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Short communication

Enantiomeric separation of 1-phenylethylamine and 1-cyclohexylethylamine in capillary electrophoresis with contactless conductivity detection

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Abstract

Contactless conductivity detection was employed for the detection of the enantiomers of 1-phenylethylamine and 1-cyclohexylethylamine which were separated in capillary electrophoresis with unprecedented high resolutions R_s of 2.3 and 3.3, respectively, by using a combination of dimethyl-β-cyclodextrin and the chiral crown ether $18C6H_4$ as chiral selectors in a citric acid buffer of pH 2.4. The conductivity measurement enabled the direct detection, i.e. without having to derivatize or resort to indirect methods, of all species including the non-UV-absorbing enantiomers of cyclohexylamine. Detection limits of 0.5 μM were achieved and the determination of enantiomeric ratios of up to 99:1 was found possible.

Keywords: Enantiomeric separations; 1-Phenylethylamine; 1-Cyclohexylethylamine; Capillary electrophoresis; Contactless conductivity detection; Cyclodextrin; $18C6H_4$

1. Introduction

Analytical enantiomeric separations have become more important in recent times as there is a continuing trend towards the use of enantiomerically pure compounds [1]. Quantitative determinations are often carried out by HPLC, which however requires the use of specially designed columns with immobilized chiral selectors. Furthermore the sample throughput is commonly not adequate [2]. Capillary electrophoresis (CE) is an attractive alternative method. Enantiomeric separation is achieved by simply dissolving a small amount of a chiral selector in the buffer solution, and the analysis times are significantly reduced. This has therefore become an important field of application of capillary electrophoresis (for recent reviews see [3–7]). The most commonly employed chiral selectors are derivatives of cyclodextrins (CDs) and the chiral crown ether 18-crown-6-tetracarboxylic acid ($18C6H_4$).

The development of a method for the enantiomeric separation of the optical isomers of 1-phenylethylamine (α-methylbenzylamine) and 1-cyclohexylethylamine is reported herein. These compounds have been widely used as intermediates for industrial asymmetric synthesis. Their enantiomeric separation is difficult due to the shortness of the substituents on the chiral centres and to our knowledge, only a few reports on CE-methods for the phenylethylamines have appeared [8–12]. Only Mori et al. [10] and Armstrong et al. [11] achieved a useful resolution. The enantiomeric separation of the 1-cyclohexylethylamines was studied solely by Reetz et al. [12], using fluorescence detection, as the compounds are not accessible by the UV-detection employed by the other workers. However, Reetz et al. needed to derivatize the analytes.

The limitations of optical detection can be overcome with the relatively new method of axial capacitively coupled contactless conductivity detection (C^4D). This C^4D detector was introduced in 1998 and consists of a pair of short tubular electrodes which encompass the capillary [13,14]. The conductivity is probed by applying a sine voltage to the first electrode and picking up the resulting cell current at the second electrode. The chief advantages of this detector are its universality to all ionic species, its robustness and its low cost. It has been thoroughly characterized and its operation is straightforward [15–19]. The main requirement for a sensitive and stable output is the use of buffers of low conductivity. Many applications to inorganic as well as organic cations and anions have been reported (for recent reviews see [20,21]). It has also been shown recently by our group that this mode of detection is useful in the electrophoretic

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separation of enantiomers [22]. Non-UV-absorbing amino acids could be analysed directly with good sensitivity without having to resort to chemical derivatization for fluorescence detection.

2. Experimental

2.1. Instrumentation

The capillary electrophoresis instrument was purpose-built around a commercial high voltage power supply module (CZE 2000R, Start Spellman, Pulborough, UK). The detector is based on two electrodes of 4 mm length, consisting of steel tubing with an internal diameter of about 400 μm, and a detection gap of 1 mm. A sine wave voltage of 100 kHz and an amplitude of 400 V_{pp} (peak-to-peak) was used for cell excitation. The cell current was converted to a voltage, which was then rectified, low-pass filtered and digitized with a MacLab/4e data acquisition system (AD Instruments, Castle Hill, Australia). More details can be found elsewhere [23,24]. All electropherograms were inverted for presentation of the peaks in the normal orientation.

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-cyclodextrin) and dimethyl-β-cyclodextrin (DM-β-CD), which were purchased from Acros (Geel, Belgium). All solutions were degassed by ultrasonication and filtered through 0.2 μm nylon filters before use. The fused-silica capillary of 10 μm I.D. and 375 μm O.D. (Polymicro Technologies, Phoenix, AZ, USA) and total and effective lengths of 48 and 43 cm was preconditioned with a 0.1 M sodium hydroxide solution before rinsing with water followed by flushing with the running buffer. 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 obtained by diluting stock solutions (containing 20 mM of the amine in water) with the background electrolyte solution to ensure injection under non-stacking conditions.

3. Results and discussion

3.1. Cyclodextrins

Reetz et al. [12] reported at least partial separation for both compounds with different β- and γ-cyclodextrins at pH 9.1. Our attempts with seven different CDs, i.e. native α-, β-, and γ-cyclodextrins, HP-α-, β-, and γ-cyclodextrins, and DM-β-cyclodextrin, with different buffer solutions between pH-values of 2.4 and 9.1 were futile for both compounds. The reason for this discrepancy must be the fact that Reetz et al. had derivatized the analytes with fluorescein in order to render them accessible to their detection technique and it can be assumed that interaction of the fluorescein moiety with the cavity of the cyclodextrin was taking place.

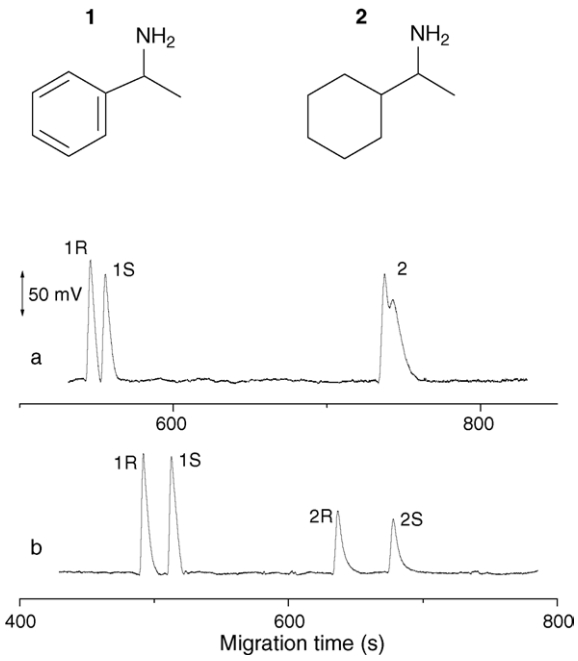

Fig. 1. Electropherograms for a mixture of (*R*/*S*)-1-phenylethylamine (1) and (*R*/*S*)-1-cyclohexylethylamine (2) for different conditions: (a) 25 mM citric acid with 10 mM DM-β-CD and 5 mM 18-crown-6; (b) in 25 mM citric acid with 5 mM DM-β-CD and 5 mM 18C6H_4 (pH 2.4).

3.2. Cyclodextrins with 18-crown-6

The combination of a cyclodextrin and the non-chiral crown ether 18-crown-6 has reportedly led to successful enantiomeric separations where cyclodextrins on their own have not been adequate, possibly through the formation of ternary, sandwich-type complexes [9,11,25,26]. As illustrated in Fig. 1a, we were able to reproduce the results reported by Armstrong et al. [11] with DM-β-CD, and an R_s value of 0.8 was achieved with HP-β-CD and 18-crown-6 in our case. The conductivity detection employed by us also allowed the examination of 1-cyclohexylethylamine but none of the combinations of 18-crown-6 with either of the cyclodextrins HP-α-CD, HP-β-CD, HP-γ-CD and DM-β-CD was found to lead to a good resolution of this non-aromatic compound.

3.3. Chiral crown ether (18C6H_4)

The chiral crown ether (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (18C6H_4) was found not to be adequate for the enantiomeric separation of either of the two species. Kuhn et al. [8] also unsuccessfully attempted the same approach for the phenylethylamine enantiomers, but note that Mori et al. [10] succeeded in separating the isomers of 1-phenylethylamine in an organic, rather than aqueous, solvent employing the chiral crown ether.

3.4. Cyclodextrins with 18C6H_4

The combination of cyclodextrins with the chiral crown ether is not common but has been reported before for the enantiomeric

Table 1
Determination of enantiomeric ratios for (*R*/*S*)-1-cyclohexylethylamine and (*R*/*S*)-1-phenylethylamine using a background electrolyte solution of 25 mM citric acid containing 5 mM DM-β-CD and 5 mM 18C6H$_4$

Ratio *R*-to-*S*	Expected	Found[a]	Bias (%)	Ratio *S*-to-*R*	Expected	Found[a]	Bias (%)
1-Cyclohexylethylamine							
1:99	0.010	0.018	+80	1:99	0.010	0.019	+85
25:75	0.333	0.35	+4	25:75	0.333	0.34	+2
50:50	1.000	1.02	+2	50:50	1.000	0.98	−2
1-Phenylethylamine							
1:99	0.010	0.018	+82	1:99	0.010	0.019	+90
25:75	0.333	0.35	+6	25:75	0.333	0.32	−5
50:50	1.000	1.02	+1	50:50	1.000	0.96	−2

[a] Ratio of peak areas.

separation of a few compounds [27–29]. Our investigation of using the chiral crown ether together with DM-β-CD showed that this combination can indeed be used for the enantiomeric separation of both, the optical isomers of 1-phenylethylamine and of 1-cyclohexylamine. The results are illustrated in Fig. 1b. As evident, very good baseline resolution is obtained for both compounds. The corresponding R_s values are 2.3 and 3.3 for 1-phenylethylamine and 1-cyclohexylethylamine, respectively. In case of 1-phenylethylamine the separation is clearly better than what can be obtained with the non-chiral crown ether in combination with DM-β-CD as reported above. The application of this combination was then tested for quantification of the enantiomers. Calibration curves were acquired from 2 μM to 0.2 mM and a linear response was found for at least this range. As shown in Fig. 2 for the non-aromatic compound, it is possible to detect the enantiomers in ratios of 99:1, allowing the determination of large enantiomeric excess (ee) values. The results for the quantitative determination of different ratios for the enantiomers of both compounds are given in Table 1. The detection limits (3× signal-to-noise ratio) were determined as 0.5 μM for all four species. Due to the lack of published data, a comparison of this value with those obtained by other capillary electrophoresis methods is not possible, but it is generally comparable to what is achieved with direct UV-absorption detection for aromatic compounds.

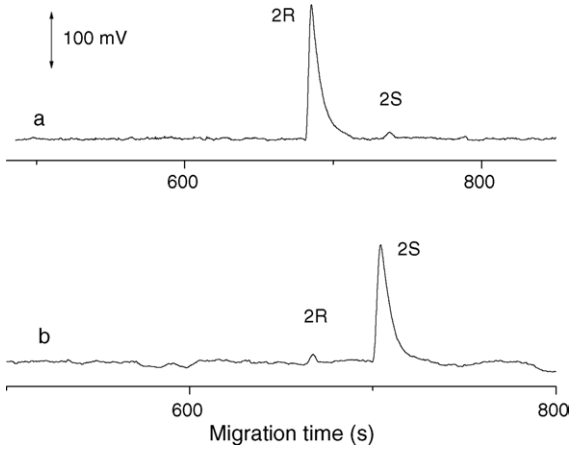

Fig. 2. Electropherograms for 0.2 mM (*R*/*S*)-1-cyclohexylethylamine (2) in 25 mM citric acid with 5 mM DM-β-CD and 5 mM 18C6H$_4$ (pH 2.4). (a) R 99%, S 1%; (b) R 1%, S 99%.

4. Conclusions

The use of a combination of two chiral selectors, the chiral crown ether and DM-β-cyclodextrin, led to unprecedented good enantiomeric separations of 1-phenyethylamine and 1-cyclohexylethylamine. Conductivity detection allowed the measurement of the non-aromatic cyclohexylamine without having to resort to optical methods based on dye displacement or derivatization. This is, to our knowledge, the first report on the separation of the underivatized enantiomers of this compound in capillary electrophoresis. The combination of the efficient separation approach and the impedance method for detection is expected to also lead to the facile enantiomeric analysis of other small, non-UV-absorbing amines, which to date has been carried out only with difficulty.

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