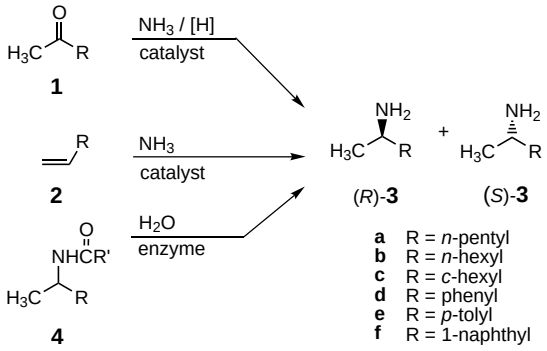
Super-High-Throughput Screening of Enantioselective Catalysts by Using Capillary Array Electrophoresis**

Manfred T. Reetz,* Klaus M. Kühling, Alfred Deege, Heike Hinrichs, and Detlev Belder*

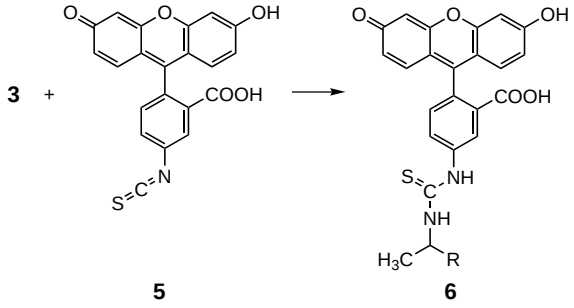
The enantiomeric purity of a product resulting from an asymmetric reaction is usually determined by direct chromatographic separation of the enantiomers by using conventional gas chromatography (GC),[1] high pressure liquid chromatography HPLC[2] or capillary electrophoresis (CE)[3] in conjunction with chiral stationary phases. However, throughput in these sequentially run assays is generally restricted to less than a few dozen determinations of the enantiomeric excess (% *ee*) per day. As a part of our investigations concerning directed evolution as a method to create enantioselective enzymes, we have been engaged in the development of high-throughput *ee*-screening systems.[4] Such assays are also crucial to the success of combinatorial methods in the development of enantioselective transition metal catalysts.[5] The currently available *ee*-screening systems developed by us[4] and other groups[6] are based on UV/Vis and fluorescence spectroscopy, circular dichroism or mass spectrometry, and all have limitations with respect to the type of substrate or reaction, throughput being limited to 300–1000 samples per day. Herein we describe a new screening system which allows for thousands of *ee* determinations per day.

The starting point of our considerations was conventional CE in which the electrophoretic separation of enantiomers is possible by the use of appropriate chiral selectors as part of the electrolyte.[3] Accordingly, soluble cyclodextrins (CDs) were employed as so-called pseudo-stationary phases. An important field of application of CE is DNA analysis and sequencing. In particular for the human–genome project various technical versions have emerged to increase throughput drastically.[7, 8] Among them is capillary array electrophoresis (CAE) in which a high number of capillaries are operated in a parallel manner so that DNA separations and sequencing can be performed automatically with high-throughput.[7] Special instruments of this kind, specifically designed for DNA analysis, are commercially available, for example, the MegaBACE system[9] consisting of 6 bundles of 16 capillaries which spatially address standard 96-well microtiter plates. Our approach to high-throughput screening of enantioselective catalysts was based on the adaptation of this technology by using chirally modified electrolytes.[10] As a specific goal we chose the *ee* determination of chiral amines.

Chiral amines of the type **3** constitute important intermediates in the synthesis of pharmaceuticals and plant protecting agents.[11] They can be prepared by various methods including the catalytic reductive amination of ketones **1**, Markovnikov addition of NH_3 to olefin **2**, or hydrolytic enzyme-catalyzed kinetic resolution of amides **4** (or reverse reaction).



In exploratory experiments we first varied the electrophoretic conditions of a conventional, single-capillary CE system for the enantiomeric separation of the chiral amines **3**. To make parallel detection possible by laser-induced fluorescence (LIF) in a capillary array system, the amines **3** were treated with the standard fluorescein isothiocyanate[12] (FITC) **5**, which results in the formation of the derivatives **6**.



Although complete optimization of the electrolyte composition was not carried out for all the compounds, the experiments do show that the use of various commercially available cyclodextrin derivatives as chiral selectors does lead to an acceptable antipode separation of the substrates (Table 1).

As part of a model study we attempted the antipode separation of the amine derivative **6c** with the help of CAE. To this end the parameters obtained in the conventional single-capillary system had to be adapted for application in the multi-capillary MegaBase system. As a result of the special characteristics of this system which was developed for DNA analyses (only electrokinetic injection and detection solely at the anode), the electrolyte system had to be adapted for our purposes. Electrolytes which led to excellent results in single-capillary CE systems provided very unstable electrophoretic runs with large fluctuations in the electric current. The results obtained, which are not very reproducible, can be

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Table 1. Results of chiral CE separations of the amine derivatives **6** at various electrolyte compositions.[a]

Buffer[b]	**6a**	**6b**	**6c**	**6d**	**6e**	**6f**
40 mmol CHES, 40 mmol γ-CD+15% CH_3CN	B	B	–	n.m.	–	–
20 mmol borate, 20 mmol DM-β-CD	–	–	–	–	–	–
40 mmol CHES, 20 mmol DM-β-CD	–	–	–	–	–	–
40 mmol CHES, 5 mmol DM-β-CD	–	–	–	P	–	–
40 mmol CHES, 40 mmol HE-β-CD	–	–	–	–	–	–
40 mmol CHES, 20 mmol HE-β-CD	P	–	–	P	–	–
40 mmol CHES, 10 mmol HE-β-CD	P	–	–	P	P	–
40 mmol CHES, 5 mmol HE-β-CD	aB	P	P	aB	aB	–
40 mmol CHES, 2.5 mmol HE-β-CD	aB	P	P	P	aB	–
40 mmol CHES, 5 mmol HP-α-CD	–	–	–	–	–	–
40 mmol CHES, 20 mmol HP-β-CD	–	–	–	P	–	–
40 mmol CHES, 5 mmol HP-β-CD	P	–	P	aB	P	–
40 mmol CHES, 20 mmol HP-β-CD+15% CH_3CN	P	–	–	P	–	P
40 mmol CHES, 25 mmol HP-γ-CD	–	–	P	–	–	–
40 mmol CHES, 6.25 mmol HP-γ-CD	–	–	B	–	P	aB
40 mmol CHES, 25 mmol HP-γ-CD+15% CH_3CN	–	–	B	–	–	–
40 mmol CHES, 10 mmol NH_2-β-CD	–	–	–	–	–	–
40 mmol CHES, 10 mmol β-CD	–	–	–	–	–	–

[a] P: partial separation; B: base-line separation; aB: almost base-line separation; –: no separation; n.m.: not measured. [b] DM: heptakis(2,6-di-*O*-methyl); HE: hydroxyethyl; HP: hydroxypropyl; NH_2: 6^A-amino-6^A-desoxy; CHES: 2-(*N*-cyclohexylamino)ethanesulfonic acid.

explained in part by the conjecture that the electrokinetic injection of the anionic analytes, even at only weak cathodic electroosmotic flow (EOF), as found in the polyacrylamide coated capillaries used, is problematic. In contrast, reproducible results of higher quality were obtained by using a viscous electrolyte containing a linear polyacrylamide (LPA) as an electrolyte additive which reduces the EOF. The electrolyte consists of γ-CD (6.25 mM) dissolved in CHES buffer (40 mM) at pH 9.1, in ratio 5:1 with the highly viscous LPA buffer (from Amersham Pharmacia). The voltage per capillary was adjusted to -10 kV at 8 μA at a sampling voltage of -2 kV for 9 s.

Initially, 12 solutions of (+)/(−) mixtures of **6c** were analyzed by the adapted MegaBACE system.[9] Figure 1 shows the electropherograms obtained in parallel and Table 2 the *ee* values determined by CAE. As a control, the enantiomeric purities of the corresponding non-derivatized amines (+)/(−)-**3c** were checked by using GC with a chiral stationary phase (Ivadex-1/PS086 d_f: 0.15 μm, i.D.: 0.25, l: 25 m). The agreement is very good, although integration of the signal corresponding to the (+)-isomer in the CAE experiments was made difficult by an impurity, especially at low (+)/(−) ratios (Table 2, entry 1).

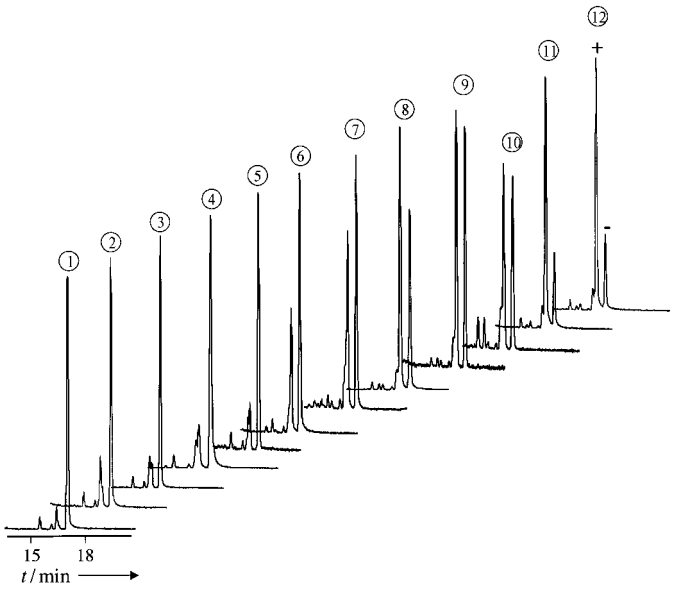

Figure 1. CAE separation of representative samples of the amine derivatives **6c**.

Table 2. Comparison of the experimentally determined enantiomeric excess of samples of (+)/(−)-**3c** by GC and the corresponding enantiomeric excess of (+)/(−)-**6c** determined by CAE.

Sample	*ee* **3c** (GC) [%]	*ee* **6c** (CAE) [%]
1	98	>95
2	88	90
3	84	82
4	74	70
5	74	70
6	38	38
7	18	16
8	18	16
9	0	0
10	0	2
11	54	52
12	54	52

This unoptimized antipode separation requires about 19 minutes. Upon using the automated 96-array system at least 7000 *ee* determinations are possible per day. By optimizing the experimental parameters, such as increasing the electric field strength and/or control of the electroosmotic flow by the use of special capillaries,[3g] considerably shorter analysis times can be reached so that a daily throughput of 15000 to 30000 *ee* determinations is realistic. Such super-high-throughput screening of enantioselectivity is not possible using any other system currently available. As CAE has many advantages, including the use of very small amounts of sample, practically no utilization of solvents, absence of high-pressure pumps and valves as well as variable use of different and relatively cheap chiral phases, the present assay appears particularly attractive.

Another possibility to increase throughput of CE makes use of chip technology in which photolithographic techniques are preferably applied to create one or more capillaries on microchips.[8] Such CE microchips are made of plastic or glass and have been used in the analysis of oligonucleotides, DNA-sequence fragments, amino acids, DNA restriction fragments as well as PCR products. These highly miniaturized systems allow for extremely rapid separations within seconds or minutes. We have therefore started to study separations of enantiomers on CE microchips.[13] Whereas in the separation of biomolecules in aqueous electrolytes plastic or glass chips can be utilized (as in the case of amino acids)[14] we observed that in the separation of conventional organic compounds, in electrolytes that contain organic solvents, on plastic (polymethyl methacrylate (PMMA)) based microchips the system is not chemically stable enough. We have therefore concentrated on glass chips.

Since appropriate CE instruments based on microchips are not yet commercially available, we first built our own

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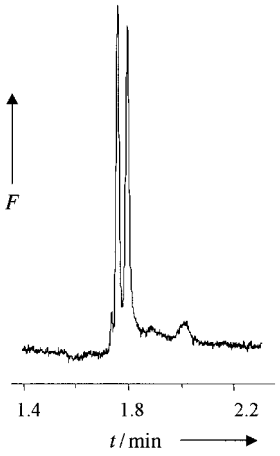

Figure 2. Chiral electrophoretic separation of the racemic amine derivative **6c** on a CE microchip; F = fluorescence intensity.

configuration equipped with two high-voltage sources, high-voltage switches, and an LIF-detector (argon-ion laser). In initial experiments, by using CE glass chips from Micralyne (Edmonton, Canada), we obtained very promising results concerning chiral separations of FITC-derivatized amines. In the case of **6c** practically complete basis-line separation of the antipodes was achieved within two minutes (Figure 2). In doing so we used the same electrolyte as before (Figure 1), but without LPA. For sample injection a voltage of 1 kV was applied at the electrodes, at the buffer inlet, buffer outlet, as well as the sample inlet, whereas the sample outlet was kept at the ground-level potential. For separation the voltage was switched so that the voltage at the four electrodes was as follows: buffer inlet 1 kV, sample inlet 0.6 kV, sample outlet 0.6 kV, buffer outlet 0 V.

Further development of this analytical instrument with the capability of higher electric-field strength leading to the possibility of chiral separation within seconds is in progress. Thus, a second CE-based method for super-high-throughput screening of enantioselective catalysts can be anticipated. Another goal is to carry out the actual catalytic reactions on microchips and to couple this process directly with such a CE-screening system.

Our study shows that the development of capillary electrophoresis in the form of CAE for the determination of enantiomeric purity can be used in a truly high-throughput manner (> 7000 *ee* determinations per day). This result as well as the optimization of the methods described herein are of great significance for the further development of combinatorial asymmetric transition metal catalysis[5] and directed evolution of enantioselective enzymes.[4, 15]

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