ELSEVIER

Journal of Chromatography A, 895 (2000) 51–65

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Chiral capillary electrophoresis as predictor for separation of drug enantiomers in continuous flow zone electrophoresis

Mathias Wind[a], Peter Hoffmann[a], Horst Wagner[a], Wolfgang Thormann[b,*]

[a]*Institut für Anorganische und Analytische Chemie und Radiochemie, Fachrichtung 11.4 der Universität des Saarlandes, Postfach 151150, D-66041 Saarbrücken, Germany*

[b]*Department of Clinical Pharmacology, University of Berne, Murtenstrasse 35, CH-3010 Berne, Switzerland*

Abstract

Separation of the enantiomers of chlorpheniramine and methadone in acidic buffers containing carboxymethyl-β-cyclodextrin (CMCD) as chiral selector was investigated by capillary zone electrophoresis. For a range of pH and CMCD concentrations, the mobility difference and resolution of the enantiomers were determined. Then, conditions known to provide well resolved enantiomers and optimized chiral separation were applied to chiral continuous flow electrophoresis. In that approach, a thin film of fluid flowing between two parallel plates is employed as carrier for electrophoresis. The electrolytes and the sample are continuously admitted at one end of the electrophoresis chamber and are fractionated by an array of outlet tubes at the other. The number of pure enantiomeric fractions obtained by chiral continuous flow electrophoresis was found to be directly dependent on the enantiomeric mobility difference. For racemic chlorpheniramine separated in a betaine–acetic acid buffer at a total throughput of 5 mg/h, complete enantiomeric separation is shown to require a mobility difference of about $3 \cdot 10^{-9}$ m^2/V s. Furthermore, compared to the previous investigations with hydroxypropyl-β-cyclodextrin, CMCD was found to permit improved fractionation of methadone enantiomers. With a total racemic drug throughput of about 15 mg/h, continuous flow zone electrophoresis processing with CMCD as chiral selector is shown to have the potential of providing pure enantiomers on a mg/h scale. The results indicate that chiral capillary zone electrophoresis data can be employed as predictor for preparative scale chiral separations based upon continuous flow zone electrophoresis.

Keywords: Enantiomer separation; Buffer composition; Preparative electrophoresis; Capillary electrophoresis; Chlorpheniramine; Methadone

1. Introduction

As the significance of stereochemical considerations in drug metabolism and pharmacokinetics has become a major issue for both the pharmaceutical industry and the regulatory authorities, a high demand for chiral separation techniques and chiral analytical assays emerged in the past two decades [1–4]. Although chiral separations and stereospecific drug monitoring are widely and successfully accomplished via use of chromatographic methods, chiral capillary electromigration methods have recently undergone a spectacular development and have been shown to provide high-resolution at low cost in an environment-controlled age [5–10]. For enantiomeric separation under electrokinetic conditions, a chiral selector [such as a cyclodextrin (CD)] and proper

*Corresponding author. Tel.: +41-31-6323-288; fax: +41-31-6324-997.

E-mail address: wolfgang.thormann@ikp.unibe.ch (W. Thormann).

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00410-6

buffer conditions (pH, ionic strength, micelles, additives, etc.) are required. Compared to chiral high-performance liquid chromatography (HPLC), chiral capillary electrophoresis (CE) provides higher efficiency and is simpler, faster and cheaper, and consumes no or a much smaller amount of organic solvents. Chiral CE not only represents a complementary tool to the widely applied chromatographic methods, it also offers the possibility of bringing chiral separations and analyses into the routine arena. Not surprisingly, enantioselective determination of drugs in body fluids by chiral CE has become a popular approach for assessment of drug purity, the stereoselectivity of drug metabolism and bioanalysis of enantiomers of illicit and banned substances [11–14].

Enantiomerically pure substances can be obtained via asymmetric synthesis of the desired isomer or by synthesis of both compounds and resolution of a racemic mixture into individual isomers. Commonly used purification methods include recrystallization and chromatography, the latter approach being more widely applied, namely via formation of diastereomers or via direct separation on chiral stationary phases [4,15]. Like all methods, the chromatographic approach suffers from certain drawbacks, including high cost of stationary phases, dilution of sample, consumption of large amounts of mobile phase and difficulties in reusing the mobile phase. In addition, most chromatographic procedures are necessarily batch, as opposed to continuous, procedures and it is difficult to monitor and control a separation while it is in progress. Preparative free fluid electrophoresis is a largely unexplored methodology that circumvents many of these drawbacks [16–18]. Recently, preparative free fluid electrophoretic processing of racemic methadone by recycling isotachophoresis [19], continuous flow isotachophoresis [20] and continuous flow zone electrophoresis [20] in presence of hydroxypropyl-β-CD (OHP-β-CD) was found to provide partial separation of the two enantiomers. Interval flow electrophoresis, however, resulted in complete separation and a mg/h recovery of pure enantiomers [20]. In another approach, the preparative-scale continuous flow isoelectric focusing separation of the enantiomers of dansylated phenylalanine has been demonstrated with a mg/h throughput [21]. Data obtained indicate that the chosen approaches with mg recoveries of purified enantiomers are attractive and merit further consideration. Furthermore, the use of chiral CE for micropreparative separations with recovery of μg quantities of enantiomers using flow-counterbalanced capillary zone electrophoresis [22] and capillary isotachophoresis [23] have recently been described.

Continuous or free flow electrophoresis is based upon a thin film of fluid flowing between two parallel plates. The electrolytes and the sample are continuously admitted at one end of the electrophoresis chamber and are fractionated by an array of outlet tubes at the other. In the work presented here, separation of chlorpheniramine (CPAM) and methadone (for chemical structures refer to Fig. 1) enantiomers in presence of carboxymethyl-β-CD (CMCD) as chiral selector was first investigated by CE and then applied to the continuous preparative separation under conditions of continuous flow zone electrophoresis using the Octopus apparatus.

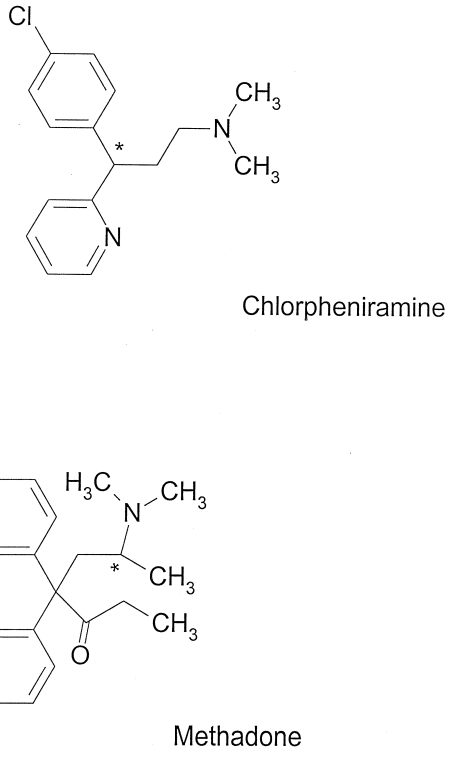

Fig. 1. Chemical structures of chlorpheniramine and methadone. Asterisks mark the chiral C atoms.

2. Experimental

2.1. Chemicals, standard solutions and preparation of buffers

All chemicals used were of analytical or research grade. Betaine was from Fluka (Buchs, Switzerland). Racemic CPAM (as maleate) was purchased from Sigma (St. Louis, MO, USA), racemic methadone (as chloride) of European Pharmacopoeia quality was from the hospital pharmacy (Inselspital, Berne, Switzerland), CMCD (quality: CY-2006; degree of substitution~3–3.5) was from Cyclolab (Budapest, Hungary) and OHP-β-CD (degree of substitution~ 0.6) was from Fluka. Aqueous standard solutions were prepared. All chiral buffers were prepared daily and used immediately. pH measurements were made in the presence of the chiral selector.

2.2. Capillary electrophoresis instrumentation and running conditions

The HP^{3D} capillary electrophoresis system (Hewlett-Packard, Waldbronn, Germany) equipped with a bare fused-silica capillary of 44 cm (37 cm effective length)×50 μm I.D. was employed. The applied voltage was 25 kV, the chamber air was thermostatted at 25°C, pressure injection of sample was effected at 50 mbar for 3 or 5 s, and detection was performed at 200 nm. Before each experiment the capillary was rinsed with 0.1 *M* NaOH for 3 min, water for 1 min, and running buffer for 7 min. If not stated otherwise, the buffer used was composed of 100 m*M* phosphoric acid, the chiral selector and triethanolamine (adjusted to the desired pH).

2.3. Continuous flow instrumentation and operation conditions

An overall schematic representation of the instrumental setup used for continuous flow zone electrophoresis is given in Ref. [20]. Electrolytes and the sample are continuously admitted at one end of the electrophoresis chamber and are fractionated by an array of outlet tubes at the other end. Continuous flow experiments were performed on an Octopus apparatus (Dr. Weber, Kirchheim, Germany) having chamber dimensions for length, width and fluid layer thickness of 500, 100 and 0.4 mm, respectively, an electrode length of 450 mm and 96 equidistant outlet ports. The back plate comprises a thermostatted aluminum slab that was covered by a glass plate with a thin silver layer (mirror) on the back side, whereas the front plate was made from plexiglass. The latter part comprises all inlet and outlet ports. A 0.4 mm poly(vinyl chloride) (PVC) spacer was used to define the gap between the two plates. The instrument was operated in the horizontal position and the electrode chambers were separated from the separation chamber by PP60 membranes (Dr. Weber). Buffer and counterflow were continuously infused using a Model IPC-12 peristaltic pump (Ismatec, Munich, Germany) and buffer flow through the electrode chambers was maintained by a membrane pump Model MD-100 (KNF-Neuberger, Freiburg, Germany). Sample was continuously infused using a peristaltic pump. Fractions (about 0.4 ml) were collected into 96-well plates. The power supply was a Model 2197 (LKB, Bromma, Sweden). The cooling temperature of the recirculation fluid was maintained at about 5°C.

The separation buffer containing the chiral selector was infused into the chamber through five inlet ports (flow: 3.3 ml/min; residence time: 9 min) and both electrode chambers were continuously flushed with 0.1 *M* K_2HPO_4. Water was used as counterflow for fraction collection (8.2 ml/min; about 3.5-fold dilution of each fraction). The running buffer was composed of 20 m*M* betaine, the chiral selector and acetic acid (adjusted to required pH). If not stated otherwise, the sample was composed of 15 m*M* CPAM or methadone, dissolved in water, and continuously infused at 0.02 ml/min through an inlet placed at the position that corresponded to fraction 22. Sample collection was commenced after an equilibration time interval of about 20 min.

2.4. Analysis of collected fractions

Fractions were analyzed by chiral CE as described above. Quantitation of CPAM was executed via five-level calibration in the range between 5 and 250 μ*M* per enantiomer. Accordingly, methadone was quantitated with six standards in the 5–1000 μ*M* range.

Calibration graphs were found to be linear ($y=18.18x+0.21$ and $y=15.68x+2.86$ for CPAM and methadone, respectively) with r values of 0.998 (CPAM) and 0.997 (methadone). The detection limits ($S/N=3$) were determined to be 3 and 4 mM, respectively. Thus, at a 100 mM drug level, enantiomeric purity >97% could not be elucidated with this CE assay.

3. Results and discussion

3.1. CE separation of chlorpheniramine and methadone enantiomers with CMCD as chiral selector

CPAM and methadone have pK_a values of about 9.2 and 8.3, respectively, and are thus positively charged at acidic and neutral pH values. Enantiomeric separation of cationic substances is best performed at low pH [24–26]. CMCD is characterized with ~3–3.5 carboxylic groups, has a pK_a of 3.5 to 4 and can thus be used in two different modes [10]. At pH<3.5, the carboxylic acid groups become completely protonated, resulting in an electrically neutral chiral selector. At pH>4, the carboxylic acids become completely dissociated, resulting in a negatively charged derivative which migrates towards the anode. One of the main advantages of this pH dependent charge of CMCD is the possibility to reverse the migration order of enantiomers. This is exemplified by the electropherograms presented in Fig. 2. These data were obtained with CPAM at pH 3.0 and 4.0 under application of normal and reversed polarity, respectively. Assignment of enantiomers is based upon comparison with data from the literature [22,27]. At pH 3, CPAM–CMCD complexes are cations and thus migrate toward the cathode with complexed (−)-CPAM migrating faster than complexed (+)-CPAM. At pH 4, the complexes are anions and thus electromigrate towards the anode. Furthermore, electroosmotic displacement (towards the cathode) was found to be much lower than electromigrational transport towards the anode. Thus, application of reversed polarity was required and (+)-CPAM was detected prior to (−)-CPAM (Fig. 2B). The CMCD concentration dependent isoelectric

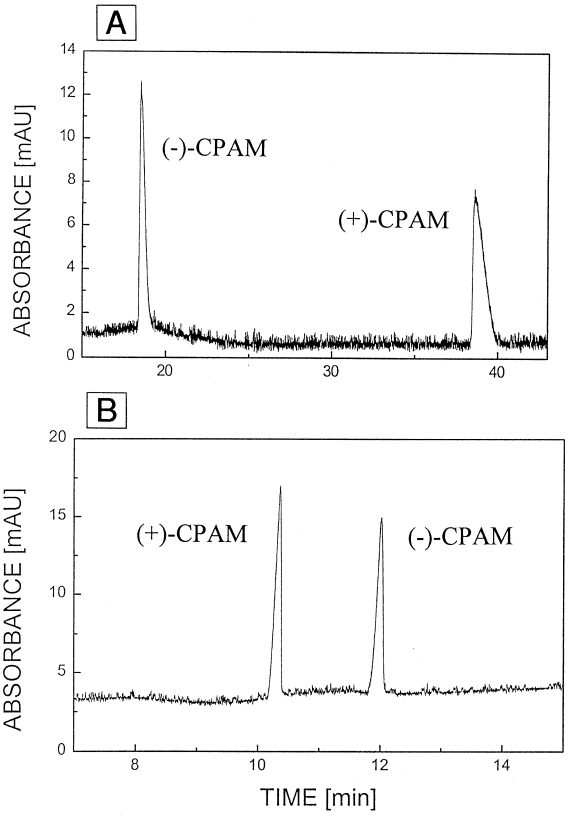

Fig. 2. Enantiomeric separation of racemic CPAM obtained by CE with a 100 mM phosphate–triethanolamine buffer at (A) pH 3.0 and (B) pH 4.0 having 5 mM CMCD as chiral selector. Solute detection occurred towards (A) the cathodic capillary end (normal polarity mode) and (B) towards the anodic capillary end (reversed polarity mode). Sample solutions comprised 400 μM of the drug. Injections occurred at 50 mbar during 3 s. The currents were 66 and 80 μA, respectively.

points for the CPAM–CMCD complexes were estimated to be between 3 and 3.5.

Enantiomeric resolution (R_s) can be characterized by:

$$R_s = 2(t_2 - t_1)/(W_1 + W_2) \quad (1)$$

where t_i and W_i represent the detection time and peak width of enantiomer i, respectively. Firstly and secondly detected enantiomers are denoted with subscripts 1 and 2, respectively. An R_s value of ≥1.4 represents baseline resolution. In the presented example, the peak resolution R_s was determined to be better than 20 and 6 for the data at pH 3 and 4,

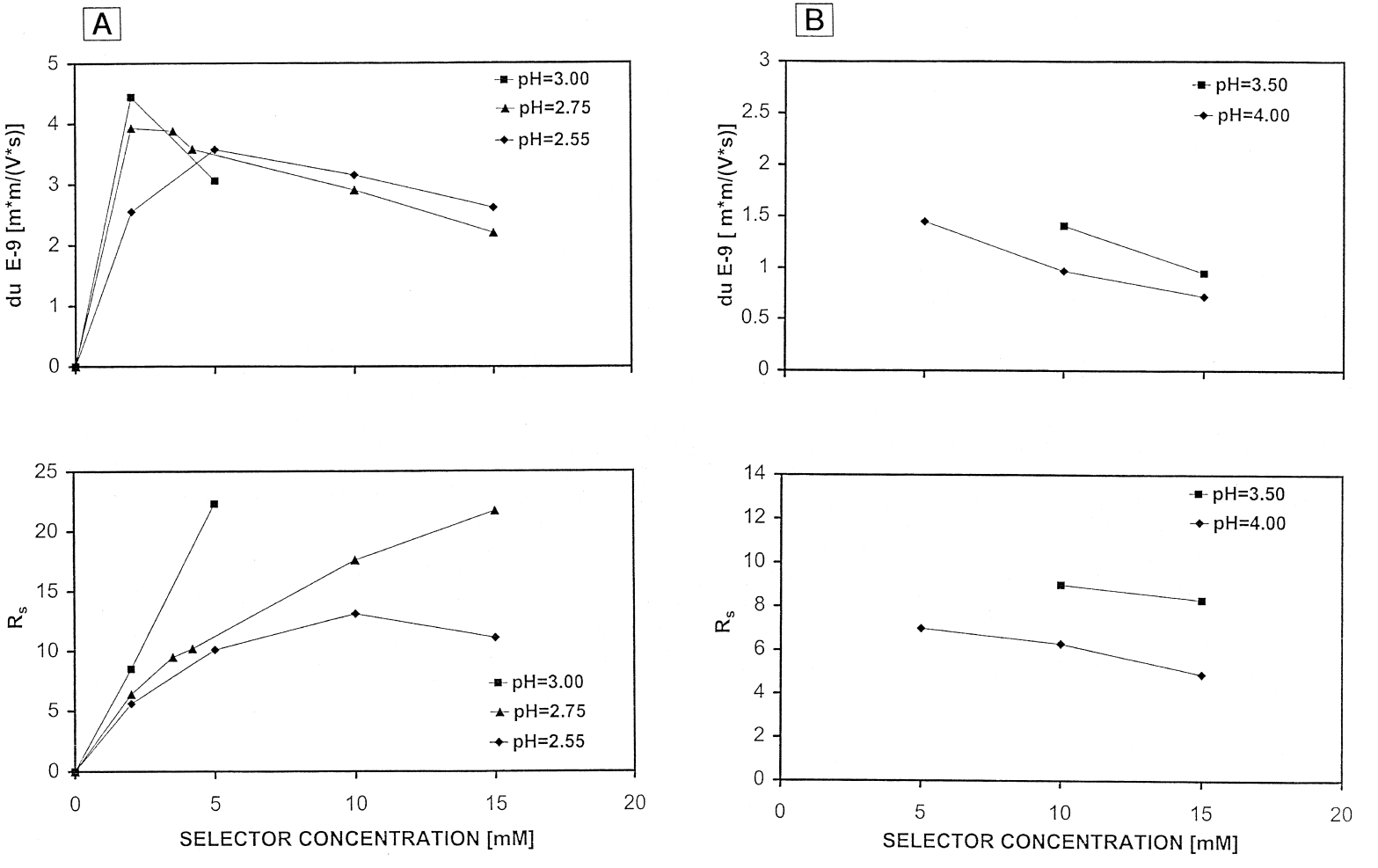

Fig. 3. Enantiomeric mobility difference d*u* and resolution R_s for CPAM under (A) cathodic and (B) anodic migration conditions as functions of selector concentration and buffer pH. Data were elucidated by CE using a 100 m*M* phosphate–triethanolamine buffer.

respectively (Fig. 3). Furthermore, enantiomeric separation depends on the difference of the electrophoretic mobilities of the two enantiomers, a quantity that can be calculated according to:

$$du = L_{eff}/E \cdot [(t_2 - t_1)/(t_1 \cdot t_2)] \quad (2)$$

where L_{eff} and E represent the effective column length (distance between initial sample and detector) and the applied electric field, respectively. For the separation of CPAM enantiomers presented in Fig. 2, the mobility difference du was found to be highest at a pH around 3 and with a CMCD concentration around 2 mM (Fig. 3). For CPAM, data produced under reversed polarity conditions (pH≥3.5, Figs.

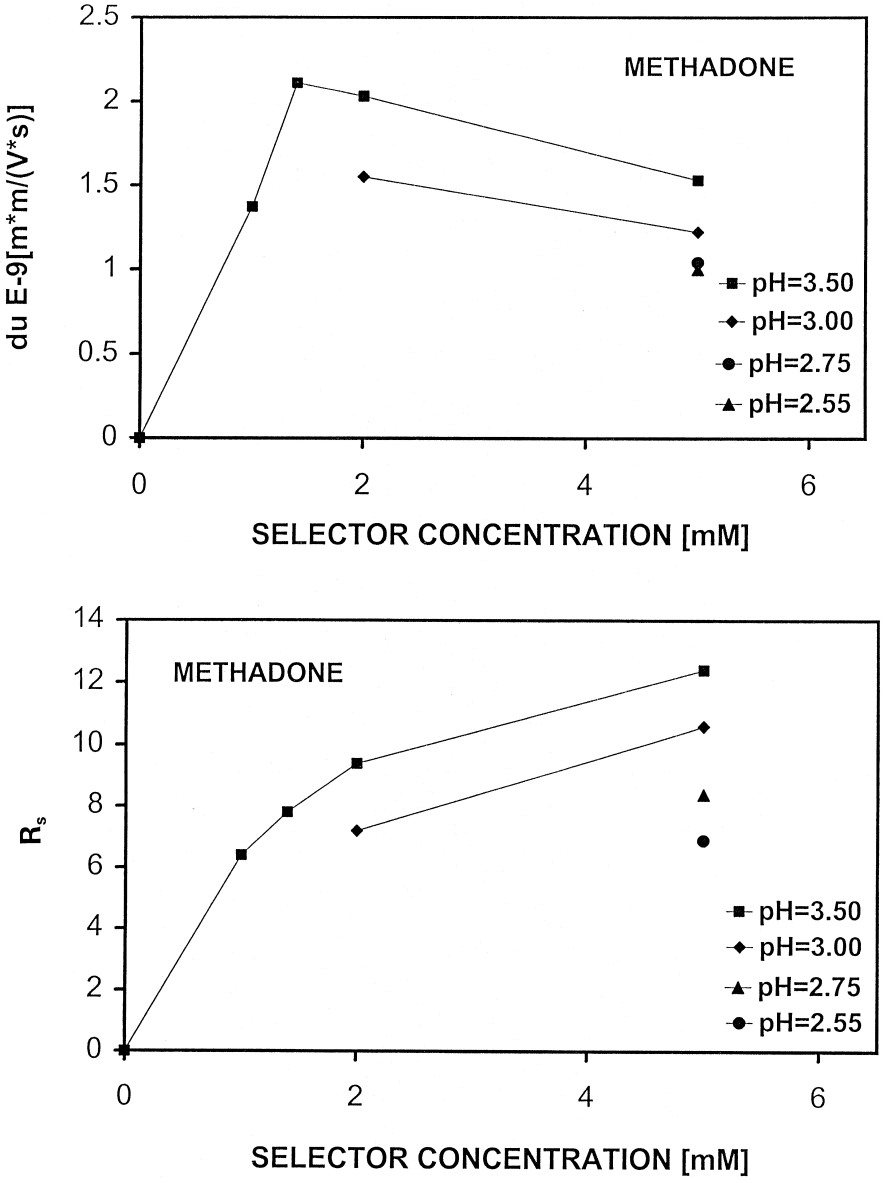

Fig. 4. Enantiomeric mobility difference du and resolution R_s for methadone under cathodic migration conditions as functions of selector concentration and buffer pH. Data were elucidated by CE using a 100 mM phosphate–triethanolamine buffer.

2B and 3B) were found to be characterized by lower resolution compared to those obtained under normal polarity (pH≤3, Figs. 2A and 3A).

The behavior of the methadone enantiomers in presence of CMCD was investigated in the same way as described for CPAM (Fig. 4). It was interesting to find that methadone–CMCD complexes migrated cationically even at pH 3.5. As was reported before for another chiral selector [20], *R*-(−)-methadone was determined to migrate faster than *S*-(+)-methadone. With a 5 m*M* CMCD concentration, the isoelectric point was estimated to be around 3.7. Thus, it can be concluded that complexation of methadone enantiomers with CMCD is weaker than that between CPAM and CMCD. Optimized conditions in terms of d*u* and R_s values for the chiral separation of methadone enantiomers can be elucidated from the data presented in Fig. 4. Highest values for d*u* and R_s were obtained at pH 3.5 and CMCD concentrations around 2 and 5 m*M*, respectively. Comparison of the data presented in Fig. 4 with those of Fig. 3A reveals that d*u* values for methadone enantiomers under optimized conditions are smaller than those observed for CPAM.

3.2. Chiral continuous flow zone electrophoresis of CPAM enantiomers

Conditions for continuous flow zone electrophoresis were first established via processing of methylene blue at various voltages (Fig. 5). Continuous flow electrophoresis performed in the Octopus with its 0.4 mm fluid film requires a buffer of lower conductivity than is typically used in CE. Therefore, the 100 m*M* phosphate–triethanolamine buffer employed for CE could not be applied to preparative separations. A buffer composed of 20 m*M* betaine–acetic acid (pH 3, conductivity $\kappa = 0.21$ mS/cm) was used instead. The residence time in the electrophoresis chamber was 9 min. No precaution for electrokinetic membrane effects via infusion of a higher buffer concentration along the membranes was used. At pH 3, methylene blue is positively charged and thus migrates towards the cathode. Without applied voltage, methylene blue was recovered as narrow peak in channels 21 to 25, i.e., mainly in the channels that correspond to the sample inlet (channel 22). Application of a voltage resulted in cationic sample transport and thus recovery in

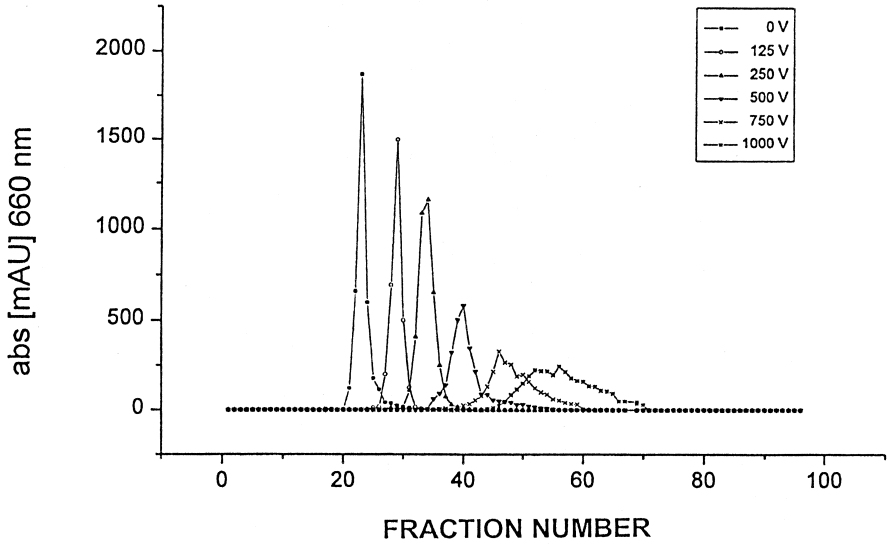

Fig. 5. Voltage dependent continuous flow zone electrophoretic data obtained with methylene blue using a buffer composed of 20 m*M* betaine and acetic acid (pH 3, $\kappa = 0.21$ mS/cm) and a residence time of 9 min. The cathode is to the right. The recovered fractions were analyzed photometrically at 660 nm.

higher channel numbers. Moreover, along with power application, voltage dependent zone broadening was observed. The distributions shown in Fig. 5 illustrate that voltages >450 V should not be used for that configuration and buffer. This is in agreement with the general experience that currents should be <100 mA (see below).

Chiral separations of CPAM enantiomers were executed with the 20 m*M* betaine–acetic acid buffer in the presence of CMCD. Typical data for the enantiomeric composition of the collected fractions are presented in Fig. 6. In these runs, the residence time was 9 min, the voltage was kept constant at 450 V and equilibrium was attained after about 12 min of power application. From that time point on the currents were 83, 108, 79 and 97 mA, respectively (applied electric power between 35.6 and 48.6 W) and fraction collection was executed during about 4 min. The rate of counterflow of water used was 8.2 ml/min and the total buffer flow through the electrophoresis chamber was 3.3 ml/min. Thus, at the collection outline ports, the chamber fluid was continuously diluted about 3.5-fold by water. The sample was composed of 15 m*M* racemic CPAM dissolved in water and the infusion rate was 0.02 ml/min (total throughput: about 5 mg/h). The data presented in Fig. 6 reveal that separation of the two enantiomers is obtained under various buffer conditions. The enantiomeric composition of each fraction was analyzed by CE. Typical electropherograms are presented in Fig. 7. For the data of Fig. 6A, fraction 52 (F52, upper panel of Fig. 7) was found to contain (−)-CPAM only whereas fraction 42 (F42, lower panel of Fig. 7) was considered to be pure for (+)-CPAM. In that context it is important to realize that the CE assay used had a detection limit of 3 μ*M*. Thus, for a concentration of 100 μ*M* of one CPAM enantiomer and no detection of the second enantiomer, the purity of the fraction was >97%.

In the cationic migration mode (panels A to C), (−)-CPAM was recovered in higher fractions than (+)-CPAM. Not surprisingly, the opposite was found to be true for the case of anionic migration shown in panel D. Furthermore, a higher degree of separation of the enantiomers under the conditions of cationic migration was observed. This does not come as a surprise as the d*u* values determined by CE (Fig. 3) were significantly larger in this mode of operation. Thus, it appears that chiral CE data can be employed as predictor for separations under continuous flow conditions in the Octopus apparatus. This has previously been reported for electrophoretic separations in an achiral environment, including the behavior of peptides in continuous flow zone electrophoresis [28] and of proteins in preparative recycling isotachophoresis [29]. For characterization of the enantiomeric separation, a quality factor QF defined as:

$$\mathrm{QF} = \frac{\text{number of fractions with pure enantiomers}}{\text{total number of fractions containing the drug}} \quad (3)$$

was introduced. Complete separation of the enantiomers is characterized with a QF value of 1. QF values for the data presented in panels A to D of Fig. 6 were determined to be 0.82, 0.67, 0.92 and 0.30, respectively. Correlation of QF values determined from the continuous flow electrophoresis experiments with the d*u* values determined by CE (cf. Eq. (2) and data presented in Fig. 3) were found to follow a linear relationship ($r^2 = 0.996$) (upper graph in Fig. 8A). No such relationship was noted for the correlation of QF with R_s (lower graph in Fig. 8A). The same was found to be true for d*u* and R_s values that were determined employing the 20 m*M* betaine–acetic acid buffer (Fig. 8B) instead of the 100 m*M* phosphate–triethanolamine buffer that was used to generate the data presented in Figs. 2 and 3. Although the betaine–acetic acid buffer was not well suited for CE measurements (conductivity too low; electropherograms not shown), the d*u* vs. QF relationship was also found to be linear ($r^2 = 0.967$). Furthermore, d*u* values determined with this buffer were found to be somewhat smaller than those observed in the 100 m*M* phosphate–triethanolamine buffer. Full separation is obtained with a d*u* value of about $3 \cdot 10^{-9}$ cm^2/V s. The corresponding value for the phosphate–triethanolamine buffer is about $5 \cdot 10^{-9}$ cm^2/V s.

The continuous flow electrophoretic data presented in Fig. 6 were generated with a residence time of 9 min. For the conditions of Fig. 6A, a residence time of 9 min was found to provide the highest QF value, namely 0.82. Having residence times of 7 and 12 min and otherwise identical conditions as for Fig. 6A, the QF values for both cases were determined to be 0.67 (distributions not shown). Furthermore, as

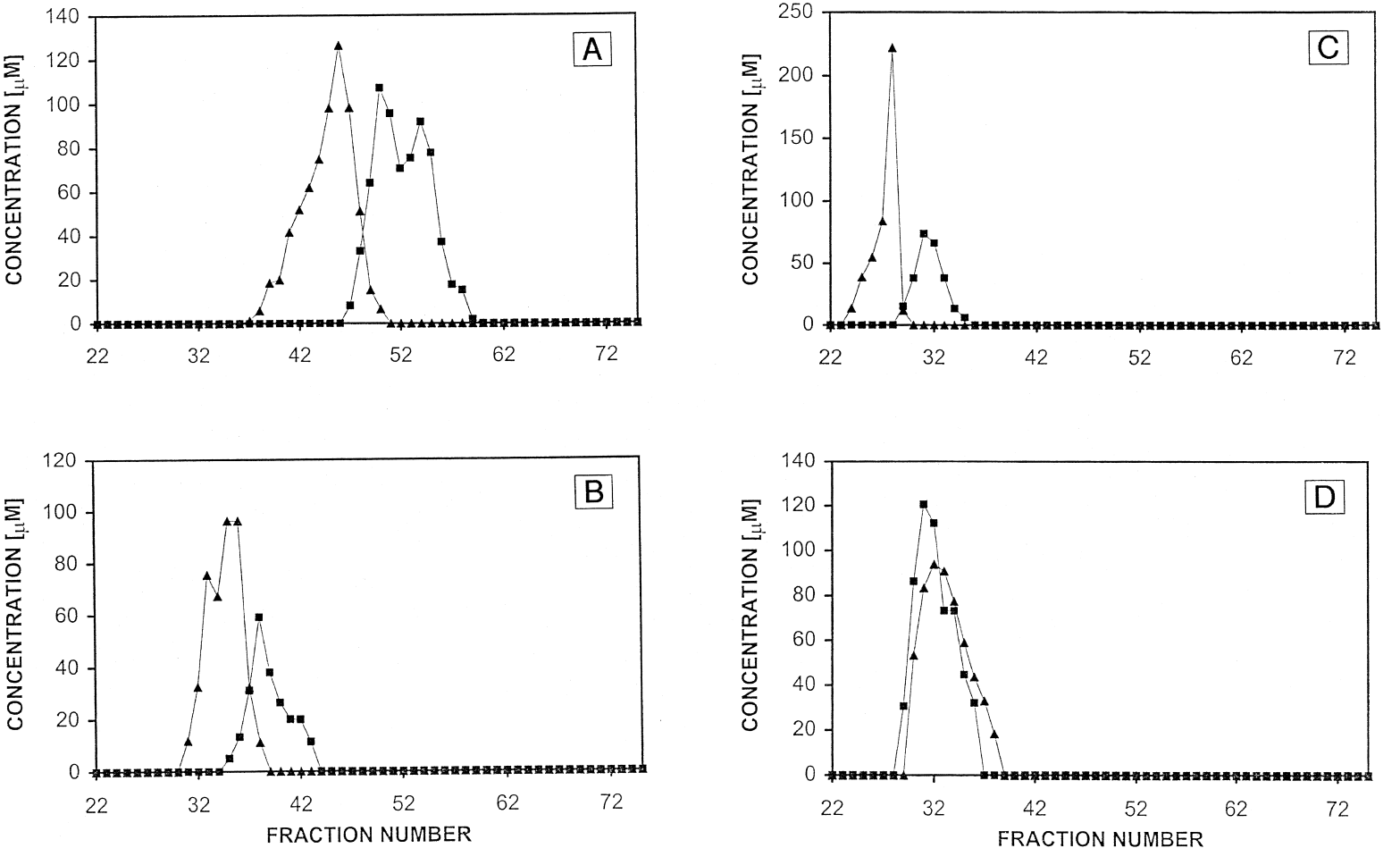

Fig. 6. Chiral continuous flow data for CPAM obtained at 450 V and a residence time of 9 min. For the data of panels A to C the cathode was on the right side (normal polarity) whereas for the data of panel D the anode was on the right side (reversed polarity). CMCD concentrations and pH were (A) 2 m*M* and 2.75, (B) 5 m*M* and 2.75, (C) 2 m*M* and 3.0 and (D) 5 m*M* and 4.0, respectively. The currents during sample collection were 83, 108, 79 and 97 mA, respectively. The graphs show the enantiomeric composition of the fractions as assessed by chiral CE. The distributions denoted by ■ and ▲ refer to (−)-CPAM and (+)-CPAM, respectively.

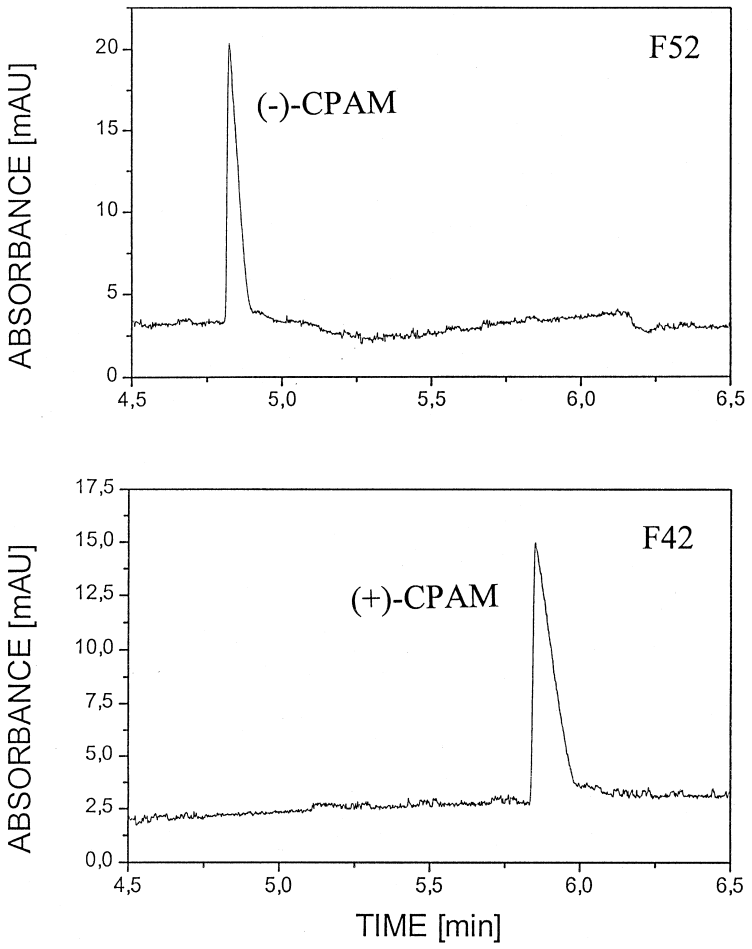

Fig. 7. Electropherograms of fractions 52 (upper panel) and 42 (lower panel) from the data of Fig. 6A. Fractions were analyzed by chiral CE employing a 100 m*M* phosphate–triethanolamine buffer of pH 2.75 containing 1 m*M* CMCD as chiral selector.

observed previously for the separation of methadone enantiomers in presence of OHP-β-CD as chiral selector [20], addition of small amounts of hydroxypropylmethylcellulose (HPMC) to the buffer was found not to improve the separation of the CPAM enantiomers. Having an HPMC concentration of 0.02% (w/v), a residence time of 12 min and otherwise identical conditions as for Fig. 6A, the QF value was determined to be 0.71 (data not shown). Therefore, all other experiments were performed with a residence time of 9 min and without addition of HPMC or any other viscosity enhancing agent. Using this configuration with a sample solution composed of 15 m*M* racemic CPAM, the recovery of pure CPAM enantiomers was calculated to be in the order of 700 to 800 μg/h.

3.3. Chiral continuous flow electrophoresis of methadone enantiomers

The separation of methadone enantiomers by continuous flow electrophoresis in the 20 m*M* betaine–acetic acid buffer with CMCD as chiral selector was also studied. As was the case with CPAM, chiral CE data could be successfully used as predictor. Under cationic migration (pH$<$3.7) and independent of the buffer used (phosphate vs. betaine buffer), *R*-(−)-methadone was determined to migrate

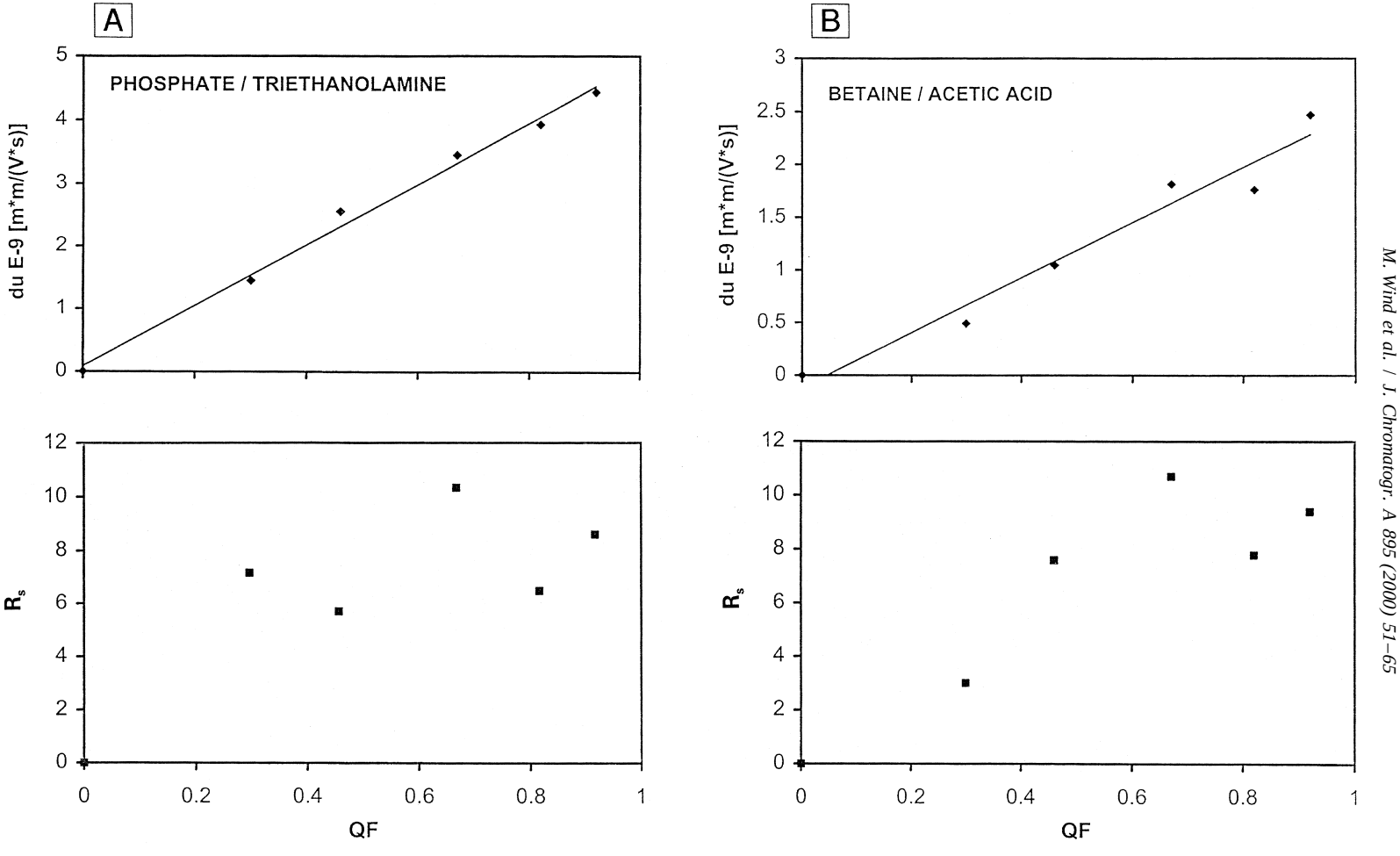

Fig. 8. Relationships between enantiomeric mobility difference d*u* and resolution R_s of CPAM enantiomers assessed by CE in (A) the 100 m*M* phosphate–triethanolamine buffer and (B) the 20 m*M* betaine–acetic acid buffer and the quality factor QF determined by continuous flow electrophoresis.

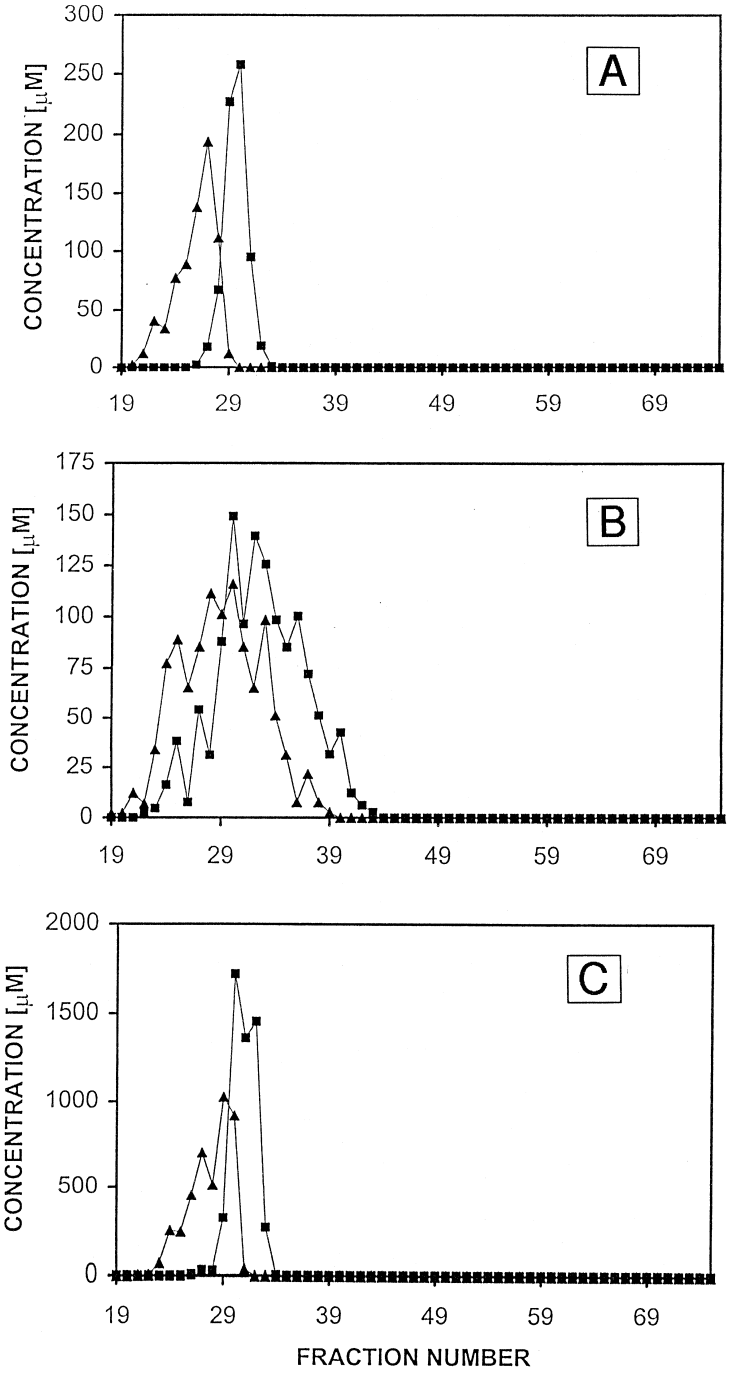

Fig. 9. Chiral continuous flow data for methadone obtained with a residence time of 9 min, a buffer pH of 3.5 and a CMCD concentration of 1.3 m*M*. For the data of panels A to C the applied voltages were 450 V, 700 V and 450 V, respectively (cathode to the right). The currents during sample collection were about 63, 82 and 62 mA, respectively. The graphs show the enantiomeric composition of the fractions as assessed by chiral CE. The sample concentrations were 15, 15 and 50 m*M*, respectively. The distributions denoted by ■ and ▲ refer to *R*-(−)-methadone and *S*-(+)-methadone, respectively.

ahead of *S*-(+)-methadone. This is the same order as previously observed with the neutral OHP-β-CD as chiral selector [20]. Having an applied voltage of 450 V (as was used for the data presented in Fig. 6), optimized continuous flow electrophoretic data were obtained for a buffer pH of 3.5 and a CMCD concentration of 1.3 m*M* (Fig. 9A), this corresponding to the conditions with the highest d*u* value as assessed by chiral CE (Fig. 4). The recovered methadone enantiomer distributions are depicted in panel A of Fig. 9 and typical electropherograms of pure fractions are presented in Fig. 10. The sample was composed of 15 m*M* racemic methadone dissolved in water and the infusion rate was 0.02 ml/min (throughput per enantiomer: about 2.8 mg/h). The data presented in Fig. 9A reveal that considerable separation of the two enantiomers is obtained. *R*-(−)-Methadone was recovered in higher fractions than *S*-(+)-methadone. The quality factor QF for that configuration was calculated to be 0.71. This value is lower than that obtained for CPAM under optimized conditions. This does not come as a

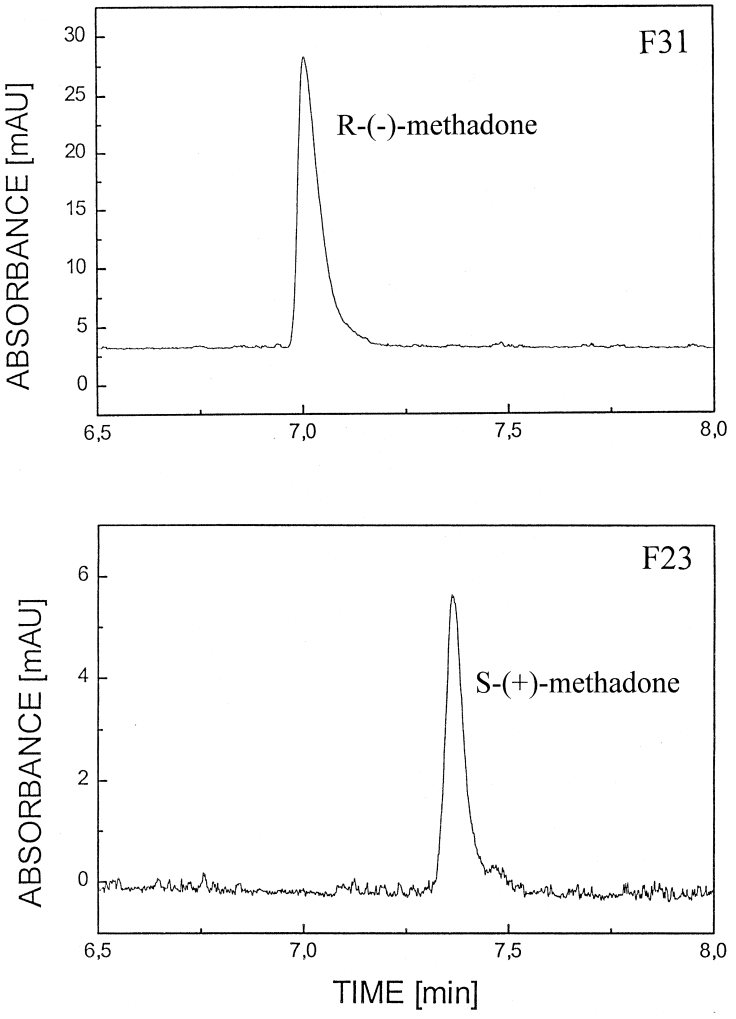

Fig. 10. Typical electropherograms obtained with fractions containing pure *R*-(−)-methadone (upper panel) and *S*-(+)-methadone (lower panel). Fractions were analyzed by chiral CE employing a 100 m*M* phosphate–triethanolamine buffer of pH 2.75 containing 1.3 m*M* OHP-β-CD as chiral selector.

surprise, as the highest d*u* value observed for methadone was significantly lower than that determined for CPAM (compare data of Figs. 3 and 4).

The data shown in panel A of Fig. 9 were obtained at a constant 450 V (28.4 W). Fractionation under a constant 700 V (57.4 W) provided the data presented in Fig. 9B. As already documented with the continuous flow electrophoretic data obtained with methylene blue (Fig. 5), the power increase was determined to distribute the methadone enantiomers across a larger number of fractions. As a consequence, peak concentrations were lower. Furthermore, enantiomer separation under otherwise identical conditions was observed to be much decreased. The QF value became 0.28, this being less than half compared to that characteristic for the 450 V run. Thus, an increase in applied power does not lead to improved separation of the enantiomers.

From an economical point of view, an increase of the sample throughput is of high interest. Thus, the separation of methadone enantiomers was also investigated by admitting a higher sample load. The data presented in panel C of Fig. 9 were obtained with a sample solution of 50 m*M* racemic methadone and otherwise identical conditions as for Fig. 9A. With this 3 1/3 higher total sample throughput (16.7 mg/h of racemic methadone), the concentrations of the methadone enantiomers in the collected fractions became higher. The QF factor, however, was found to become reduced to 0.57. Nevertheless, pure *R*-(−)-methadone was collected in fractions 32–34 with concentrations of 1.46, 0.27 and 0.004 m*M*, respectively. Fractions 21–25 provided pure *S*-(+)-methadone with the highest concentration of 0.25 m*M* in fraction 23. Thus, using this configuration via continuous collection of the product stream resulting from the combination of fractions 32 and 33, production of the pharmacological more potent *R*-enantiomer of methadone on the 3.8 mg/h scale would be possible. This compares well to the calculated recovery of 1.1 mg/h of *R*-(−)-methadone through fractions 29–32 of Fig. 9A and favorably with the preparative scale data reported previously using OHP-β-CD as chiral selector [20].

For the separation of methadone enantiomers and CMCD as chiral selector, the highest d*u* value observed was $2.1 \cdot 10^{-9}$ $m^2/V\,s$ (value for pH 3.5 and 1.3 m*M* CMCD, Fig. 4). This is about twice as high as was obtained at pH 3 [20] and pH 2.75 (this work, data not shown) in the presence of 5 m*M* OHP-β-CD as chiral selector (the optimized concentration for separation of methadone enantiomers). Thus, CMCD appears to be the better chiral selector for fractionation of methadone enantiomers by free fluid preparative zone electrophoresis. Furthermore, as was observed with CPAM, d*u* values determined in the 20 m*M* betaine–acetic acid buffer were found to be somewhat smaller. For pH 3.5 and a CMCD concentration of 1.2 m*M*, d*u* was found to be $1.3 \cdot 10^{-9}$ $m^2/V\,s$.

4. Conclusions

The data presented in this paper indicate that chiral CE can be employed as predictor for chiral preparative scale continuous flow zone electrophoretic separations. Conditions leading to highest mobility differences in CE were also found to provide best enantiomeric separations in continuous flow electrophoresis. For CPAM and methadone enantiomers in presence of CMCD as chiral selector, cationic complexes were found to provide higher mobility differences compared to electrophoretic conditions under which the complexes migrate anionically. Optimized continuous flow zone electrophoretic separations were obtained with a residence time of 9 min and an applied voltage of 450 V. There was no need to admit a viscosity enhancing agent to the buffer and to take special precaution to avoid electrokinetic membrane effects. Continuous flow zone electrophoretic processing with CMCD as chiral selector is shown to have the potential for purification of drug enantiomers on the mg/h scale.

Acknowledgements

This work was partly sponsored by the Swiss National Science Foundation.

References

[1] J. Caldwell, J. Chromatogr. A 694 (1995) 39.
[2] J. Caldwell, J. Chromatogr. A 719 (1996) 3.
[3] A.M. Krstulovic (Ed.), Chiral Separations by HPLC –

Applications to Pharmaceutical Compounds, Ellis Horwood, Chichester, 1989.
[4] E.R. Francotte, Chimia 51 (1997) 717, and references cited therein.
[5] J. Snopek, I. Jelínek, E. Smolková-Keulemansová, J. Chromatogr. 609 (1992) 1.
[6] T.J. Ward, Anal. Chem. 66 (1994) 633A.
[7] H. Nishi, S. Terabe, J. Chromatogr. A 694 (1995) 245.
[8] S. Fanali, J. Chromatogr. A 735 (1996) 77.
[9] G. Gübitz, M.G. Schmid, J. Chromatogr. A 792 (1997) 179.
[10] K. Verleysen, P. Sandra, Electrophoresis 19 (1998) 2798.
[11] K.D. Altria, Analysis of Pharmaceuticals by Capillary Electrophoresis, Vieweg, Wiesbaden, 1998.
[12] I.S. Lurie, R.F.X. Klein, T.A. Dal Cason, M.J. LeBelle, R. Brenneisen, R.E. Weinberger, Anal. Chem. 66 (1994) 4019.
[13] J. Bojarski, H.Y. Aboul-Enein, Electrophoresis 18 (1997) 965.
[14] S. Zaugg, W. Thormann, J. Chromatogr. A 875 (2000) 27.
[15] E. Francotte, J. Chromatogr. A 666 (1994) 565.
[16] M. Bier, N.B. Egen, G.E. Twitty, R.A. Mosher, W. Thormann, in: C.J. King, J.D. Navratil (Eds.), Chemical Separations, Principles, Vol. 1, Litarvan Literature, Denver, CO, 1986, p. 133.
[17] H. Wagner, Nature 341 (1989) 669.
[18] W. Thormann, in: J.C. Janson, L. Ridén (Eds.), Protein Purification – Principles, High Resolution Methods and Applications, Wiley–VCH, New York, 1998, p. 651.
[19] M. Lanz, J. Caslavska, W. Thormann, Electrophoresis 19 (1998) 1081.
[20] P. Hoffmann, H. Wagner, G. Weber, M. Lanz, J. Caslavska, W. Thormann, Anal. Chem. 71 (1999) 1840.
[21] P. Glukhovskiy, G. Vigh, Anal. Chem. 71 (1999) 3814.
[22] B. Chankvetadze, N. Burjanadze, D. Bergenthal, G. Blaschke, Electrophoresis 20 (1999) 2680.
[23] D. Kaniansky, E. Šimunicova, E. Ölvecká, A. Ferancová, Electrophoresis 20 (1999) 2786.
[24] A. Guttman, Electrophoresis 16 (1995) 1900.
[25] M. Fillet, I. Bechet, P. Hubert, J. Crommen, J. Pharm. Biomed. Anal. 14 (1996) 1107.
[26] M. Fillet, P. Hubert, J. Crommen, Electrophoresis 19 (1998) 2834.
[27] B. Chankvetadze, G. Pintore, N. Burjanadze, D. Bergenthal, D. Strickmann, R. Cerri, G. Blaschke, Electrophoresis 19 (1998) 2101.
[28] V. Kašicka, Z. Prusik, P. Sazelova, J. Jiraček, T. Barth, J. Chromatogr. A 796 (1998) 211.
[29] J.E. Sloan, R.A. Mosher, W. Thormann, M.A. Firestone, M. Bier, in: R. Burgess (Ed.), Protein Purification: Micro to Macro, UCLA Symposia on Molecular and Cellular Biology, New Series, Vol. 68, A.R. Liss, New York, 1987, p. 329.