# Separation of chiral drugs with β-CD phosphate by capillary electrophoresis

Xue Qin REN[1], Xiao Dan SU[1], Ai Jin HUANG[1], Yi Liang SUN[1]*, Zeng Pei SUN[2]

[1] Department of Chemistry, Peking University, Beijing 100871
[2] National Institute for the Control of Pharmaceutical and Biological Products, Beijing 100050

**Abstract:** β-Cyclodextrin phosphate (β-CD-phosphate) was used as a selector for separating chiral drugs by capillary electrophoresis (CE). A solution comprising of 120 mmol/L Britton-Robinson buffer (BRB) containing 10 mmol/L β-CD phosphate with the pH adjusted to 7.0 was used as the background electrolyte (BGE), and a small amount of analyte was injected (600v/1s). Triethylamine, diethylamine, triethanolamine, diethanolamine, Tris added as modifier were compared. Isoprenaline, methoxamine, oxprenolol, practolol were successfully resolved.

**Keywords**: Capillary electrophoresis, chiral separation, β-cyclodextrin-phosphate, isoprenaline, methoxamine, oxprenolol, practolol.

In capillary electrophoresis (CE) chiral separation is accomplished by adding suitable selector in the running electrolyte[1,2]. The type of selector is of primary importance for achieving successful resolution. Selector concentration has considerable influence as well[3,4]. Charged cyclodextrins were first introduced for chiral separation of amino acids[5] and used for enantioseparation of drugs by Terabe group[6]. Many kinds of charged CDs are now commercially available[7-11]. The charged CD commonly used nowadays are carboxymethyl-β-CD (CM-β-CD), β-CD-phosphate, γ-CD-phosphate, sulfobutylether-β-cyclodextrin (SBE-β-CD). Compared with neutral CDs the charged CDs have better solubility in aqueous solution and wider pH range for use. β-CD phosphate is suitable to be used in pH above 2.0 and has been employed recently for enantioseparation of chiral drugs[12,13]. In this report four chiral drugs previously not reported for β-CD phosphate were separated. The use of amines in the background electrolyte (BGE) as modifier was also examined.

## Experimental

*1 Apparatus and CE conditions*

Experiments were carried out at ambient temperature on a laboratory-assembled CE system. A non-coated fused silica capillary of 60 cm length (effective length 40cm) with 50μm I.D. × 375 μm O.D. (Yongnian Optical Fiber Factory, Hebei, China) was used as a separation channel. A laboratory-made high-voltage power supply that can provide voltage from 0 to 30 kV was used to drive the separation. On-column detection was

performed at cathode on a CV$^4$ UV detector (ISCO, Lincoln, NE, U.S.A.) at a wavelength of 214 nm with a rise time of 0.8s. Electropherograms were taken on a SE120 recorder (ABB Goerz Instruments, Vienna, Austria) with a TL-9302 workstation (Taili Corporation, Beijing, China). A small fan was used to dissipate the Joule heating generated by power. Injection was performed under pressure mode at anode at ambient temperature. 15 kV was used as the applied voltage. Before each run 1mol/L NaOH was used to rinse the fresh column overnight, followed with re-distilled water and the BGE solution successively for 30 min each.

*2. Reagents and materials*

All the chiral drugs, including atenolol, bepridil, bencynonate, benzhexol, esmolol, isoprenaline, lobeline, methoxamine, methylphenidate, norpseudoephedrine, oxprenolol, practolol, prenylamine, propranolol, ondansetron, synephrine, terazosin, timolol, were provided by National Institute for the Control of Pharmaceuticals and Biological Products (Beijing, China). β-CD phosphate was purchased from Cyclolab Co. (Budapest, Hungary) with a concentration of 10 mmol/L and DS (degree of substitution) 6-10. All other chemicals were of analytical grade. The Britton-Robinson buffer (BRB), comprising of a mixture of acetic acid, phosphoric acid and boric acid, 40 mmol/L for each, was adjusted to various pH values with 5 mol/L NaOH$^3$ or the additive amines mentioned below. The drug stock solutions were prepared at 1.0 mg/ml concentration and diluted with the buffer prior to use. All the solutions were filtered through a 0.45$\mu$m syringe type cellulose acetate membrane filter and sonicated for 20 minutes before use.

*3. Calculation*

For resolution $R_s$ >1.5 the following equation is used for its calculation:

$$R_s = 1.18\times (t_2 - t_1) / (w_{1/2\,(1)} + w_{1/2(2)}) \qquad (1)$$

where $t_1$ and $t_2$ are the migration times (min) of the two enantiomers, $w_{1/2\,(1)}$ and $w_{1/2(2)}$ the peak widths at their half heights (min), respectively. For resolution <1.5, resolution is estimated from the depth of valley in the electrorpherogram according to reference[14].

**Results and Discussion**

*1 Preliminary screening*

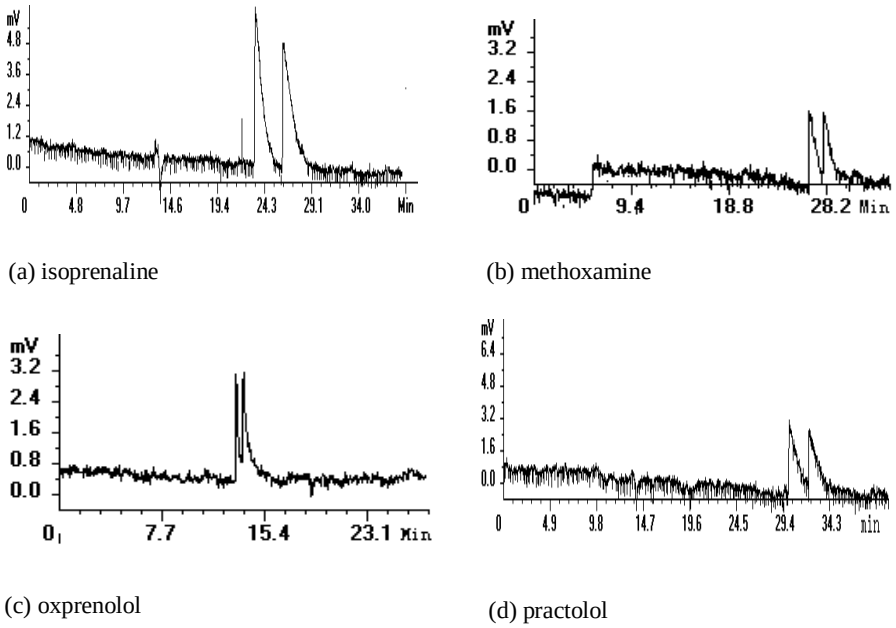
Preliminary screening tests were performed for chiral drugs with BGE comprising of BRB (40 mmol/Lphosphoric acid, 40 mmol/Lactic acid and 40 mmol/Lboric acid) with its pH adjusted to 7.0 with NaOH and anode injection in a small amount (600v/1s). Isoprenaline, methoxamine, oxprenolol, practolol showed signs of separation ( Rs>0.6 ) and further endeavors were attempted to improve resolution.

*2 Effect of additive on resolution*

It is well known that besides selector species, injection amount, concentration of BGE,

pH value, organic modifier exert more or less influence on resolution. In the present case the effect of organic modifier on resolution was examined. Under favorable experimental conditions [small injection amount (600v/1s) at pH 7.0, sufficiently high buffer concentration], column length (from 20cm to 40cm) showed no obvious influence on resolution. The effect of additive on resolution was examined. Five organic modifiers, *i.e.*, diethylamine, triethylamine, diethanolamine, triethanolamine and Tris were used as additive and compared. From **Table 1** it can be seen that the effect of additive is rather unpredictable, no general rule can be followed for selecting an additive for a particular drug. The addition of triethanolamine and Tris into BGE proved to be favorable in improving the resolution of isoprenaline and practolol, respectively.

**Figure 1** Separation of chiral drugs by β-CD phosphate with and without modifier




(a) isoprenaline (b) methoxamine

(c) oxprenolol (d) practolol

(a) triethanolamine; (b)(c) without additive; (d) Tris.

Condition: running voltage, 15 kV; injection, electromigration mode, 600V/1s; wavelength, 214 nm; column, fused non-coated silica, total length, 60 cm; effective length, 40 cm; temperature, ambient; BGE, BRB at pH7.0 with 10 mmol/L β-CD phosphate.

## Conclusion

β-CD phosphate was used as a selector for separating four chiral drugs, isoprenaline, methoxamine, oxprenolol, practolol with success. Triethanolamine and Tris were added as organic modifier to enhance the resolution of isoprenaline and practolol, respectively.

**Table 1** Separation of four drugs with and without modifiers

| Drugs | Isoprenaline | Methoxamine | Oxprenolol | Practolol |
|---|---|---|---|---|
| No modifier | * | 1.1 | 1.2 | 0.7 |
| | 37.40/30.87 | 28.02/26.62 | 13.83/13.32 | 28.88/27.98 |
| Triethylamine | 2.2 | 0.6 | 1.0 | 1.0 |
| | 25.96/27.25 | 16.20/15.95 | 12.13/11.68 | 25.58/24.35 |
| Diethylamine | 1.7 | 0.9 | 1.0 | 0.8 |
| | 17.38/15.13 | 17.53/16.82 | 11.65/11.18 | 26.88/25.53 |
| Triethanolamine | 3.0 | 0.8 | no peak | 0.9 |
| | 33.52/28.07 | 16.02/15.35 | | 26.17/24.75 |
| Diethanolamine | 2.3 | 0.7 | 0.9 | 1.0 |
| | 26.20/23.30 | 17.48/16.75 | 12.30/11.70 | 12.25/11.78 |
| Tris | 1.5 | 0.8 | 1.0 | 1.1 |
| | 22.60/20.65 | 18.67/17.70 | 13.13/12.45 | 32.18/30.08 |

* The first eluting peak did not return back to baseline.
The upper value denotes the resolution while the lower the migration time.
The experimental conditions are the same as in Figure 1

## Acknowledgment

The financial support of this work by the Doctoral Program Foundation of the National Ministry of Education of China is gratefully acknowledged.

## References

1. S. Fanali, J. *Chromatogr.A*, **1997,** *792*, 227.
2. B. Chankvetadze, G. Endresz, G. Blaschke, *J. Chromatogr. A*, **1995**,*700*, 43.
3. S.A.C. Wren, R.C. Rowe, *J. Chromatogr. A*, **1992**, ***603***, 235.
4. S.A.C. Wren, R.C. Rowe, *J. Chromatogr. A*, **1992**, *635*, 113.
5. S. Terabe, H. Ozaki, K. Otsuka, T. Ando, *J. Chromatogr.A*, **1985**, *332*, 211.
6. Y. Tanaka, M. Yanagawa, S. Terabe, *J. HRC*, **1996,** *19*, 421.
7. K. Ishibuchi, S. Izumoto, H. Nishi, T. Sato, *Electrophoresis*, **1997,** *18*, 1007.
8. S. Fanali, C. Desiderio, Z. Aturki, *J. Chromatogr. A*, **1997,** *772*, 185.
9. Y. Tanaka, S. Terabe, *J. Chromatogr. A*, **1997,** *781*, 151.
10. J.B. Vincent, A.D. Sokolowski, T.V. Nguyen, G. Vigh, *Anal. Chem.*, **1997**, ***69***, 4226.
11. G. Schulte, B. Chankvetadze, G. Blaschke, *J. Chromatogr. A*, **1997**, *771*, 259.
12. F. Wang, M.G. Khaledi, *Anal. Chem*., **1996,** *68*, 3460.
13. K.H. Gahm, L.W. Chang, D.W.Armstrong, *J. Chromatogr. A*, **1997**, *759*, 149.
14. L.R. Snyder, J.J. Kirkland, "*Introduction to Modern Liquid chromatography*", 2nd Ed. John `Wiley & Sons, **1979**, p.38.

Received 13 September 1999