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Enantiomer separation by capillary electrophoresis utilizing carboxymethyl derivatives of polysaccharides as chiral selectors

Hiroyuki Nishi *, Yukari Kuwahara

Analytical Chemistry Department, Product Technology Development Laboratory, Tanabe Seiyaku Co. Ltd., 16-89, Kashima 3-chome, Yodogawa-ku, Osaka 532-8505, Japan

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Dedicated to Professor Dr Gottfried Blaschke on the occasion of his 65th birthday.

Abstract

Enantiomer separations of various drugs by capillary electrophoresis (CE) were investigated utilizing carboxymethyl (CM) derivatives of some polysaccharides. Three types of CM-polysaccharides, namely CM-dextran, -amylose and -cellulose were employed as chiral selectors in the CE enantiomer separation. Capability of enantiomer separation by these CM-polysaccharides was compared with that by polysaccharides without CM residues (i.e. native or neutral polysaccharides). Among three selectors employed, CM-dextran and -cellulose showed a relatively wide capability of enantiomer separation. Modification of polysaccharides seems to lead to the enhancement of the capability of enantiomer separation. Degree of substitution greatly affected the capability of enantiomer separation of these polysaccharide derivatives as in the β-cyclodextrins derivatives.

Keywords: Capillary electrophoresis; Enantiomer separation; Dextran; Dextrin; Cellulose; Carboxymethylpolysaccharides

1. Introduction

Capillary electrophoresis (CE) offers many advantages such as high separation efficiency, short analysis time, reduced operating costs, instrumental simplicity etc. compared with other analytical separation methods. One of the most successful application areas of CE is in drug analysis, where the focus is on relatively small synthetic drugs. Various CE methods (modes), including micellar electrokinetic chromatography (MEKC), have been developed and employed for the assay of active ingredients in formulations, purity testing of the main component, ion analysis, enantiomer separations etc. [1–7]. CE methods have been already discussed by the regulatory authorities [8–12]. And the first USP monographs describing CE were published in 1997 [9,10]. A draft general

* Corresponding author. Tel.: +81-6-6300-2658; fax: +81-6-6300-2629.
E-mail address: nishi-h@tanabe.co.jp (H. Nishi).

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chapter on CE has been published in European Pharmacopoeia (EP) Forum [11].

Among various applications, separation and quantitation of enantiomers are key applications in drug analysis. CE enantiomer separation has several attractive features such as simple and fast method development in addition to the above-mentioned advantages. One of the USP monographs mentioned above employs enantioselective assay of active ingredient in formulation using cyclodextrin (CD) as a chiral selector [9]. Various chiral additives such as CDs, polysaccharides, proteins, crown ethers, chiral surfactants etc. have been found to be useful for the CE enantiomer separation.

Polysaccharides are one of the promising chiral additives in the CE enantiomer separation as in the HPLC enantiomer separation, where most of the chiral columns utilize polysaccharide derivatives as a chiral moiety. Many ionic polysaccharides such as heparin [13–15], chondroitin sulfates [16–18], dextran sulfate [19], λ-carrageenan [20] etc. and neutral polysaccharides such as dextran [21,22], dextrin [21–28], laminaran [29], pullulan [29], etc. have been successfully employed for the CE enantiomer separation.

In this paper, we employed three types of carboxymethylated (CM) derivatives of polysaccharides, namely CM-dextran, -amylose and -cellulose, as novel chiral selectors in the CE enantiomer separation. We have already published that dextrin and dextran are useful chiral selectors in the CE enantiomer separation [21]. Capability of enantiomer separation of these three CM-derivatives of polysaccharides for the 12 drugs are compared one another and with those without CM-residues (i.e. native neutral polysaccharides).

Polysaccharides are typically complex mixtures of homologues and isomers which can vary greatly from lot to lot. Therefore, especially in the case of natural (ionic) polysaccharides and derivatized polysaccharides, different selectivity (separation factor: α) in the enantiomer separation may be obtained from 'the same' polysaccharides. On the other hand, most of these polysaccharides are easily obtained commercially and are interesting compounds because some of them are biological components, which may contribute to recognition of chirality of drugs in the body.

2. Experimental

2.1. Materials

CM-derivatives of polysaccharides used were as follows: CM-dextran (two types), -amylose and -cellulose. CM-amylose (sodium salt) was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA) and CM-cellulose (sodium salt) of ultra low viscosity grade was obtained from Fluka (Buchs, Switzerland). These were used without any purification. According to the specifications of Fluka, the degree of substitution (DS) in CM-cellulose is 0.60–0.95. Other data such as average molecular mass and DS for CM-amylose were not available. However, judging from the sodium content (19.2%), almost all hydroxy groups seem to be substituted in CM-amylose (DS; around 3). Average molecular mass of CM-cellulose determined by gel permeation chromatography (GPC) using a TSKgel G4000PWXL column was ca. 40 000. That of CM-amylose seemed to be much lower ($< 10\,000$).

On the other hand, two types (different DS of CM) of CM-dextran (sodium salt) were synthesized in our company from commercially available dextrans (obtained from Pharmacia Biotech, Uppsala, Sweden) whose average molecular mass were around 120 000–150 000, although CM-dextran can be obtained from the commercial sources. DS of CM residue determined by the titration in the two CM-dextrans was 0.5 and 0.9, respectively. Average molecular mass was confirmed by GPC analysis.

Structures of drugs investigated in this study are shown in Fig. 1. Totally, 12 drugs enantiomers were selected from our previous study [21]. Most of these enantiomers have been successfully enantioseparated by simply adding dextrin or dextran as chiral selectors to buffer solutions. Diltiazem, clentiazem, diltiazem derivative, trimetoquinol, sulconazole, timepidium, denopamine were obtained from our laboratory. Other drugs such as laudanosine, laudanosoline,

norlaudanosoline, primaquine and verapamil were purchased from the commercial sources. Active forms of diltiazem (2*S*,3*S*-form), clentiazem (2*S*,3*S*-form), denopamine (*R*-form) and trimetoquinol (*S*-form) were obtained from Tanabe Seiyaku Co. Ltd.

HPLC grade methanol from Katayama Kagaku Kogyo (Osaka, Japan) was used to prepare stock standard solutions. Potassium phosphate, phosphoric acid and all other chemicals were of analytical reagent grade from Katayama Kagaku Kogyo. Purified water by Milli-RO 60 water system (Millipore Japan, Tokyo, Japan) was used to prepare buffer solutions.

2.2. Apparatus

All CE separations were carried out using a Beckman P/ACE system 5510 equipped with a photodiode array detector (Beckman Instruments, Fullerton, CA, USA). Uncoated fused-silica capillary tubes (75 μm ID, effective length 20–40 cm) were purchased from Beckman. The capillary was thermostated at a constant temperature of 20 °C with a liquid coolant. The applied voltage was held constant at 15–25 kV. Detection wavelength was adjusted at 220 nm. Sample introduction was performed by the pressure (0.5 psi, 1 psi = 6894.76 Pa, 2–5 s). The instrument control and data collections were performed with a personal computer (COMPAQ ProLinea 4/33).

Average molecular mass of two CM-dextrans, -cellulose and -amylose was determined by GPC analysis. The GPC column used was G4000PWXL (7.8 mm i.d., 300 mm length, TOSOH, Tokyo, Japan) and the mobile phase was 0.2 M phosphate buffer (pH 6.9). Detection was performed by using a refractive index detector

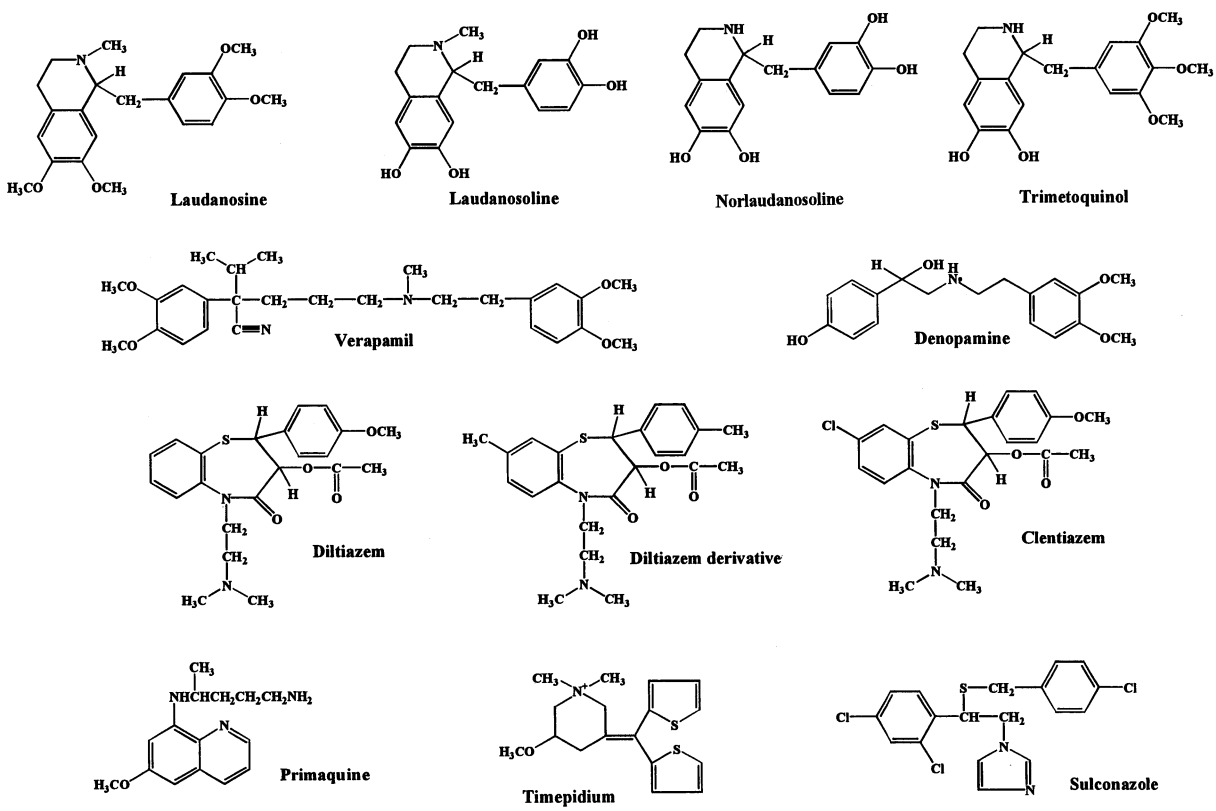

Fig. 1. Chemical structures of drugs investigated.

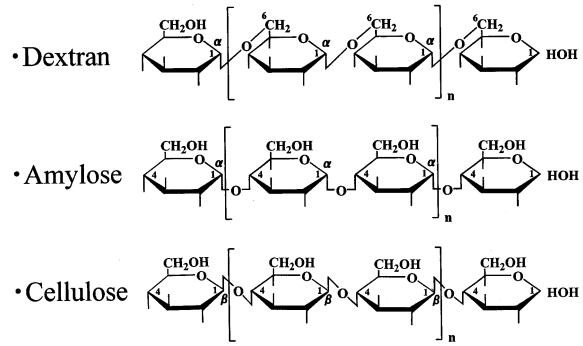

Fig. 2. Unit structures of dextran, amylose (dextrin) and cellulose.

(Shodex RI-71, Showa denko, Tokyo, Japan). A HPLC apparatus for GPC analysis was Shimadzu LC-10A (Shimadzu, Kyoto, Japan).

2.3. Procedure

The buffer solutions of pH 2.5 were prepared by adding a diluted phosphoric acid solution to 20 or 50 mM potassium phosphate solution. Then one of CM-polysaccharides (sodium salt) was added to the buffer solution. These solutions were passed through a membrane filter of 0.45 or 5 μm pore size (Gelman Science Japan, Tokyo, Japan) and degassed by sonnication with a Branson Model B-2200 ultrasonic cleaner (Yamato, Tokyo, Japan) prior to use. At the beginning of each experiment, the capillary was washed with a 0.1 N NaOH solution for 10 min followed by water for 10 min, then the running buffer solution for 20 min. Before each injection, the capillary was washed with the buffer for 1 min. The stock standard solutions for the drug enantiomers were prepared in methanol with an approximate concentration of ca. 1.0 mg/ml. The stock standard solutions were diluted with water to a concentration of ca. 0.1 mg/ml for the CE injection. The sample solutions were injected on the anodic end by the pressure mode of the apparatus. Apparent separation factor (α) for enantiomer separation was calculated by t_2/t_1, where t_1 and t_2 are migration times of the first migrated enantiomer and the secondly migrated enantiomer, respectively. Under the experimental conditions employed (acidic conditions), the velocity of the electroosmotic flow can be neglected compared with that of analytes.

3. Results and discussion

3.1. Enantiomer separation by three CM-polysaccharides

Dextrans are polymers in which the D-glucose units are connected almost extensively by α-(1,6)-linkages. Hydrolysis of starch, which contains amyloses (linear polysaccharides with molecular mass ca. 5×10^5–2×10^6) and amylopectins (non-linear polysaccharides with molecular mass ca. 15×10^6–400×10^6), yields a mixture of dextrins where D-glucose units are connected by α-(1,4)-linkages. These two polysaccharides are soluble in water. On the other hand, cellulose is found in the protective cell walls of plants, particularly in stalks, stems, trunks, and all the woody portions of plant tissues. Cellulose, in which the D-glucose units are connected by β-(1,4)-linkages, is a water-insoluble polysaccharide. Through the introduction of CM residue to cellulose, cellulose can be solubilized in water. Unit structures for these neutral polysaccharides are shown in Fig. 2.

Among these neutral polysaccharides (except cellulose), dextrins showed a wide capability of enantiomer separation, probably due to their helical structures [30]. A full turn in the helix requires at least six glucose units. A schematic illustration is shown in Fig. 3. Among the 12 drugs in Fig. 1, enantiomers of diltiazem, clentiazem, sulconazole, primaquine, verapamil and timepidium were separated by dextrin (3–15% addition), and enantiomers of trimetoquinol, laudanosoline and norlaudanosoline were separated by dextran (3–15% addition) [21] (see, Table 1).

For the strict comparison of the capability of enantiomer separation, the concentration of CM-polysaccharides should be adjusted at the same % or molar concentration. In addition to the concentration, molecular mass, molecular mass distribution, DS of CM residue etc. also must be controlled. However, preparation of solutions at the same concentration (for example, 3%) was

difficult due to the high viscosity. The low concentrations below 3% (i.e. 1 or 2%) were not effective in the enantiomer separation by CE with dextran or dextrin [21]. The practically usable maximum concentrations for each CM-polysaccharide were 1% for CM-amylose, 2% for CM-cellulose and 5% for CM-dextran.

Enantiomer separations by CE with CM-polysacchrides were then investigated by simply adding commercially obtained CM-amylose (1%), -cellulose (2%) or -dextran (3%) of DS 0.5 and the capability of enantiomer separation was compared. Results are summarized in Table 1. Separation of enantiomers of diltiazem by CE with CM-dextran is shown in Fig. 4 together with CE by 10% dextran and by 10% dextrin. Some other separation examples are shown in Fig. 5. Enantiomers of diltiazem, clentiazem and diltiazem

• Dextrin

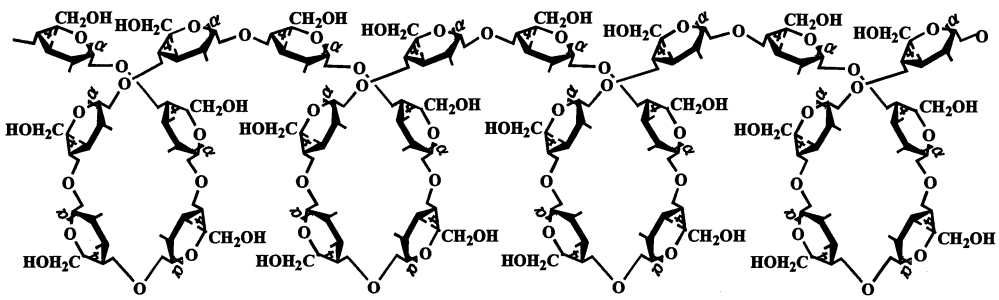

Fig. 3. A schematic illustration of a helical structure in dextrin.

Table 1
Results of enantiomer separations by the CE with three CM derivates of polysaccharides

Analytes	Dextrin[a] 3–15% (25–30 kV)	Dextran[a] 15% (30 kV)	CM-amylose 1% (15 kV)	CM-cellulose 2% (25 kV)	CM-dextran (DS = 0.5) 3% 25 kV
Diltiazem	○	×	○1.01[b] (8.86, 8.95)[c]	○1.02 (7.80, 7.99)	○1.01 (7.08, 7.15)
Clentiazem	○	×	○1.03 (10.05, 10.33)	○1.03 (8.38, 8.67)	○1.01 (7.74, 7.84)
Diltizazem derivative	−	−	○ 1.02 (11.99, 12.24)	○ 1.04 (8.03, 8.33)	○1.01 (7.37, 7.47)
Trimetoquinol	×	○	× (11.10)	○ 1.04 (9.22, 9.60)	○1.01 (7.94, 8.05)
Laudanosine	×	×	× (10.26)	○ 1.01 (7.59, 7.63)	○1.01 (6.62, 6.67)
Laudanosoline	×	○	× (10.64)	○ 1.06 (9.27, 9.78)	○1.01 (7.88, 7.97)
Norlaudanosoline	×	○	× (11.43)	○ 1.10 (10.84, 11.95)	○1.04 (8.63, 8.95)
Sulconazole	○	×	× (10.89)	× (8.67)	N.D.
Timepidium	○	×	× (9.42)	× (6.33)	N.D.
Primaquine	○	×	× (9.46)	× (8.62)	×(7.36)
Verpamil	○	×	× (12.22)	× (8.05)	×(7.04)
Denopamine	−	×	× (11.82)	× (8.46)	N.D.

○, successful; ×, not successful; −, not examined; N.D., not detected.
[a] From Ref. [21].
[b] Separation factor α.
[c] Migration time in min.

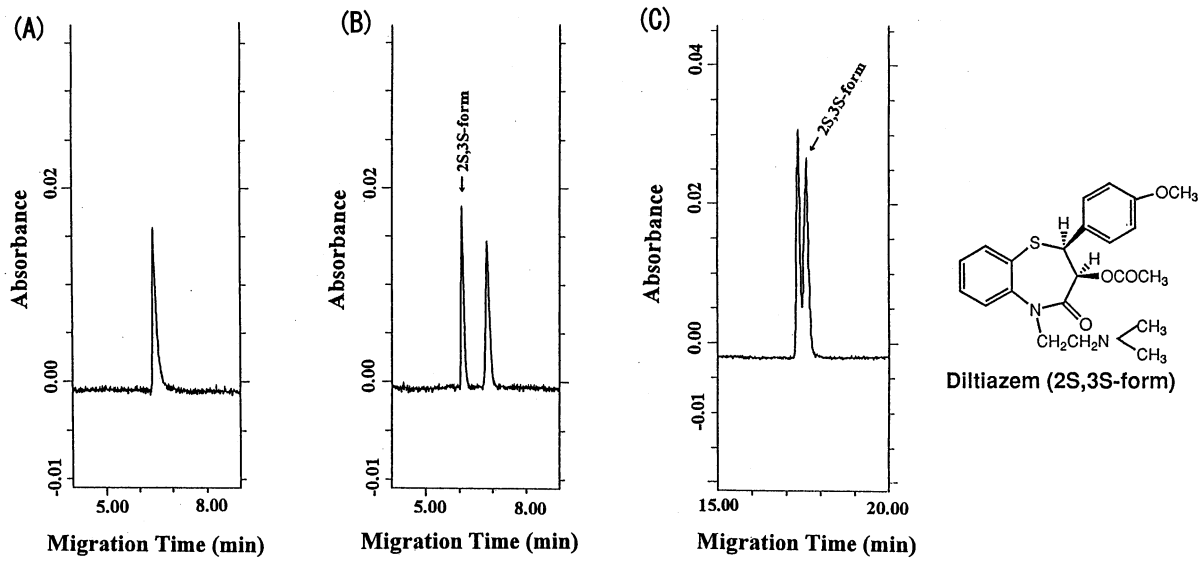

Fig. 4. Separation of enantiomers of diltiazem by CE with: (A) 10% dextran; (B) 10% dextrin; and (C) 5% CM-dextran (DS = 0.5). Buffers, 50 mM phosphate buffer (pH 2.5) for (A) and (B), 20 mM phosphate buffer (pH 2.5) for (C). Capillary, effective length 20 cm and 75 μm I.D. for (A) and (B), effective length 40 cm and 75 μm I.D. for (C). Applied voltage, 25 kV for (A) and (B), 15 kV for (C). Detection was at 220 nm. Temperature, 20 °C.

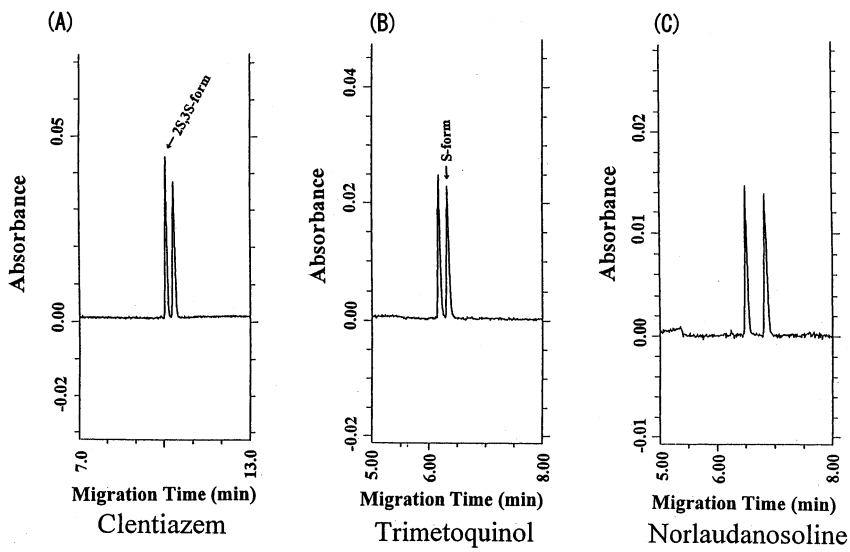

Fig. 5. Enantiomer separations by CE: (A) clentiazem with 1% CM-amylose; (B) trimetoquinol; and (C) norlaudanosoline with 1% CM-cellulose. Buffer, 20 mM phosphate buffer (pH 2.5) containing CM-polysaccharide. Applied voltage, 15 kV for (A), 25 kV for (B) and (C). Capillary effective length, 40 cm, 75 μm I.D. Detection was at 220 nm. Temperature, 20 °C.

derivative, which were not separated by dextran [21], were successfully separated by CE with CM-dextran. Introduction of CM residue enabled enantiomer separations of these analytes. Judging from the results in Table 1, CM-cellulose and -dextran seem to have a relatively wide capability

of enantiomer separation, although these two CM-polysaccharides cannot form a helical structure as in dextrin derivatives. Relatively steric bulky CM residue to the sugar unit probably enhanced the interaction with the analytes, leading to the successful enantiomer separation. The larger migration times of the analytes in CM-dextran, compared with native dextrans, will support this interpretation.

As for the migration order of enantiomers of diltiazem and clentiazem, (2*S*,3*S*)-form migrated faster than the corresponding enantiomer, (2*R*,3*R*)-form, in CM-amylose and -cellulose. These results corresponded to the migration order in the CE enantiomer separation with dextrin [21]. On the other hand, the reverse order was observed in CM-dextran. *R*-form of trimetoquinol migrated faster than *S*-form in CM-dextran and -cellulose as in the CE enantiomer separation with dextran [21].

Table 2
Effect of DS on the enenatioselectivity

Analytes	CM-dextran (3%)	
	DS = 0.5	DS = 0.9
Diltiazem	○ 1.01 (7.08, 7.15)[a]	× (7.60)
Clentiazem	○ 1.01 (7.74, 7.84)	× (10.02)
Diltizazem derivative	○ 1.01 (7.37, 7.47)	× (7.10)
Trimetoquinol	○ 1.01 (7.94, 8.05)	○ 1.02 (13.05, 13.34)
Laudanosine	○ 1.01 (6.62, 6.67)	○ 1.02 (7.69, 7.89)
Laudanosoline	○ 1.01 (7.88, 7.97)	○ 1.03 (13.97, 14.34)
Norlaudanosoline	○ 1.04 (8.63, 8.95)	○ 1.14 (17.85, 20.29)
Sulconazole	N.D.	N.D.
Timepidium	N.D.	× (5.76)
Primaquine	× (7.36)	× (9.24)
Verpamil	× (7.04)	× (7.00)
Denopamine	N.D.	N.D.

○, successful; ×, not successful; N.D., not detected.
[a] Separation factor α (migration time in min).

3.2. Effect of degree of substitution of carboxymethylated residue in CM-dextran

Effect of DS in CM-dextran on the enantioselectivity was investigated by using two CM-dextrans whose DS values were 0.5 and 0.9. Concentration of the selector was fixed at 3%. Results are summarized in Table 2. Enantiomers of diltiazem, clentiazem, and diltiazem derivative, which were all successfully separated by CM-dextran with DS 0.5, were not separated by CM-dextran with DS 0.9.

On the other hand, CM-dextran with DS 0.9 was effective for enantiomer separations of trimetoquinol, laudanosine, laudanosoline and norlaudanosoline, referring to the separation factor (α) values. For these four enantiomeric pairs, a significant increase of the migration time was observed (about two times). This might lead to the improvement of the enantiomer separation. Probably an increase of CM residue in dextran contributed to the enhancement of the interaction between the analytes and CM-dextran through the hydrogen bonding etc.

These results indicate that small change in the DS value (also in the microstructure) affects the enantiomer separation greatly. The same observations have been already reported in the CE enantiomer separation employing methylated β-CDs as chiral selectors [31–33]. Therefore, it is important to control (or to know) the DS value for the reproducible enantiomer separation (recognition) in CE.

As for the position of CM residue in the sugar unit (D-glucose), some reports have been published [34,35]. We tried to investigate the position of CM residue in the CM-dextrans used in this study, by employing the previously reported method [34]. As a result, among three hydroxy groups in D-glucose (2, 3 and 4 position) (see Fig. 2), substitution in 2-position seems to take place preferentially (data not shown).

3.3. Effect of the concentration of CM-dextran

Effect of the concentration of CM-polysaccahrides on the enantioselectivity was investigated by employing CM-dextrans. Results for CM-dex-

Table 3
Effect of the concentration on the enantioselectivity

Analytes	CM-dextran (DS = 0.5)			
	1%	2%	3%	5%
Diltiazem	× (5.49)	○ 1.01 (6.17, 6.21)	○ 1.01 (7.08, 7.15)	○ 1.01 (7.38, 7.43)
Clentiazem	○ 1.01 (5.95, 5.98)[a]	○ 1.01 6.69, 6.77)	○ 1.01 (7.74, 7.84)	○ 1.01 (8.00, 8.09)
Diltizazem derivative	○ 1.01 (5.82, 5.86)	○ 1.01 (6.28, 6.34)	○ 1.01 (7.37, 7.47)	○ 1.01 (7.53, 7.63)
Trimetoquinol	○ 1.01 (5.81, 5.84)	○ 1.01 (6.90, 6.98)	○ 1.01 (7.94, 8.05)	○ 1.02 (7.98, 8.13)
Laudanosine	× (5.28)	○ 1.01 (6.01, 6.04)	○ 1.01 (6.62, 6.67)	○ 1.01 (6.83, 6.90)
Laudanosoline	○ 1.01 (5.58, 5.62)	○ 1.01 (6.88, 6.96)	○ 1.01 (7.88, 7.97)	○ 1.02 (7.99, 8.12)
Norlaudanosoline	○ 1.01 (5.87, 5.97)	○ 1.03 (7.53, 7.76)	○ 1.04 (8.63, 8.95)	○ 1.05 (10.25, 10.79)
Sulconazole	N.D.	N.D.	N.D.	N.D.
Timepidium	N.D.	N.D.	N.D.	N.D.
Primaquine	× (5.48)	× (7.10)	× (7.36)	× (7.90)
Verpamil	× (5.85)	× (6.67)	× (7.04)	× (7.44)
Denopamine	N.D.	N.D.	N.D.	N.D.

○, successful; ×, not successful; N.D, not detected.
[a] Separation factor α (migration time in min).

tran with DS 0.5 are summarized in Table 3. With an increase of the concentration up to 5%, increases of the migration times and the separation factor values were observed as in other polysaccharides [13–19,21]. The same results were obtained for CM-dextran with DS 0.9 (data not shown). The optimum concentratin of CM-dextrans for the enantiomer separation of these analytes seems to be much higher than 5%. However, due to the high viscosity, applicable maximum concentration was 5% for CM-dextrans. On the other hand, in CM-cellulose, separation of enantiomers of trimetoquinol by 1% was better than that by 2% (data not shown).

4. Conclusion

CE is now established as a viable option for the analysis of pharmaceuticals. The CE enantiomer separation is one of the most successful application areas as shown in the previous review papers and books [36–42]. This method can have benefits in terms of method robustness, cost and time. Various chiral selectors including polysaccharides have been employed for the CE enantiomer separation. Although not quite as versatile and powerful as CDs, inexpensive polysaccharides showed remarkable enantioselectivity in some cases.

A preliminary study of commercially available CM-derivatives of polysaccharides (CM-dextran can be obtained from the commercial sources) showed that these derivatives may have potential as chiral selectors in CE. Furthermore, as in CDs, it was found that small modification or introduction of some residues to polysaccharides lead to the change of enantioselectivity greatly.

In the case of polysaccharides as chiral selectors, the ionic character is important. Therefore the choice of pH and the concentration of the selector are very principal for the improvement in selectivity. The optimization of the separations could be achieved by the proper choice of the experimental conditions. Further study on enantiomer separation by CM-polysacchrides under the alkaline conditions (ionic form) is in progress.

References

[1] A.S. Cohen, S. Terabe, Z. Deyl (Eds.), Capillary Electrophoretic Separations of Drugs, Elsevier, Amsterdam, 1996.
[2] K.D. Altria, M.A. Kelly, B.J. Clark, Trends Anal. Chem. 17 (1998) 204–226.
[3] M.F. Miola, M.J. Snoeden, K.D. Altria, J. Pharm. Biomed. Anal. 18 (1998) 785–797.
[4] K.D. Altria, Analysis of Pharmaceuticals by Capillary Electrophoresis, Vieweg, Wiesbaden, Germany, 1998.

[5] M.G. Khaledi (Ed.), High Performance Capillary Electrophoresis, John Wiley & Sons, New York, USA, 1998.
[6] J. Caseavska, W. Thormann (Eds.), Paper Symp., Electrophoresis, vol. 19, 1998, pp. 2695–3051.
[7] H. Nishi, Electrophoresis 20 (1999) 3237–3258.
[8] *Pharm. Forum* 22 (1996) 1727–1735.
[9] *Pharm. Forum* 23 (1997) 3991–3992.
[10] *Pharm. Forum* 23 (1997) 3992–3993.
[11] *Pharmeuropa* 9 (1997) 179–184.
[12] USP-NF, second supplement, <727> Capillary Electrophoresis, 1999, pp. 2896–2900.
[13] A.M. Stalcup, N.M. Agyei, Anal. Chem. 66 (1994) 3054–3059.
[14] N.M. Agyei, K.H. Gahm, A.M. Stalcup, Anal. Chim. Acta 307 (1995) 185–191.
[15] Y. Jin, A.M. Stalcup, Electrophoresis 19 (1998) 2119–2123.
[16] H. Nishi, K. Nakamura, H. Nakai, T. Sato, Anal. Chem. 67 (1995) 2334–2341.
[17] H. Nishi, S. Terabe, J. Chromatogr. Sci. 33 (1995) 698–702.
[18] R. Gotti, V. Cavrini, V. Andrisano, G. Mascellani, J. Chromatogr. A 814 (1998) 205–211.
[19] H. Nishi, K. Nakamura, H. Nakai, T. Sato, S. Terabe, Electrophoresis 15 (1994) 1335–1340.
[20] G. Beck, S. Neau, Chirality 8 (1996) 503–510.
[21] H. Nishi, S. Izumoto, K. Nakamura, H. Nakai, T. Sato, Chromatographia 42 (1996) 617–630.
[22] H. Nishi, J. Chromatogr. A 735 (1996) 345–351.
[23] A. D'Hulst, N. Verbeke, J. Chromatogr. 608 (1992) 275–287.
[24] A. D'Hulst, N. Verbeke, Chirality 6 (1994) 225–229.
[25] M. Stefansson, M. Novotny, J. Am. Chem. Soc. 115 (1993) 11 573–11 580.
[26] H. Soini, M. Stefansson, M.-L. Riekkola, M. Novotny, Anal. Chem. 66 (1994) 3477–3484.
[27] A. D'Hulst, N. Verbeke, J. Chromatogr. A 735 (1996) 283–293.
[28] A. D'Hulst, N. Verbeke, Electrophoresis 15 (1994) 854–863.
[29] B. Chankvetadze, M. Saito, E. Yashima, Y. Okamoto, J. Chromatogr. A 773 (1997) 331–338.
[30] R. Rundle, J. Am. Chem. Soc. 66 (1944) 2116–2120.
[31] K. Otsuka, S. Honda, J. Kato, S. Terabe, K. Kimata, N. Tanaka, J. Pharm. Biomed. Anal. 21 (1999) 75–81.
[32] G. Weseloh, H. Bartsch, W.A. Konig, J. Microcolu. Sep. 7 (1995) 355–363.
[33] M. Yoshinaga, M. Tanaka, J. Chromatogr. A 679 (1994) 359–365.
[34] S. Ukai, A. Honda, K. Nagai, T. Kiho, J. Chromatogr. 513 (1990) 338–343.
[35] T.I. Usmanov, U.G. Karimova, A. Sarymsakow, Vysokomol. Soedin., Ser. A 32 (1990) 1176–1183.
[36] S. Terabe, K. Otsuka, H. Nishi, J. Chromatogr. A 666 (1994) 295–319.
[37] B. Chankvetadze, Capillary Electrophoresis in Chiral Analysis, John Wiley & Sons, New York, USA, 1997.
[38] G. Gubitz, M.G. Schmid, J. Chromatogr. A 792 (1997) 179–225.
[39] S. Fanali, J. Chromatogr. A 792 (1997) 227–267.
[40] K. Verleysen, P. Sandra, Electrophoresis 19 (1998) 2798–2833.
[41] H. Nishi, S. Terabe (Eds.), Applications of Chiral Capillary Electrophoresis, Special vol., J. Chromatogr. A 875 (2000) 3–484.
[42] H. Nishi, J. Chromatogr. A 792 (1997) 327–347.