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Separation of enantiomers in capillary electrophoresis with contactless conductivity detection

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Abstract

Contactless conductivity detection is successfully demonstrated for the enantiomeric separation of basic drugs and amino acids in capillary electrophoresis (CE). Derivatization of the compounds or the addition of a visualization agent as for indirect optical detection schemes were not needed. Non-charged chiral selectors were employed, hydroxypropylated cyclodextrin (CD) for the more lipophilic basic drugs and 18-crown-6-tetracarboxylic acid ($18C6H_4$) for the amino acids. Acidic buffer solutions based on lactic or citric acid were used. The detection limits were determined as 0.3 μM for pseudoephedrine as an example of a basic drug and were in the range from 2.5 to 20 μM for the amino acids.

Keywords: Enantiomeric separations; Basic drugs; Amino acids; Capillary electrophoresis; Contactless conductivity detection

1. Introduction

Most pharmaceutical compounds have chiral centres and their effects are due to interactions with chiral biological compounds. Different enantiomers of the Pharmaceuticals therefore usually have different pharmacological properties in terms of activity, toxicity, transport mechanism and metabolic route. For this reason drugs are administered in enantiomeric pure form. The determination of the enantiomeric purity of intermediates used for, and products of, enantioselective syntheses is therefore an important analytical task. This is often carried out with separation methods such as HPLC or GC (see for example, the following review [1]). However, the approach is challenging as the fundamental chromatographic separation mechanisms are not adequate for distinction between enantiomers because their physical and chemical properties are too similar. Therefore, special chiral reagents have to be bonded onto the chromatographic column.

The suitability of capillary electrophoresis (CE) for enantiomeric separation was first demonstrated by Gassmann et al. [2], and this approach has become an important technique. Besides the general advantages of CE, high separation efficiency and short analysis times, the method is less costly than chromatography as the preparation of special chiral columns is not necessary. In CE small amounts of reagents are dissolved in the background electrolyte solution, which interact with the analytes, thus modify their electrophoretic mobilities and act as enantiomeric selectors. A range of selectors has been used, such as cyclodextrins (CDs), a chiral crown ether, macrocyclic antibiotics, polysaccarides and chiral surfactants. The use of CDs, introduced by Terabe [3], is the most common approach in CE to obtain enantiomeric separations and a range of modified CDs have been used for this purpose. A number of recent reviews are available (see for example, [4–8]).

In CE, UV or fluorescence detectors are most often used and commercially available. Therefore, in the enantiomeric separation of non-UV absorbing compounds, such as alkylamines and most amino acids, these are often measured after derivatization [9–12], or by indirect methods in which a chromophore is displaced by the analyte leading to peaks due to a reduction in the background absorbance [13,14]. However, derivatization adds an additional step to the analytical process and may also affect the interaction of the analytes with

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the chiral selector. Indirect methods generally show relatively poor sensitivity.

Electrochemical detection techniques are a good alternative, in particular the recently developed capactively coupled contactless conductivity detector (C^4D) has a number of advantages. The technique is universal in capillary electrophoresis in that all ionic compounds can be detected without derivatization or indirect approaches. Except for the electrodes, no physical parts are needed, all the other components consist of inexpensive electronic circuitry. The contactless approach features also unprecedented ease of the cell arrangement and inherent prevention of electrode fouling. Handling of the separation capillary is facile as it is not necessary to remove the polymeric cladding to create a detection window. For these reasons, contactless conductivity detection has been adopted by a number of research groups and the successful application of this detector for organic molecules of different classes, incuding basic drugs [15] and amino acids [15–18] has been reported. Recent reviews on contactless conductivity detection are available [19,20]. However, to our knowledge, the use of C^4D in chiral separations has not been previously explored, and a study of this application is presented here.

2. Experimental

2.1. Instrumentation

Separations were carried out on an instrument, which was built in-house and is based on a high voltage power supply with interchangeable polarity (CZE 2000R) from Start Spellman (Pulborough, UK). The contactless conductivity detector consists of two tubular electrodes of 4 mm length separated by a gap of 1 mm and a Faradaic shield. Cell excitation was carried out with a sine-wave with a frequency of 100 kHz and a peak-to-peak amplitude of 450 V. The resulting current signal was amplified, rectified and low pass filtered with a circuitry described elsewhere [21,22] before passing to a MacLab/4e data acquisition system (AD Instruments, Castle Hill, Australia) for recording of the electropherograms. All electropherograms were inverted for presentation of the peaks in the normal orientation. Detection limits are reported as the concentrations giving peak heights corresponding to three times the baseline noise.

2.2. Reagents and methods

All chemicals were of analytical reagent grade and were obtained from Fluka (Buchs, Switzerland) with the exception of hydroxypropyl-β-cyclodextrin (HP-β-CD) which was purchased from Acros (Geel, Belgium) and sulphated β-cyclodextrin (HS-β-CD), which was purchased from Aldrich (Buchs, Switzerland). All solutions were degassed by ultrasonication and filtered through 0.2 μm nylon filters before use. Fused-silica capillaries of 10 μm i.d. and 375 μm o.d. were used for performing the electrophoretic separations. These were purchased from Polymicro Technologies (Phoenix, AZ, USA) and were preconditioned with a 0.1 M sodium hydroxide solution before flushing with water followed by flushing with the running buffer. All capillaries had a total length of 48 and 43 cm effective length. Sample injection was carried out electrokinetically at 5.0 kV for 7 s, the separation voltage was 15 kV unless stated otherwise. Standard solutions were diluted with background electrolyte solution to ensure injection under non-stacking conditions.

3. Results and discussion

3.1. Basic drugs

In order to explore the feasibility of using contactless conductivity detection in enantiomeric separations first experiments were conducted with a relatively well-established method, the separation of basic drugs with cyclodextrins as chiral selector. Frequently, sulfated cyclodextrins are used for this purpose (see for example, [23,24]) and therefore the use of such an enantiomeric selector was first investigated. It was however found, that it was not possible to obtain a stable baseline when including sulfated β-CD into an electrolyte solution consisting of 20 mM lactic acid. This was ascribed to the fact that at the high concentration of 1% at which the modifier has to be used, this highly charged substance leads to a marked increase of conductivity of the running buffer (from 692 to 3260 $\mu S\,cm^{-1}$ for HS-β-CD), which is not compatible with conductivity detection. Lower concentrations of the sulfated cyclodextrins on the other hand are not adequate for achieving enantiomeric separations. It appears that the use of cyclodextrins must thus be limited to non-charged derivatives when employing conductivity detection.

Further trials were therefore carried out with the neutral hydroxypropyl-β-cyclodextrin, the use of which had been described previously for the purpose of the enantiomeric separation of basic drugs employing optical detection (see for example, [25,26]), and this was indeed successful. The separation of the enantiomers of adrenaline as an example for the use of HP-β-CD with C^4D is shown in Fig. 1. The four electropherograms were obtained with different concentrations of the enantiomeric selector. As evident, the concentration in the range from 10 to 40 mM has a strong effect on the separation, which is expected from the previous results reported in the literature (see for example, [26]). Interesting is also the effect of the concentration on the sensitivity, which is in contrast to results obtained with optical detection. Peak heights are smaller for the higher concentrations of the cyclodextrin. Presumably this is caused by the partial complexation of the analyte by the selector, which lowers the sensitivity to conductometric detection. A similar effect was observed previously for amperometric detection as well [27]. Besides the concentration of the chiral selector, the overall buffer composition also strongly influences the separation. In Fig. 2, the

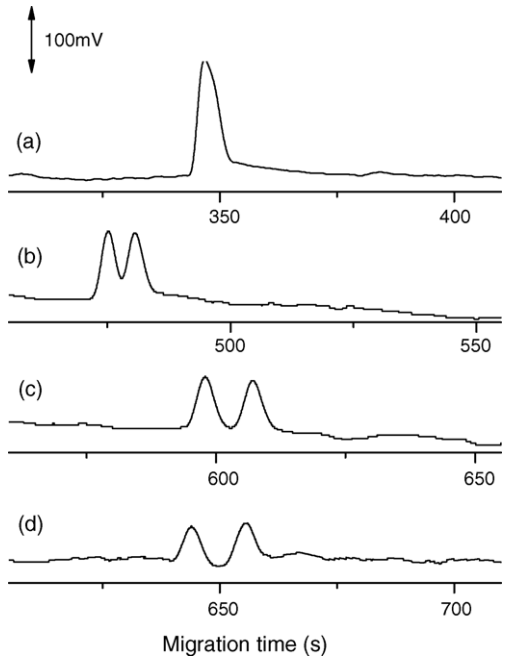

Fig. 1. Influence of the concentration of HP-β-CD on the resolution of D- and L-adrenaline (100 μM): (a) 10 mM; (b) 20 mM; (c) 30 mM; and (d) 40 mM HP-β-CD in 100 mM lactic acid, 5 mM L-histidine at pH 2.75. Capillary: fused silica (48/43 cm × 10 μm i.d.), separation voltage: 15 kV, and injection: 7 s/5 kV.

effect of adding histidine to the lactic acid buffer is illustrated. The addition of this compound leads to an increase of the pH-value from 2.40 to 2.75. However, the effect cannot be completely reconciled with a rise in the pH-value alone as an alteration of the concentration of lactic acid in the absence

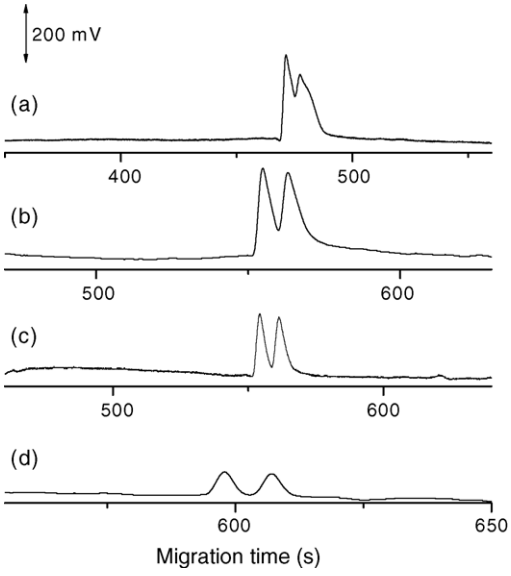

Fig. 2. Influence of the pH-value and the concentration of histidine on the resolution of D- and L-adrenaline (100 μM) in buffers of 100 mM lactic acid and various concentration of L-His containing 30 mM HP-β-CD: (a) pH 2.4; (b) 1 mM His, pH 2.5; (c) 2.5 mM His, pH 2.6; and (d) 5 mM His, pH 2.75. Other condition as for Fig. 1.

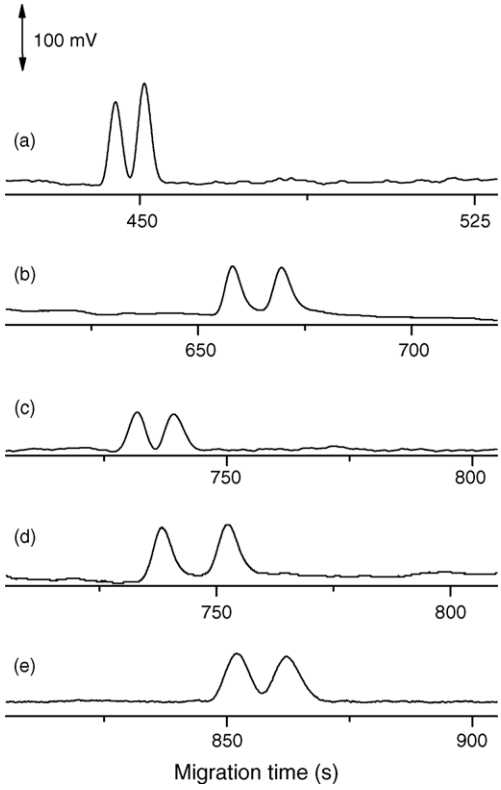

Fig. 3. Enantiomeric separation of: (a) doxylamine using 40 mM HP-β-CD in 100 mM lactic acid and 5 mM L-His at pH 2.75; (b) ephedrine; (c) noradrenaline; (d) isoproterenol using 30 mM HP-β-CD in 100 mM lactic acid with 5 mM L-His at pH 2.75; and (e) propranolol using 10 mM HP-β-CD in 60 mM lactic acid with 7.5 mM L-His at pH 3.06. All at 100 μM. Other conditions as for Fig. 1.

of histidine had a much less pronounced effect. Again, an effect on the peak height is noted.

The optimized buffer system (lactic acid, histidine, and HP-β-CD) was then applied to the separation of several further basic drugs. In Fig. 3, the enantiometric separation of doxylamine, ephedrine, isoproterenol, noradrenaline, and propanolol is shown. At the conditions employed, a good separation of all samples could be achieved. The resolution values, R, were determined as 1.34, 0.95, 1.21, 1.36, 0.96, 0.94 for adrenaline, doxylamine, ephedrine, isoproterenol, noradrenaline, and propanolol, respectively.

In enantiomeric separations it is often important to accurately measure the enantiomeric excess (ee). The separation of pseudoephedrine, as a further example, at enantiomeric ratios of 99:1 and 1:99 is illustrated in Fig. 4. The resolution for the separation of lS,2S-(+)- and lR,2R-(−)-pseudoephedrine was determined as $R = 1.86$. The quantitative results for pseudoephedrine are reported in Table 1 and are showing a good accuracy. The detection limits for the two enantiomers of pseudoephedrine were determined as 3.0×10^{-7} M and 3.8×10^{-7} M for the lS,2S-(+)- and lR,2R-(−)-pseudoephedrine, respectively. Calibration curves were linear up to at least 1.0×10^{-4} M, that is over more than two orders of magnitude, using peak areas for quantitation. The

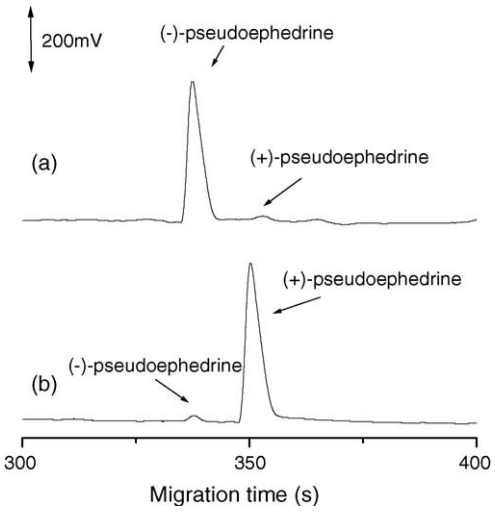

Fig. 4. Enantiometric separation of lS,2S-(+)- and lR,2R-(−)-pseudoephedrine at ratios of 1:99 and 99:1. Total concentration: 100 μM. Buffer: 20 mM lactic acid and 20 mM HP-β-CD at pH 2.45. Other conditions as for Fig. 1.

R-values of the regression analysis were 0.9989 and 0.9999 for the + and − forms, respectively.

3.2. Amino acids

Although enantiomers of amino acids have been separated with cyclodextrins, the reported results appear to be limited to species bearing a phenol group or to species which had been labelled for UV- or fluorescence detection [8]. Our attempts to carry out the separation of underivatized amino acids with HP-β-CD were not successful, presumably due to unsufficient lipophilicity of the analytes. For this reason the chiral crown ether (+)-(18-crown-6)-2,3,ll,12-tetracarboxylic acid ($I8C6H_4$) was adopted as chiral selector. This use of this compound had previously been reported for the successful separation of some amino acids [28–31]. These applications had, however, been limited to UV-absorbing amino acids, or necessitated analyte derivatization or undirect detection with a chromophore added to the buffer.

The successful enantiomeric separation of six underivatized amino acids using $I8C6H_4$ and contactless conductivity detection is illustrated in Fig. 5. Note that aspartic acid is one of the species. It was reported previously that this compound could not be separated using $I8C6H_4$ and the failure was ascribed to electronic repulsion between the crown ether and the side chain of the amino acid [30]. Enantiomeric separation was achieved only after one of the carboxylic acids of the amino acid was blocked by derivatization to form an ester. However, our result indicates that the previous explanation is not sufficient. A possible interaction between the acidic part of aspartic acid and benzyl trimethyl ammonium chloride, which had been included in the buffer for indirect UV-detection, might have to be taken into account.

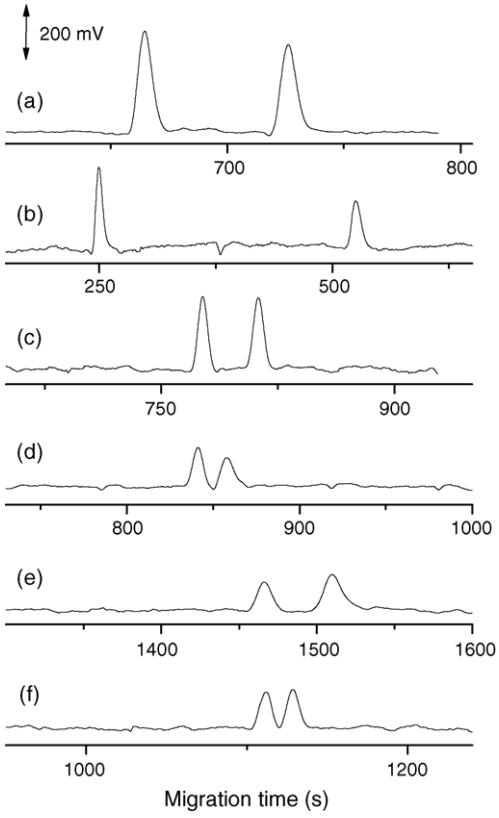

Fig. 5. Enantiomeric separation of amino acids at 250 μM using 10 mM $I8C6H_4$ in 25 mM citric acid at pH 2.1: (a) arginine; (b) serine; (c) threonine; (d) methionine; (e) tryptophan; and (f) aspartic acid. Other conditions as for Fig. 1 except for serine (separation at 25 kV).

The method is not restricted to the species shown in Fig. 5. A total of nine amino acids, including examples from all classes of these species, were investigated. The following *R*-values were obtained: arginine 3.1, valine 1.2, serine 7.7, phenylalanine 1.5, tyrosine 1.7, aspartic acid 1.1, threonine 2.3, methionine 1.1 and tryptophan 1.9. Note the particularly strong separation of the enantiomers of serine. The reason for this is not known. The separation was therefore successful for

Table 1
Determination of the enantiomeric ratio for lS,2S-(+)-and lR,2R-(−)-pseudoephedrine

Ratio 1R,2R to 1S,2S	1R,2Rin 1S,2S		Bias (%)	Ratio 1S,2S to 1R,2R	1S,2S in 1R,2R		Bias (%)
	Added	Found			Added	Found	
1: 99	0.0101	0.019	+88	1: 99	0.0101	0.018	+80
10: 90	0.1111	0.119	+6.8	15: 85	0.1765	0.182	+2.8
25: 75	0.3	0.318	+6.0	25: 75	0.300	0.320	+6.6
50: 50	1	1.05	+5.0	50: 50	1.00	0.948	−5.2

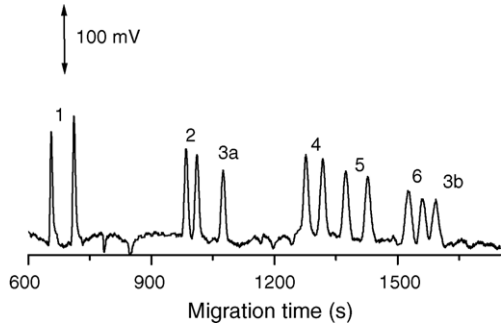

Fig. 6. Enantiomeric separation of a mixture of amino acids (2 mM) using 10 mM $I8C6H_4$ in 10 mM citrate/Tris at pH 2.2: (1) arginine; (2) valine; (3a and 3b) serine; (4) phenylalanine; (5) tyrosine; (6) aspartic acid. Other condition as for Fig. 1.

all species studied, and it is expected that the approach can be extended to all amino acids. The detection limits ranged between 2.5 and 20 μM for the species examined. The concurrent enantiomeric separation of a mixture of six amino acids is illustrated in Fig. 6. Good resolution was achieved in all cases.

4. Conclusions

Contactless conductivity detection was found suitable for enantiomeric separations in capillary electrophoresis and to our knowledge this is the first report on this application. The method is particular useful for non-UV-absorbing compounds as derivatization is not needed and any possible complications due to dyes added for indirect optical detection can be avoided. The enantiomeric separation of the non-absorbing amino acids demonstrated is thus unprecedented in its simplicity. Absorbing and non-absorbing species may also be determined in the same run, due to the universality of the conductometric detection method.

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