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Effect of charged and uncharged chiral additives on the resolution of amlodipine enantiomers in liquid chromatography and capillary electrophoresis

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Abstract

Neutral cyclodextrins (CDs) and derivatives carrying an overall cationic or anionic charge are widely utilised as chiral additives in liquid chromatography (LC) and particularly in capillary electrophoresis (CE). Five neutral CDs (α-CD, β-CD, γ-CD, hydroxypropyl-β-CD (HP-β-CD) and hydroxyethyl-β-CD (HE-β-CD)) and the anionic sulphobutylether-β-CD (SBE-β-CD) and carboxymethyl-β-CD (CM-β-CD) have been examined for the resolution of amlodipine enantiomers both in LC and in CE. In LC, although the neutral CDs yielded no enantioselectivity for amlodipine, CM-β-CD did show enantioselectivity (R_s=1.1, t_R=35 min), albeit with poor peak shape. It was found, however, that the anionic SBE-β-CD gives a robust separation (R_s=1.7, t_{R2}=21.5 min) when using acetonitrile–20 m*M* NaH_2PO_4 (pH 3.95), containing 20 m*M* SBE-β-CD (35:100, v/v). A spectrophotometric continuous-variation plot method was used to determine that the amlodipine–SBE-β-CD complex has predominantly a 1:1 stoichiometry in solution. Stability constants have been calculated for this 1:1 complex in LC. For each amlodipine enantiomer with the anionic SBE-β-CD, these were 596 and 561 M^{-1}, respectively. The enantiomers of amlodipine are also separated in CE using hydroxypropyl-β-CD (20 m*M*) and the anionic SBE-β-CD (1 m*M*) and CM-β-CD (2.5 m*M*) where the anionic CDs were shown to offer an enhanced enantioselectivity over the neutral CD. The amlodipine enantiomer migration order in CE is found to be the same for both anionic and neutral CDs. Further examination of the enantiomer migration order indicated that the binding constant for the *S* enantiomer to the SBE-β-CD was greater than that for the corresponding *R* enantiomer. It was then possible, by examining the enantiomer retention, to conclude that in LC the enantioselective interaction between each amlodipine enantiomer and the anionic SBE-β-CD occurs in the bulk mobile phase and not on a dynamically coated chiral stationary phase.

Keywords: Enantiomer separation; Buffer composition; Chiral selectors; Mobile phase composition; Amlodipine; Cyclodextrins

1. Introduction

The use of cyclodextrins (CDs) for chiral drug discrimination in separative and spectroscopic studies is now well established [1,2]. CDs have been employed effectively as chiral selectors in gas chromatography (GC) [3], liquid chromatography (LC) [4,5], capillary electrophoresis (CE) [6] and capillary electrochromatography (CEC) [7]. CDs have also been employed in nuclear magnetic resonance (NMR) studies as chiral shift reagents [2].

CDs can be modified chemically at any number of

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the primary or secondary hydroxyl sites on a CD ring (β-CD has 21 hydroxyl sites for potential chemical modification). Traditionally CDs have been modified with neutral derivatives which often showed enhanced solubility and enantioselectivity over the underivatised native CDs [8]. This trend of chemical modification has now continued to include CDs that have been modified with an ionic derivative [9].

The use of ionic CDs (both cationic and anionic) as chiral selectors in LC, CE and NMR have shown great potential as efficient chiral selectors, offering enhanced discrimination for many chiral drugs [1,10]. Additionally, these CDs have been shown to be effective in controlling enantiomer migration order in CE [11]. This method for controlling enantiomer migration order in CE may be considered in developing methods for the quantification of drug enantiomers where migration of the distomer before the eutomer may be preferred. It is also important to note that a number of reports have also appeared describing the critical effect of the degree of substitution (DS i.e. the average number of derivatives per CD molecule) of these ionic CDs on enantioselectivy [12–14]. It will therefore be crucial to the success and sustained use of these ionic CDs that reliable, reproducible and well characterised ionic CDs are universally utilised.

The current work examines the anionic sulphobutylether-β-cyclodextrin (SBE-β-CD) as a chiral mobile phase additive in LC, for the separation of racemic amlodipine, a calcium channel blocker. The effect of higher concentrations of the anionic cyclodextrin SBE-β-CD, pH, and organic modifier in terms of separation robustness are compared to those reported [15]. Additionally, five neutral CDs and the anionic carboxymethyl-β-cyclodextrin (CM-β-CD) are also examined as chiral selectors in LC for the enantiomeric separation of amlodipine. The method of continuous-variation plots [16] using spectrophotometry was employed to establish the stoichiometry of the amlodipine–SBE-β-CD complex. Stability constants are also calculated in LC according to the methods developed by Sybilska et al. [17] for the amlodipine–SBE-β-CD complex. The effect of anionic CDs, compared with neutral CDs on the enantiomer migration order of amlodipine, is also examined in CE and compared to the retention order in LC to identify the possible enantioselective mechanism taking place. The observed migration order of amlodipine enantiomers when using the neutral hydroxypropyl-β-cyclodextrin (HP-β-CD) is compared to that observed when using both the anionic CDs.

2. Experimental

2.1. Materials

Racemic amlodipine (as the maleate salt) and the SBE-β-CD sodium salt were used as received from Pfizer (Kent, UK). The SBE-β-CD was characterised by NMR, microanalysis and CE, at Pfizer Central Research as having a DS of 6.5. α-CD, γ-CD and CM-β-CD sodium salt (DS=5.3) were used as received from Wacker (Walton-on-Thames, UK). β-CD, HP-β-CD (DS=4.2) and HE-β-CD (MS=11.2) were used as received from Aldrich. (Gillingham, UK). Organic solvents, methanol (MeOH) and acetonitrile (MeCN) (AnalaR grade) were purchased from Fisons (Loughborough, UK). Sodium dihydrogenorthophosphate and potassium dihydrogenorthophosphate buffers were purchased from BDH (Poole, UK) and pH adjusted with orthophosphoric acid or sodium hydroxide as appropriate. All mobile phases were filtered using a 0.45-μm filter purchased from Anachem (Bedfordshire, UK) and degassed by sonication under vacuum.

2.2. Apparatus

Liquid chromatography was carried out using an LKB 2150 HPLC pump (LKB, Sweden) at a constant flow-rate of 1.0 ml/min. An LKB 2151 variable-wavelength UV detector pump (LKB) was used to measure the absorbance of amlodipine, ~100 μg/ml, at 238 nm and the chromatograms were recorded on a Kipp and Zonen BD40 chart recorder (Kipp and Zonen, Netherlands). The column dimension was 150×4.6 mm I.D., packed with 5-μm Hypersil BDS C_8 packing material (kind gifts from Hypersil, Cheshire, UK). The temperature was maintained constant at 17°C using a temperature-controlled oven (Kariba Instruments, Jones Chromatography, Hengoed, UK).

Capillary electrophoresis was carried out using a

Beckman P/ACE 5510 (Fullerton, CA, USA) equipped with a UV detector operated at 214 nm. The electrophoretic experiments were performed in an uncoated fused-silica capillary 57 cm×50 μm I.D. (50 cm effective length) obtained from Beckman Instruments (High Wycombe, UK). The temperature was maintained constant throughout at 17°C.

Continuous-variation plots using spectrophotometry were carried out using the HP 8452A diode array spectrophotometer attached to a HP Vectra E5/12 computer and colour graphics monitor (Hewlett-Packard, Stockport, UK)

2.3. Methods

In LC, amlodipine (~100 μg/ml) was prepared each day in water from a 1 mg/ml stock solution prepared in water. Aqueous phases were prepared and pH controlled before any CD was added at the desired concentration. Organic solvents were then mixed with the aqueous phase, filtered and degassed for 15 min, to obtain the final mobile phase.

In CE, the capillary was conditioned initially for 1 h with 1 *M* NaOH and 20 min with water. Sodium dihydrogen orthophosphate dihydrate adjusted to the appropriate pH with orthophosphoric acid and sodium hydroxide as appropriate was used as electrolyte. CDs were prepared in this electrolyte at the required concentration for the final run buffer. The capillary was washed for 1 min with 0.1 *M* NaOH and 3 min with run buffer prior to each run. Amlodipine (~100 μg/ml) was prepared each day in deionised water from a 1 mg/ml stock solution prepared in electrolyte. Samples were prepared in 10% buffer to favour sample stacking and were introduced to the capillary by pressure injection. All samples and buffers were sonicated and filtered through a 0.45-μm filter before use.

Continuous-variation plots were generated according to the methods of Likussar and Boltz [16]. Stock solutions (0.1 m*M*) of amlodipine and SBE-β-CD were prepared in distilled water. Different ratios of amlodipine complexed with SBE-β-CD were prepared from the stock solutions by mixing different volumes of the equimolar stock solutions. Thus each solution has the same total molar concentration but with different mole fractions of each component. The prepared solutions were placed in a 1-cm path length silica cuvette. UV spectra were obtained on the eleven solutions containing the two components in the following molar ratios; 1:0, 9:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:9 and 0:1. In the characteristic triangular continuous-variation plots, the function used was [AL]/{[AL]+[CD]} on the ordinate, against [CD]/{[AL]+[CD]} on the abscissa. However, since [AL] and [CD] are expressed as mole fractions with a constant total molarity, then these parameters reduce to give a plot of ($\Delta A_{\lambda\ 238\ nm}$). [AL] i.e. change in amlodipine absorbance at λ_{max} (238 nm) on addition of CD, multiplied by amlodipine concentration, versus [CD].

3. Results

3.1. Liquid chromatography

Initially in LC, the aim was to compare the utility of five neutral CDs (α-, β-, γ-, HP-β-CD and HE-β-CD) with two anionic CDs (CM-β-CD and SBE-β-CD) as chiral selectors for racemic amlodipine. It was decided to investigate the CD chiral selectors as chiral mobile phase additives for the separation of amlodipine enantiomers since earlier work in our laboratories [18] had utilised neutral CD-bonded chiral stationary phases for the separation of the same enantiomers. Table 1 describes the chromatographic conditions and results for the separation of amlodipine enantiomers using the five neutral CDs. With one exception, HP-β-CD, a decrease in retention factor (k') was observed after the addition of each CD to the mobile phase. This indicates that in the interaction between amlodipine and these neutral CDs the adsorption of an analyte–CD complex onto

Table 1
Retention time (t_R), retention factor (k') and separation factor (α) for amlodipine enantiomers when using neutral CDs in the mobile phase

CD additive	t_R (min)	k'	α
None	44.0	26.5	0
20 m*M* α-CD	42.2	26.3	0
10 m*M* β-CD	41.0	24.6	0
20 m*M* γ-CD	38.4	22.1	0
20 m*M* HP-β-CD	51.0	30.8	0
20 m*M* HE-β-CD	37.2	19.6	0

the hydrophobic stationary phase is less than that of the corresponding free molecule, which is consistent with the earlier work [19]. However, no enantiomeric separation of amlodipine was observed when any of the neutral CDs was used as a chiral mobile phase additive.

The addition of the anionic CM-β-CD to the mobile phase, however, did allow the complete enantioseparation of amlodipine (R_s=1.1, t_{R2}=35 min), albeit with poor peak shape and a relatively long retention time. This separation was reminiscent of early work on chiral mobile phase additives where poor peak shapes were obtained with long retention times. Attempts were made to improve the peak shape for this separation but they only resulted in a loss of enantioselectivity.

It was also possible to separate amlodipine enantiomers when using the anionic SBE-β-CD as a chiral mobile phase additive. In earlier work, a central composite design (CCD) had been employed to optimise SBE-β-CD concentration over a limited range (2.3–2.9 m*M*), pH and acetonitrile content of the mobile phase [15]. These earlier results represented the first chiral separation ever achieved on adding an anionic CD to an LC system. It was found, however, that this separation was not very robust and the separation was extremely sensitive to all three parameters optimised (SBE-β-CD concentration, pH and acetonitrile content in the mobile phase).

This separation has now been examined further using higher concentrations of the anionic SBE-β-CD. Fig. 1a and b show the effect of SBE-β-CD concentration on peak retention factor (k') and the Kaiser peak separation index (P_i), respectively, for each amlodipine enantiomer. The Kaiser peak separation index, (P_i), is defined as the average valley depth expressed as a ratio to the average peak height of the two enantiomeric peaks. Fig. 1a indicates that the adsorption of the amlodipine–CD complex to the hydrophobic stationary phase is smaller than that for the corresponding free molecule. This is consistent with earlier reports when using CDs as mobile phase additives for the resolution of chiral drugs [20]. Fig. 1a and b corroborate the earlier reports that the enantioseparation of amlodipine is extremely sensitive to CD concentration, particularly over the range of concentrations studied (2.3–2.9 m*M*) [15]. It can also be seen, however, that the chiral separation

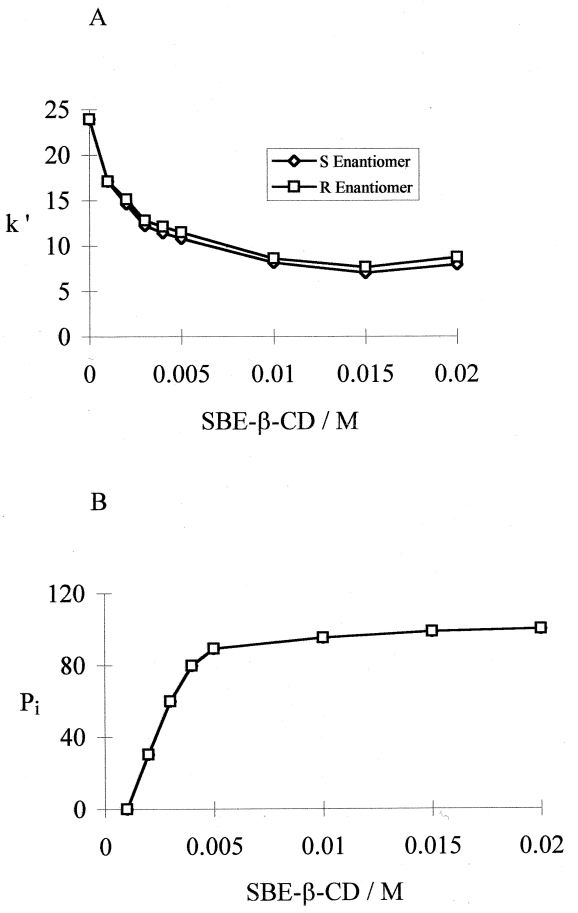

Fig. 1. Plot of (a) retention factor (k') and (b) the Kaiser peak separation index (P_i) vs. SBE-β-CD concentration using: MeCN–20 m*M* KH_2PO_4 (pH 3.95) (35:100, v/v)

becomes less sensitive at higher concentrations of the anionic SBE-β-CD (10 m*M*). In fact, the separation also becomes less sensitive to pH and organic modifier (type and content) when SBE-β-CD is employed at higher concentrations as shown in Table 2. It can be seen that the effect of varying pH and organic modifier (type and content) has little effect on the separation factor of amlodipine enantiomers when 10 m*M* SBE-β-CD additive is used. Fig. 2 shows the separation of amlodipine enantiomers in LC when using a higher concentration of CD compared with that used in the optimised conditions. This represents a complete separation (R_s=1.7, t_{R2}= 21.5) and yields significantly more robust conditions.

The use of neutral CDs in LC, both as mobile

Table 2
Effect of variation of pH and organic modifier on robustness for recently reported optimised conditions [15]

Organic modifier	Ratio, organic modifier–aqueous phase[a] (v/v)	pH	SBE-β-CD (m*M*)[b]	α
MeCN	35:100	3.95	10	1.06
MeCN	35:100	2.95	10	1.07
MeCN	35:100	5.0	10	1.08
MeCN	30:100	3.95	10	1.12
MeOH	43:100[c]	3.95	10[d]	1.06

[a] Aqueous phase, 20 m*M* KH_2PO_4.
[b] Concentration in aqueous phase before addition of organic modifier.
[c] Volume of MeOH corresponds to the solvent strength of 35:100 MeCN–aqueous phase.
[d] CD concentration adjusted to be equal to the final concentration in eluent when using MeCN.

phase additives and as chiral stationary phases, for the separation of drug enantiomers is now well established [17]. However, the use of charged CDs in LC is a relatively recent development, either as mobile phase additives [15,21] or as chiral stationary phases [22]. Thus little has been reported on the actual enantioselective mechanisms involved.

Sybilska et al. pioneered the use of neutral CDs as mobile phase additives in LC and later went on to describe various possible enantioselective mechanisms, based on a rationalisation of the interactions observed between free enantiomers, a CD additive and a hydrophobic stationary phase [17]. Sybilska's hypothetical basis for chiral resolution using neutral CDs in the mobile phase in LC describes the following possible mechanisms:

(1) Differences in the stability constants of the CD complexes formed in the mobile phase;

(2) Differences in adsorption of CD complexes onto the surface of the hydrophobic stationary phase itself;

(3) Differences in the interaction of free enantiomeric solutes with a CD layer that is adsorbed from the mobile phase onto the reversed-phase surface.

Mechanisms 1 and 2 correspond to complexation equilibria established in the bulk mobile phase solution. The third mechanism however, would involve the formation of a dynamic chiral stationary phase, and would be expected to mimic the resolution mechanism involved when the CD is covalently bonded to a silica surface.

It is very difficult to characterise which of the enantioselective mechanisms apply when considering neutral CDs added to the mobile phase. There have been reports of enantioselectivity resulting from a dynamically coated CD stationary phase, where a HP-γ-CD added to the mobile phase had adsorbed onto the hydrophobic stationary phase, and would still yield the separation of the drug enantiomers with an achiral mobile phase [23]. These observations favour enantioselective mechanism 3. However, it is possible that this is not the universal enantioselective mechanism for neutral CD chiral mobile phase additives.

When utilising the anionic SBE-β-CD, it is not known which of the enantioselective mechanisms described above is taking place for amlodipine. However, when an identical mobile phase to that described previously [15] was examined containing no CD, without undertaking any washing procedures, no enantioselectivity was observed. Additionally, given the anionic nature of the SBE-β-CD, it is unlikely that any adsorption of this CD to the stationary phase would take place, since any residual anionic silanol activity would be expected to result in a charge repulsion effect.

The stoichiometry between amlodipine and the anionic SBE-β-CD was determined by continuous-variation plot method using UV spectrophotometry. Fig. 3 indicates that the continuous-variation plot has

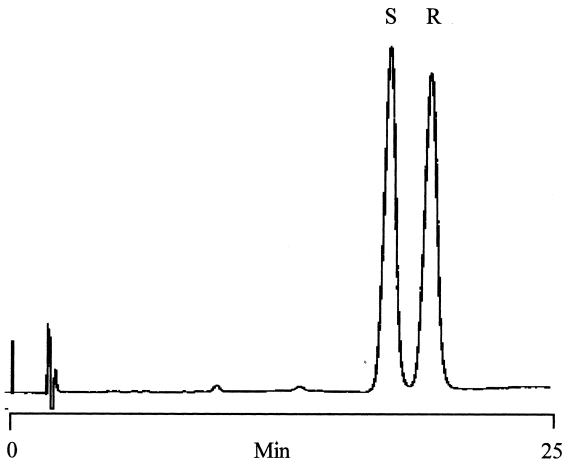

Fig. 2. Separation of amlodipine enantiomers in LC using SBE-β-CD: MeCN–20 m*M* KH_2PO_4 (pH 3.95) 20 m*M* SBE-β-CD (35:100, v/v).

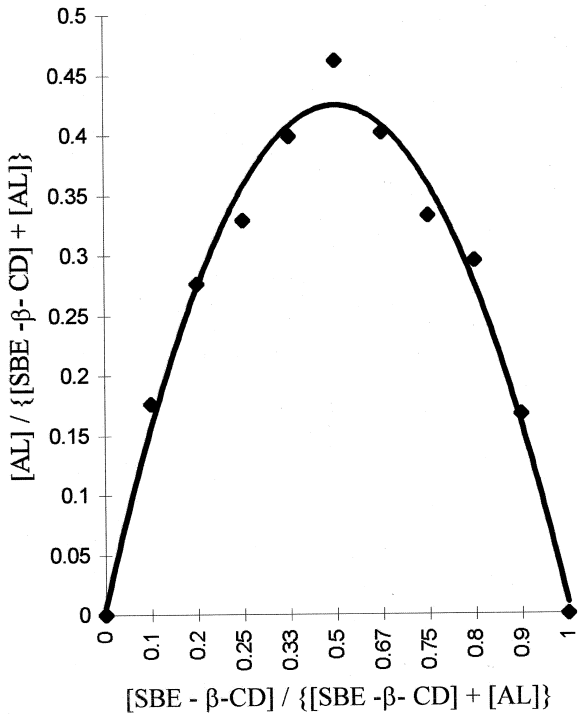

Fig. 3. Spectrophotometery continuous-variation plot for the amlodipine–SBE-β-CD complex.

a maximum corresponding to the amlodipine-anionic SBE-β-CD molar fraction in solution of 0.5:0.5. This indicates that a 1:1 complex stoichiometry between amlodipine and the anionic SBE-β-CD is predominant in solution. According to Likussar and Boltz [16], the shape of the curve may also be used to interpret the stability of the complex. A broad peak often indicates a weak complex whereas a sharp peak indicates a strong complex [16,24]. The continuous-variation plot in Fig. 3 is relatively sharp which may indicate a strong complex between amlodipine and the anionic SBE-β-CD. In fact recently, relatively broad continuous-variation plots were reported for a range of neutral CDs with amlodipine, which may thus corroborate the present LC and CE data, where greater enantioselectivity was obtained for SBE-β-CD compared to that for any neutral CD [18].

Eq. (1) developed by Sybilska, describes a simple reversed-phase system, where the mobile phase contains a CD additive, and permits calculation of the stability constant, K_G, for a guest molecule–CD complex of stoichiometry 1:1 (such as that of amlodipine and SBE-β-CD determined above) for each enantiomer [17]:

$$k' = \frac{1}{K_G} \cdot \frac{k'_G - k'}{[CD]} + k'_{G-CD} \quad (1)$$

where k'_G and k'_{G-CD} are the retention factors of the free guest molecule and of its CD complex on the stationary phase, respectively. If a linear relationship exists between k' vs. $(k'_G - k')/[CD]$ this would allow the calculation of stability constants (K_G) for each enantiomer, and the retention factors (k'_{G-CD}) of these complexes on the hydrophobic stationary phase.

Fig. 1a shows the relationship between retention factors (k') for each amlodipine enantiomer as a function of SBE-β-CD concentration. When the same data as in Fig. 1a for k', is plotted as a function of $(k'_G - k')/[CD]$, a linear relationship is observed, which allows stability constants (K_G) to be calculated for each amlodipine enantiomer with the anionic SBE-β-CD according to Eq. (1).

The calculated stability constants were 596±70 M^{-1} and 561±40 M^{-1} for each amlodipine enantiomer, respectively. Unfortunately, it was not possible to calculate stability constants for this drug with any neutral CD using this method since no enantioselectivity was observed for these CDs (Table 1). However, the data for amlodipine (cationic at pH 3.95) with the anionic SBE-β-CD, compare favourably with stability constants calculated recently for the cationic doxazosin enantiomers with another anionic CD, carboxymethyl-β-CD (CM-β-CD), 647±55 and 594±45 M^{-1}, using similar methods [21].

These data for amlodipine in LC indicate a contrast in enantioselectivity between the SBE-β-CD and the neutral CDs as chiral mobile phase additives. This suggests that the charge is a significant factor to be considered. In fact, the role of charge was recently investigated during the interaction of charged and uncharged drugs with neutral HP-β-CD and the anionically charged SBE-β-CD. Binding studies were carried out using the phase solubility method for a number of charged and uncharged drugs with these two CDs [25]. The authors concluded that: ‘the binding constants for the neutral

forms of the drugs studied were always greater with SBE-β-CD than with HP-β-CD, while the binding constants for the cationic drugs studied with SBE-β-CD were significantly higher than those for HP-β-CD′.

It may then be concluded that the fundamental enantioselective mechanism for interaction between SBE-β-CD or CM-β-CD and amlodipine is more complex than a simple inclusion process and that charge may well be playing a significant role. This is also confirmed by one- and two-dimensional NMR studies recently reported for this drug–CD mixture [26].

3.2. Capillary electrophoresis

The separation of amlodipine enantiomers was also explored in CE when using both neutral and anionic CDs. An uncoated fused-silica capillary with cathodic electroosmotic flow (EOF) was used throughout these studies. Fig. 4a shows the enantioseparation of amlodipine when using 20 m*M* of the neutral HP-β-CD. In this case, since amlodipine is cationic, the mobility of the enantiomers will be in the direction of the cathodic EOF. It can be seen that the binding constant for the second migrating *S* enantiomer of amlodipine with the neutral HP-β-CD is stronger of that of the *R* enantiomer (i.e. $K_S > K_R$).

It was found that the anionic CM-β-CD and SBE-β-CD, offered an enhanced separation for amlodipine enantiomers over the neutral CD and that the concentration required was lower, as shown in Fig. 4b and c. No reversal of enantiomer migration order was observed for the enantiomers of amlodipine in CE on addition of the anionic CDs or for the neutral CD. Despite the fact that the overall amlodipine–CD complex when using anionic CDs is characterised by an anionic charge and will thus migrate in the anodic direction (i.e. opposite to that observed when using the neutral CD), the migration order is still the same (i.e. *R* before *S*). This indicates that the binding constant for the *S* enantiomer of amlodipine with the anionic SBE-β-CD is stronger than that of the *R* enantiomer (i.e. $K_S > K_R$) and thus the migration order is *R* before *S*.

Interestingly, the enantiomer elution order for amlodipine when using the anionic SBE-β-CD as a mobile phase additive in LC is *S* before *R*. This would indicate that the interactions between amlodipine and the anionic SBE-β-CD occur in the mobile phase itself, according to the mechanisms 1 and 2 above. The anionic SBE-β-CD predominantly stays in the mobile phase also predicted above, due to repulsion from any free silanol anionic activity. The stronger binding *S* enantiomer to the SBE-β-CD is carried forward with the mobile phase with the CD more rapidly than the weaker binding *R* enantiomer, which is retained by the hydrophobic stationary phase (i.e. $K_S > K_R$).

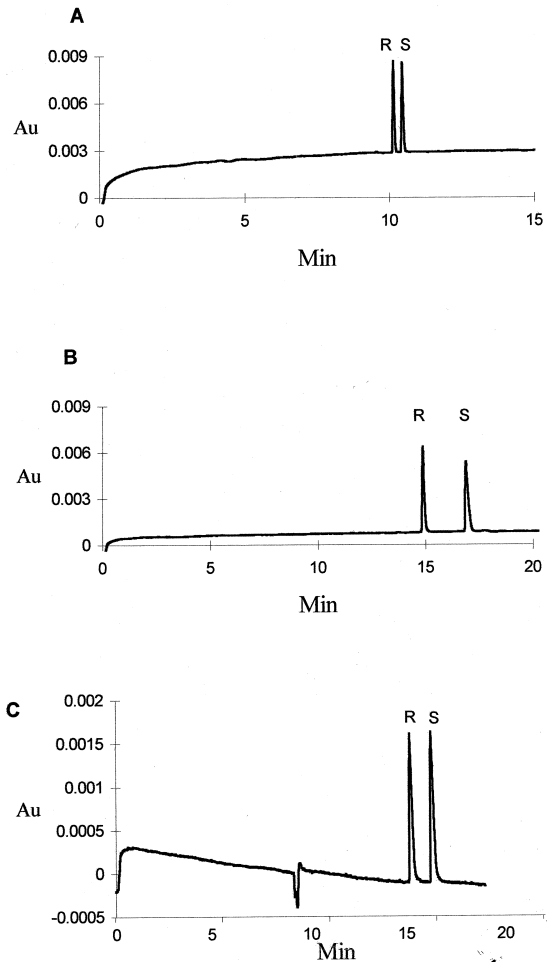

Fig. 4. Separation of amlodipine enantiomers in CE using 50 m*M* NaH_2PO_4 electrolyte containing (a) 20 m*M* HP-β-CD (pH 3.0) at 20 kV (b) 2.5 m*M* CM-β-CD (pH 3.0) at 15 kV and (c) 1.0 m*M* SBE-β-CD (pH 7.0) at 15 kV.

4. Discussion

It has been shown in LC that the addition of the anionic CM-β-CD and SBE-β-CD to the mobile phase offers a complete resolution of amlodipine enantiomers. This contrasts with the five neutral CDs, where no enantioselectivity was observed. A set of optimised conditions reported earlier [15] for a limited range of SBE-β-CD concentration was examined further, utilising higher CD concentrations while maintaining the original optimised pH and organic modifier conditions. It was found that a significantly enhanced separation was obtained in less than half the analysis time by examining the CD at higher concentration levels. This enhanced separation was also significantly more robust than the original optimised conditions, where minor changes in the separation factor were observed, for small changes in the pH and organic modifier concentration of the mobile phase.

The continuous-variation plot using spectrophotometry was used for determining the stoichiometry of the amlodipine–SBE-β-CD complex. It was found that this complex had predominantly a 1:1 stoichiometry in solution. The method for determining stability constants for a 1:1 complex between drug enantiomers with a CD added to the mobile phase in LC, as proposed by Sybilska [17], was then carried out for each amlodipine enantiomer with the anionic SBE-β-CD. These stability constants compared favourably with those calculated earlier for doxazosin enantiomers with the anionic CM-β-CD, but could not be directly compared to any existing physical data.

It was found in CE, that a complete enantioseparation for amlodipine was possible when using the neutral HP-β-CD, the anionic CM-β-CD and the anionic SBE-β-CD as electrolyte additives. The separation obtained for the anionic CDs, however, was significantly enhanced over that obtained for the neutral CD, coupled with a significantly lower concentration of the anionic CDs employed. On examination of the enantiomer migration order for both the neutral and the anionic CDs, it was possible to show that the binding constant for the *S* amlodipine enantiomer to the SBE-β-CD was stronger than that for the corresponding *R* enantiomer. It was then possible, by examining the retention order for amlodipine enantiomers in LC, to assign which of the two possible enantioselective mechanisms proposed by Sybilska [17] was taking place for this drug–CD system, where CDs were added to the mobile phase. It can be concluded that the enantioselective mechanism (or enantioselective interactions) between amlodipine and SBE-β-CD was taking place in the bulk mobile phase and not on a dynamically coated CD chiral stationary phase. This finding also supports earlier proposals for this drug–CD system [15] that the adsorption of the anionic SBE-β-CD to the hydrophobic stationary phase was thought unlikely, since a charge repulsion effect from any residual anionic free silanol activity with the anionic SBE-β-CD would be expected to take place.

5. Conclusions

Two anionic CDs in LC were shown to afford the complete separation of amlodipine enantiomers, compared to the lack of observed enantioselectivity when using five selected neutral CDs. Moreover, the use of the SBE-β-CD at higher concentrations than those previously examined [15] was shown to offer a significantly more robust chiral separation. From binding constants calculated in LC for the 1:1 complex between the cationic amlodipine and anionic SBE-β-CD, and from the observed enantiomer migration order in CE, it was possible to determine that the enantioselective mechanism of interaction was most probably occurring in the bulk mobile phase, in agreement with a mechanism proposed earlier by Sybilska et al. [17].

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