ELSEVIER

Journal of Chromatography A, 680 (1994) 157–162

JOURNAL OF CHROMATOGRAPHY A

Practical aspects of chiral separations of pharmaceuticals by capillary electrophoresis
I. Separation optimization

Andras Guttman*, Nelson Cooke

Beckman Instruments, Inc., 2500 Harbor Boulevard, Fullerton, CA 92634, USA

Abstract

Capillary electrophoresis employing chiral selectors has been shown to be a useful analytical method to separate enantiomers. In our model system hydroxypropyl-β-cyclodextrin was used as chiral selector for the separation of racemic propranolol. Results are presented regarding the effect of different operational variables such as buffer pH, concentration of chiral selector, applied electric field and temperature on the chiral separation. Based on the experimental data, the operational variables were optimized to attain maximum resolving power with minimal analysis time.

1. Introduction

Conventionally, instrumental chiral separations have been achieved by gas chromatography (GC) [1] and by high-performance liquid chromatography (HPLC) [2,3]. In recent years, there has been considerable activity in the separation and characterization of racemic pharmaceuticals by high-performance capillary electrophoresis (HPCE) with particular interest paid to using this technique in modern pharmaceutical analytical laboratories [4–9]. HPCE represents an instrumental approach to electrophoresis with the advantages of fast analysis time, automation, on-column injection and detection. The method is similar to HPLC in that analytes are detected as they pass through the detection window, allowing for quantitation [10]. The great resolving power of this method is based on the high separation efficiency, *i.e.* high theoretical plate counts inherent with CE. It is important to note here, that CE-based chiral separation methods were just recently accepted in inter-company cross-validation reported by Altria *et al.* [11].

As in GC [1,12] and HPLC [2,3], the versatility of HPCE can be extended via incorporation of chiral selectivity into the electromigration process. It has previously been shown that native and derivatized cyclodextrins can be successfully employed in chiral separations using isotachophoresis [13–16], capillary zone electrophoresis [17–20], micellar electrokinetic chromatography [21,22] and capillary gel electrophoresis [23,24]. Cyclodextrins (CDs) are non-ionic cyclic polysaccharides containing glucose units shaped like a toroid or hollow truncated cone. The cavity is relatively hydrophobic while the external faces are hydrophilic, with the edge of the torus of the larger circumference containing secondary hydroxyl groups connected to the chiral carbons

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(94)00283-F

[25]. These secondary hydroxyl groups can be derivatized in order to increase the size of the cavity or the solubility of the cyclodextrin, *e.g.*, via permethylation, hydroxypropylation.

In the chiral recognition process it is believed that the non-polar portion of the solute molecule *i.e.*, the naphthyl group in the propranolol, distributes inside the cavity of the cyclodextrin and form hydrophobic interactions with the inner hydrophobic moiety. The hydroxyl and amino groups of the propranolol form hydrogen bonds with the hydroxyl groups at the rim of the toroid. Enantioselective recognition arises from these hydrogen bonds at the entrance of the cavity with the chiral glucose moiety, while the complex is stabilized by the host–guest complexation in the cyclodextrin cavity [23].

According to the theory of Rawjee and Vigh [26], when gels or polymer networks are used in CE of enantiomers using CDs as chiral selectors, better separation can be achieved when only the dissociated form of the solute (Type II) or both the dissociated and non-dissociated form of the solute (Type III) complex selectively with the chiral selector. To take advantage of this phenomenon a non-cross-linked hydrophilic polymer network was used in our experiments as a buffer additive using hydroxypropyl-β-cyclodextrin (HP-β-CD) as chiral selector for the separation of racemic propranolol (Type III with HP-β-CD). The use of a polymer network is also advantageous in that suppresses the ζ potential of the inner capillary wall, thus reducing the electroosmotic flow. In this way, the tendency of bulk electroosmotic flow to decrease electrophoretic mobility differences can be minimized [27]. In chiral separations with very low selectivity values this can be an important factor in achieving better enantiomeric separation.

The research described in this report details the effects of the different operational variables such as separation buffer pH, chiral selector concentration, applied electric field strength and separation temperature on the resolution of racemic propranolol. Based on our results, we propose a new optimization scheme that involves changing the electrophoretic operating variables systematically to attain the best available enantiomeric separation in CE of racemic molecules.

2. Materials and methods

2.1. Apparatus

All of the experiments on P/ACE System 2210 capillary electrophoresis apparatus (Beckman Instruments, Fullerton, CA, USA) were performed with the anode on the injection side and the cathode on the detection side. Capillary columns of 25 μm I.D. (Polymicro Technologies, Phoenix, AZ, USA) were used in these experiments in order to achieve the highest available efficiency, thus, good resolution due to the small injected amount of the dilute sample. Capillaries with 20 cm effective length (27 cm total length) were used in the experiments (Beckman). The separations were monitored on column at 230 nm wavelength. The temperature of the coolant liquid in the P/ACE instrument was controlled from 20–50°C to $\pm$ 0.1°C. The samples were injected by the pressure injection mode of the system, typically for 5 s at 0.5 p.s.i. (1 p.s.i. = 6894.76 Pa). The electropherograms were acquired and stored on an IBM 486/66 computer using the System Gold software package (Beckman).

2.2. Chemicals

The racemic (*R*,*S*) propranolol (pK = 9.47) (Sigma, St. Louis, MO, USA) was dissolved in deionized water at a concentration of 10 μg/ml. Ultrapure-grade ϵ-aminocaproic acid, 2-(N-morpholino)ethanesulfonic acid (MES), 3-[N-tris-(hydroxymethyl)methylamino] - 2 - hydroxypropanesulfonic acid (TAPSO), 3-[(1,1-dimethyl-2-hydroxyethyl)amino] - 2 - hydroxypropanesulfonic acid (AMPSO), methanesulfonic acid and tetrabutylammonium hydroxyde (TBAH) were used in the experiments (ICN, Costa Mesa, CA, USA). Buffers were prepared of ϵ-aminocaproic acid, and MES, TAPSO AMPSO adjusted to the proper pH of 3 or 4 by methanesulfonic acid and pH 5, 6, 7, 8 or 9 by TBAH, respectively. HP-β-CD with an average substitution rate of 4.9, was purchased from American Maize Products, Hammond, IN, USA. All buffer solutions contained 0.4% hydrophilic polymeric additive

and were filtered through a 0.8-μm pore size filter (Schleicher & Schuell, Keene, NH, USA) and carefully vacuum degassed at 100 mbar before use.

3. Results and discussion

Rawjee and Vigh's three-dimensional peak resolution model [26] has been put into practice in a simple optimization scheme, where the effects of the major operation parameters; pH, chiral selector concentration, applied electric field strength and separation temperature were consecutively studied in capillary gel electrophoresis. According to this theory, when performing chiral separation of enantiomeric drugs containing one asymmetric center and having only one charged functional group in the molecule (acidic or basic [28,29]), separation buffer pH and the chiral selector concentration are the two most important parameters defining chiral selectivity. Using *R,S*-propranolol as a test compound, the effect of the running buffer pH was first studied on the enantiomeric separation. All the other separation variables were maintained at the starting level of 15 m*M* HP-β-CD, 700 V/cm, applied electric field strength and 20°C running temperature, over the range of pH optimization experiments. Thus, pH buffers containing 15 m*M* HP-β-CD were prepared as described in section 2. In this way, gel-buffer solutions with pH ranging from 3 to 9 were formulated. In order to get the closest mobility match between the solute ions and the buffering and co-ions, buffer pH values were adjusted by using TBAH and methanesulfonic acid [30]. As seen in Fig. 1, the resolution shows a maximum at pH 7.0 for the racemic mixture of propranolol ($R_s = 1.7$), using the starting conditions were given above. As Fig. 1 shows, care needs to be taken in adjusting the pH of the separation buffer solution around the pH optimum of 7.0 since even a minor shift to lower or higher pH values can cause significant differences in the resolution of the enantiomers. The larger decrease in resolution that occurs below pH 6 and above pH 8 might be due to the lack of chiral selectivity at those pH values. It is important to note that

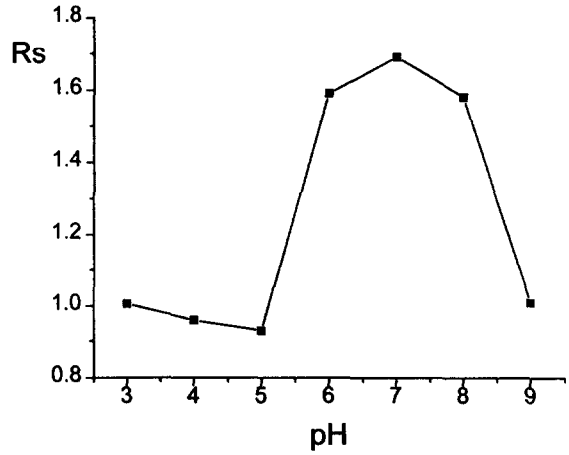

Fig. 1. Separation of *R* and *S* propranolol as a function of the pH ranging from 3 to 9. Conditions: column length: 20 cm (effective, 27 cm total); gel–buffer: 200 m*M* ϵ-aminocaproic acid–methanesulfonic acid (pH 3, 4) and MES–, TAPSO– and AMPSO–tetrabutylammonium hydroxide (pH 5, 6, 7, 8, 9) with 15 m*M* HP-β-CD, containing 0.4% hydrophilic polymeric additive; field strength: 700 V/cm; temperature: 20°C; detection: UV 230 nm.

when phosphate buffers are used significantly lower resolution values ($R_s = 0.8$–1.0) were attained in the pH 6–8 range. This lower resolution might be caused by a drop in efficiency due to the mobility mismatch between the solute and the higher mobility running buffer ions and the competition between the HPO_4^{2-} ions and the solute molecules for binding to the chiral selector [30].

The effect of the concentration of the chiral selector on enantiomeric resolution was investigated next, keeping the pH of the running buffer at the previously defined optimal value of pH 7.0 and maintaining all the other separation parameters constant as described above. The migration times increase with rising HP-β-CD concentration due to two simultaneously occurring phenomena. One is that the solute resides longer in the complex, so it moves slower than the free solute because of its increased mass-to-charge ratio. The other is that the viscosity of the buffer increases with the elevating HP-β-CD concentration [5] which decreases mobility of the solute. Fig. 2 shows the relationship between the

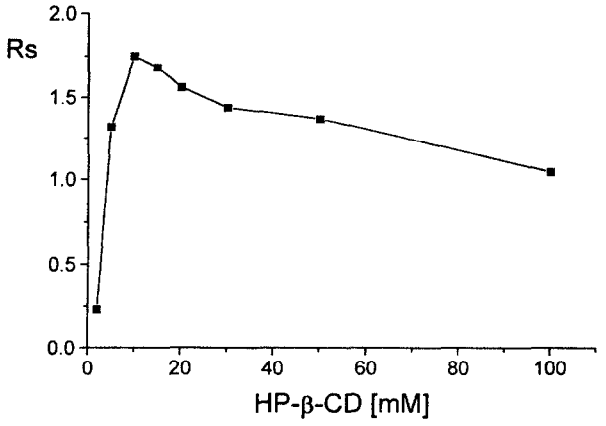

Fig. 2. Relationship between the chiral selector concentration and the resolution of the propranolol enantiomers. Conditions as in Fig. 1, with pH 7.0 gel–buffer and HP-β-CD concentration 2.5–100 m*M*.

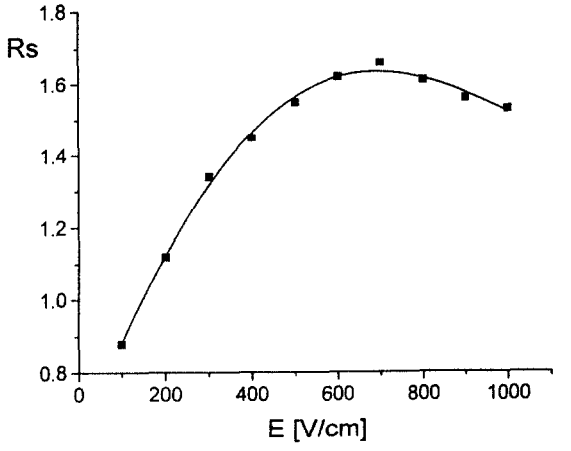

Fig. 3. Effect of the applied field strength on the resolution of propranolol enantiomers. Conditions as in Fig. 2, but HP-β-CD concentration is 10 m*M*; applied electric field 100–1000 V/cm.

resolution and the HP-β-CD concentration in the CE separation of *R* and *S* propranolol. As Fig. 2 shows, there is an optimum in the HP-β-CD concentration at 10 m*M* in the separation of the racemic propranolol. This is the point where a further increase in the chiral selector concentration leads to a resolution decrease. The same effect was found earlier by Wren and Rowe [5] using permethylated β-CDs for the separation of several important β-blockers.

The resolution drop caused by the mobility mismatch and competition of buffer components for the chiral selector is not considered here and in the remaining optimization experiments since the running buffer concentration and pH were maintained at the same level.

Considering the two optimum values found above for the running buffer pH (pH 7.0) and the chiral selector concentration (10 m*M* HP-β-CD), the effect of the applied electric field on the chiral resolving power was studied next (Fig. 3). The plot in Fig. 3 follows the theoretical resolution versus square root electric field strength (E) relationship in capillary electrophoresis, reported earlier by Karger *et al.* [10]. Note that resolution starts to decline beyond 700 V/cm, where the excessive Joule heat probably cannot be removed efficiently from the capillary column. This curve suggests the use of a maximum of 700 V/cm field strength for the separation of this particular solute and buffer composition. The resulting migration time is still very short, less than 5 min.

Fig. 4 shows the effect of the separation temperature on the resolution of the propranolol enantiomers, maintaining all the other running variables at the previously recognized optimal levels, *i.e.*, pH 7.0, 10 m*M* HP-β-CD. However, at elevated temperatures with the use of 700 V/cm applied electric field strength in these

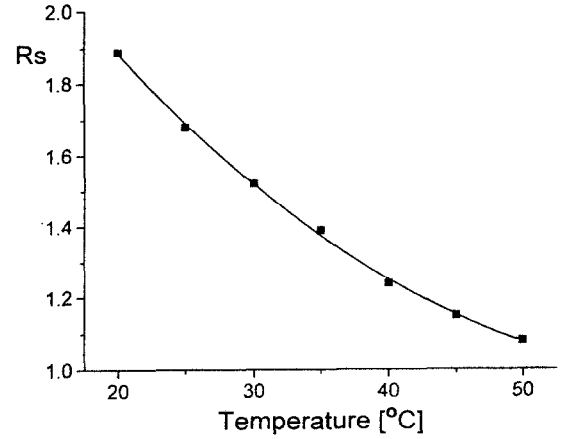

Fig. 4. Relationship between the separation temperature and the resolution of the propranolol enantiomers. Conditions as in Fig. 3, but electric field strength 700 V/cm, temperature 20–50°C.

experiments, one should take into account the running buffer conductivity increase resulting in higher current, caused by the elevated temperature, thus increasing the Joule heat is being developed. In Fig. 4, the temperature *versus* resolution plot suggests that with this particular solute the best resolution can be attained at the lowest running temperature used; in this case 20°C. The use of a lower separation temperature is also favorable for the solute–chiral selector complexation [25] and also decreases diffusion. However, further decrease in separation temperature would increase significantly the analysis time. It is important to note here that temperature has quite a remarkable effect on the separation of the propranolol enantiomers. The resolving power drops almost by 50% over the 30°C temperature window while analysis time decreased only by 28%.

4. Conclusions

We describe a general systematic approach to optimize enantiomeric separations in CE which involves pH, chiral selector, field strength and temperature optimization steps. It is shown that using this approach for the separation of propranolol enantiomers, the use of pH 7.0 running buffer with 10 m*M* HP-β-CD concentration, applying 700 V/cm field strength at 20°C were found to be as optimal separation conditions (Fig. 5). Using these parameters resolution of $R_s = 1.75$ was attained in less than 5 min using a 20 cm polymer network-filled capillary column. Extension of this method to other compounds will be reported in future papers.

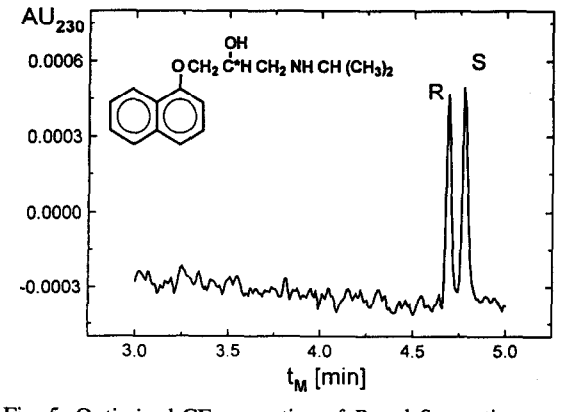

Fig. 5. Optimized CE separation of *R* and *S* enantiomers of propranolol. Conditions: column length: 20 cm (effective); separation gel–buffer: 200 m*M* TAPSO–tetrabutylammonium hydroxide (pH 7.0) with 10 m*M* HP-β-CD, containing 0.4% hydrophilic polymeric additive; electric field strength: 700 V/cm; temperature: 20°C; detection UV 230 nm. t_M = Migration time.

Acknowledgement

The authors wish to acknowledge Professor Gyula Vigh for his stimulating discussions. The authors also thank Ms. Julie Ficke and Dr. Jeff Allen for reviewing the manuscript before submission.

References

[1] E. Smolkova-Keulemansova, *J. Chromatogr.*, 251 (1982) 17.
[2] D. Armstrong, *Anal. Chem.*, 59 (1987) 84A.
[3] S. Ahuja, *Chiral Separations by Liquid Chromatography*, American Chemical Society, Washington, DC, 1991.
[4] K.D. Altria, A.R. Walsh and N.W. Smith, *J. Chromatogr.*, 645 (1993) 193.
[5] A.C. Wren and R.C. Rowe, *J. Chromatogr.*, 635 (1993) 113.
[6] R. Kuhn, F. Stoecklin and F. Erni, *Chromatographia*, 33 (1992) 32.
[7] S. Fanali, *J. Chromatogr.*, 545 (1991) 437.
[8] S. Fanali, *J. Chromatogr.*, 470 (1989) 123.
[9] J. Snopek, I. Jelinek and E. Smolkova-Keulemansova, *J. Chromatogr.*, 609 (1992) 1.
[10] B.L. Karger, A.S. Cohen, A. Guttman, *J. Chromatogr.*, 492 (1989) 585.
[11] K.D. Altria, R.C. Harden, M. Hart, J. Hevizi and P.A. Hailey, *J. Chromatogr.*, 641 (1993) 147.
[12] V. Schurig and H.P. Nowotny, *J. Chromatogr.*, 441 (1988) 155.
[13] I. Jelinek, J. Snopek and E. Smolkova-Keulemansova, *J. Chromatogr.*, 439 (1988) 386.
[14] J. Jelinek, J. Snopek and E. Smolkova-Keulemansove, *J. Chromatogr.*, 438 (1988) 211.
[15] S. Fanali and M. Sinibaldi, *J. Chromatogr.*, 442 (1988) 371.
[16] J. Jelinek, J. Dohnal, J. Snopek and E. Smolkova-Keulemansova, *J. Chromatogr.*, 464 (1989) 139.
[17] S. Terabe, *Trends Anal. Chem.*, 8 (1989) 129.

[18] A. Fanali, *J. Chromatogr.*, 474 (1989) 441.
[19] A.C. Wren and R.C. Rowe, *J. Chromatogr.*, 603 (1992) 235.
[20] J. Snopek, I. Jelinek and E. Smolkova-Keulemansova, *J. Chromatogr.*, 452 (1988) 571.
[21] S. Terabe, H. Ozaki,K. Otsuka and T. Ando, *J. Chromatogr.*, 332 (1985) 211.
[22] H. Nishi and M. Matsuo, *J. Liq. Chromatogr.*, 14 (1991) 973.
[23] A. Guttman, A. Paulus, A. Cohen, N. Grinberg and B.L. Karger, *J. Chromatogr.*, 448 (1988) 41.
[24] I.D. Cruzado and Gy. Vigh, *J. Chromatogr.*, 608 (1992) 421.
[25] J. Szejtli, *Cyclodextrins and their Inclusion Complexes*, Akademia Kiadó, Budapest, 1982.
[26] Y.Y. Rawjee and Gy. Vigh, *Anal. Chem.*, 66 (1994) 416.
[27] M.J. Sepaniak, R.O. Cole and B.K. Clark, *J. Liq. Chromatogr.*, 15 (1992) 1023.
[28] Y.Y. Rawjee D. Staerk and Gy. Vigh, *J. Chromatogr.*, 635 (1993) 291.
[29] Y.Y. Rawjee, R. Williams and Gy. Vigh, *J. Chromatogr. A*, 652 (1993) 233.
[30] Y.Y. Rawjee and Gy. Vigh, *J. Chromatogr. A*, 680 (1994) 599.