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Review

Alternative methods providing enhanced sensitivity and selectivity in capillary electroseparation experiments

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Abstract

The study of alternative and novel techniques for altering selectivity and enhancing sensitivity as well as injection and detection protocols are important in the ongoing development of capillary electroseparation protocols. Some recent research from our laboratory in these fields is presented and discussed in this review. To improve sensitivity an off-line sample enrichment technique utilising solvent evaporation in a levitated drop or an on-line solid-phase extraction protocol was used. The selectivity was tuned by the use of protein gels or molecularly imprinted polymer mediated capillary electrochromatography. Furthermore, a picolitre droplet injection method is described as well as a detection protocol based on laser-induced fluorescence imaging.

Keywords: Reviews; Sensitivity; Selectivity; Electrochromatography; Capillary electrophoresis

Contents

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1. Introduction

Research into the enhancement of sensitivity and the development of novel approaches to obtain and alter selectivity are important to further develop and expand the use of capillary electroseparation methods in general. Novel injection and detection protocols are also important in that sense. Some recent efforts in the field of sensitivity and selectivity in capillary electroseparation methods as well as work on injection and detection protocols in our laboratory are reviewed in this article.

2. Sample enrichment techniques in capillary electrophoresis

Despite the great resolution power, the use of capillary electrophoresis (CE) is hampered by its low detection sensitivity, which is inherent in the short optical path length [the same as the capillary inside diameter (I.D.)], when on-line absorption detectors are used. To increase sensitivity, either larger sample volumes or more sensitive detection schemes must be used. To exploit that the sample introduced in CE is below 20 nL, an initial sample volume of 20 μl is sufficient if a concentration factor of 1000 is needed. It is a challenge to handle and concentrate such small volumes.

2.1. Off-line enrichment by solvent evaporation in a levitated drop

Sample volumes in the microlitre range can be enriched by solvent evaporation by the aid of acoustical levitation, Fig. 1 [1,2] and then directly injected into a CE capillary. By this containerless approach, the sample is easily accessed and effects such as contamination by compounds desorbing from, and analyte adsorption on container walls are reduced. The drop can be trapped in the standing ultrasonic wave at several positions where the sound pressure node is nearby [3]. Positioning of the sample in the levitator was automatically done using a flow-through microdispenser [4,5]. The orifice of the flow-through liquid microdispenser was placed approximately 6 mm from the sound pressure node [2]. The droplet diameter ejected through the orifice was 50 μm corresponding to a volume of 65 pL. During the enrichment procedure the droplets were

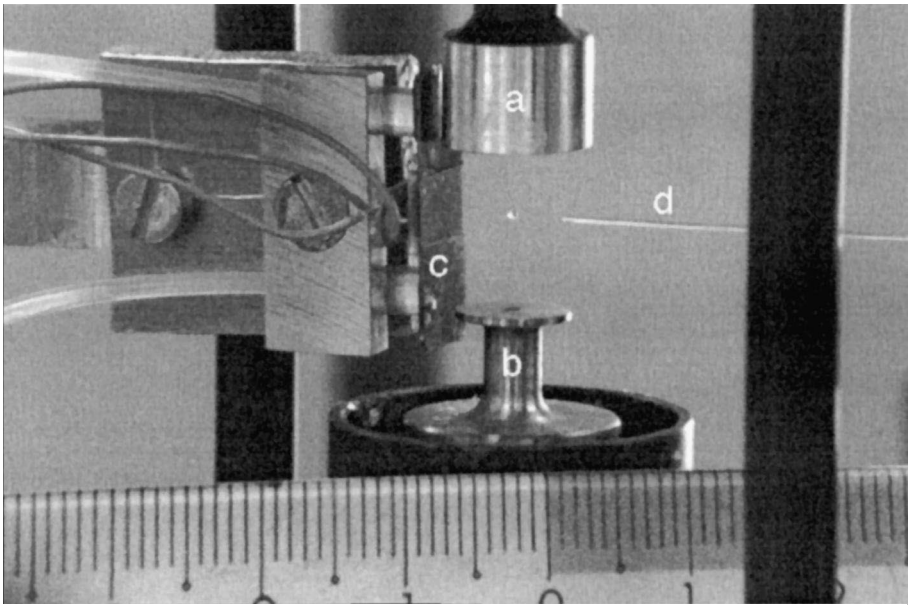

Fig. 1. Droplet levitated in a sound pressure node of a standing ultrasonic wave. a=Reflector, b=ultrasound transducer, c=flow-through liquid microdispenser and d=capillary. From Ref. [2] with permission.

ejected at a frequency of 100 Hz equal to 0.4 μl/min. Counting the number of droplets ejected and accumulated in the levitated drop controlled the initial sample volume. The final volume of the levitated drop after evaporation was in the low nanolitre range because the final drop diameter was in between the capillary I.D. (50 μm) and O.D. (375 μm). The capillary detection end was lowered and attachment of the drop to the capillary inlet tip did the sample introduction. Through solvent evaporation in the levitated drop, a concentration factor of two orders of magnitude was reached in about 30 min using an initial sample volume of about 2 μl. The concentration limit of detection using UV absorbance detection for the model compounds dansyl-valine and dansyl-glycine dissolved in ethanol was 15 n*M* using enrichment compared to 2.5 μ*M* without. The drawback is that not only the analytes of interest will be enriched but also any other non-volatile components present in the sample. This may lead to high ion concentrations, which will impair CE performance.

2.2. On-line solid-phase extraction–capillary electrophoresis

To eliminate the need for solvent evaporation and to minimise sample handling, solid-phase extraction (SPE) has been done on-line in CE using a microcartridge at the inlet, Fig. 2 [6–17]. In this way, high concentration factors can be approached even if the sample volume is in the microlitre range because the desorption volume is in the nanolitre range. By optimisation of the SPE–CE procedure, both high concentration factors and high efficiencies were obtained [17]. The enrichment capillary was made from sorbent particles, glass-fibre filters, epoxy glue and fused-silica capillaries of two different I.D.s. The extractor was made small enough to be used in a commercial instrument and it was easily placed inside a CE cartridge. The extractor capillary was packed with 12 μm sorbent particles using a high-pressure slurry packing procedure. The glass-fibre filters were easily punched from a large diameter filter disk using the 200-μm I.D. capillary in which

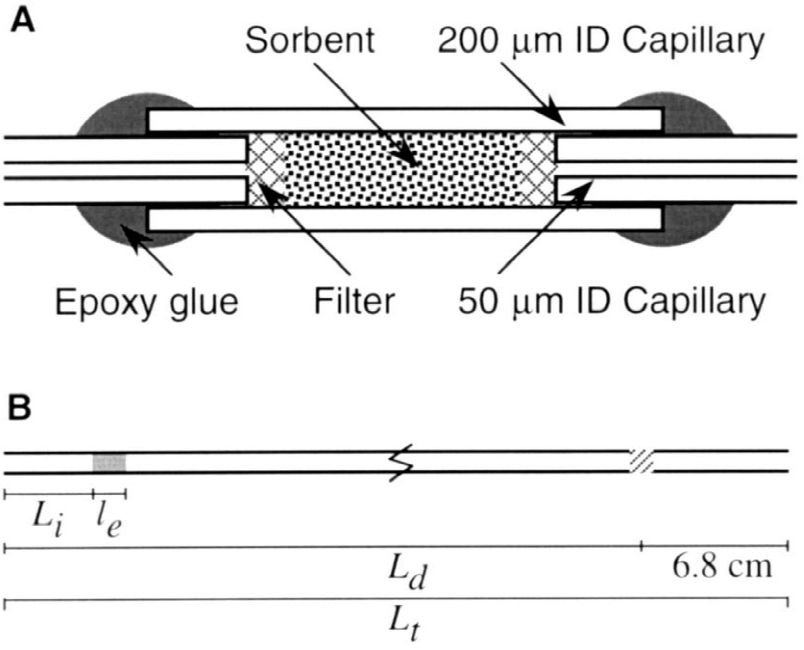

Fig. 2. Cross-section of (A) the extractor and (B) the enrichment capillary where L_t (28–58 cm) is the enrichment capillary total length, L_d (21.2–52.2 cm) is the length to the detector, L_i (5.4 cm) is the length of the inlet capillary and l_e (1–3 mm) is the extractor length. From Ref. [17] with permission.

the filters were to be used. The glass-fibre filters were compressed to minimise dead volumes to a final length less than 100 μm. By the use of dilute slurry and a small slurry reservoir, the final length of the packed bed could be made as short as 1–3 mm. The flow through the enrichment capillary was about 75% of the flow through a capillary without extractor. There are numerous different sorbent particles available and the construction of the enrichment capillary does not put any particular constraints on which sorbent to be used as long as it can be suspended in slurry and is mechanically stable. A restricted-access media, alkyl-diol silica [18] was used as sorbent in this study. Using silica-based sorbents the enrichment capillary cannot be regenerated using sodium hydroxide, though. Impregnated and particle-loaded membranes have also been used [9,19]. A new type of sorbents used in SPE is molecularly imprinted polymers (MIPs) [20]. Such polymers can be prepared in situ [21]. Their use in SPE–CE is promising but has not yet been demonstrated.

The sample enrichment procedure follows the general steps used in off-line SPE, i.e. wetting, conditioning, sorption, washing and desorption, Fig. 3. Methanol was not only used to wet the sorbent, it also minimised carry-over effects. This was important because the enrichment capillaries were reused. They were stable for more than 200 runs if non-proteinaceous samples were applied. The sample was introduced using the pressure facilities of the CE instrument. In a 58 cm×50 μm capillary without

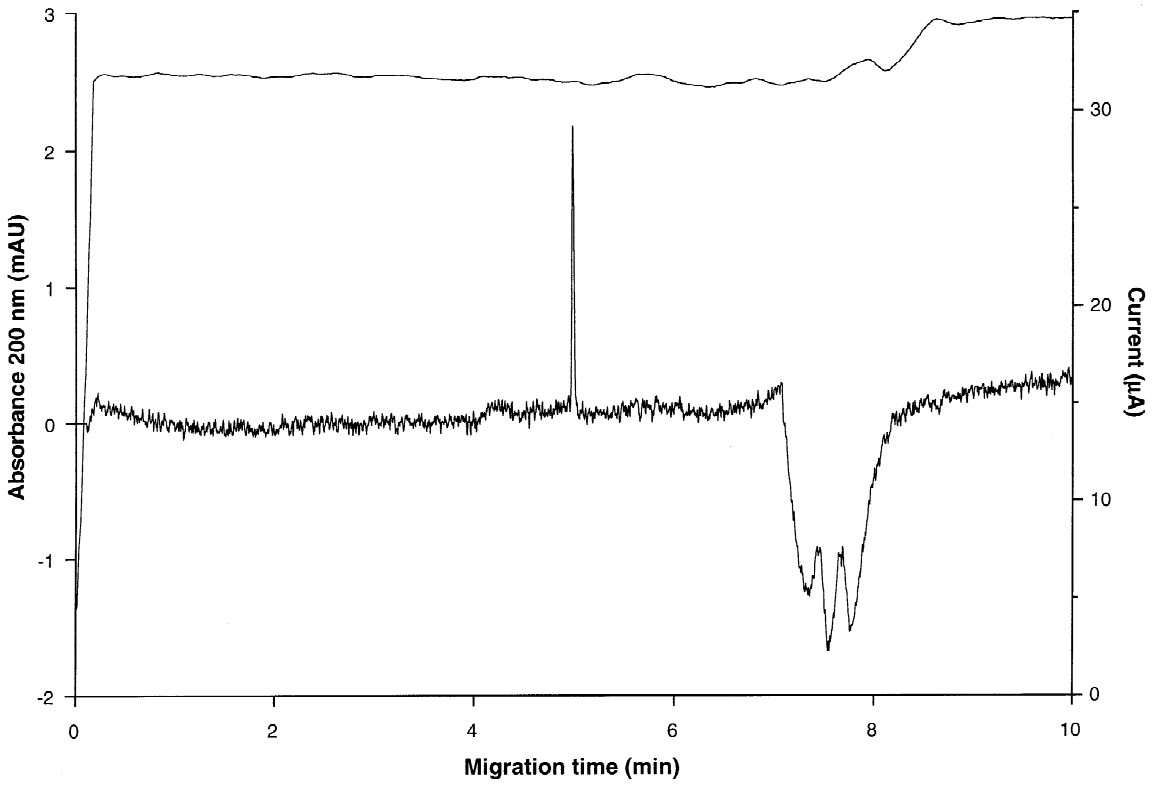

Fig. 3. Representative electropherogram of terbutaline demonstrating that high (250 000) separation efficiency was maintained during the on-line enrichment procedure. Enrichment capillary: L_t 58.0 cm, L_d 51.2 cm, L_i 5.4 cm, l_e 1.25 mm, wash: water, 1.4 min, 20 p.s.i.; wetting: methanol, 2.1 min, 20 p.s.i.; conditioning: water, 2.1 min, 20 p.s.i.; injection: 100 n*M* terbutaline in water, 1.0 min, 20 p.s.i.; wash/filling: 40 m*M* potassium phosphate (pH 6.4), 0.7 min, 20 p.s.i.; desorption: acetonitrile, 30 s, 0.5 p.s.i. followed by 40 m*M* potassium phosphate (pH 6.4), 3.0 min, 0.5 p.s.i.; voltage: 20 kV; detection wavelength: 200 nm; temperature: 25°C. From Ref. [17] with permission.

extractor, the volumetric flow is approximately 2.5 μl/min using 20 p.s.i. at 25°C (1 p.s.i.=6894.76 Pa). It is thus time-consuming to introduce the large volumes encountered when striving for high concentration factors. The post-sorption washing conditions were critical and the effects from large volumes and different washing solutions must be investigated. A good desorbing solvent in SPE–CE should induce complete desorption in a small volume and has optimal electrophoretic properties [maximal stacking and minimal disturbance of electroosmotic flow (EOF)]. Organic solvents are used for desorption using reversed-phase sorbents which, in addition, promotes further enrichment by stacking. The desorbing solvent volume needed was approximately half the extractor empty volume. This equates to 20–40 nL. The desorbing solvent was preferably pushed through the extractor using a low external pressure. For the basic drug terbutaline used as a model compound, an initial concentration factor of 1000 was demonstrated. The initial concentration factor was calculated as sample volume introduced divided by desorption volume. The low nanomolar range is thus within reach using UV absorbance detection. For terbutaline in water, the breakthrough volume was about 45 μl. The concentration factor can be much increased with more hydrophobic analytes, which allow larger sample volumes to be introduced before breakthrough. Because terbutaline was desorbed using organic solvent e.g. acetonitrile, additional on-line concentration took place by stacking. This can increase the concentration about 10 times further leading to a final concentration factor of 10 000.

3. Selectivity

3.1. Protein based monolithic stationary phases

The low-molecular-mass binding characteristics of the transport protein albumin have been utilised for a long time to resolve chiral compounds [22–26]. Serum albumin's interaction and binding characteristics for small molecules, was better understood when its three-dimensional structure was elucidated [27]. A methodology for creating monolithic protein based columns for capillary electrochromatography (CEC), was early developed by us [28]. Later this methodology was also adapted for other proteins such as, cellulase and monoclonal antibodies [29,30]. The proteins were cross-linked in the capillary by the bifunctional cross-linking agent glutaraldehyde. In order to allow UV detection the capillary was only partially gel-filled. This was achieved by carefully timing, during the filling of protein-glutaraldehyde mixture into the capillary column. This mild cross-linking retains the three-dimensional structure of the protein-based selector, which is favourable for chiral recognition as well as for other macromolecular studies. The resulting monolithic opaque protein stationary phase was not allowed to reach the detection area in the column. In later work [31] all the monolithic electrochromatography columns were developed using the pressure facilities of the CE instrument. The efficiencies obtained for the last eluted enantiomers (Figs. 4–6) were from 5000 to 80 000 plates/m and with a chiral resolution of $1.6 \leq R_s \leq 4.2$. One of the weak points of the monolithic protein CEC columns is their susceptibility to air and drying during use i.e. they should be treated like cross-linked polyacrylamide gel columns. The optimal handling procedure was by creating the column in the CE instrument and then run it in situ without external manual handling [31]. The small consumption of selector as well as sample makes the described methodology ideal for the screening of the interaction of different small molecules with macromolecules i.e. drug–protein interaction as well as macromolecules enzymatic behaviour on various substrates.

3.1.1. Protein based pseudo-stationary phases

A novel system for selective recognition based on latex-immobilised selectors for electrochromatography is under development at our laboratory. By covalent attachment of selector protein to micro and sub-micron particles, successful recognition of antigen from immobilised monoclonal antibodies was obtained [32]. Two different latex bound monoclonal antibodies directed towards the same antigen were used aiming for specific selection and single molecule detection. Chiral recognition was also successfully achieved by electrochromatography, performed with latex immobilised protein (unpublished results).

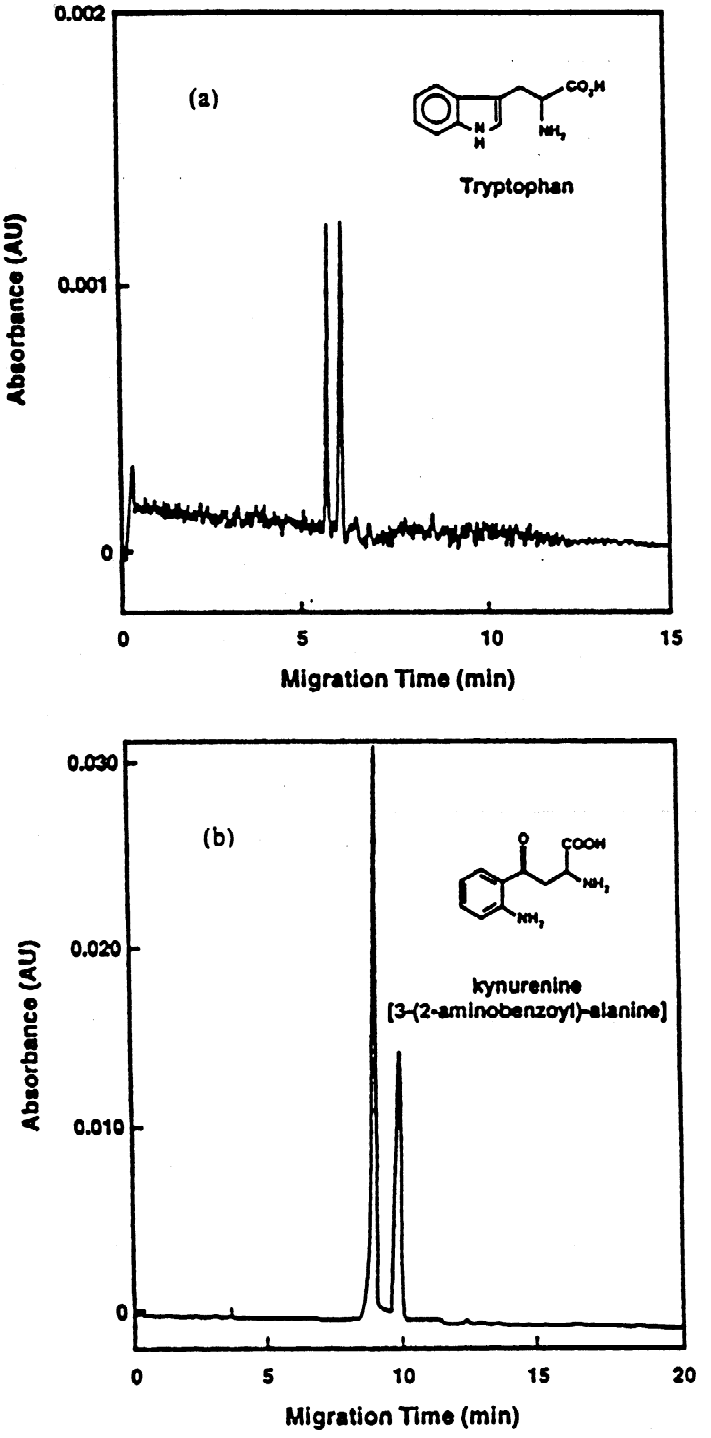

Fig. 4. (A) Separation of 70 μ*M* *rac*-tryptophan on a monolithic bovine serum albumin column. Conditions; voltage: 3 kV (~128 V/cm), current: 60 μA, gel length: 16.5 cm, total capillary length: 23.5 cm, run buffer: 50 m*M* potassium phosphate (pH 8.2), sample buffer: 20 m*M* potassium phosphate (pH 6.4), sample injection: 3 kV, 3 s. (B) Separation of 250 μ*M* racemic kynurenine. Conditions; voltage: 3.5 kV (~150 V/cm), current: 67 μA, otherwise as above. From Ref. [48], with permission.

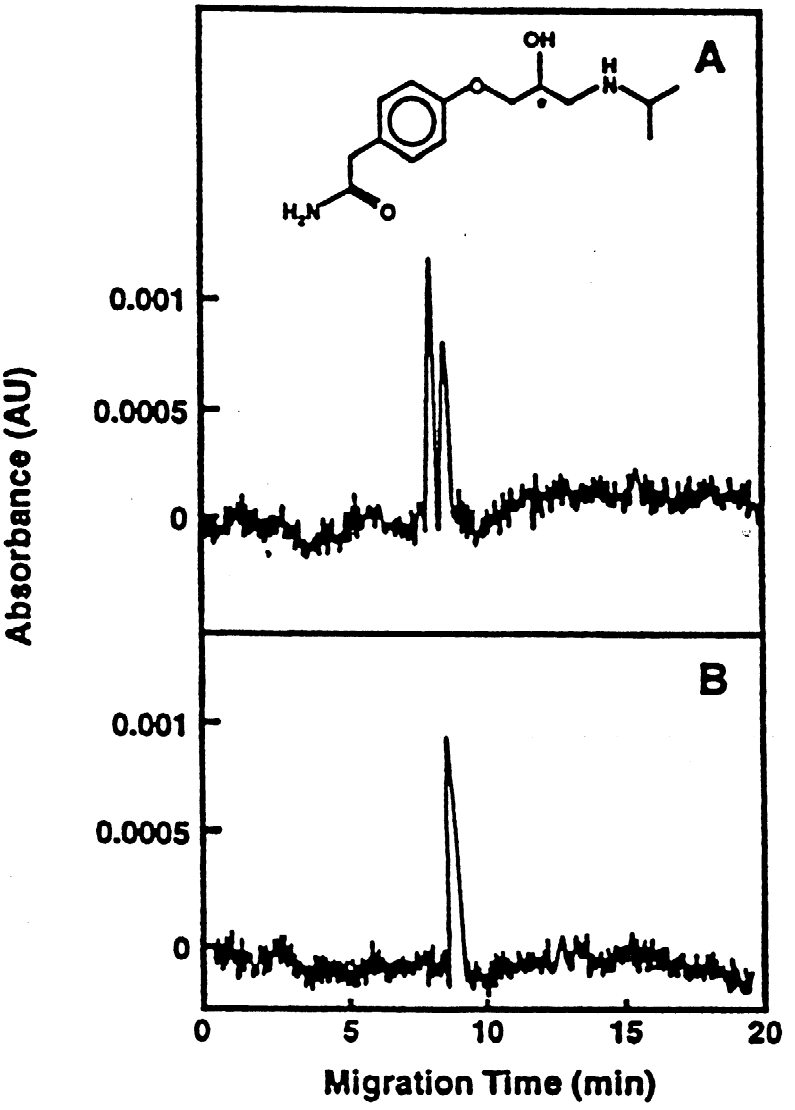

Fig. 5. Separation of *rac*-metoprolol on a monolithic cellulase/BSA column. (A) 0.15 m*M* *rac*-metoprolol. (B) 0.075 m*M* S-metoprolol (identification of racemate). Conditions; voltage: 3 kV (~130 V/cm), current: 40 μA, gel length: 16.5 cm, total capillary length: 23.5 cm, buffer: 50 m*M* potassium phosphate (pH 6.8) with 1% (v/v) 2-propanol, injection: 2 kV, 3 s. From Ref. [48] with permission.

3.2. Molecular imprinting mediated capillary electrochromatography

The ever ongoing research into novel stationary phases for chromatographic separations of chemical entities have sparked interest in molecular imprinting [33–37] as a facile technique for the preparation of phases with pre-determined selectivity. Imprinted polymers can be made selective for one of the enantiomers of a chiral compound, a specific compound or a class of compounds (Fig. 7). Most molecularly imprinted polymer (MIP) studies have focused on chiral separation problems and many different racemic compounds have been successfully resolved, including drugs [38–40], amino acid derivatives [33–35] and sugars [33–35]. In our labora-

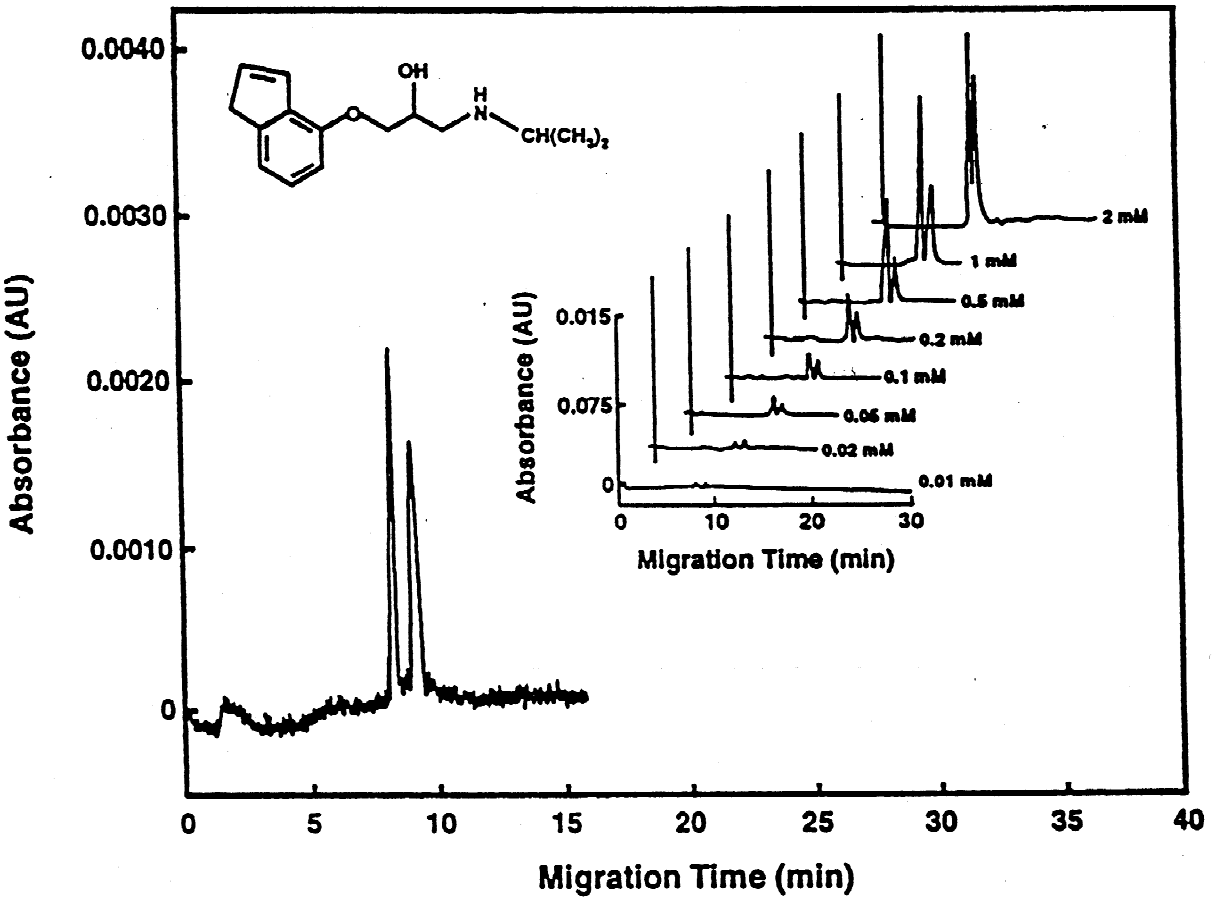

Fig. 6. Separation of 0.1 m*M rac*-pindolol on a monolithic cellulase/BSA column. Inset: Sample loading capacity 0.01–2 m*M*. Conditions; voltage: 3.5 kV (~150 V/cm), current: 45 μA, gel length: 16.5 cm, total capillary length: 23.5 cm, buffer: 50 m*M* potassium phosphate (pH 6.8) with 1% (v/v) 2-propanol, injection: 2 kV, 3 s. From Ref. [48] with permission.

tory, the focus is to investigate, employ and develop molecularly imprinted polymers for use as stationary phases in capillary electrochromatographic applications. Research efforts in this area were recently reviewed [41].

3.2.1. In situ prepared molecularly imprinted polymer monoliths

Our approach to MIP-based CEC utilises capillary columns filled with a monolithic, superporous imprinted polymer [21,31,42]. The morphology of a certain molecularly imprinted polymer monolith is depicted in Fig. 8. Using this system, enantiomer separations of predetermined elution order, with baseline resolution were carried out in less than 2 min. The MIP-filled capillaries are obtained by an in situ photo-initiated polymerisation process. The capillary is filled with a pre-polymerisation mixture of imprint molecule, functional and crosslinking monomers (methacrylic acid and trimethylolpropane trimethacrylate, respectively), radical initiator (2,2′-azobis(isobutyronitrile) and solvent (toluene). Both ends of the capillary are sealed and the polymerisation is performed by placing the capillary under a UV-source (350 nm) at −20°C. The polymerisation reaction is terminated by hydrodynamically flush remaining monomer, radical initiator, and imprint molecule out of the capillary column. In the same way the solvent is exchanged for electrolyte. After a short time of equilibration the capillary column is ready for use. The inner surface of the capillary is derivatised with methacryloxypropyltrimethoxy-

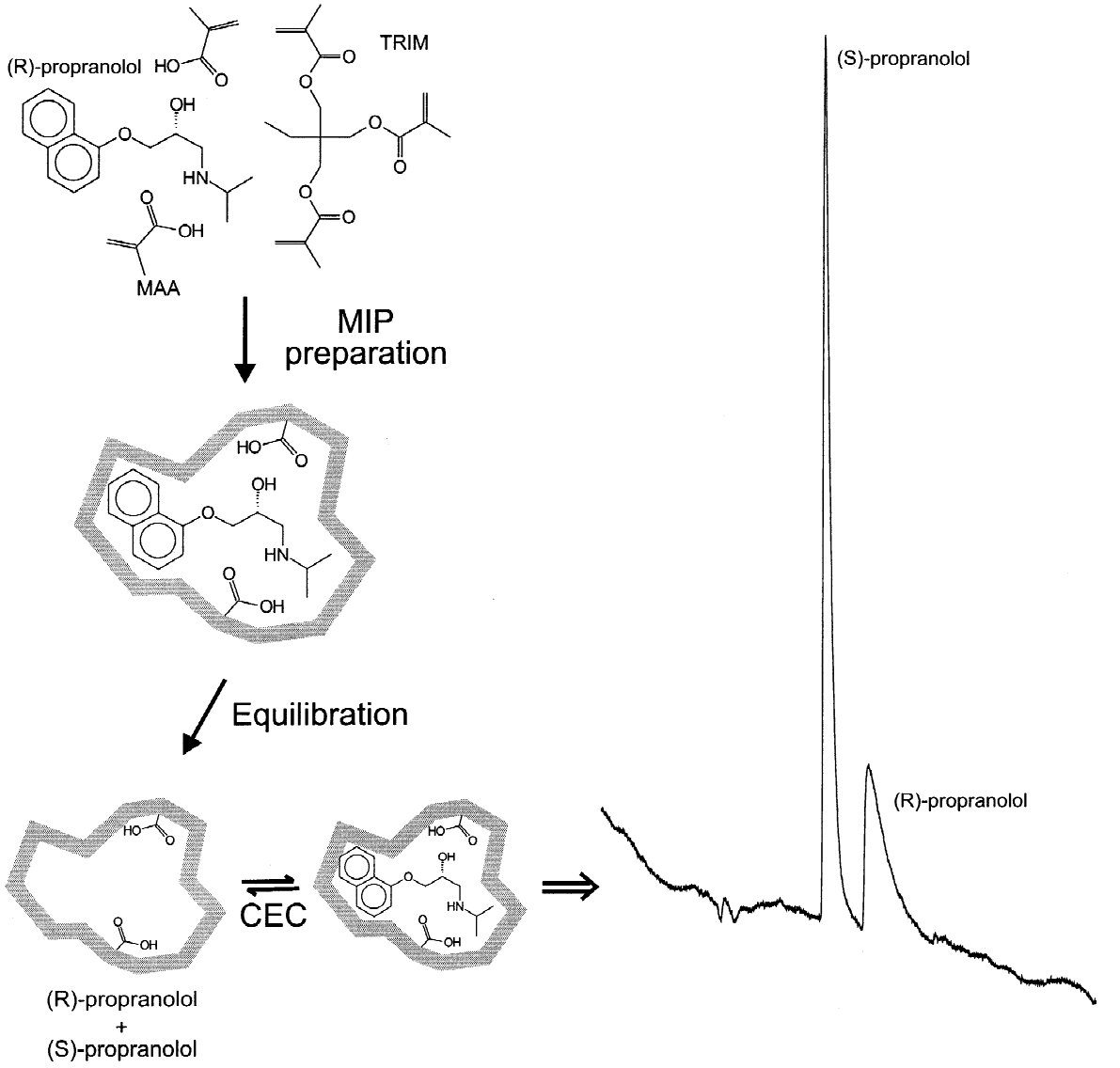

Fig. 7. Schematic of molecular imprinting of (R)-propranolol using methacrylic acid (MAA) as the functional monomer and trimethylolpropane trimethacrylate (TRIM) as the cross-linking monomer. The enantio-selectivity of a given MIP is pre-determined by the enantiomer of the ligand that was present during its preparation, here the R-form of propranolol. Since the imprinted enantiomer possesses the higher affinity for the MIP, the separation is done with predictable elution order of the enantiomers. From Ref. [41] with permission.

silane, which participate in the polymerisation reaction. The polymer monolith is thus covalently attached to the inner wall of the capillary (Fig. 8), thereby preventing elution of the polymer during electrochromatography. The technique is simple and quick, and enables imprint-based separation systems to be operational within 3 h of the start of capillary preparation. The success of this approach relies on the polymer-filled capillaries possessing good flow-through properties, because before use the organic solvent employed for polymerisation must be replaced by an electrically conducting electrolyte. This method requires, however, a careful timing of the polymerisation reaction to establish the optimal reaction time for the system used. If the reaction time is too long, the polymer will be dense and

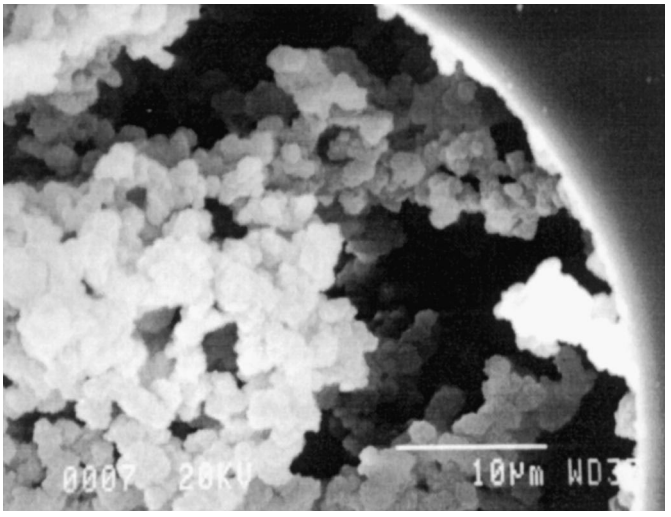

Fig. 8. SEM of a super-porous polymer-filled capillary column. Micron-sized globular units of macro-porous molecularly imprinted polymer are surrounded by interconnected super-pores. These super-pores are in the range of 1–7 μm in this case. The covalent attachments of the polymer to the capillary wall can be seen. From Ref. [41] with permission.

hydrodynamic flushing will be impossible. A too short reaction time will result in a low amount of MIP in the capillary column. Once established, the success rate of producing capillary columns with a super-porous monolithic stationary phase is 100%.

In a slightly modified approach, the MIPs can be rendered super-porous by the use of 1–25% isooctane as a porogenic agent [31,42]. The porogen is composed of a mixture of solvents in which the porogenic agent (isooctane) acts as a bad solvent for the growing polymer chains, whereas the other (toluene for example) acts as a good one. In the choice of porogen it is important that no or minimal solvent-caused disturbance of the imprinting procedure occurs. Imprinted super-porous polymer monoliths can be prepared using several crosslinking monomers [trimethylolpropane trimethacrylate (TRIM), pentaerythritol triacrylate (PETRA), pentaerythritol tetraacrylate (PETEA), or ethylene glycol dimethacrylate (EDMA)] and functional monomers (methacrylic acid (MAA) and 2-vinylpyridine (2VPy)) using a porogen composed of 1–25% (v/v) isooctane in toluene. It is important to optimise the volume ratio of isooctane to toluene ratio in the pre-polymerisation mixture to obtain capillary columns of optimal flow-through characteristics.

Regarding the detection protocols used with capillary columns with molecularly imprinted stationary phases, on-column UV-absorbance detection have been used exclusively. Some sort of open tubular area without imprinted polymer is normally prepared to perform detection. It has been shown however, that UV detection can be performed through the imprinted polymer [21] in some cases. A nice feature with the photo-induced polymerisation procedure is that a part of the capillary column can be covered during the polymerisation reaction, thus preventing polymer to be formed in that area. This is utilised to readily prepare detection windows on the molecularly imprinted polymer capillary columns.

Furthermore, the microcolumn format of these MIP columns leads to minimal consumption of chemicals, including the imprint species. Typically 10–100 nmol of imprint species are required for a polymer the size of a capillary column, which is about 1–10 μl in volume.

3.2.2. Electrochromatographic separations

As yet, research into the field of MIP-CEC has focused on the adaptation of imprinted polymers to electrochromatography, and the preparation of MIP stationary phases within fused-silica capillaries. The optimisation of separation parameters have been addressed less. Most often, the electrolyte is com-

posed of 70–90% of an organic modifier, such as acetonitrile or acetone, and an aqueous buffer at low pH. In one study, using (S)-ropivacaine imprinted polymer filled capillaries, it was found that an electrolyte composed of 80% acetonitrile and 20% aqueous buffer was suitable for CEC separation of the enantiomers of ropivacaine [42]. The enantiomer separation increased with increasing pH, at least in the pH-range 2–6.5, as well as with increasing volume ratio of acetonitrile to aqueous buffer. In most cases, however, improved selectivity occurred at the expense of peak broadening. It was observed that the resolution could be improved by increasing the capillary temperature. Baseline separations of the enantiomers of propranolol and metoprolol were achieved using an electrolyte composed of 80% (v/v) acetonitrile and 20% (v/v) 4 or 2 *M* ammonium acetate buffer pH 3.0 respectively. Also, the (S)-enantiomer of propranolol could be separated and detected using an (R)-propranolol-imprinted column from a non-racemic sample containing but 1% (S)-propranolol.

The low efficiency obtained so far using molecularly imprinted stationary phases in chromatography probably mainly originates from the imprinting procedure itself. It is argued that this process yields a population of imprints with varying quality. Thus, an imprinted polymer matrix consists of imprints exhibiting all from high affinity to low affinity to an analyte. The result may be unfavourable analyte association and dissociation kinetics to the imprinted phase. The future research into the preparation of uniform imprints will most probably improve the chromatographic efficiency. One can also argue that unfavourable non-specific interaction of the analyte to the polymer matrix may contribute to bad efficiency. New polymer matrices may in that case overcome such problems. As expected, however, the CEC-based separations show better efficiency than compared to the LC-based ones.

4. Novel injection and detection protocols

4.1. Picolitre droplet injection for capillary electrophoresis

To address the problem of injection in CE, we investigated the use of a silicon based flow-through liquid microdispenser, see Fig. 1, for direct sampling into CE columns. Each droplet injected contained 140 pL of sample solution. The injection process and subsequent separation were followed in real time by a laser-induced fluorescence (LIF)/charge-coupled device (CCD) imaging system. The display showed simultaneously a dual magnification of the separation capillary and the injection site. The standard deviation for the droplet to droplet volume was less than 3% (unpublished results).

4.2. Laser-induced fluorescence imaging as detection in capillary electrophoresis

Detection of fluorescence from analytes, which are separated, is an often used detection technique. This technique is far more sensitive than for example absorbance based methods. With modern lasers and CCD cameras it is possible to construct compact and advanced detection system based on laser-induced fluorescence. Another advantage with such a system is that the camera is normally connected to a computer with which the user can do visualisation, evaluation, signal processing etc. Therefore, a system based on this concept was constructed to image a larger part of the capillary. In contrast to ordinary end-point detection, detection in this manner makes it possible to investigate the course of events during the propagating separation. The first generation of our system used normal focusing optics to image the capillary onto the CCD camera. The system was used for the study of CE separation of double-stranded (ds) DNA [43], isotachophoretic preconcentration [44] and frits for CEC [45]. To enhance the limit of detection, and also making the set-up more compact, an improved light collection unit was constructed. The idea was to use optical fibres close to the capillary for collection of the fluorescent light. The limit of detection was 10 times lower for this system compared with the first system [46,47]. A computer program for investigation of the analytes' separation behaviour was also constructed. Another purpose of the program was to post process the collected data to decrease the limit of detection even further.

The system set-up is depicted in Fig. 9. Light from a light source is focused into an optical fibre. Lasers

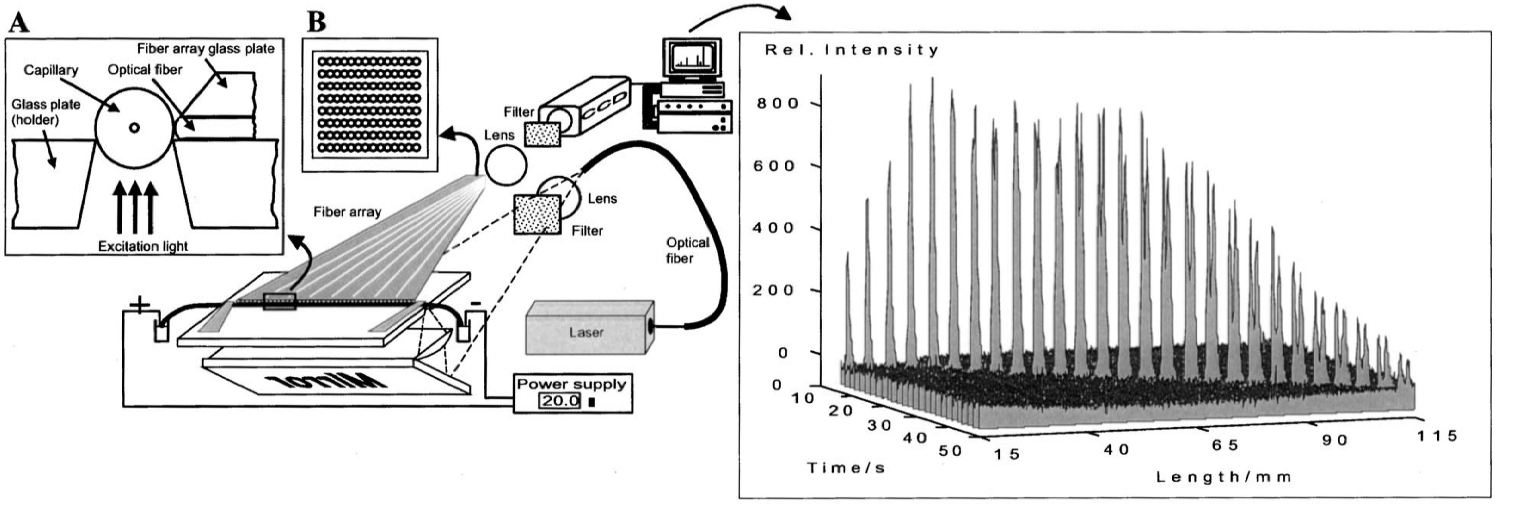

Fig. 9. Survey of set-up for fluorescence imaging of capillary electrophoresis using the fibre array. To the right is a three-dimensional image of 10 μ*M* dansyl-Asp (dissolved in methanol and diluted in water) Inset A: Fibre array collecting end, close up of capillary and fibre array alignment. Inset B: Fibre array output end, the interface to the camera consists of eight rows of optical fibres.

are preferably used due to their monochromatic light. The excitation light out from the fibre is focused onto 10 cm of the capillary using different lenses. To accurately align the excitation light onto the capillary, the lens, which is used to focus the excitation light, is placed on a micromanipulator. The fluorescence originating from the optical fibre is eliminated with a filter. The capillary is placed between two glass plates (Fig. 9, inset A) to make a gap for the excitation light, which is focused from below. Before the capillary is aligned, a 10.5-cm detection window is made. Part of the fluorescence from the analytes inside the capillary is collected with the optical fibre array. This array is placed as close as possible to the capillary. The array used today consists of 800 fibres. The fibre ends, which are toward the capillary, are glued beside each other on a glass substrate. The other end is divided into eight rows to make a more compact output end (Fig. 9, inset B). This 10×10-mm output end can easily be imaged onto the CCD camera with lenses of a diameter of 5 cm. A filter was placed in front of the camera to prevent scattered light from the laser from reaching the CCD camera.

The laser power was typically 50 mW, resulting in an intensity of approximately 0.4 mW/mm^2. Higher intensities are normally used in single-point detection but high intensities may photo bleach the sample, which must be prevented while imaging the analytes during the migration through the capillary. No bleaching effects were observed during imaging of the 10-cm-long detection window. A 50 μm I.D.

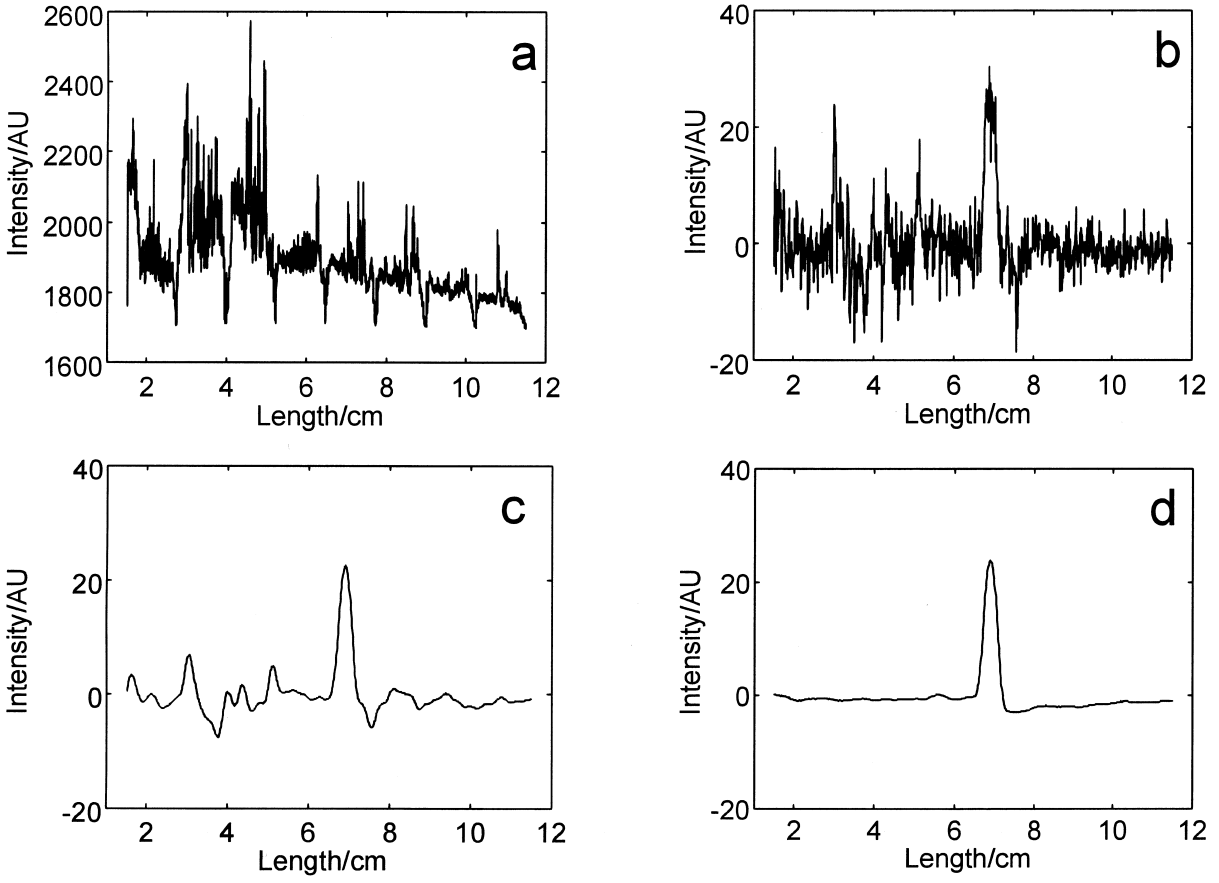

Fig. 10. Electropherograms of 2 n*M* dichlorofluorescein dissolved in the electrolyte 50 m*M* sodium borate at pH 9.0. (a) Raw data (550 pixels/cm). The high intensity is from the background fluorescence. (b) After background correction. (c) Data after low pass filtering. (d) To maximise the S/N ratio in the centre of the detection window, 79 frames were summarised in a moving process.

separation capillary was used. This puts high demands on correct alignment and focusing. First, the excitation light must be properly aligned and focused onto the capillary. Second, the fibre array must be aligned along the capillary and to collect as much fluorescence as possible it has to be very close to the capillary. Correct alignment will also reduce the background fluorescence. The focusing of the image onto the camera is also an important step. Bad focusing will result in low intensities and too wide band zones. To evaluate the performance of the system, enantiomer separations were imaged. Excellent imaging of separations was achieved for dansyl-Asp, and partial enantiomer resolution was obtained for dansyl-Glu and dansyl-Val [47].

All the data collected can be used for post-processing (Fig. 10) and evaluation of the analytes' behaviour (Fig. 9). The first task is to correct for imaging aberrations introduced by the excitation profile and the different lenses. An image of a capillary, uniformly filled with a fluorophore dissolved in buffer, is used for this purpose. After this, the background is corrected in the same manner, but the capillary is filled only with buffer. The program is also used to increase the signal-to-noise ratio. Each image is processed with a digital low-pass filter along the spatial dimension. The filter parameters depend strongly on the zone width of the analytes. Another technique to increase the S/N ratio is to summarise several images while the analytes moves over the 10-cm-wide detection window, like a moving average. This means that the signal from a specific peak, collected from all images, was summarised, producing a higher S/N ratio (Fig. 10). Another feature of the program is to display the data in a way so important properties of the analytes' behaviour are visualised. Migration time, analyte velocity, band broadening, and separation efficiencies are some properties of a separation that can be extracted. It is thus possible to investigate how the separation parameters are changing during time and/or position.

The fibre array based LIF imaging system, with the combination of computer-aided digital signal processing, is an improved method for evaluation of the analytes' behaviour during the separation process. Both the set-up and the software are under development. Additional information at web site: http://www.teknlk.lth.se/imaging.

5. Concluding remarks and future outlook

We believe that focusing on fundamental aspects such as methods to alter selectivity, methods to enhance sensitivity, and the development of detection and injection protocols are of uttermost importance to the further development of miniaturised, high throughput analysis systems. This is driven by the urge to be able to analyse complex samples of lower and lower concentration and volume. A strong development in the area also requires, we believe novel approaches and unorthodox pathways, to explore all aspects of electroseparations in general.

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