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# Optimization of the separation and detection of the enantiomers of isoproterenol in microdialysis samples by cyclodextrin-modified capillary electrophoresis using electrochemical detection

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**Abstract**

Isoproterenol is a chiral catecholamine with a half-life of elimination of less than 10 min. In order to study the pharmacokinetics of this compound using microdialysis sampling, an analytical method was needed which could resolve the individual enantiomers of isoproterenol and required less than 1 $\mu$l of sample. A capillary electrophoretic method using a run buffer containing methyl-O-$\beta$-cyclodextrin as a chiral recognition agent was developed which could resolve the enantiomers of isoproterenol. The detection limits using UV absorbance detection were found to be too high to determine the concentration of isoproterenol in plasma for a sufficient time following administration to establish the pharmacokinetics. The detection limits were decreased three orders of magnitude to 3 ng/ml by using an amperometric detector. The detection limits were decreased to 0.6 ng/ml using an on-column concentration technique in which peak stacking was accomplished by following the sample injection with a plug of acid.

*Keywords:* Enantiomer separation; Isoproterenol

## 1. Introduction

Capillary electrophoresis (CE) has been shown to be a versatile approach to the separation of the individual enantiomers of chiral compounds by the addition of a chiral recognition agent to the electrophoresis run buffer. Chiral bile acids [1], surfactants [2], metal complexes [3], crown ethers [4], oligopolysaccharides [5] and cyclodextrins [6–8] have all been used as run buffer modifiers to provide chiral selectivity to CE separations. Because the chiral recognition agent is added to the electrophoresis run buffer rather then bonded to a stationary phase as in chiral liquid chromatography, optimization of the type and concentration of the agent is more facile. In addition, the mixed mode separation of chiral CE more readily provides both resolution of the analyte from other sample components as well as resolution of the individual enantiomers.

Microdialysis sampling provides a technique for continuously monitoring the concentration of analytes in a conscious animal with high temporal resolution [9–12]. At a typical microdialysis perfusion rate of 1 $\mu$l/min, at least 5 min are needed to collect sufficient sample for most LC analyses. To achieve higher temporal resolution, correspondingly less sample is available for analysis. The nanoliter sample volume requirements of CE therefore offer

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significant advantages for the analysis of samples obtained by microdialysis sampling [13–16].

This report describes the development of a CE analysis method for the determination of the individual enantiomers of a chiral compound, isoproterenol, designed for use with microdialysis sampling. The half-life of isoproterenol is less than 10 min [17], therefore, a 1-min sampling frequency is desired to sufficiently define the pharmacokinetic curve. Using a dialysis perfusion flow-rate of 0.5 $\mu$l/min, this sampling frequency provides only 500 nl of sample for analysis. For this reason, no off-line sample preconcentration is possible and the analytical method must provide detection limits sufficient to detect isoproterenol as it is being eliminated. The analytical method must also be capable of analyzing highly ionic sample because the microdialysis perfusate must closely match the plasma in ionic composition.

The chiral separation was achieved using a cyclodextrin modifier in the electrophoresis run buffer. Several cyclodextrins were evaluated in order to optimize the separation. While UV absorbance detection was used for optimization of the separation, this approach did not provide sufficient detection limits for the pharmacokinetic experiments. In order to achieve lower detection limits, amperometric detection was used. This required some modification of the CE separation to maintain compatibility. The detection limits were further decreased by the development of a peak-stacking approach, using injection of a plug of acid following the sample injection.

## 2. Experimental

### 2.1. Chemicals and reagents

Disodium ethylenediaminetetraacetic acid dihydrate (EDTA), phosphoric acid, monochloroacetic acid, glacial acetic acid, hydrochloric acid and 90% formic acid were obtained from Fisher Scientific (Fairlawn, NJ, USA). Racemic isoproterenol·HCl, (+)-isoproterenol bitartrate, (−)-isoproterenol bitartrate, 3,4-dihydroxybenzylamine, N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid] (HEPES), $\beta$-cyclodextrin ($\beta$CD), 2-hydroxypropyl-$\beta$-cyclodextrin (2OH$\beta$CD), and methyl-O-$\beta$-cyclodextrin (M$\beta$CD) were obtained from Sigma (St. Louis, MO, USA). All other chemicals were of reagent grade or better and were used as received.

Ringer's solution consisted of 155 m*M* NaCl, 2.3 m*M* $CaCl_2$ and 5.5 m*M* KCl. The EDTA–bisulfite stabilizer solution consisted of 8 m*M* disodium EDTA and 0.1 m*M* sodium bisulfite. All buffers were prepared by titrating a solution of the free acid, at the desired concentration, with either 8 *M* NaOH or 8 *M* LiOH, as specified in the text, to give the desired pH. The alkaline EDTA solution was prepared by titrating a 0.5 m*M* disodium EDTA solution with NaOH to pH 13. All solutions were prepared with Nanopure water (Barnstead, Dubuque, IA, USA), filtered through a 0.22-$\mu$m filter.

### 2.2. CE

For CE experiments using UV absorbance detection, an ISCO 3850 capillary electrophoresis unit was used (ISCO, Lincoln, NE, USA). A 65 cm length of 50 $\mu$m I.D. fused silica (Polymicro Technologies, Phoenix, AZ, USA) was used as the separation capillary. The length from injection end to the detection window was 40 cm. Detection was at a wavelength of 220 nm. Samples were introduced by vacuum injection for 8 s. The separation voltage was adjusted as specified in the text. The capillary was manually flushed between injections with 500 $\mu$l of water, 500 $\mu$l of 3% (v/v) Microcleaning solution (International Products, Trenton, NJ, USA), 500 $\mu$l of water, and then 500 $\mu$l of run buffer. The composition of the run buffer was varied to achieve optimal separation conditions as described in the text.

For CE experiments using amperometric detection a CE–electrochemical detection system (CEEC), built in-house, was used [18]. Amperometric detection was performed using a 1 mm long, 33 $\mu$m diameter carbon fiber as the working electrode. A potential of +0.65 V versus the Ag/AgCl reference was used. Electrical isolation of the detector from the CE was accomplished using an end-column electrical decoupler [19]. The run buffer consisted of 0.1 *M* lithium acetate, pH 4.75, 0.1 g/ml methyl-O-$\beta$-cyclodextrin and 0.5 m*M* disodium EDTA. Prior to each run, the capillary was flushed with the alkaline

EDTA solution for 10 min and then with the run buffer for 1 min, at a pressure of 40 p.s.i. Samples were injected either by normal electrokinetic injection or with acid stacking. Electrokinetic injection was for 3 s at 18 kV. pH-mediated peak stacking involved electrokinetic injection of the sample for 15 s at 18 kV, followed immediately by electrokinetic injection of 0.1 *M* HCl for 20 s at 18 kV.

### 2.3. Microdialysis sampling

Microdialysis samples were collected from the jugular vein of anesthetized Sprague–Dawley rats, as described previously [14]. A flexible cannula-style microdialysis probe with a 5 mm length of Cuprophan dialysis fiber was used for sampling. Isoproterenol was administered at a dose of 10 mg/kg i.v. The dialysis perfusate solution was pumped at a flow-rate of 0.5 $\mu$l/min using a CMA 100 microinfusion pump (Bioanalytical Systems, West Lafayette, IN, USA).

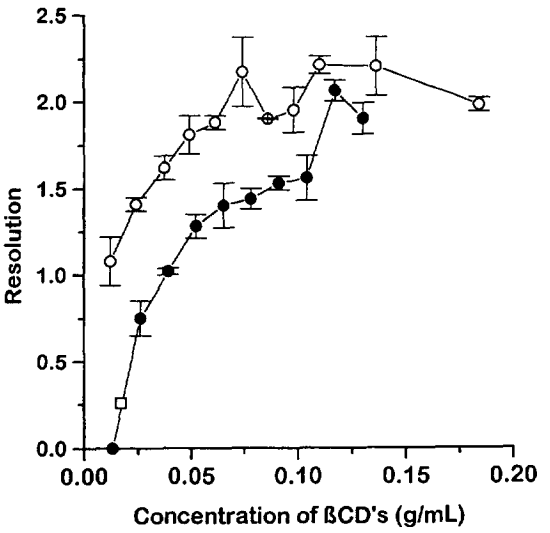


Fig. 1. Resolution of the enantiomers of isoproterenol as a function of cyclodextrin concentration. (●) M$\beta$CD; (○) 2OH$\beta$CD; (□) $\beta$CD. The run buffer was 125 m*M* sodium acetate, pH 4.76. Error bars represent one standard deviation of 3 injections.

## 3. Results and discussion

### 3.1. Optimization of the CE separation

Because the enantiomers of a chiral compound have identical free solution electrophoretic mobilities, a chiral recognition agent, such as a cyclodextrin, must be added to the run buffer to achieve resolution. The apparent electrophoretic mobility of a compound that forms an inclusion complex with the cyclodextrin is then the weighted average of the free solution electrophoretic mobility and the electrophoretic mobility of the inclusion complex. Resolution of enantiomers can be achieved if the binding constant of the individual enantiomers to the cyclodextrin differ sufficiently [20]. Therefore, the first step in the optimization of the chiral CE separation of the enantiomers of isoproterenol was to evaluate various cyclodextrins. The resolution and elution time of isoproterenol as a function of cyclodextrin concentration are shown in Fig. 1 and Fig. 2. Isoproterenol is a cation over the pH range studied (pH 2.1 to 7.5) and therefore migrates faster than neutral compounds such as the cyclodextrins. Therefore, longer elution times are indicative of greater binding to the cyclodextrin. The binding of isoproterenol was strongest to M$\beta$CD and weakest to $\beta$CD. At any given concentration of cyclodextrin, the extent of resolution of the enantiomers of isoproterenol was greatest for M$\beta$CD and least for

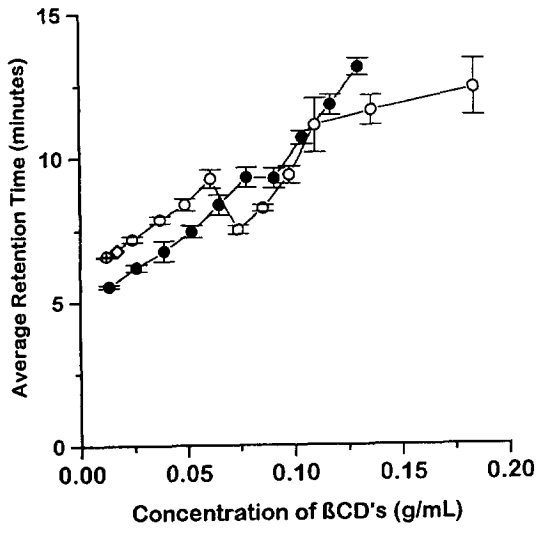


Fig. 2. Retention time as a function of cyclodextrin concentration. Symbols and conditions as in Fig. 1.

βCD. βCD was not evaluated further because of its poor resolving power and limited solubility.

The effect of the run buffer pH on the separation of the enantiomers of isoproterenol was then evaluated. While the charge state of both isoproterenol and the cyclodextrins remains unchanged over the pH range studied, the electroosmotic flow is pH-dependent. It has been shown that high electroosmotic flow typically results in poorer resolution in chiral CE [6]. This is because the separation is dependent upon transport between the cyclodextrin and the run buffer, if the elution time is too short insufficient time is provided to resolve the enantiomers. As seen in Table 1, lower run buffer pH resulted in slower electroosmotic flow and greater resolution of the enantiomers of isoproterenol. However, the slower electroosmotic flow also resulted in much longer analysis times and lower separation efficiencies (Table 1). As the ultimate use of this method was for pharmacokinetic investigations, where large numbers of samples would be generated, optimization of analysis time was a critical concern. A good compromise between resolution and analysis time was a pH 4.75 run buffer. MβCD provided better resolution and higher separation efficiencies than did 2OHβCD and was therefore chosen as the cyclodextrin for use in the analysis of isoproterenol. An acetate run buffer of pH 4.75, using MβCD, provided a resolution of 2.4 with an analysis time of 10.4 min. In order to cover the wide pH range studied, a variety of buffer types were used. While there may be some effect of buffer type on the CE separation, it is expected that the major effect will be due to changes in the pH. This was verified by changing the identity of the cation for the acetate buffer. While the electrophoretic current was highly dependent upon the cation, the separation was not affected.

The run buffer ionic strength affects resolution in chiral CE by increasing the hydrophobic interaction of the analyte with the cyclodextrin [7]. In addition, the ionic strength of the sample, relative to that of the run buffer, affects the separation efficiency in CE. The effect of run buffer ionic strength on the separation with the sample ionic strength held constant is shown in Fig. 3. Low run buffer ionic strength results in both poor efficiency and resolution. Increasing the run buffer concentration to 300 m*M* significantly enhanced both parameters. Increasing the concentration above 300 m*M* had little effect. The sensitivity of the system, based on peak height, was also a function of the run buffer ionic strength (Fig. 4). Sensitivity increased up to a run buffer concentration of 200 m*M* and then decreased at higher concentrations. The optimal run buffer concentration was selected as 250 m*M*.

The final parameter investigated was the electrophoretic field strength. In free solution, resolution increases as field strength increases, however, the mass transfer controlled nature of chiral CE reverses

Table 1
Effect of pH

| Buffer type | pH | $R_S$ | $N$ (× 1000) | $t_R$ (mean)[a] |
|---|---|---|---|---|
| *MβCD as chiral selector* | | | | |
| Phosphate | 2.14 | 3.8 ± 0.2 | 60 ± 8 | 20.5 ± 1.5 |
| Monochloroacetate | 2.87 | 3.0 ± 0.6 | 79 ± 24 | 14.4 ± 1.6 |
| Formate | 3.75 | 3.1 ± 0.6 | 111 ± 30 | 12.6 ± 1.1 |
| Acetate | 4.75 | 2.4 ± 0.6 | 112 ± 40 | 10.4 ± 0.9 |
| HEPES | 7.50 | 0.8 ± 0.1 | 46 ± 12 | 5.7 ± 0.4 |
| *2OHβCD as chiral selector* | | | | |
| Phosphate | 2.14 | 3.5 ± 0.4 | 87 ± 35 | 20.3 ± 1.3 |
| Monochloroacetate | 2.87 | 3.2 ± 0.3 | 104 ± 22 | 17.6 ± 0.5 |
| Formate | 3.75 | 2.3 ± 0.1 | 89 ± 1 | 12.9 ± 0.2 |
| Acetate | 4.75 | 1.8 ± 0.3 | 74 ± 24 | 9.4 ± 0.3 |
| HEPES | 7.50 | 0.8 ± 0.1 | 54 ± 26 | 5.6 ± 0.3 |

Values are the mean ± one standard deviation of 3 injections.
[a]Average elution time of both enantiomers.

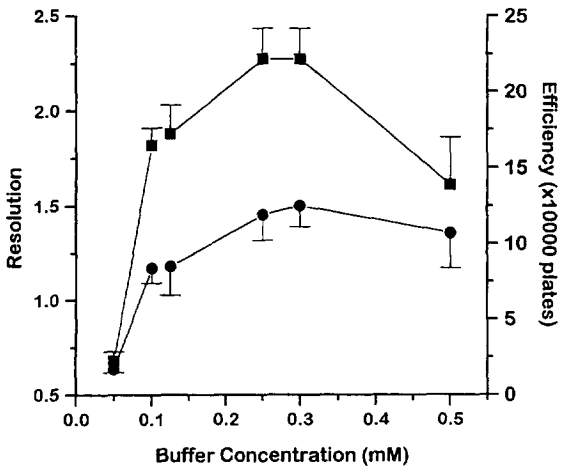


Fig. 3. Resolution and efficiency as a function of the run buffer concentration. (■) Resolution and (●) efficiency. The run buffer contained 0.1 g/ml MβCD in sodium acetate buffer, pH 4.76. Error bars represent one standard deviation of 3 injections.

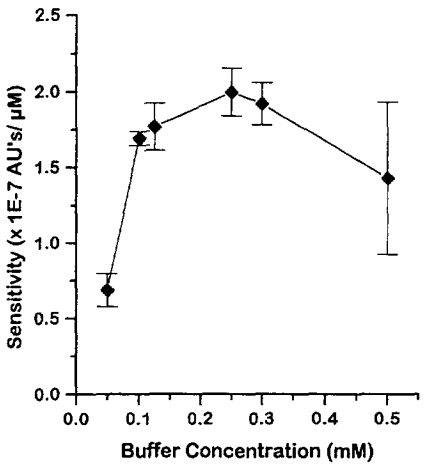


Fig. 4. Average sensitivity of 100 μM (−) and (+) isoproterenol (ISP), as a function of run buffer concentration. Conditions as in Fig. 3. Error bars represent one standard deviation of 3 injections.

this dependence [7]. As seen in Table 2, better resolution of the enantiomers of isoproterenol is achieved at lower field strength, although decreasing the field strength results in a loss of separation efficiency.

The optimized separation conditions were determined to be a run buffer of 250 m*M* sodium acetate buffer, pH 4.75, containing 0.1 g/ml MβCD and using a voltage of 20 kV. Fig. 5A shows a typical electropherogram of racemic isoproterenol under these conditions. The stereochemical identities were determined from analysis of the individual enantiomers. Under these conditions, with an 8 s vacuum injection, the response was linear from 7.4 to 106 μg/ml (slope = 0.98 mAU/mg/ml, intercept = 0.38 μAU, $r$ = 0.999), with a detection limit of 2.8 μg/ml, at a $S/N$ of 3.

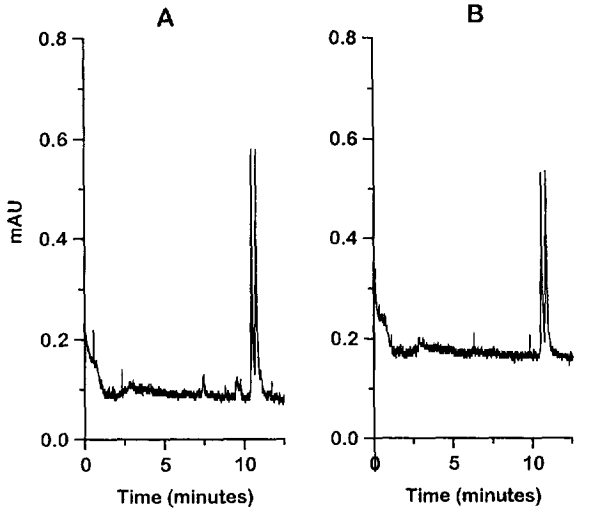


Fig. 5. CE–UV electropherograms of isoproterenol. (A) Standard containing 100 μ*M* of (−)- and (+)-isoproterenol (ISP) bitartrate dissolved in Ringers–8.0 m*M* $Na_2EDTA$–97 μ*M* $NaHSO_3$; (B) Microdialysate acquired from a rat 5 min after dosing. Conditions are given in the text.

Table 2
Resolution and efficiency as a function of field strength

| $V$ (kV) | $E$ ($V/L$) | $R_S$ | $N$ (× 1000) |
|---|---|---|---|
| 20 | 308 | 2.7 ± 0.2 | 67 ± 10 |
| 25 | 385 | 2.4 ± 0.2 | 77 ± 11 |
| 30 | 462 | 2.1 ± 0.1 | 75 ± 10 |

Values are the mean ± one standard deviation for 3 injections.

Table 3
Electrophoretic current as a function of buffer type and electrophoretic voltage

| Buffer type[a] | pH | Electrophoretic current ($\mu$A) | | |
|---|---|---|---|---|
| | | 30 kV | 20 kV | 10 kV |
| Sodium phosphate | 2.14 | 144 | 59 | 23 |
| Sodium formate | 3.78 | 258 | 84 | 30 |
| Sodium acetate | 4.76 | 195 | 68 | 24 |
| Lithium acetate | 4.76 | 92 | 41 | 17 |
| Sodium phosphate | 7.20 | Offscale[a] | 144 | 73 |
| HEPES | 7.50 | 34 | 17 | 7 |

Conditions: A 65 cm × 50 $\mu$m I.D. uncoated fused-silica capillary was used. Each buffer was 125 m*M* and was prepared at the pH =p$K_a$.
[a]Maximum measurable current was 300 $\mu$A.

### 3.2. Analysis of microdialysis samples

The optimized chiral CE method was applied to the analysis of intravenous microdialysis samples collected following administration of racemic isoproterenol. A typical electropherogram of a microdialysis sample collected over the first 5 min after dosing is shown in Fig. 5B. The enantiomers of isoproterenol are resolved from each other and from all endogenous compounds. Unfortunately, the detection limits were not sufficient to follow the concentration of isoproterenol for long enough to establish pharmacokinetic parameters. Only the first three samples contained detectable concentrations of isoproterenol. These initial results indicated that detection limits of less than 10 ng/ml were required in order to determine the pharmacokinetics of isoproterenol.

### 3.3. CEEC

Isoproterenol, a catecholamine, is oxidizable at modest potential. Using a carbon fiber electrode, a potential of +0.65 V versus Ag/AgCl is on the limiting current plateau and was chosen for detection. The electrochemical detector must be shielded from the electrophoretic current. This was accomplished using a cast Nafion end-column electrical decoupler [19]. The electrical decoupler is most effective if the electrophoretic current is kept below 50 $\mu$A. The electrophoretic currents of various buffers that were considered for this separation are shown in Table 3 and Table 4. The 250 m*M* sodium acetate buffer at pH 4.75, used with UV detection, produced unacceptably large electrophoretic currents. The electrophoretic current could be decreased, by decreasing the concentration of the run buffer.

Table 4
Electrophoretic current as a function of buffer concentration

| Buffer type | Concentration (m*M*) | Electrophoretic current ($\mu$A) | | |
|---|---|---|---|---|
| | | 30 kV | 20 kV | 10 kV |
| Sodium acetate | 50 | 19 | 12 | 6 |
| Sodium acetate | 100 | 40 | 23 | 11 |
| Sodium acetate | 125 | 61 | 33 | 15 |
| Sodium acetate | 250 | 154 | 66 | 28 |
| Sodium acetate | 300 | 220 | 80[b] | 32 |
| Sodium acetate | 500 | Offscale[a] | 167[c] | 52 |
| Lithium acetate | 100 | 22 | 14 | 6 |

Conditions: A 65 cm × 50 $\mu$m I.D. uncoated fused-silica capillary was used. Each buffer was prepared at pH = 4.75 and contained 0.1 g/ml of M$\beta$CD.
[a]Maximum measurable current was 300 $\mu$A.
[b]The current increases by 5-$\mu$A during a 25-min analysis, due to Joule heating.
[c]The current increases by 18 $\mu$A during a 25-min analysis, due to Joule heating.

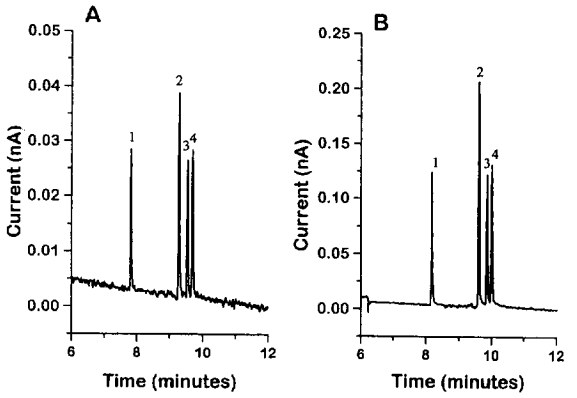


Fig 6. CE–EC electropherograms of standard solutions using normal electrokinetic injection (A) and acid stacked electrokinetic injection (B). Peaks: 1=DHBA; 2=5NMHT; 3=(−)-isoproterenol (ISP); 4=(+)-ISP.

Decreasing the buffer concentration to 100 m*M* still provided baseline resolution of the enantiomers of isoproterenol but resulted in a decrease in the electrophoretic current to 40 $\mu$A. The electrophoretic current was further decreased to 22 $\mu$A, without changing the separation, by switching to a lithium acetate buffer. The lower current is a result of the lower mobility of lithium ions relative to sodium ions. EDTA was added to the run buffer, to complex metals which can result in noise with the electrochemical detector. With these minor changes, the system was compatible with electrochemical detection. The final CEEC run buffer consisted of 100 m*M* lithium acetate, pH 4.75, with 0.5 m*M* EDTA and 0.1 g/ml M$\beta$CD. Fig. 6A shows a typical electropherogram of racemic isoproterenol under these conditions. With a 3-s electrokinetic injection, the response was linear from 2.5 ng/ml to 12.7 $\mu$g/ml (slope = 0.46 pA/mg/l, intercept = 1.2 pA, $r$ = 0.999), with a detection limit of 2.5 ng/ml at a *S/N* of 3.

### 3.4. pH-mediated peak stacking

The ionic strength of the sample matrix relative to the CE run buffer affects the separation efficiency and the maximum volume that can be injected. For CEEC, the maximum run buffer ionic strength is limited by the acceptable electrophoretic current. Therefore, only changes in the sample ionic strength are practical. As this method is intended for use with microdialysis sampling, the sample is initially a Ringer's solution with an ionic strength of 168 m*M*. As seen in Table 5, dilution of this sample in order to reduce the ionic strength results in improved separation efficiency. However, the gain in sensitivity from improved peak shape is more than offset by loss due to dilution. Therefore, another approach was necessary to improve sensitivity.

Peak stacking has been accomplished by injecting the sample in a solution that is much lower in ionic strength than the run buffer. The limitation of having the sample in Ringer's solution precluded this type of peak stacking. However, by injecting a plug of acidic solution directly after the sample in Ringer's solution, the separation efficiency was dramatically improved (Table 6). The observed peak stacking may be due to titration of the run buffer's anions in the highly acidic region. The high mobility of protons in an electric field will cause this low pH

Table 5
Dependence of chiral resolution, separation efficiency and sensitivity on the ionic strength of the sample matrix with normal electrokinetic injection

| Dilution factor of Ringer's matrix[a] | Ionic strength of sample (m*M*) | Resolution | Separation efficiency[b] (*N*/1000) | Absolute sensitivity[b] (pA/n*M*) | Overall sensitivity[c] (pA/n*M*) |
|---|---|---|---|---|---|
| 4:5 | 159 | 1.47 | 211 | 0.0650 | 0.0520 |
| 1:2 | 108 | 1.52 | 250 | 0.0863 | 0.0431 |
| 1:3 | 80 | 1.88 | 375 | 0.0952 | 0.0317 |
| 1:4 | 66 | 2.04 | 373 | 0.0952 | 0.0238 |
| 1:10 | 41 | 2.19 | 395 | 0.132 | 0.0132 |

[a]Each solution was made so that the final concentration of the stabilizer was 8.0 m*M* $Na_2EDTA$ and 97 $\mu M$ $NaHSO_3$.
[b]Separation efficiency and sensitivity were calculated from the peaks of (−)-isoproterenol.
[c]Overall sensitivity = absolute sensitivity × dilution factor.

Table 6
Dependence of chiral resolution, separation efficiency and sensitivity on the ionic strength of the sample matrix with acid sample stacking

| Dilution factor of Ringer's matrix[a] | Ionic strength of the sample matrix (m*M*) | Resolution | Separation efficiency[b] (*N*/1000) | Absolute sensitivity[b] (pA/n*M*) | Overall sensitivity[c] (pA/n*M*) |
|---|---|---|---|---|---|
| 4:5 | 159 | 1.96 | 395 | 0.342 | 0.274 |
| 1:2 | 108 | 1.88 | 364 | 0.365 | 0.183 |
| 1:3 | 80 | 1.86 | 341 | 0.389 | 0.128 |
| 1:4 | 66 | 1.80 | 317 | 0.448 | 0.112 |
| 1:10 | 41 | 1.79 | 301 | 0.535 | 0.0535 |

[a]Each solution was made so that the final concentration of the stabilizer was 8.0 m*M* $Na_2EDTA$ and 97 $\mu M$ $NaHSO_3$.
[b]Separation efficiency and sensitivity were calculated from the peaks of (−)-isoproterenol.
[c]Overall sensitivity = absolute sensitivity × dilution factor.

region to migrate through the sample zone, effectively lowering the ionic strength in this region. Although the precise mechanism of this pH-mediated peak stacking is not known, the loss in efficiency due to the higher ionic strength of the sample relative to the run buffer was overcome using this approach. In fact, pH-mediated peak stacking proved to be more effective when the ionic strength of the sample was higher. Using pH-mediated peak stacking, the injection time could be increased from 3 to 15 s, without loss of separation efficiency or chiral resolution. This results in a five-fold increase in sensitivity using acid peak stacking and the elimination of sample ionic strength effects (Fig. 6B).

### 3.5. Optimized CE method

Isoproterenol is easily oxidized, therefore, stability of the samples is of concern. In order to stabilize the samples, the dialysate was diluted one to three with a stabilizer solution containing EDTA and bisulfite. Stability of the migration time has been a concern with CE analysis. An internal standard was included in the stabilizer solution, in order to compensate for migration time and detector response drift. 3,4-Dihydroxybenzylamine (DHBA) was found to be a good internal standard, being a catecholamine with similar electrochemistry to ISP, yet having a sufficiently different migration time so that it does not interfere. Using DHBA as an internal standard, the precision of analysis increased from 3.2% R.S.D. to 1.4%.

The final optimized method was to dilute the microdialysis sample one to three with an EDTA–bisulfite stabilizer solution, containing 0.8 m*M* DHBA. This sample was electrokinetically injected for 15 s at 18 kV followed by acid peak stacking, by injection of 0.1 *M* HCl for 20 s at 18 kV. The buffer consisted of 100 m*M* lithium acetate, pH 4.75, with 0.5 m*M* EDTA and 0.1 g/ml M$\beta$CD. Under these conditions, a detection limit of 0.6 ng/ml of isoproterenol in the dialysis sample prior to dilution with the stabilizer solution was achieved ($S/N$ = 3). This is almost four orders of magnitude lower than could be achieved using UV detection. The response was linear up to 1.1 $\mu$g/ml, using pH-mediated peak stacking. The linear range could be extended to 12.7 $\mu$g/ml by using normal electrokinetic injection without pH-mediated peak stacking. The linear range and detection limit are sufficient to follow the elimination of isoproterenol in microdialysis samples following an i.v. dose in the therapeutic range.

### 3.6. Analysis of microdialysis samples

The optimized chiral CEEC method was applied to the analysis of intravenous microdialysis samples collected following administration of racemic isoproterenol. Typical electropherograms of microdialysis samples collected prior to, 6 min and 54 min after dosing with racemic isoproterenol are shown in Fig. 7. The enantiomers of isoproterenol are resolved from each other and all endogenous compounds. The improved detection limits of the CEEC system, coupled to the chiral resolution provided by the cyclodextrin, allow for the monitoring of the individual enantiomers of isoproterenol in microdialysis samples collected for over 6 half-

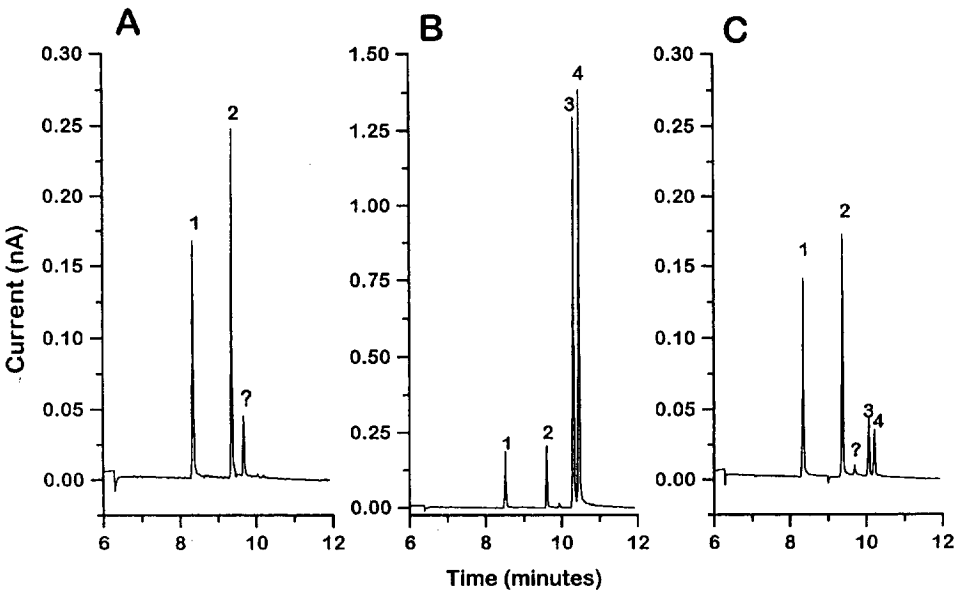


Fig. 7. Typical CE–EC electropherograms from a pharmacokinetic experiment. (A) blank sample acquired prior to administering an ISP dose; (B) microdialysate acquired 6 min after dosing and (C) microdialysate acquired 54 min after dosing. Peak identities as in Fig. 6.

lives. This is sufficient to establish the pharmacokinetics of each enantiomer.

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