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Determination of trace enantiomeric impurities in chiral compounds by capillary electrophoresis with uncoated, cationic, anionic and neutral surface modified capillaries

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Abstract

The usefulness of commercially available polyamine, sulphonic acid and neutral coated capillaries is demonstrated for the resolution of chiral drugs. The methods, using cyclodextrins and their derivatives are developed from conventional fused-silica capillary procedures without any changes to the procedures. Different coatings can be used to adjust the order of elution of enantiomers when one of the enantiomers is present as a trace level impurity. Limit of quantitation levels of $<0.1\%$ (m/m) have been illustrated for D- or L-tryptophan in the presence of the other enantiomer with acceptable levels of reproducibility. Similar levels have been detected for warfarin and propranolol, using a polyamine-coated capillary. The coated capillaries were stable to long-term changes in the electrolyte and its pH value and the methods were interchangeable with capillaries obtained from different batches.

Keywords: Enantiomer separation; Pharmaceutical analysis; Coated capillaries; Propranolol; Warfarin; Tryptophan

1. Introduction

The resolution of chiral, drugs, metabolites and related substances continues to be an important area in pharmaceutical analysis. In many instances pharmaceutical companies decide to progress only single enantiomers as new chemical entities. There are a number of analytical techniques which give reliable and stable assays for chiral drugs, as racemic mixtures or when variable enantiomeric ratios exist. Capillary electrophoresis (CE) has been shown to be particularly successful in this area [1–4].

In most cases, direct separation of the chiral drug has been obtained by capillary zone electrophoresis (CZE) through the addition of an enantioselector to the electrolyte. However, alternative approaches of immobilising the chiral selectors onto the capillary wall [5] or packing a chiral stationary phase into the capillary [6], are increasing in popularity. In these separations a diverse range of cyclodextrin (CD) additives has been employed including charged and uncharged CDs [7] and chemically modified CD derivatives [8,9].

Despite these successful CE applications the resolution of the enantiomers of basic chiral drugs can still suffer from problems such as peak dispersion and peak tailing which can be caused by analyte–surface interactions with the capillary wall. This tailing even occurs at low pH for strongly basic drugs. In order to reduce these surface effects and/or

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improve resolution, procedures for partial or full suppression of the wall interactions have been demonstrated. Modification or deactivation of the wall capillary surface has been carried out through 'dynamic' or 'permanent' surface coating. In dynamic modification surfactants or polymers are incorporated into the electrolyte. Interaction of these additives can be as a loose attachment or the modifier can become strongly coated onto the capillary wall [10].

Permanent coating is generally carried out through covalent bonding to the capillary surface or through adsorption with physical or chemical immobilisation procedures such as cross-linking. Modification of the fused-silica surfaces is generally associated with the suppression, or even reversal, of the electroosmotic flow (EOF). In the first case limiting the EOF has the advantage of reducing the nonseparative migration speed and has been shown to improve chiral resolutions of basic drugs at the expense of longer analysis times [11,12]. An alternative is to generate a positive charge on the capillary wall by permanently coating the capillary with cationic polyamines [13]. The cationic basic drugs are therefore repelled from the wall, which reduces the interaction effects. The positive charge on the capillary wall then generates a strong reversed EOF flow. Positively charged capillary walls can be dynamically generated using suitable additives to the electrolyte. These additives include long-chain cationic surfactants [14,15], as tetradecyltrimethylammonium bromide (TTAB).

Highly negatively charged capillary walls can be generated with sulphonic acid functions. The sulphonic acid group is fully ionised over a wide pH range (pK_a~2), compared to the pK_a of a silanol group, which is around pH 5–6, which reduces the pH dependence on the EOF.

Coated capillaries offer the possibility of manipulating and improving chiral drug separations and can be important in single enantiomer purity studies where it is helpful if the minor enantiomer migrates before the principal component.

Although coated capillaries and different wall immobilised commercial capillaries have been available for some years their stability and restriction on operating pH range have made it difficult to obtain an optimum separation of components. However, advances in the development of stable coatings have been illustrated such as polyamine-coated capillaries [10] which were stable over an extended time period under a wide range of pH conditions not only for basic drugs but also neutral and acidic compounds.

The object of this study was to evaluate the applicability of a range of coated capillaries to the determination of trace level enantiomers in both acidic and basic drugs. Three different commercially available coated capillary types were evaluated, a polyamine cationic capillary surface, a sulphonic acid anionic coated and a neutral surface coated capillary which was used for comparison and gave a low EOF over a wide pH range.

The results of the studies are compared with those from a conventional uncoated fused-silica capillary. The chiral test compounds were propranolol, warfarin and tryptophan which gave enantioselective migration in the presence of CDs and their derivatives under different CE conditions.

2. Experimental

A Beckman P/ACE system 2210 (Beckman Instruments (UK) Ltd, High Wycombe, UK) was used in all experiments. Positively charged capillaries were generated using eCAP capillaries with a Polyamine Regenerator solution, and neutral coated capillaries, 37 cm (30 cm to the detector)×50 μm I.D., which were obtained from Beckman. The polyamine coating was dynamically generated by first creating a fully ionised silica surface by washing with 1 *M* sodium hydroxide (2 min) and then flushing with concentrated polyamine regeneration solution (2 min), and rinsing with the test buffer. The samples were analysed on the amine capillary using reversed polarity. Anionic sulphonic acid capillaries (37 cm (30 cm to the detector)×75 μm) were purchased from Electro-Kinetic Technologies (EKT, Capital HPLC, Bathgate, UK) while the uncoated capillaries were obtained from Composite Metal Services (Hallow, UK).

The chiral selectors heptakis (2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD), and hydroxypropyl β-cyclodextrin (HP-β-CD) were purchased from Sigma–Aldrich (Poole, UK). α-Cyclodextrin was purchased from Fluka (Gillingham, UK). Propranolol hydrochloride, tryptophan, and warfarin were obtained from Sigma–Aldrich.

The capillaries were used within the pH ranges recommended by the suppliers. The uncoated capillaries were washed for 1.5 h with 1 *M* sodium hydroxide, 1 h with 0.1 *M* sodium hydroxide and 30 min with water, to fully activate the surface silanols prior to initial use. Propranolol hydrochloride samples were dissolved in double distilled water, racemic tryptophan (and its individual enantiomers) were prepared in methanol–water (30:70, v/v) and warfarin in methanol–water (45:55 v/v). Sample concentrations were 100 μg/ml.

3. Result and discussion

3.1. Chiral resolution of propranolol

Samples of the racemic aminoalcohol, propranolol hydrochloride were efficiently resolved on both the uncoated and polyamine coated capillary using a 40 m*M* Tris–phosphoric acid electrolyte containing 10 m*M* HP-β-CD. Maximum resolution was obtained at pH 2.4 (adjusted with concentrated orthophosphoric acid). A negative voltage was used with the amine coated capillary due to the reversed direction of the EOF. The same electrolyte at pH 3.0 (adjusted with concentrated orthophosphoric acid) gave separation with the neutral capillary. On this capillary the operating pH was limited, due to the supplier suggested pH range restriction of 3–8.

Using these capillaries, the *R*-enantiomer of propranolol was successfully determined, when present as the minor component (1:99, m/m), in *S*-propanolol. Fig. 1a–c shows the resolutions obtained on the uncoated, amine coated and neutral coated capillaries. The migration order for the uncoated and neutral capillaries was *S,R* and for the amine capillary a reversed order of *R,S* applies as the negative voltage was due to the reversed EOF direction. The longer analytical time for the neutral coated capillary (Fig. 1c) was due to the large reduction of EOF. Quantitation of the *R*-enantiomer below 1% (m/m) levels was difficult using the uncoated or neutral capillary as peak tailing occurred on the uncoated capillary, due to some interactions of the drug cation with capillary silanols. However, detection at $<0.5\%$ (m/m) level was possible with the polyamine capillary and with further optimisation it was expected that 0.1% would be achievable. The anionic coated capillary was also examined for this separation, but this gave very poor performance as the positively charged propanolol has strong undesired interactions with the highly negatively charged sulphonic acid groups.

Wren [16] reported that variations in CD concentration can maximise mobility differences for chiral resolutions. However, the resolution of propranolol on the polyamine capillary was unaffected by the concentration of HP-β-CD over the range of concentration 5–20 m*M*, whereas the uncoated capillary gave a distinct resolution maxima at 10 m*M* for HP-β-CD. Therefore, it was concluded that the polyamine coated capillary would provide a more robust assay than the uncoated capillary.

3.2. Chiral resolution of tryptophan

Trace levels of D-tryptophan present in L-tryptophan were determined on the three capillary types using an electrolyte of 40 m*M* Tris–phosphoric acid containing 50 m*M* α-CD. Limits of detection (LOD) for D-tryptophan of 0.5% and 0.35% (m/m) were determined for the uncoated and neutral capillaries. The LOD was measured as a peak giving a signal 3 times the baseline noise. The resolution was improved in the neutral capillary by the reduction in residual EOF but at the expense of a significant increase in analysis time. The polyamine coated capillary again gave a reversed migration order, with the D-enantiomer being the first migrating peak.

The tryptophan separation was further optimised by examining the buffer concentration (20–60 m*M*), the level of chiral selector, voltage level and temperature. A buffer concentration of 40 m*M* was chosen as giving the best resolution, above this concentration the current rose above an acceptable level. An optimum was found at 75 m*M* α-CD for both the uncoated and polyamine coated capillaries. An increase in temperature resulted in a decrease in the binding constants between CD and the analytes and produced a diminished resolution. In order to improve the capability to transfer the method between laboratories the operating temperature was set at 30°C. The LODs were reassessed for the optimised conditions. The polyamine capillary improved the LOD of the minor D-enantiomer to 0.03% (m/m)

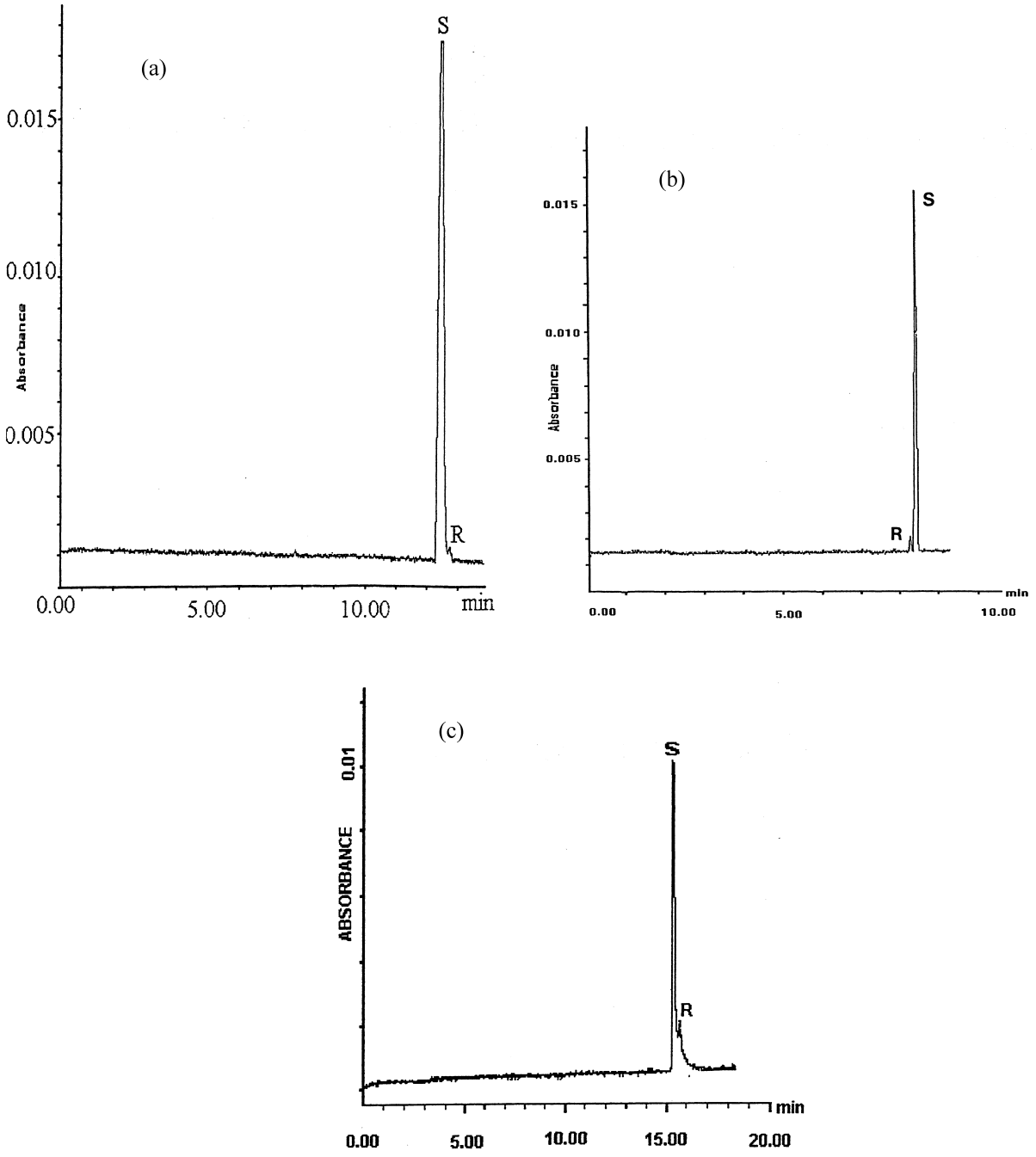

Fig. 1. Determination of trace levels of *R*-propranolol in *S*-propranolol (1:99%, m/m) with three different coated capillaries. The capillaries were (a) conventional fused-silica capillary, (b) polyamine-coated capillary, (c) neutral polymer coated capillary. Operating conditions: 40 m*M* Tris–phosphoric acid buffer containing 10 m*M* hydroxypropyl-β-CD (pH 2.4), capillary dimensions 37 cm (30 cm to detector)×50 μm, detection wavelength 200 nm; and injection time, 2 s, with an operating temperature of 22°C. Applied voltage was +15 kV.

(LOD) and limit of quantitation (LOQ) of 0.1% m/m (10× baseline noise) (Fig. 2a) whilst an LOD of 0.3% (m/m) was obtained on the uncoated capillary.

To check the polyamine coated capillary method for suitability for routine analysis, the method was validated for selectivity, precision, linearity and reproducibility. Aspartame was used as an internal standard to improve injection precision as it was intended to routinely assay both the content and enantiomeric purity of tryptophan samples. Fig. 2b shows the resolution of tryptophan and aspartame.

Spiking with the individually pure enantiomers, was used to demonstrate selectivity and migration order. Linearity of detector response (peak area ratios) for L-tryptophan was tested over the range 250–700 μg/ml which represented 50–150% m/m) of the concentration of the D-enantiomer present. Six concentrations were taken in the range and each contained the aspartame internal standard at 0.2 mg/ml. The linearity data for L-tryptophan to aspartame was $y=0.005x+0.0266$ ($r=0.999$, $n=6$).

The method was capable of detecting 0.05% (m/m) of either enantiomer in the presence of the major enantiomer. For the L-enantiomer this is contrary to the normal expectation that improved performance will result with closely migrating peaks when the minor enantiomer is the first detected peak. From the data the LOQ for the L-tryptophan in D-tryptophan was established at 0.1% (m/m) with a repeatability of R.S.D.=7.29% ($n=10$). For the racemic mixture the repeatability of the L-enantiomer main peak area was R.S.D.=0.55% ($n=10$) at a concentration of 500 μg/ml which is the nominal concentration set in the assay method for the major component.

3.3. Chiral resolution of warfarin

Resolution of a racemic sample of warfarin, an acidic drug, was performed using the four capillary types. The buffer was methanol–100 m*M* sodium dihydrogen orthophosphate (pH 8.4) containing 8 m*M* dimethyl-β-CD (2:98 v/v) and the applied voltage was 10 kV (Fig. 3a–d). Variations in the operating conditions were needed to transfer the method between the capillary types. A reversed voltage polarity was used for both the cationic and neutral capillaries. The anionic capillary was only

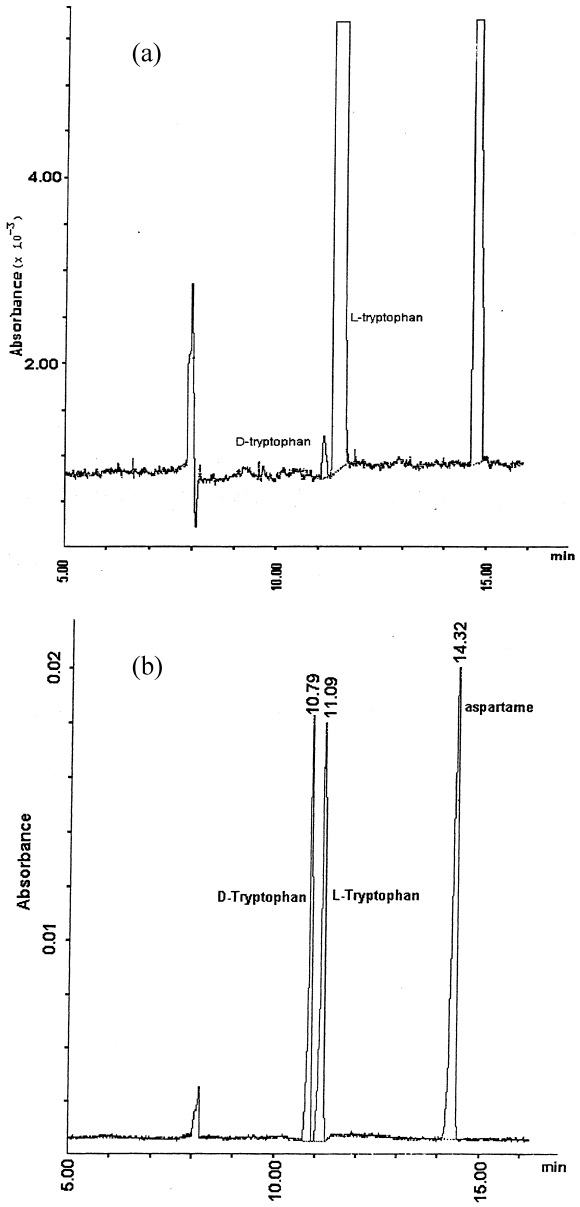

Fig. 2. Optimised method for detection of D-tryptophan in L-tryptophan with the polyamine-coated capillary. (a) Detection of 0.05% (m/m) of D- in L-tryptophan (b) resolution of racemic tryptophan, with the presence of the internal standard, aspartame. Operating conditions: 40 m*M* Tris–phosphoric acid containing 75 m*M* α-CD. The capillary was 37 cm (30 cm to detector)×50 μm, with an applied voltage −10 kV, detection wavelength 214 nm, injection time 2 s, and operating temperature 30°C.

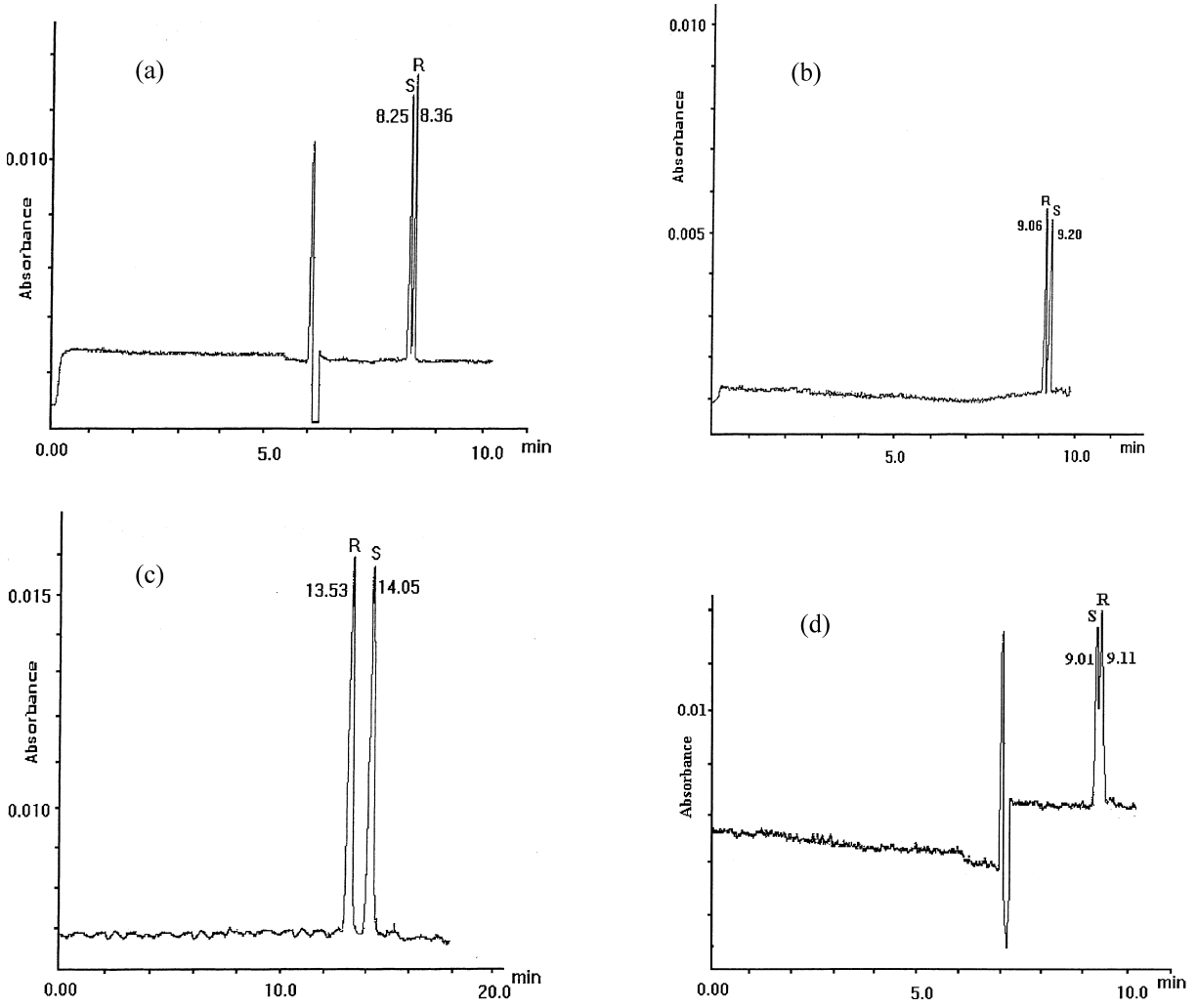

Fig. 3. Resolution of racemic warfarin using a conventional fused-silica capillary with polyamine, neutral and sulphonic acid capillary types. (a) Conventional fused-silica capillary 37 cm (30 cm)×50 μm (b) polyamine-coated capillary 37 cm (30 cm)×75 μm, (c) neutral capillary 37 cm (30 cm) ×75 μm, anionic capillary 37 cm (30 cm)×75 μm. All the capillaries were run at 10 kV except the sulphonic acid capillary, which was at +8 kV. Operating parameters were: methanol–100 m*M* sodium dihydrogen orthophosphate containing 8 m*M* dimethyl-β-CD (pH 8.4) (2:98, v/v), detection wavelength was 200 nm; injection time 2 s, and operating temperature 22°C.

commercially available with an I.D. of 75 μm and therefore a reduced applied voltage (8 kV) was used to prevent excessive current generation. The migration order of the individual warfarin enantiomers was *S* followed by *R* for both the uncoated and anionic coated capillaries. A reversed migration order of *R* then *S* was obtained using the amine or neutral capillaries where a reversal of the polarity was used with these capillaries. The neutral capillary (Fig. 3c) gave the best resolution but with the longest analysis time. Therefore, at high pH with the range of treated capillary surfaces the analyst can choose the migration order, with all the methods giving relatively fast resolution times.

3.4. Selection of capillary type

Table 1 gives the suggested usage of the various

Table 1
Applications of capillary type

Capillary coating	Sample type	Voltage/pH	Comments
Amine	Base	Negative, low	Best capillary type for basic drugs
	Acid	Negative, high	Peak tailing due to ionic interaction
Neutral	Base	Positive, low	Resolution but some tailing due to residual silanols
	Acid	Negative, high	Good separation with long analysis time
Anionic	Base	Positive, low	Peak tailing due to ionic interaction
	Acid	Positive, high	Rapid chiral separation with good stability to pH changes

coated capillary types evaluated. The polyamine capillary is especially useful for low pH separations of basic compounds as peak tailing effects are minimised. The neutral capillary is useful for increasing resolution of acidic compounds and offers improvements at low pH compared to an uncoated capillary. The anionic capillary has the advantage that the EOF is pH independent, which improves the robustness of methods for routine use but seems to be inappropriate for separation of basic compounds at low pH values as strong interactions occur with the coating.

4. Conclusion

The experimental programme shows that it is possible to manipulate both the resolution and migration order of a chiral separation using a range of capillary types. This is particularly useful when one of the enantiomers is present as a trace level impurity. The same parameters could be applied both at high and low pH conditions to achieve a degree of chiral separation, when using uncoated, neutral, anionic and cationic coated capillaries. However, the peak efficiencies and LODs were variable between the capillary types tested.

The polyamine coated capillary was shown to be most useful in the resolution of basic enantiomers. This was primarily due to the elimination of peak tailing effects arising from charge interactions. Acidic drugs were found to be usefully separated with the neutral capillary at high pH and reversed polarity. The sulphonic acid coated capillaries introduce the possibility of improved method robustness as the EOF generated with these capillaries is pH independent.

Therefore, a chiral CE method using a coated capillary with CDs and their derivatives may be extended from a method developed on an uncoated capillary. Although the same method has been shown to be applicable for all the capillary types for the best conditions, some adjustment of the operating conditions is required. Of these the CD concentration should be examined when switching between capillary types. Using these chiral CE methods above detection limits of 0.03% (m/m) have been demonstrated which for the minor enantiomer are at least equivalent to HPLC determinations. The coated capillaries proved to be rugged and robust to changes in pH and under different electrolyte conditions.

It may be possible to develop a scheme involving a range of capillary types, which could be used to rapidly screen drugs for chiral separation, whether these drugs are acidic or basic. This screening possibility is currently being fully studied.

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