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Research Article

Simultaneous contactless conductivity detection and UV detection for the study of separation of tamsulosin enantiomers in discontinuous electrolyte systems by CE

This work shows the potential of using discontinuous electrolyte systems for the separation of tamsulosin enantiomers by CE. Sulfated β-cyclodextrin was used as a chiral selector. In acidic electrolytes, sulfated β-cyclodextrin migrates as an anion and the analyte (tamsulosin) migrates as a cation. Due to this, four experimental arrangements were proposed. These arrangements differ in composition of electrolytes in the inlet compartment, in the capillary and in the outlet compartment. The separation of tamsulosin enantiomers in acetate buffers with sodium and Tris counterions was studied. Simultaneous contactless conductivity detection and UV detection were used for the study of the separation mechanism in these systems. Mobilities of sulfated β-cyclodextrin were used for the calculation of the time when the analyte migrates through the BGE zone with the selector. The simulation program Simul 4.0 was used for the calculations of the concentration profiles of the electrolyte components dependent on the time of the separation. The mechanism of enantioseparation in these arrangements was suggested.

Keywords: CE / Chiral separation / Contactless conductivity detection / Discontinuous systems / Sulfated β-cyclodextrin DOI 10.1002/elps.200600063

1 Introduction

CE is a very popular technique for analyzing a variety of compounds. One of the main fields where CE plays the dominant role is separation of optical isomers [1–3]. An interesting way to optimize the chiral separation set-up is by using discontinuous electrolyte systems. For the first time these systems were used in ITP, where the discontinuality is created in mobilities of co-ions in the leading electrolyte and the terminating one, which cause the main property of ITP, the same velocity of boundaries in the steady-state [4].

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Abbreviations: CC, contactless conductivity; **PFT**, partial filling technique; **S-β-CD**, sulfated β-cyclodextrin

The discontinualities are also formed in preconcentration techniques [5]. The concentration discontinuality between the loaded sample zone and BGE zones can cause a stacking effect. When analyte ions migrate into the BGE zone with lower electric field strength, they get slower and stacked [6, 7]. Another technique, transient ITP, uses the Kohlrausch regulation function; the sample zone is concentrated to the concentration of the leading electrolyte; then the terminating electrolyte is changed to the leading one and conventional zone electrophoresis is carried out [8]. The next main preconcentration technique, sweeping, also deals with the discontinuous systems. After sample loading the inlet vial is changed with the vial which contains BGE with micelles. After switching the voltage, analytes are swept into a narrow zone. Then the common MEKC technique proceeds [9]. The junction of two electrolytes with different pH is a basic characteristic of the dynamic pH junction preconcentration technique [10–12].

Discontinuous electrolytes are also used in partial filling techniques (PFTs). Valtcheva [13] first used PFT for chiral separation of some drugs by using cellobiohydrolase I as a protein selector, which migrates in the opposite direction to analytes. Nowadays, the use of PFT in chiral separation involves UV-absorbing selectors and their interferences with the analyte in UV detection [14] as well as with non-volatile additives in CE-MS techniques [15]. Amini [16] reviewed the three main approaches for PFT. The selector plug migrates away from the detector, the selector is stationary in the capillary and the selector plug migrates slowly in the same direction as enantiomers. PFTs are also used for the evaluation of formation constants between analytes and selectors [17].

An interesting use for discontinuous electrolyte systems is in the formation of gradients. Tesařová *et al.* [18] published the separation of enantiomers of a coumarin derivative by hydroxypropyl-β-CD-modified MEKC. SDS was used for the micelle formation. Gradient was generated by variation of the composition of electrolytes in the individual compartments of the separation system (inlet vial, capillary and outlet vial). It was noted that the variation of experimental arrangements affected both the migration times and the resolution of the enantiomers. Also, a mathematical model was proposed for the confirmation of the observed data [18].

The description of processes which take place in these systems is very difficult because of the many constituents of the BGE and complex processes that can affect the separation. It was shown that most of the changes in discontinuous electrolyte systems are reflected in changes of concentrations and mobilities of BGE components, *i.e.*, in the conductivities of the zones. It seemed promising to use conductivity detection, namely, contactless conductivity (CC) detection for the study of these processes. Some fundamental aspects of CC detection in CE are given in works published by Kubáň *et al.* [19–21] or by Brito-Neto *et al.* [22, 23]. The first type of CC detector was constructed for ITP by Gaš *et al.* [24]. Today, the CC detector is used for the detection of both inorganic [25, 26] and organic ions [27–29]. Gong *et al.* [30] recently published the first use of the CC detector for detection of separated chiral species. Zemann [31] reviewed the importance of optimization of BGE conductivity, advising use of a BGE with a pH range of 5–9 where the H_3O^+ and OH^- ions do not interfere. CC detection is also used in nonaqueous CE with highly UV-absorbing electrolytes [32–35].

Detection in CE can be improved by using simultaneous CC detectors and spectrophotometric detectors. Tan *et al.* [36] reported on the simultaneous fluorescence and CC detection for determination of labeled amino acids and small proteins [36]. However, CC detectors are mostly hyphenated with UV detectors [37, 38]. The UV detection window can be at the same spot as a detection cell of the CC detector [39, 40] or it can be placed differently [41]. The UV detector gives information about changes in UV absorption and the CC detector gives information about appropriate changes in conductivity and therefore about the migration of established zones.

This work shows the separation of tamsulosin enantiomers (for the structure see Fig. 1 and for more details see our previous works [42, 43]) in suggested discontinuous electrolyte systems. The discontinuity was created by variation of the presence of sulfated β-cyclodextrin (S-β-CD) in individual compartments of the separation system. The study of the influence of these systems on both migration times and resolution is included. Simultaneous CC and UV detection was used to gain insight on the separation mechanism. The calculation of the selector mobilities and effective migration times is also presented.

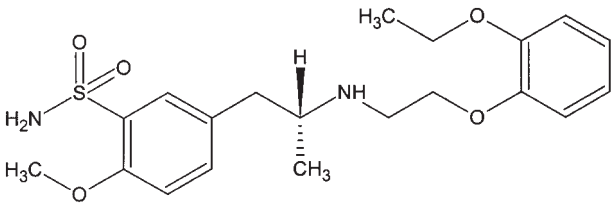

Figure 1. Structure of *(R)*-tamsulosin.

2 Materials and methods

2.1 Chemicals

Standards of tamsulosin enantiomers were kindly provided by Farmak (Olomouc, Czech Republic). Electrolyte components (acetic acid, sodium hydroxide, Tris, and sodium salt of S-β-CD) were obtained from Sigma (St. Louis, MO, USA). All the chemicals used were of analytical grade purity. Deionized water 18 $M\Omega \cdot cm^{-1}$ (Elga Bucks, Elga, UK) was used for the preparation of all solutions.

2.2 CE

Experiments were performed on an electrophoresis instrument made in our laboratory. A Jasco CE-975 UV detector (detection wavelength was set at 210 nm), Spellman CZE1000R power supply (Spellman, New York, USA), GFG-8217A high-frequency generator (Goodwill Instrument, USA) and 50-μm id uncoated fused-silica capillaries (Caco-Sila Tubing and Optical Fibers, Slovakia) were used. A CC detector was kindly provided by Pro-

fessor Opekar (Charles University, Prague, Czech Republic). Analyses were performed at laboratory temperature (25°C ± 1°C).

Electrolytes were prepared by dissolving an appropriate amount of acetic acid in water, adjusted to pH 4.0 with sodium hydroxide or Tris. The selector was then added (addition of the selector does not cause any change in pH, see our previous work [42]). Standards of (*R*)- and (*S*)-tamsulosin (10^{-4} mol·dm^{-3}) were prepared by dissolving tamsulosin in the ten times diluted electrolytes without the selector. The velocity of the EOF was measured by the water peak.

The capillary was conditioned every day before experiments with 0.1 M NaOH for 5 min, followed by deionized water for 5 min, 0.1 M HCl for 5 min, deionized water for 5 min and electrolyte without the selector for 15 min. Between the experiments the capillary was rinsed with 0.1 M HCl for 2 min, deionized water for 2 min and electrolyte without the chiral selector for 2 min.

2.3 Computer simulations

Simulations were performed using the freeware programs Simul 4.0 and Peakmaster 5.1, products of the research group of Professor Gaš (Charles University, Prague, Czech Republic), which are available online at http://www.natur.cuni.cz/gas. The mathematical background of these programs is described in a number of papers [44–48]. The input parameters were the same as in the real experiments.

3 Results and discussion

3.1 Separations in discontinuous electrolyte systems

Maier *et al.* [42] published the set-up for the separation of tamsulosin enantiomers in acidic electrolytes with S-β-CD as a chiral selector. In acidic electrolytes, tamsulosin is positively charged and migrates in the same direction as the EOF. The selector S-β-CD migrates in the opposite direction, to the anode. Therefore four experimental arrangements were suggested (Fig. 2). In the first one (a), the inlet vial, the capillary and the outlet vial are filled by the BGE containing S-β-CD. The next three arrangements differ in the presence of the electrolyte with S-β-CD in individual compartments. System (b) contains the BGE with S-β-CD in the capillary and in the outlet vial, while the inlet vial contains only the BGE without the selector. In arrangement (c) the BGE with S-β-CD is in the outlet vial only and in arrangement (d) the BGE with S-β-CD is only in the capillary.

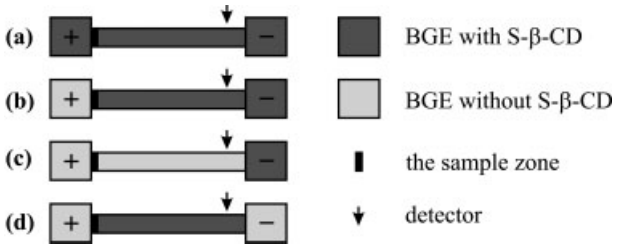

Figure 2. Suggested experimental arrangements.

These separation systems differ in conductivities of the generated zones, in the duration of interaction of the analytes with the chiral selector and also in the consumption of the selector. The influence of the systems on resolution was studied in 100 mM acetate/Na pH 4.0 and 100 mM acetate/Tris pH 4.0. The concentration of the S-β-CD varied from 1 to 3 g/L.

Figures 3 and 4 give examples of separations of the tamsulosin enantiomers. In all analyses the decrease of absorbance in system (c) and the increase of absorbance in system (d) at about 2 min were observed. It is caused by the migration of S-β-CD into the BGE without the selector (system (c)), that causes the decrease of absorbance. In system (d) the migration of S-β-CD from the detector causes the increase of absorbance due to the removal of the less absorbing electrolyte component. Tables 1 and 2 show the observed migration times of the enantiomers and their resolution values. In system (b) the highest resolution was observed. It is caused by the lower conductivity of the electrolyte in the inlet vial (there is no S-β-CD) in contrast to system (a) where S-β-CD is present in the all compartments. With higher concentrations of S-β-CD, no signal was observed, probably due to the formation of strong anionic complexes of enantiomers and the selector with small mobilities; the dispersion is high and the peaks cannot be distinguished from the baseline.

The influence of a counter-ion (sodium and Tris) was studied. In the case of Tris ions, the dynamic coating of a capillary inner surface causes the lower EOF velocity and therefore higher resolution of enantiomers. The influence of the suggested arrangements on electroosmotic mobilities as well as enantiomer mobilities was tested. EOF has the same velocity in the zone of both the BGE with S-β-CD and the BGE without S-β-CD. The effective mobilities of tamsulosin enantiomers in the suggested systems show decreasing tendency with increasing selector concentration due to the increase in the ratio of concentration of the enantiomers bound into complexes to concentration of the free enantiomers. The complex between tamsulosin and S-β-CD is negatively charged in the concentration range 1–3 g/L of S-β-CD. This was confirmed by using the mathematical approach for the determina-

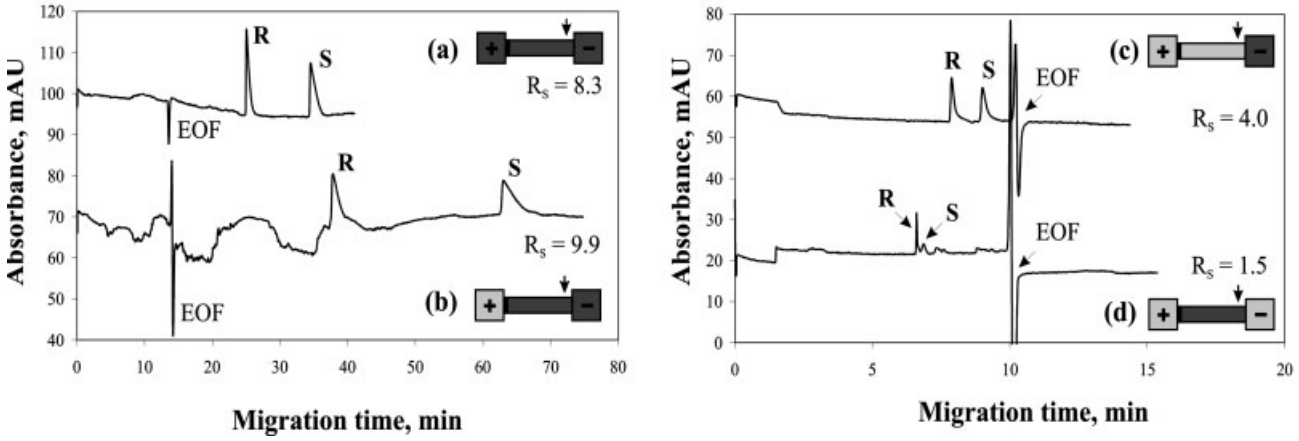

Figure 3. Separations of tamsulosin enantiomers in suggested systems in acetate/Na. BGE: 100 mM acetate/Na pH 4.0 with 3 g/L S-β-CD, capillary: 26 cm/39 cm; 15 kV.

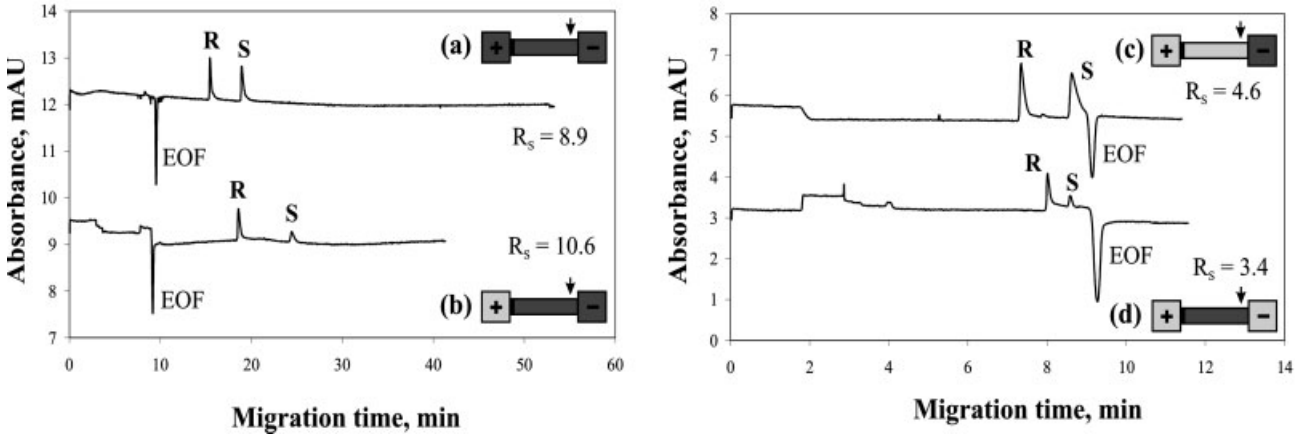

Figure 4. Separations of tamsulosin enantiomers in suggested systems in acetate/tris. BGE: 100 mM acetate/tris pH 4.0 with 3 g/L S-β-CD, capillary: 26 cm/39 cm; 15 kV.

Table 1. Resolution and effective migration times of tamsulosin enantiomers in acetate/Na ($t_{PL,S}$, $t_{PL,R}$, Δt_{PL} values are in minutes)

Conc. S-β-CD g/L	System (a)				System (b)			
	$t_{PL,R}$	$t_{PL,S}$	Δt_{PL} [a)]	R_S	$t_{PL,R}$	$t_{PL,S}$	Δt_{PL}	R_S
1	7.20	7.60	0.40	1.38	7.23	7.63	0.40	1.28
2	9.23	10.17	0.94	5.30	8.79	9.76	0.97	4.97
3	28.04	39.46	11.42	11.20	33.00	52.88	19.88	11.52
Conc. S-β-CD g/L	System (c)				System (d)			
	$t_{PL,R}$	$t_{PL,S}$	Δt_{PL}	R_S	$t_{PL,R}$	$t_{PL,S}$	Δt_{PL}	R_S
1	2.50	2.60	0.10	0.48	2.93	2.97	0.04	0.68
2	2.45	2.65	0.20	1.57	3.25	3.32	0.07	1.77
3	5.87	7.13	1.26	3.74	3.50	3.60	0.10	1.31

a) $\Delta t_{PL} = t_{PL,S} - t_{PL,R}$

Table 2. Resolution and effective migration times of tamsulosin enantiomers in acetate/Tris ($t_{PL,S}$, $t_{PL,R}$, Δt_{PL} values are in minutes)

Conc. S-β-CD g/L	System (a)				System (b)			
	$t_{PL,R}$	$t_{PL,S}$	Δt_{PL}[a)]	R_S	$t_{PL,R}$	$t_{PL,S}$	Δt_{PL}	R_S
1	6.75	7.16	0.41	3.02	6.53	6.96	0.43	3.16
2	10.41	11.75	1.34	6.07	10.69	12.28	1.59	5.85
3	15.32	18.74	3.42	9.05	18.56	24.42	5.86	10.45
Conc. S-β-CD g/L	System (c)				System (d)			
	$t_{PL,R}$	$t_{PL,S}$	Δt_{PL}	R_S	$t_{PL,R}$	$t_{PL,S}$	Δt_{PL}	R_S
1	1.63	1.73	0.10	0.98	3.41	3.48	0.07	1.80
2	2.27	2.54	0.27	1.87	4.01	4.15	0.14	2.72
3	4.57	5.86	1.29	4.47	4.57	4.83	0.26	4.03

a) $\Delta t_{PL} = t_{PL,S} - t_{PL,R}$

tion of stability constants published by Barták *et al.* [49], assuming 1:1 complex formation. The mobilities of the complexes as well as the stability constants for both electrolytes are summarized in Table 3.

Table 3. Tamsulosin – S-β-CD complex formation data

	Effective mobility ($\times 10^{-9}$ $m^2V^{-1}s^{-1}$)		Stability constant (dm^3mol^{-1})	
Electrolyte		Acetate/Na		
(R)-Tamsulosin	−21.32	0.43[a)]	745	15[a)]
(S)-Tamsulosin	−22.54	0.24[a)]	792	11[a)]
Electrolyte		Acetate/Tris		
(R)-Tamsulosin	−17.78	0.58[a)]	984	23[a)]
(S)-Tamsulosin	−19.14	0.56[a)]	1050	29[a)]

a) SD for five runs

3.2 Effective migration time

Amini [17] defined an effective plug length PL_{ef} , which is the capillary length where an enantiomer migrates through the selector zone. On the basis of this definition, the effective migration time is the time when the enantiomer migrates through the selector zone. It is the maximum time for the interactions of an analyte with the selector.

In the first two systems (a) and (b) the effective migration times of both enantiomers $t_{PL,R}$ and $t_{PL,S}$ are equal to the migration times. Since the tamsulosin mobilities in the selector-free acetate electrolytes and the selector mobilities in the acetate electrolytes were determined, it is also possible to calculate the effective migration times in systems (c) and (d). Tamsulosin mobilities in the selector-free electrolytes were 16.41 (SD for five runs is 0.31) $\times 10^{-9}$ $m^2V^{-1}s^{-1}$ in acetate/Na and 15.70 (0.12) $\times 10^{-9}$ $m^2V^{-1}s^{-1}$ in acetate/Tris electrolyte.

Derivation of relations for the calculation of effective migration times is shown for the (*R*)-enantiomer only (similar relations can be derived for the (*S*)-enantiomer). The migration time of an enantiomer $t_{M,R}$ is equal to the sum of the time which an enantiomer spends in the BGE without S-β-CD t_a and the time which the enantiomer spends in the BGE with S-β-CD (the effective migration time) $t_{PL,R}$:

$$t_{M,R} = t_a + t_{PL,R} \quad (1)$$

The migration time can be expressed as the migration length divided by the migration velocity. For system (c) from Fig. 5, $t_{PL,R}$ and t_a can be re-written as:

$$t_{M,R} = \frac{l - PL_{ef}}{v_a} + \frac{PL_{ef}}{v_{PL,R}} \quad (2)$$

where v_a is the velocity of tamsulosin in the BGE without S-β-CD and $v_{PL,R}$ is the velocity of *(R)*-tamsulosin in the BGE with S-β-CD.

Because of the knowledge of the selector velocity v_{CD}, t_a can be reformulated as:

$$t_a = \frac{l - PL_{ef}}{v_a} = \frac{PL_{ef} + L - l}{v_{CD}} \quad (3)$$

From Eq. (3) it is possible to calculate the effective plug length (the same for both enantiomers), and from Eqs. (1) and (2) the effective migration times can be determined.

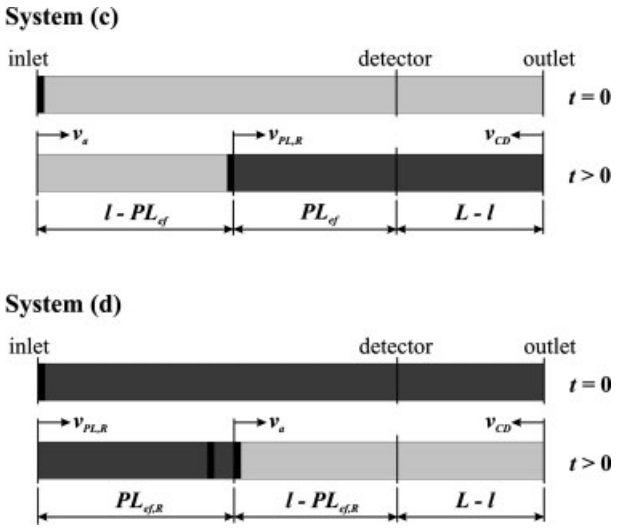

Figure 5. Schemes of systems (c) and (d) for calculating effective migration times.

For system (d), Eqs. (1) and (2) can also be applied (see Fig. 5). The migration time of the enantiomer $t_{M,R}$ can be expressed as:

$$t_{M,R} = \frac{l - PL_{ef,R}}{v_a} + \frac{L - PL_{ef,R}}{v_{CD}} \quad (4)$$

which, with the combination of Eqs. (1) and (2), allows calculation of the effective migration times. Calculated results are shown in Tables 1 and 2. Resolution values in system (d) are higher than those in system (c) for the concentrations of S-β-CD below 3 g/L. It respects the fact that differences in the velocity of the selector plug play an important role in enantioseparation.

3.3 Simultaneous CC and UV detection

Simultaneous CC detection and UV detection was used for the study of processes that take place in the suggested systems. The addition of S-β-CD sodium salt increases the specific conductivity of the BGE. Therefore the lower concentrations of the selector (0.5–1.0 g/L) were used. The input frequency of the CC detector was optimized to achieve the best S/N; 300 kHz was used in both electrolytes. In Fig. 6 there is an example of the separation of tamsulosin enantiomers in system (a). The UV detector was used for the determination of tamsulosin peaks and the system peak, which indicates the EOF. It was used for the notation of these peaks in the CCD electropherograms.

In Figs. 7 and 8 examples of the separations (records only from CC detector) in 100 mM acetate/Na pH 4.0 with 1 g/L S-β-CD and 100 mM acetate/Tris pH 4.0 with 1 g/L S-β-CD are shown, respectively.

3.4 Study of separation mechanism by model systems

Firstly, the influence of the migration of S-β-CD was studied. The electrolytes 100 mM acetate/Na pH 4.0 as BGE without the selector and 125 mM acetate/Na pH 4.0 as BGE with S-β-CD were proposed. The concentration increase modeled the appropriate ionic strength of the addition of S-β-CD.

In the model systems (c) and (d) any small changes in conductivity at about 4 min were not achieved (in comparison with the case of S-β-CD, Fig. 7, "CD-increase" and "CD-decrease").

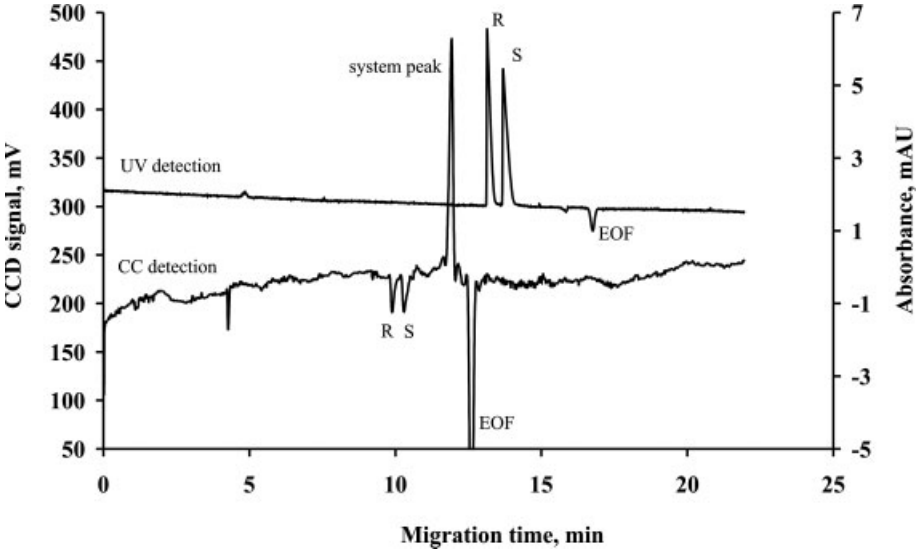

Figure 6. Example of the simultaneous CC and UV detection for the separation of tamsulosin enantiomers. BGE: 100 mM acetate/Na pH 4.0 with 1 g/L S-β-CD, capillary: 35 cm (CC detector)/47 cm (UV detector)/60 cm; 15 kV.

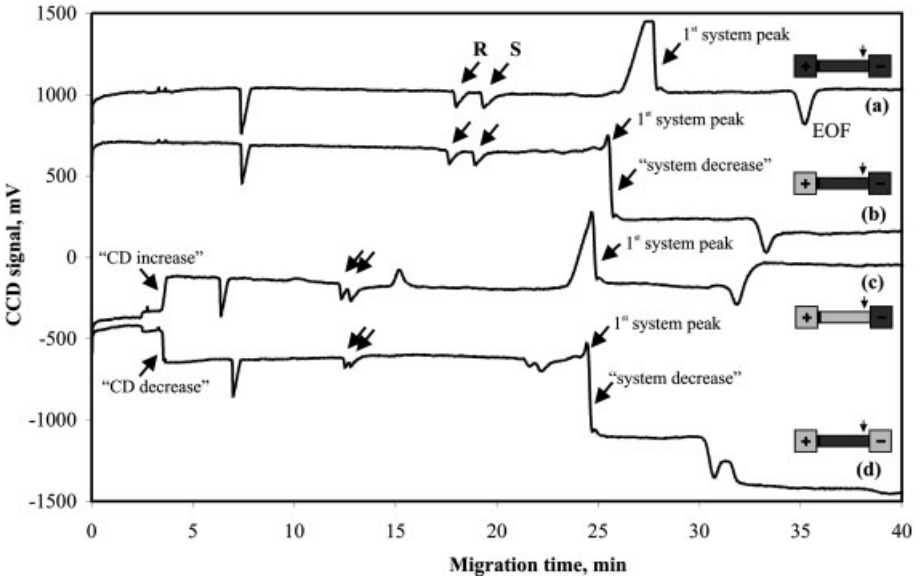

Figure 7. Separations of tamsulosin enantiomers in suggested systems in acetate/Na with using CC detection. BGE: 100 mM acetate/Na pH 4.0 with 1 g/L S-β-CD, capillary: 34 cm/59 cm; 15 kV.

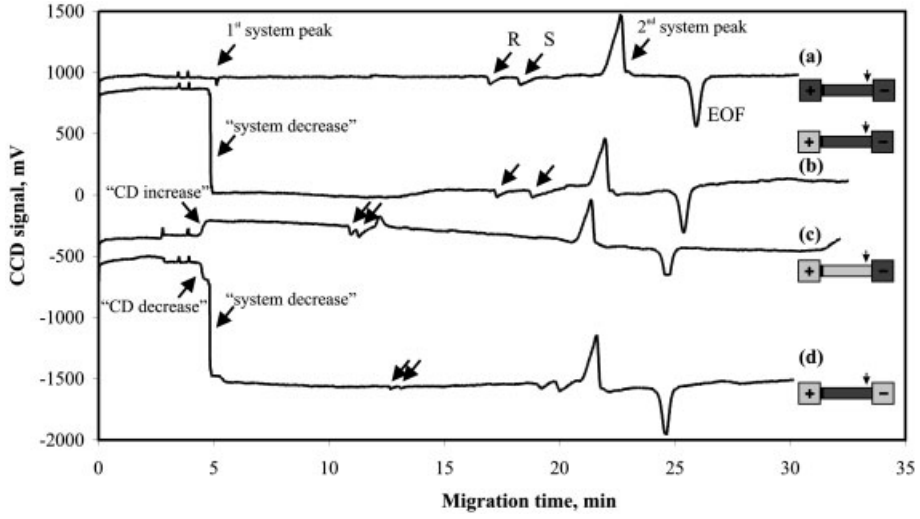

Figure 8. Separations of tamsulosin enantiomers in suggested systems in acetate/tris with using CC detection. BGE: 100 mM acetate/tris pH 4.0 with 1 g/L S-β-CD, capillary: 34 cm/59 cm; 15 kV.

It was proposed that these conductivity changes (the case of S-β-CD) are related to the selector migration. In system (c) the selector migrates from the outlet vial into the capillary and causes the increase in conductivity, while in system (d) the selector plug migrates from the end of the capillary and causes the decrease in conductivity. Both changes of conductivity occur at the same time because the described migration of S-β-CD is between the detector cell and the capillary end. From these changes it is possible to calculate the effective mobilities of S-β-CD in the acetate electrolytes. The effective mobility of S-β-CD in 100 mM acetate/Na pH 4.0 is –53.42 (SD for five runs is 0.65) $\times 10^{-9}$ $m^2V^{-1}s^{-1}$ and in 100 mM acetate/Tris pH 4.0 is -45.52 (0.99) $\times 10^{-9}$ $m^2V^{-1}s^{-1}$. The proposed model experimental arrangements were also used for the measurements without sample loading. It was found that the main conductivity decreases in systems (b) and (d) (see Figs. 7 and 8) are caused by the concentration differences between the electrolytes in the capillary and in the inlet vial.

3.5 Study of separation mechanism by mathematical simulations

Since it is possible to detect system zones with conductivity detection [50], Peakmaster 5.1 was used for the determination of system peaks. Poppe [51–53] introduced *N* electrolyte components form *N* system zones. In acetate/Tris electrolytes there are four components

without analytes: acetate, Tris, sodium and S-β-CD. It is possible to detect two cationic system zones and the system zone with its mobility close to the electroosmotic mobility. The situation in acetate/Na electrolytes is similar, with three electrolyte components, acetate, sodium and S-β-CD, which form one cationic system peak and the system peak with the mobility close to the electroosmotic mobility.

A detailed study of the separation in the suggested systems was performed with Simul 4.0 based on numerical solving of continuity equations which describe the electromigration [44]. Figures 9 and 10 show the calculated concentration profiles of the electrolyte components for acetate/Na and acetate/Tris, respectively, for systems (a) and (b). Position 0.00–0.04 simulates the sample zone. All the simulations performed assume the separation without the influence of EOF and ionization only to the first step. An ionic mobility of $-55.5 \times 10^{-9}\ m^2V^{-1}s^{-1}$ and a pK_a value of 1.0 were used in the simulations as the S-β-CD parameters. The values were estimated in accordance with our previous experiments, assuming only the first-step ionization calculation possibility of the Simul 4.0 software.

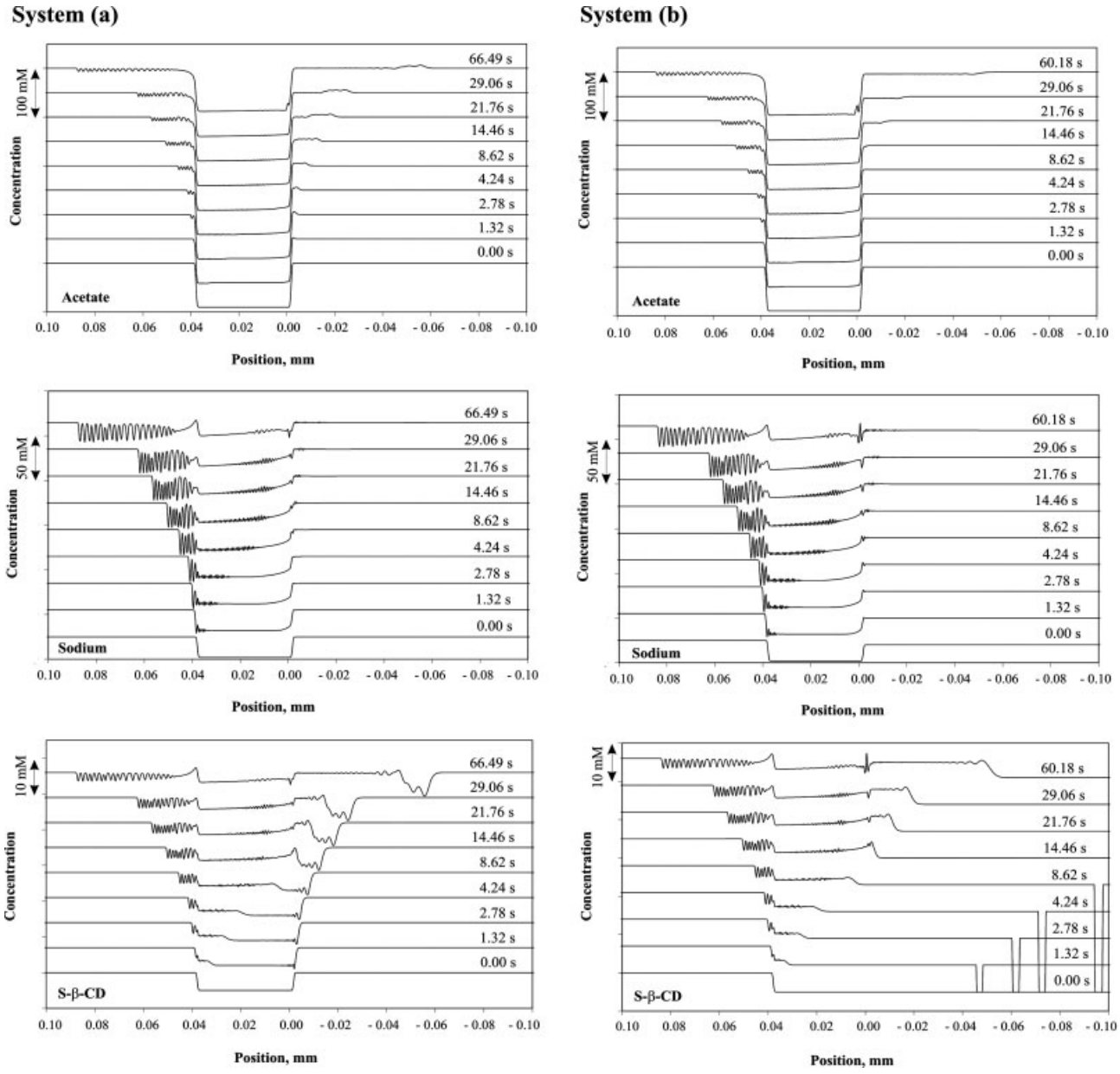

Figure 9. Simulated concentration profiles of electrolyte components in systems (a) and (b) for acetate/Na.

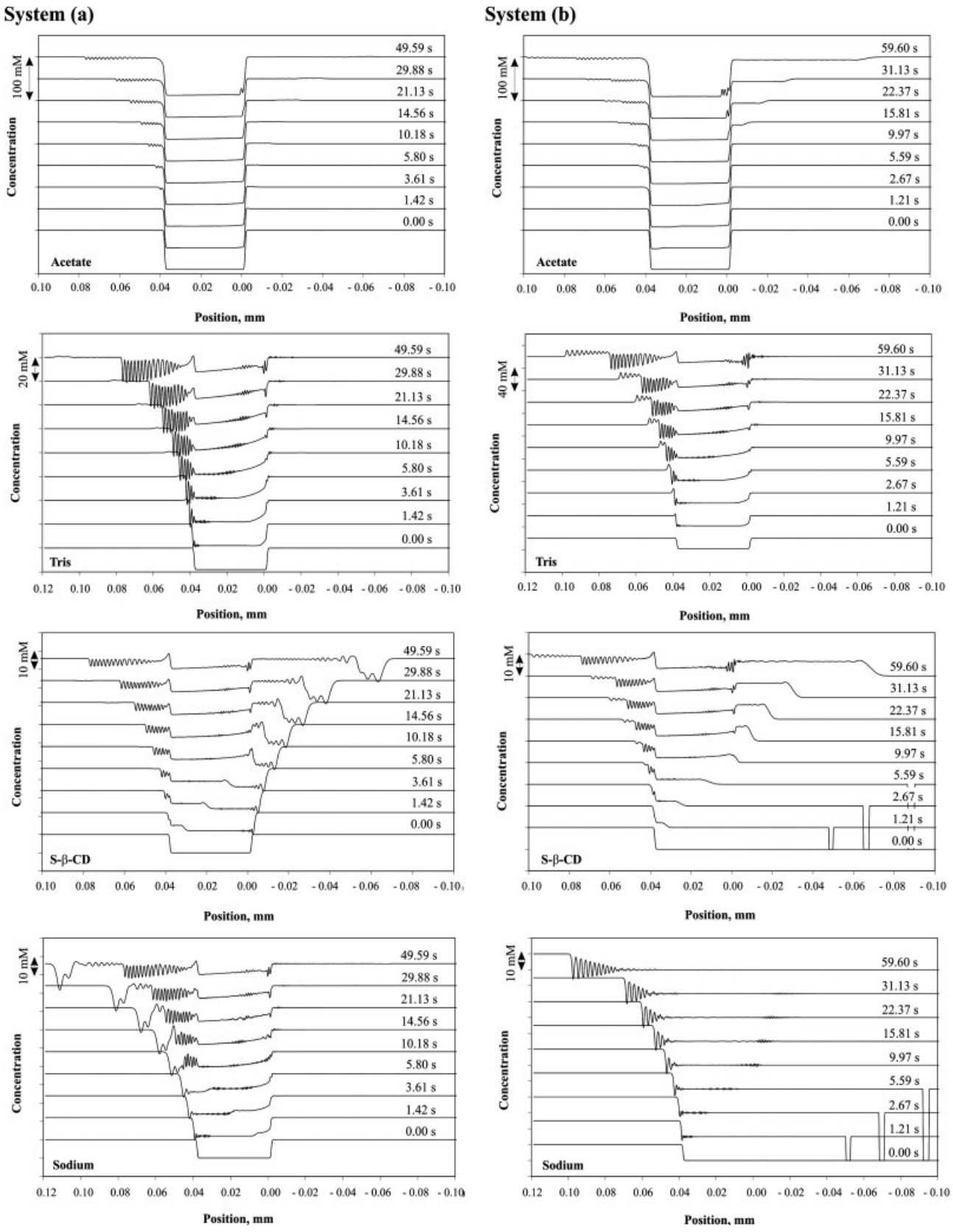

Figure 10. Simulated concentration profiles of electrolyte components in systems (a) and (b) for acetate/Tris.

Firstly, in the acetate/Tris electrolyte (Fig. 10), Tris ions are concentrated in the boundary between the BGE in the capillary and the electrolyte in the sample zone due to the Kohlrausch regulation function. In this boundary the S-β-CD ions and sodium ions are also concentrated, but in lower amount than the Tris ions. Concentration profiles for S-β-CD ions (Fig. 10) show for system (a) the formation of the fourth system zone (anionic) caused by the migration of the zone with lower S-β-CD concentration. In system (b) there are no S-β-CD ions in the inlet vial and therefore S-β-CD ions "copy" the concentration in the sample zone and then migrate into the inlet vial.

The last graphs show sodium profiles. In system (a) the migration of the zone with the lower concentration of sodium ions than that in the BGE is shown. This zone migrates fast and forms the first system peak (negative). In system (b), because of the absence of sodium in the inlet vial, sodium ions migrate out of the capillary and cause the decrease in the conductivity signal at the same time as the first system peak migrates in the system (a). These conclusions from systems (a) and (b) can be generalized to systems (c) and (d).

A similar situation is found in the acetate/Na (Fig. 9). Sodium ions provide the only exception in this electrolyte. Sodium ions are concentrated in the "BGE – sample zone" boundary only. In system (b) there is lower concentration of sodium ions in the inlet vial than in the capillary, and this lower concentration causes the decrease in the conductivity signal. The decrease is lower than that in the acetate/Tris because of the higher specific conductivity of acetate/Na electrolytes and is later due to the involvement of sodium ions in the buffering system as well as in the electroosmosis formation.

3.6 Separation mechanism in discontinuous electrolyte systems

From the experiments performed and the modeling it is possible to discuss some features of the separation mechanism in the suggested experimental arrangements. In system (a), acetate counterions (sodium or Tris) are concentrated in the "BGE – sample zone" boundary and form the system zone which can cause preconcentration of the analyte. In system (b) another boundary is formed due to the different concentrations of the sodium ions in the capillary and in the inlet vial. In acetate/Tris electrolytes this boundary migrates relatively fast and causes a decrease in conductivity and a change in the electric field strength, while the influence of sodium ions on the separation is minimal. In acetate/Na electrolytes the boundary migrates after the first system zone and therefore its influence on the separation is also minimal. The analytes can be preconcetrated in system (b) due to the formation of the boundary with the change of the electric field strength, based on both stacking and transient ITP principles. System (c) is characterized by the presence of S-β-CD in the outlet vial only. Because of the absence of S-β-CD in the sample zone, the separation of tamsulosin enantiomers starts later (after the sample zone meets the S-β-CD zone) and therefore the obtained resolution values are smaller than those in systems (a) and (b). A similar situation is found in system (d) where the selector is present in the capillary only and migrates out of the capillary to the inlet vial.

The mechanism of chiral discrimination is the same in all suggested systems. Tamsulosin molecules interact with the S-β-CD in the same way. The differences in resolution in the individual suggested systems are caused by various factors, *e.g.*, analysis time, time for the selector–select and complex formation (mainly in systems (c) and (d)), suppression of the dispersion in system (b) in comparison with system (a).

4 Concluding remarks

The separation of tamsulosin enantiomers (cationic analyte) under various experimental arrangements (Fig. 2) was studied by using an anionic chiral selector (S-β-CD). The results can be generalized as follows:

(i) Resolution values are dependent on the system used for the separation. The highest resolution is achieved in systems (a) and (b), where system (b) is more advantageous because the analytes can be preconcentrated. Another advantage of system (b) is smaller selector consumption compared to system (a). On the other hand, system (d) requires about 1000–10 000 times smaller amount of the selector (according to the volumes of the inlet and outlet vials) and it is possible to achieve resolution values of about 2.0, which can be used for analyzing racemic mixtures or for working with expensive or non-stable chiral selectors.

(ii) The mechanism of chiral resolution is the same in all systems.

(iii) The influence of the suggested systems on chiral separation is caused by the fact that the anionic selector migrates in the opposite direction to the cationic analytes and forms migrating boundaries. In systems (a) and (b) the migration of the selector does not cause any difference, because analytes are in contact with the selector zone during the entire analysis time. In systems (c) and (d) analytes are in contact with the selector zone only for a certain time,

which is affected by the velocity of migration of the selector and EOF. The differences between the times when analytes migrate through the selector zone are responsible for the resolution of optical isomers. To achieve successful chiral separation in systems (c) or (d) it is necessary to optimize the velocities of both the selector zone and the sample zone (with respect to EOF velocity) and the chiral selector concentration, *i.e.*, the concentration ratio of the analyte bound in a complex with the selector to the free analyte.

(iv) The suggested systems differ in the composition of the electrolytes in the inlet vial, in the capillary and in the outlet vial. These differences change during the separation time. The systems are discontinuous in the electric field strength, which can have preconcentration effects based on stacking and ITP principles.

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