosity (ε_r/η) of the eluent, and the variation on the packing surface charge [27,28]. In general, the low EOF velocity for the 50% and 60% (v/v) ACN is probably contributing to the low separation efficiency (N_2) for mobile phases containing ACN in the same range (Fig. 3c).

Both k_2' (Fig. 3b) and α values (Fig. 3d) decreased when the ACN percentage increased from 40 to 80% (v/v). Similar to reversed-phase HPLC, the trend of k_2' can be explained by a general decrease in polarity of the eluent, the hydrophobic interactions, and hence the distribution coefficient between the enantiomers and the CSP are reduced. Since the decreasing k_2' weakened the interaction of the enantiomers with the CSP, the α value decreased. The combined effects of decreasing k_2', α and N_2, decreased R_s significantly when %ACN increased from 40 to 60% (v/v) (Fig. 3e). However, as ACN content was increased further from 60 to 80% (v/v), the k_2' and α value decreased while N_2 increased (due to the increase in EOF) and R_s decreased slightly. This trend of R_s was most likely due to an increase in N_2 value, which seemed to offset any decrease in R_s caused by decreasing k_2' and α values. Therefore, the net result was a fairly constant R_s value over the range 60–80% ACN (v/v). Furthermore, the k_2', α and R_s curve of faster migrating enantiomers (warfarin and carprofen) showed less sensitivity to the variation of the %ACN (v/v) than slower migrating enantiomers (coumachlor). This is probably due to the lower hydrophobicity of the former two chiral analytes (Fig. 1).

Although 80% (v/v) of ACN appeared to provide the highest value of E_2 (Fig. 3f), a mobile phase containing this volume fraction is not suitable for simultaneous separation and enantioseparation of warfarin and coumachlor (Fig. 4a). In view of that, a mobile phase containing 60% ACN (v/v) seemed suitable as it provided the simultaneous separation of both warfarin and coumachlor enantiomers despite the fact that it provided moderate E_2 values. The use of 40% (v/v) ACN enhanced the enantioseparation significantly, however, it took almost 60 min to accomplish the simultaneous separation of warfarin and coumachlor enantiomers (Fig. 4c). Moreover, the lowest E_2 value indicated a very low effectiveness for 40% (v/v) ACN. Therefore, mobile phases containing 80% (v/v) and 60% (v/v) ACN were chosen to optimize the other chiral CEC parameters.

3.3. Effect of separation temperature

The influence of the temperature on the EOF velocity, k_2', N_2, α, R_s and E_2 value of the three acidic chiral analytes was investigated at three

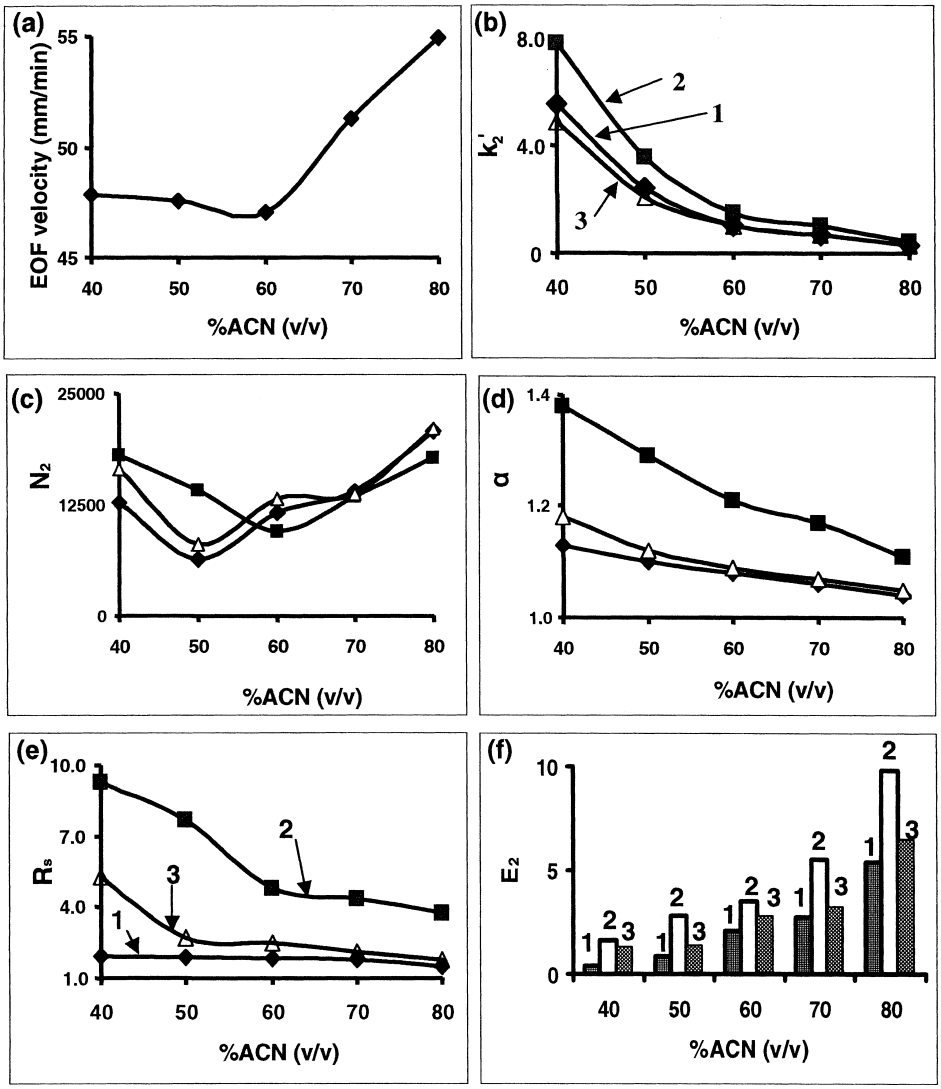

Fig. 3. Plots showing the effect of variation of the organic modifier, ACN, concentration on (a) EOF velocity, (b) k'_2, (c) N_2, (d) α, (e) R_s and (f) E_2, for the chiral separations of carprofen, coumachlor and warfarin. Conditions: mobile phase, variable ratio of ACN–water containing 2.5 m*M* phosphate buffer, pH 3.0, 25 °C, separation voltage 30 kV. Legend: carprofen 1; coumachlor 2; warfarin 3.

different column temperatures: 16.3, 25.0 and 35.0 °C.

For both 80% (v/v) ACN and 60% (v/v) ACN, a rise in temperature in packed capillary resulted in general increase in the EOF velocity, slight decrease in k'_2 as well as somewhat lower R_s and α values (data not shown). The enhancement of EOF velocity with higher temperature could be explained by the decrease of mobile phase viscosity (η) and increase of ε_r/η ratio at higher temperatures [28]. The trends in R_s values using either 80% (v/v) ACN or 60% (v/v) ACN seemed to be analyte dependent. For example, the effect of temperature on R_s values of early eluting enatiomers of carprofen and warfarin was negligible. However, compared to 80% (v/v) ACN, which showed only a slight decrease in R_s of late eluting enantiomers of coumachlor with increase in temperature, the decrease in R_s is more pro-

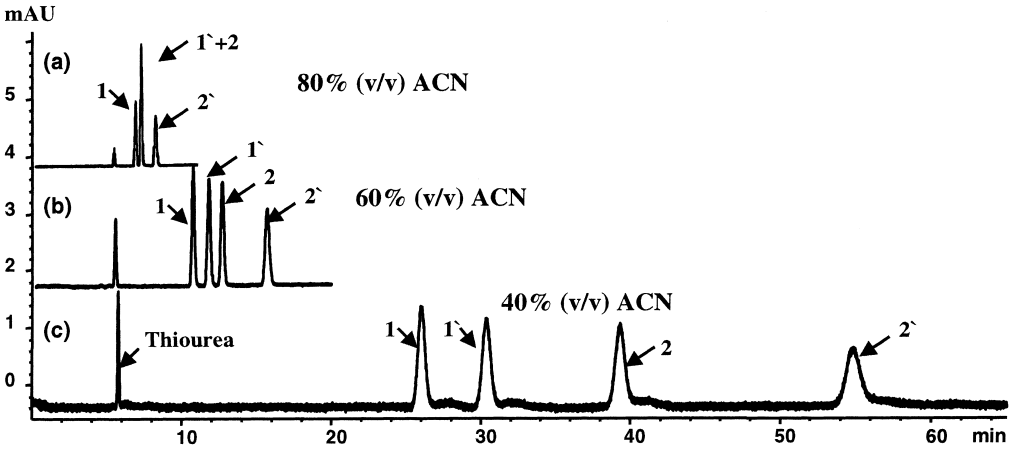

Fig. 4. Electropherograms showing the effect of %ACN on simultaneous enantio-separations of warfarin (1,1′) and coumachlor (2,2′). Conditions: mobile phase ACN–water (a) 80:20 (v/v), (b) 60:40 (v/v), (c) 40:60 (v/v), containing 2.5 m*M* phosphate buffer, pH 3.0, 25 °C, separation voltage 30 kV.

nounced for the same analyte at 60% (v/v) ACN. The influence of temperature on efficiency appeared to be mainly dependant on the concentration of ACN in the mobile phase and to a lesser extent on the type of analytes. While all three chiral analytes showed a maximum N_2 value at 25.0 °C using 80% (v/v) ACN mobile phase, the same trend was not observed with 60% (v/v) ACN mobile phase. In addition, the plots of α vs. column temperature were almost flat, indicating that, in the range studied, temperature has no significant effect on enantioselectivity. Similarly, the E_2 values of both 80% (v/v) ACN and 60% (v/v) ACN, respectively, remained fairly constant as a function of temperature. It appeared that R_s and k_2' compensate each other while α remains fairly constant with an increase in temperature.

3.4. Effects of separation voltage

The data for Van Deemter curve of thiourea (Fig. 5) was obtained by using 60% (v/v) ACN mobile phase containing 2.5 m*M* phosphate buffer (pH 3.0) with applied voltage varied from 1 to 30 kV. As expected, with increasing separation voltage, the linear flow-rate increased and plate height decreased sharply and then at a voltage higher than 10 kV, it became fairly constant. Thus, in contrast to chiral HPLC, the use of EOF in chiral CEC allows the use of a relatively high linear velocity of the mobile phase without a detrimental effect on efficiency. However, it should be noted that at a higher voltage range (20–30 kV, data not shown), the EOF velocity showed a noticeable deviation from linearity, probably due to decreasing viscosity caused by Joule heating [29].

The effects of applied voltage on the EOF velocity, k_2', N_2, α, R_s and E_2 value of the three acidic chiral analytes were also studied over the range 10–30 kV with 80 and 60% (v/v) ACN (data not

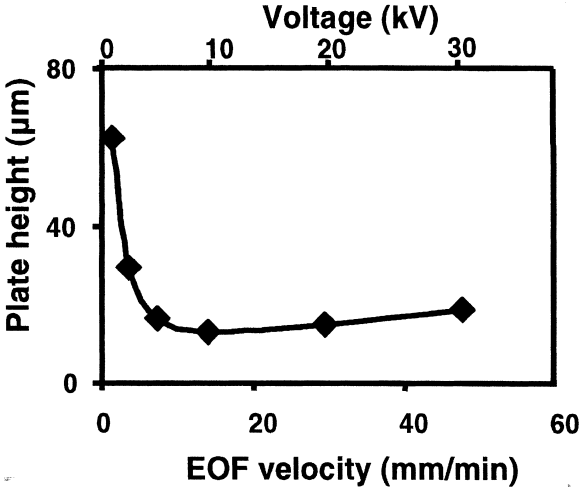

Fig. 5. Influence of separation voltage on plate height using thiourea as the EOF marker. Conditions: mobile phase, ACN–water (60:40, v/v) containing 2.5 m*M* phosphate, pH 3.0, 16.3 °C.

shown). For both mobile phases, a rise in separation voltage resulted in a linear increase of the EOF velocity. As expected, solutes migrated faster in a high electric field, with increase in applied voltage that resulted in a slight decrease of the k_2' values. For example, an 11% decrease in k_2' of warfarin was observed at 80% (v/v) ACN upon varying the voltage from 10 to 30 kV whereas the k_2' values of the same analyte were decreased by 18% at 60% (v/v) ACN. The efficiency (N_2) also decreased as the applied voltage increased at both 80% (v/v) ACN and 60% (v/v) ACN. This was probably related to an increase of EOF velocity at higher voltages. The maximum N_2 achieved at 10 kV could be explained by the minimum plate height obtained at the same voltage in the Van Deemter curve (Fig. 5). Unlike the α values, which remained constant, the R_s values showed a declining trend partially associated with the decreasing separation efficiency. Overall, 10 kV provided slightly higher E_2 values for both 80% (v/v) and 60% (v/v) ACN, probably due to high efficiency and low Joule heating effects. However, it is worth mentioning that 10 kV results in much longer analysis time than 30 kV. Therefore, using an applied voltage of 30 kV, reasonable efficiency and resolution values can be achieved with a shorter analysis time.

3.5. Effects of ionic strength

Fig. 6 shows the effects of ionic strength on the EOF velocity, k_2', N_2, α, R_s and E_2 value of the three enantiomers using 60% (v/v) ACN, pH 3.0, containing 1.0–5.0 m*M* phosphate buffer. The EOF velocity (Fig. 6a) decreased from 58 to 34 mm/min with increasing phosphate buffer concentration, primarily because of the decrease of double layer thickness and increase of viscosity [29]. Likewise, the k_2' values (Fig. 6b) also showed a decreasing trend while the ionic strength increased, consistent with the results of Rathore et al. [29], Cahours et al. [30], and Moffatt et al. [31]. This suggested that the magnitude of EOF velocity decrease was well below the magnitude of other retention decreasing effects. On the contrary, decreasing EOF velocity and k_2', N_2 (Fig. 6c) increased as phosphate buffer concentration increased from 1.0 to 5.0 m*M*. Because the ionic strength had a similar effect to the applied voltage [32]; when the phosphate buffer concentration increased, the EOF velocity decreased, thus higher efficiency was achieved. Although α remained fairly constant (Fig. 6d), high efficiency resisted the peak band-broadening, therefore the resolution increased (Fig. 6e) as the ionic strength increased.

As revealed in Fig. 6f, the increase of ionic strength also increased the E_2 value due to the increasing R_s and decreasing k_2' values. It seemed that the highest ionic strength contributed to the most effective separation. However, one should note that, besides the effects mentioned above, increasing the concentration of phosphate buffer above 5.0 m*M* also raised the electric current, which led to some drawbacks like Joule heating or bubble formation [20]. In addition, the upper limit of buffer concentration is also limited by the BGE solubility in the mobile phase. For these reasons, 5.0 m*M* phosphate buffer was selected as the optimum concentration for CEC separation of the investigated chiral analytes.

4. Conclusions

In conclusion, we demonstrated that the CEC separations of acidic enantiomers in neutral forms could be achieved using a commercially available (3*R*,4*S*)-Whelk-O 1 CSP. The facts that the best chiral separations were achieved with low pH phosphate buffer (pH 3.0) demonstrated the importance of ion-suppressed mode and BGE type for the separation of acidic enantiomer in CEC. Although there were no significant differences on the selectivity of acidic enantiomers when phosphate or MES buffer at the same pH was used, the use of MES buffer provided lower R_s values. Similar to HPLC, the use of ACN influenced the enantiomeric resolution and capacity factor of enantiomers. Temperature had little influence on selectivity and effectiveness of the enantioseparations, however, lower temperature led to higher resolution values. The other two operating parameters, applied voltage and ionic strength, affected both resolution and effectiveness through affecting the EOF velocity and separation efficiency. By carefully tuning the aforementioned parameters, chiral CEC separations were optimized. This optimized procedure for CEC revealed that the use of a pH 3.0 phosphate buffer with a concen-

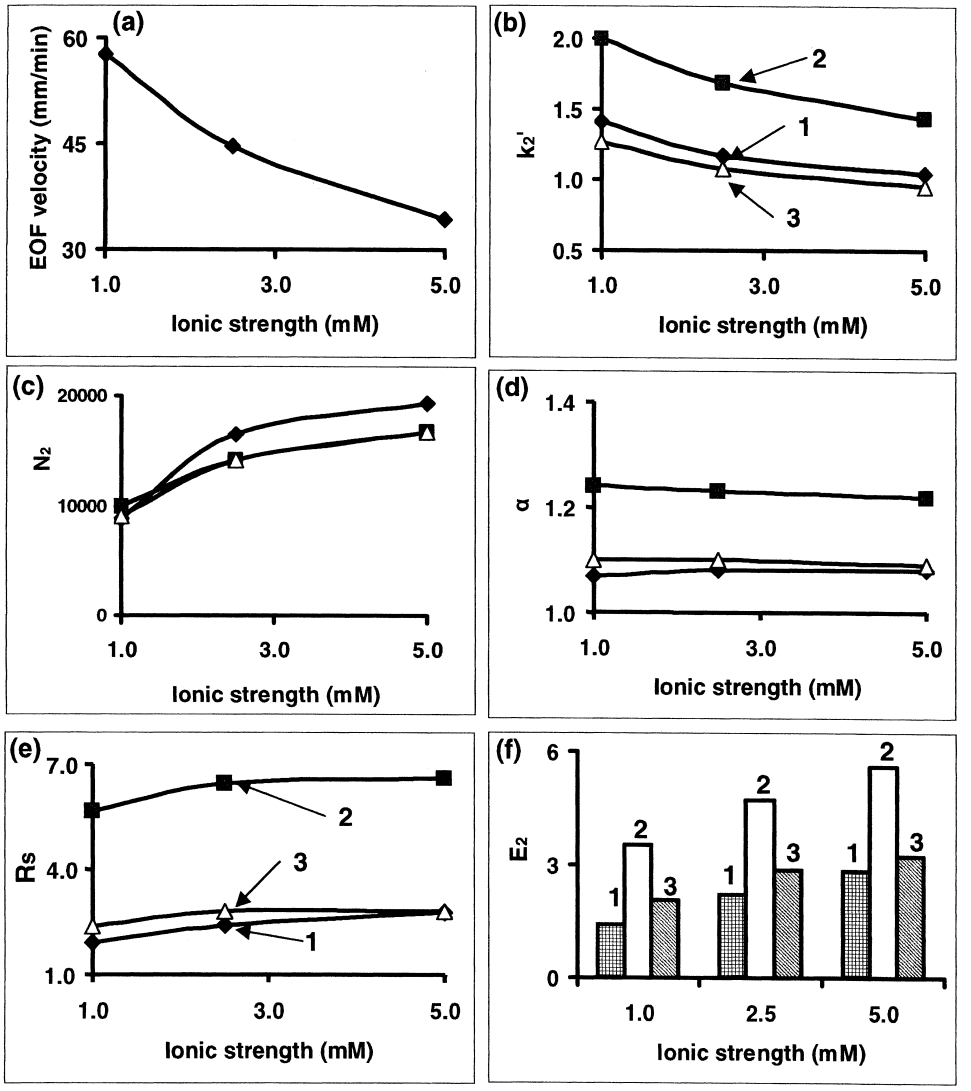

Fig. 6. Plots showing the effect of variation of the ionic strength upon (a) EOF velocity, (b) k_2', (c) N_2, (d) α, (e) R_s and (f) E_2, for the chiral separations of carprofen (1), coumachlor (2) and warfarin (3). Conditions: mobile phase ACN–water (60:40, v/v) containing various concentration of phosphate buffer, pH 3.0, 16.3 °C, separation voltage 30 kV. Legend: carprofen 1; coumachlor 2; warfarin 3.

tration of 5.0 m*M* at 60% (v/v) ACN, 30 kV and 16.3 °C provided the most effective chiral separation of the studied acidic enantiomers. Further research on the application of (3*R*,4*S*)-Whelk-O 1 CSP for chiral CEC-MS is underway in our laboratory.

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