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Journal of Chromatography A, 962 (2002) 221–231

JOURNAL OF CHROMATOGRAPHY A

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Colominic acid: a novel chiral selector for capillary electrophoresis of basic drugs

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Received 27 December 2001; received in revised form 26 April 2002; accepted 6 May 2002

Abstract

We introduced colominic acid as a new chiral selector for capillary electrophoresis of basic drugs. Use of a low concentration phosphate buffer containing this polysaccharide and a Polybrene/colominic acid double coated capillary allowed excellent separation of the enantiomers of primaquine, chloroquine and tryptophan. Other drugs giving partial enantioseparation include laudanosine and salbutamol. Capillary coating with Polybrene followed by colominic acid eliminated the problems of peak tailing and low reproducibility of migration time in uncoated capillaries. The optimum pH was in the acidic region but varied among drugs. A low capillary temperature of 16 °C and a colominic acid concentration of 9 w/v% are recommended for practical analysis of these drugs. Colominic acid preparations having higher molecular masses gave better enantioseparation, and *N*-acetylneuraminic acid, the component monosaccharide, did not give any enantioseparation.

Keywords: Enantiomer separation; Colominic acid; Basic drugs; Poly(*N*-acetylneuraminic acid)

1. Introduction

Enantioseparation has become an important topic in modern pharmaceutical analysis, because drug enantiomers often exhibit different activities to human and animal bodies. Since enantiomers possess identical chemical and physical (except chiroptical) properties in an achiral environment, their separation requires high-resolution methods. High-performance liquid chromatography (HPLC) has greatly contributed to this subject and a number of excellent systems have been established for various kinds of chiral drugs (review [1]). Recently capillary electrophoresis (CE) also plays an important role in drug chiral analysis (reviews [2,3]), and various kinds of chiral selectors have been developed, including cyclodextrins [4,5] and derivatives [5,6], macrolides [7,8], and many other small molecules [9,10]. Attention has been further extended to macromolecules such as proteins [11–13] and polysaccharides [14,19]. Polysaccharide selectors are interesting, because some members, for example chondroitin sulfates [14] and heparin [14,16], are the constituents of animal tissues and body fluids. High capability in chiral separation has been noticed for not only such polysaccharides of animal source but also many other polysac-

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0021-9673/02/$ – see front matter
PII: S0021-9673(02)00593-9

charides of plant [17] and bacterial [14,15,18] origin and even synthetic polyglycans [14,19].

Among various polysaccharide selectors chondroitin sulfate C is especially important, because it gives a remarkable resolution for enantiomers of basic drugs [14,15,18]. Nishi and coworkers [15] suggested the importance of the sulfate group and the polymer network in chondroitin sulfate C for enantioseparation of basic drugs. We also attempted elucidation of the mechanism of chiral separation by a comparative study of partially modified derivatives of chondroitin sulfate C [20]. Our result suggested that the sulfate group does not play a major role and the other substituent groups such as the carboxyl and hydroxyl groups are much more important for enantioseparation. Since the selective binding of the carboxyl group with the amino/imino or hydroxyl group through hydrogen bonding is widely observed in nature (typically in the double helix formation of DNA and specific DNA-protein binding in the transcription process), a similar mechanism can be expected for the intermolecular binding between a basic drug and this chiral selector. Based on this postulation we searched for other types of polysaccharides having the carboxyl group. As a result of this search we found that colominic acid (Col A) exhibited good enantioseparation of a few basic drugs. Unlike polygalacturonic acid [21], which has the carboxyl group at the C-5 position of the hexuronic acid residue, this newly found selector has the carboxyl group at the C-1 position in the neuraminic acid residue adjacent to the ketal group. In this paper we present details of the enantioseparation of basic drugs with this novel polysaccharide selector.

2. Experimental

2.1. Materials

Colominic acid (Col A) is a mixture of poly(*N*-acetylneuraminic acid) homologues having different degrees of polymerization (Fig. 1), produced from the bacterial broth.

Its preparations (sodium salt) were obtained from various sources, including ICN Pharmaceuticals (Chuo-ku, Tokyo, Japan), Nacalai Tesque (Nakakyo-ku, Kyoto, Japan), Sigma (St. Louis, MO, USA) and Wako Pure Chemicals (Chuo-ku, Osaka, Japan. They all were prepared from the culture fluid of *E. coli*. The average molecular masses of these preparations were 10–12, 30, 30, and 250 KDa, respectively. *N*-Acetylneuraminic acid (NANA), which is the monosaccharide unit of Col A (molecular mass, 309.3), was purchased from Wako.

Primaquine diphosphate (antimalarial drug, PRI) and laudanosine (tetanic poison, LAU) were purchased from Aldrich Chemical Company (Milwaukee, WI, USA). Tryptophan (a member of α-amino acid, TRY) was obtained from Wako. Chloroquine diphosphate (antimalarial drug, CHL), tryptophan methyl ester (TME), propranolol hydrochloride (β-blocker, PRO), salbutamol hemisulfate (β-adrenergic agonist, SAL) and doxylamine succinate (antihistaminic drug, DOX) were from Sigma. All these drug samples were racemic mixtures. The structures of these drugs are shown in Fig. 2.

Cinnamyl alcohol (CA), a marker of electroosmotic flow (EOF), used for evaluation of P/Col A double coating, was obtained from Nacalai. Polybrene (hexadimethrine bromide) used for capillary

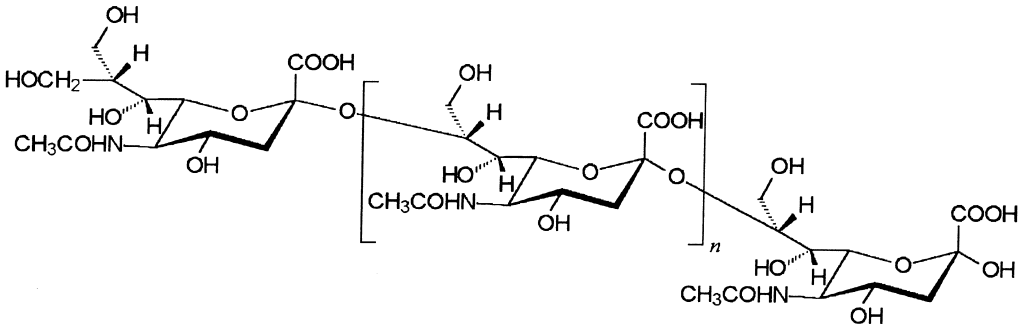

Fig. 1. Structure of Col A (a mixture of poly-NANAs).

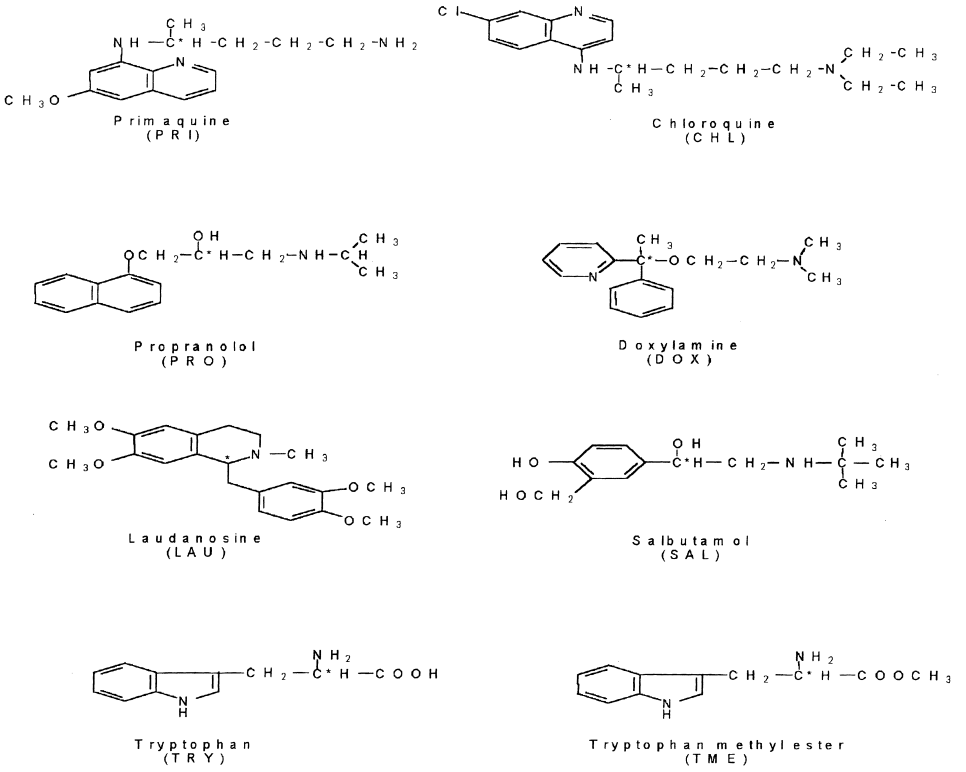

Fig. 2. Structures of basic drugs used in this study.

coating was from Aldrich. All other reagents were of the highest grade commercially available. Glassware-distilled, deionized water was used for preparation of running buffers and reagent solutions.

2.2. Apparatus

A CAPI-3100 capillary electrophoresis system of Otsuka Electronics (Hirakata-shi, Osaka, Japan) was used for CE throughout the work, which consisted of a sampling device, a power supply, a photodiode array UV detector, and a data processor. A roll of uncoated fused-silica capillary (50 μm I.D., 375 μm O.D.) was obtained from Polymicro Technologies (Phoenix, AZ, USA). A part was cut out from the roll and dynamically coated with Polybrene (P) followed by Col A by the procedure described below. The double coated capillary was fixed in a cassette, filled with running buffer, and thermostated at a temperature in a range of 16~32 °C. In a reference experiment a piece of uncoated capillary of the same length was fixed in a cassette and maintained in the same manner as for the double-coated capillary. Samples were introduced by vacuum injection (0.5 kg/cm^2) for a period in a range of 0.5–4.0 s. Enantioseparations were performed at a constant voltage in a range of 10~20 kV. The analytes were detected by measuring the UV absorbance at 259 nm (PRI), 256 nm (CHL), 220 nm (PRO), 260 nm (DOX), 230 nm (LAU), 225 nm (SAL), 280 nm (TRY), or 280 nm (TME).

2.3. Preparation of Polybrene/colominic acid (P/Col A)-double coated capillary

The double coating of the capillary was achieved by the procedure described below. A piece of capillary was rinsed with 1 *M* sodium hydroxide for 15 min followed by water for 15 min, in order to clean the capillary and enhance the dissociation of the silanol group. After this pre-conditioning, a 5 w/v% aqueous Polybrene solution was passed

through the capillary for 15 min at a constant pressure (0.5 kg/cm^2), and the capillary was allowed to stand for another 15 min for completion of coating. Thus-prepared Polybrene-coated capillary was rinsed with water for 15 min to remove the excess amount of Polybrene. A 3-w/v% aqueous Col A solution was then passed through the capillary for 15 min, and the capillary was allowed to stand for complete coupling of this anionic polymer to the Polybrene layer. Finally, the capillary was rinsed with water for 15 min to remove an excess of Col A. All these procedures were performed at room temperature.

2.4. CE

Phosphate buffers (pH 2.05, 3.00, 6.00, 7.00 and 8.00): acetate buffers (pH 4.00 and 5.00): borate buffers (pH 9.17 and 11.00) were used as the buffers for CE. Ionic strength was kept commonly at 0.05. Buffer solutions containing Col A or NANA were freshly prepared by dissolving one of the additives in a 20-m*M* phosphate buffer having a specified pH, and then adjusting pH exactly to a desired value by adding a small volume of 10 w/v% phosphoric acid or 1 *M* sodium hydroxide by using a micro syringe and a micro glass electrode. The pH values of these running buffers were always checked before and after each run. Each buffer solution was filtered through a 0.45-μm pore membrane filter and degassed by sonication before use.

The stock solution of each racemic sample was prepared at a concentration of 1 mg/ml in methanol (LAU) or water (others). Sample solutions for CE were prepared by diluting one of the stock solutions with water to a concentration of 200 μg/ml.

In all analyses the capillaries were pre-conditioned with water followed by running buffer for a short period before each run. At the end of daily experiments the capillaries were washed with 1 *M* sodium hydroxide (for analyses using an uncoated capillary) or a 3-w/v% aqueous Col A solution (for analyses using a P/Col A-double coated capillary) followed by water.

The degree of peak tailing was expressed as symmetry factor S ($W_{0.05}/2f$), where $W_{0.05}$ and f are the whole width and the front half width, respectively, of the peak at the 5% position of peak height. Separation factor (α) and resolution (R_s) were calculated by the equations, $\alpha = t_2/t_1$ and $R_s = 2\ (t_2 - t_1)/(w_2 + w_1)$, where t_1 and t_2 are the migration times of the first and second eluted enantiomers, respectively. For poorly resolved peaks w_1 and w_2 were estimated as approximate values obtained by using tangential lines along the rear and front slopes, respectively, of adjacent peaks. Symbols, w_1 and w_2, are the peak widths of the first and second eluted enantiomers, respectively. The $W_{0.05}$, f, w_1 and w_2 values were measured on printed electropherograms in which the abscissa scale was 50 cm/min. The electrophoretic mobility of EOF was expressed by the equation, $L \cdot l/V \cdot t_0$, where L, l, V, and t_0 are total capillary length, effective capillary length (the length between the inlet and the detection window), applied voltage, and migration time of cinnamyl alcohol (a neutral marker), respectively.

3. Results and discussion

The study on the effects of various factors giving influence on the enantioseparation of basic drugs with Col A was carried out first by taking PRI as a model drug, then the same procedures were extended to other drugs.

3.1. Effect of dynamic double coating on the CE of basic drugs

In our preceding paper of this series on the enantioseparation of basic drugs with chondroitin sulfate C [20] we noticed the phenomenon of heavy peak tailing of basic drugs in uncoated capillaries filled with acidic media. We could overcome this problem by dynamic double coating of capillary with Polybrene followed by chondroitin sulfate C (P/CC-double coating). In the present paper we examined the effect of analogous coating with Polybrene followed by Col A (hereafter P/Col A-double coating), because this anionic polymer can be constantly supplied from a running buffer in the present study which uses Col A-containing running buffer solutions. Table 1 compares the *S*-values of the PRI and PRO peaks in a 20-m*M* phosphate buffer (pH 2.90) between the P/Col A-double coated and uncoated capillaries.

Table 1
Comparison of *S*-values among various kinds of capillaries

Solute	*S*-value			
	P/Col A-double coated			Uncoated
	1	2	3	
PRI	1.5	1.5	1.4	17.1
PRO	2.8	2.7	2.7	18.2

Capillary, P/Col A-double coated or uncoated (total length 50.0 cm, effective length 37.5 cm; running buffer, 20 m*M* phosphate buffer (pH 2.90); applied voltage, 20 kV; capillary temperature, 20 °C; detection, UV absorption at 259 nm (PRI) or 220 nm (PRO). Each *S*-value was the average of 5 repeated estimations.

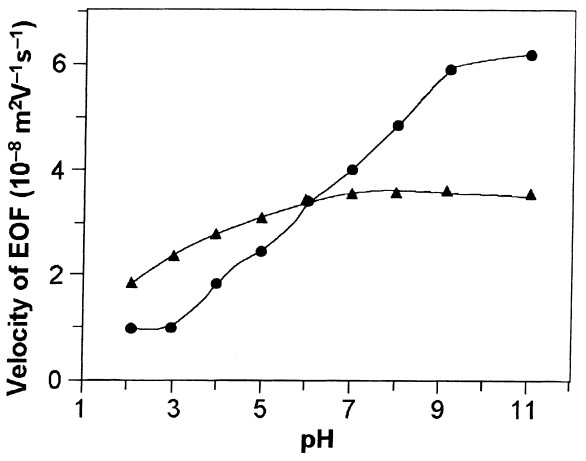

Fig. 3. pH dependence of EOF in P/Col A-double coated (▲) and uncoated (●) capillaries. Capillary: P/Col A-double coated or uncoated (total length 50.0 cm, effective length 37.5 cm). Running buffer: one of the following buffers (I = 0.05): phosphate buffer (pH 2.05, 3.00, 6.00, 7.00, 8.00), acetate buffer (pH 4.00, 5.00) or borate buffer (pH 9.17, 11.00). Applied voltage: 20 kV. Capillary temperature: 20 °C. Detection: UV absorption at 259 nm. Each plot was obtained by averaging 5 measurements.

The *S*-values of both PRI and PRO peaks in the P/Col A-double coated capillaries were obviously much smaller than in the uncoated capillary. These values were also smaller than in the P/CC-double coated capillary (data not shown).

It is notable that the *S*-value of PRI did not change greatly (1.4~1.7) over a wide pH range of 2~11. In addition the change of the velocity of EOF with pH was much smaller than in the uncoated capillary (Fig. 3). This small change of EOF is presumably due to the smaller change of negativity of the second layer (the Col A layer) with pH, and is favorable for obtaining higher reproducibility in migration time.

The velocities of EOF were compared between before and after passage (15 min) of various media through a freshly prepared P/Col A-double coated capillary at pH 3.00. The result was as follows. 1 *M* hydrochloric acid: 2.34→2.35, 1×10^{-2} *M* sodium hydroxide: 2.34→2.33, methanol: 2.36→2.39, acetonitrile: 2.30→2.31 (The unit was 10^{-8} m^2 V^{-1} s^{-1} and the number of repetitions was 5 in all measurements). Thus, there was no practical change of EOF after such treatments. Considerable change ($2.31\rightarrow1.86\times10^{-8}$ m^2 V^{-1} s^{-1}) was observed for the first time by rinse with such a strong alkali as 1×10^{-1} *M* sodium hydroxide. The durability of the P/Col A-double coated capillary was quite high, the migration times of PRI and CA being almost unchanged even after 100 times repetitions. All these data demonstrate the robustness of the P/Col A-double coated capillaries.

Table 2 compares the reproducibility of the migration time of PRI between the P/Col A-double coated and uncoated capillaries. It also includes the reproducibility data for CA, as a neutral marker. The relative standard deviations of the migration times of

Table 2
Reproducibility of the migration times of PRI and CA in the P/Col A-double coated and uncoated capillaries ($n=5$)

pH	P/Col A double coated				Uncoated			
	PRI		EOF		PRI		EOF	
	t (min)	RSD (%)	*t* (min)	RSD (%)	*t* (min)	RSD (%)	*t* (min)	RSD (%)
3.00	2.83	1.6	6.67	1.6	3.68	7.5	15.64	8.0
6.00	2.83	1.1	4.51	1.4	2.86	4.3	4.57	4.1
9.17	2.81	1.2	4.27	1.5	2.00	3.1	2.63	3.2

Capillary, P/Col A-double coated or uncoated (total length 50.0 cm, effective length 37.5 cm); running buffer ($I=0.05$), phosphate buffer at pH 3.00 or 6.00, or borate buffer at pH 9.17; applied voltage 20 kV; capillary temperature, 20 °C; detection, UV absorption at 259 nm.

PRI and CA for the P/Col A-double coated capillary were less than 1.6%, whereas those for the uncoated capillary were as high as 3~8%. This result clearly shows the priority of the P/Col A-double coated capillary in reproducibility of migration time.

3.2. Effect of capillary temperature on chiral separation of PRI

The effects of capillary temperature on the migration times and separation of PRI enantiomers were investigated in a P/Col A-double coated capillary using a 20-m*M* phosphate buffer (pH 4.0) containing Col A to a concentration of 6 w/v%. The results are shown in Fig. 4.

Over a temperature range of 16~32 °C the migration times of PRI enantiomers decreased as the temperature rose. In this system the electrophoretic migration of PRI and EOF are commonly toward the cathode, and the electrophoretic migration of Col A as chiral selector is toward the anode. Although temperature effect on the velocity of PRI due to viscosity change cannot be neglected, the decrease in migration time with temperature rise implies weakening of the interaction between PRI and Col A. Temperature rise also caused a decrease in separation factor (α) and resolution (R_s). Based on this result we performed all other experiments at a capillary temperature of 16 °C, a practical lower limit.

3.3. Effect of buffer pH on chiral separation of PRI

Fig. 5 shows the pH dependence of the migration times and separation of PRI enantiomers, obtained in a P/Col A-double coated capillary using a 20-m*M* phosphate buffer containing Col A to a concentration of 6 w/v%, over a pH range of 3.0~4.6. The migration times of PRI enantiomers increased at first as pH increased and reached a maximum value at pH 4.0. Then the migration times gradually decreased.

In response to this change α and R_s gave convex curves, giving the maximum values of 1.015 and 1.10, respectively at pH 4.0.

3.4. Effect of Col A concentration on chiral separation of PRI

Fig. 6 shows the effect of Col A concentration on the migration times and separation of PRI enantiomers, in a P/Col A double coated capillary using a 20-m*M* phosphate buffer (pH 4.0) containing Col A. The examined range of the Col A concentration was 4.0~13.0%.

The migration times of the PRI enantiomers

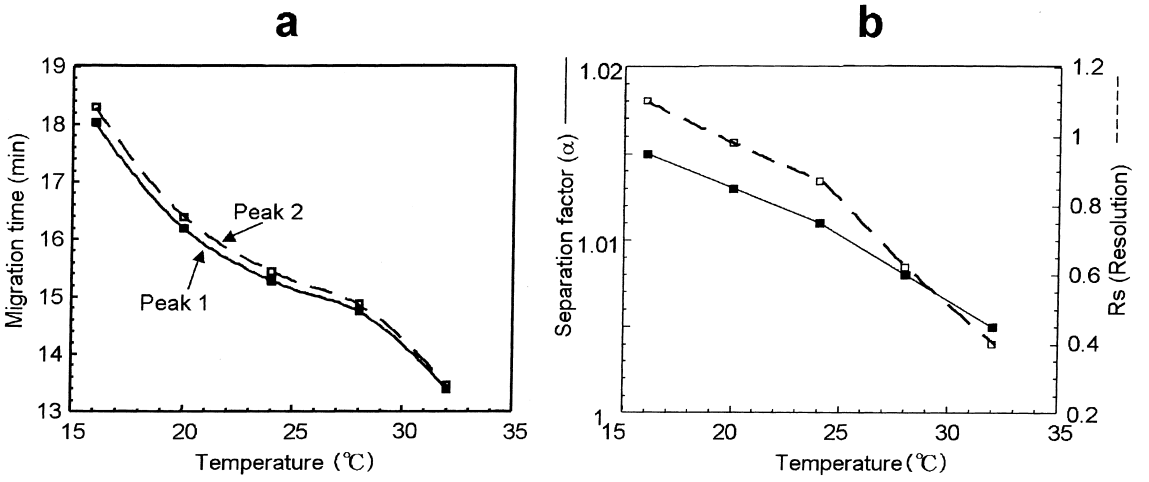

Fig. 4. Effect of capillary temperature on the migration times and separation of the PRI enantiomers. Capillary, P/Col A double coated (total length 50.0 cm, effective length 37.5 cm); running buffer, 20 m*M* phosphate buffer (pH 4.0) containing Col A (Nacalai) to a concentration of 6 w/v%; applied voltage, 8 kV; detection, UV absorption at 259 nm.

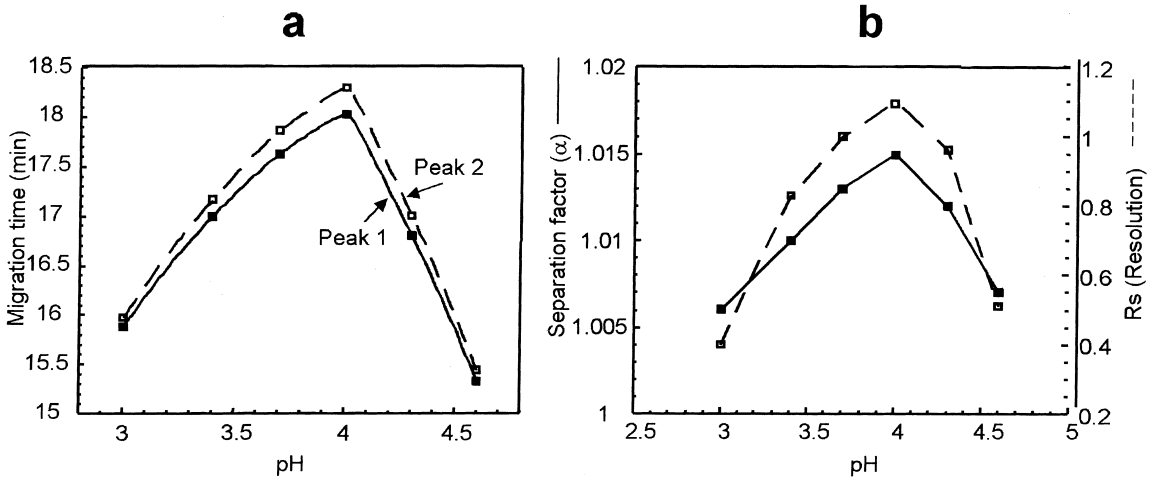

Fig. 5. pH dependence of the migration times and separation of the PRI enantiomers. The analytical conditions as in Fig. 4, except that buffer pH was changed to 3.00, 3.40, 3.70, 4.00, 4.30 and 4.60, and capillary temperature was maintained at 16 °C.

tended to increase as Col A concentration increased, naturally because the molar fraction of the PRI-Col A adduct increased with the increase of Col A concentration, giving longer migration times of the PRI peaks. The effect of increased viscosity cannot be neglected, because this will cause similar tendency. Separation factor α and Resolution R_s increased as Col A concentration increased, in parallel with the increase with migration time, but reached maximum values of 1.024 and 2.19, respectively, at 9 w/v%. At higher Col A concentration α and R_s decreased presumably due to enhanced peak broadening. Thus, the additive concentration of 9 w/v% was proved to be optimum for the separation of the PRI enantiomers.

3.5. Effect of the average molecular mass of Col A on chiral separation

Col A is a mixture of 2→8 linked NANA

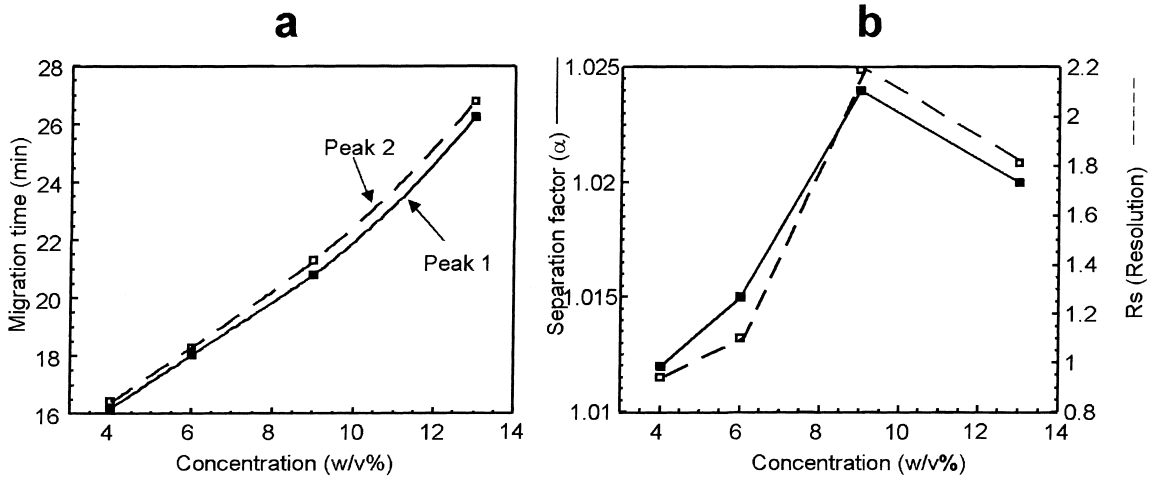

Fig. 6. Effect of Col A concentration on the migration times and separation of the PRI enantiomers. The analytical conditions as in Fig. 4, except that the Col A concentration was varied among 4.0, 6.0, 9.0, and 13.0 w/v%, and capillary temperature was maintained at 16 °C.

polymers produced from bacterial broth, but the distribution of degree of polymerization is varied among preparations. We examined the relationship between the average molecular mass (AMM) of Col A and enantioselectivity of drugs. Fig. 7 shows the dependence of α as well as R_s between PRI enantiomers on AMM of Col A preparations reported by the suppliers.

Since the AMM of the ICN, Nacalai, Sigma and Wako preparations were 10–12, 30, 30 and 250 KDa, respectively, it is clearly shown that α as well as R_s increased as AMM increased. The migration times of the enantiomers also increased significantly with the increase in AMM. The same tendency was observed for CHL, TRY and LAU. For reference the selectivity of NANA, the monosaccharide component of this polysaccharide, was also examined under the same conditions, but it did not show any enantioselectivity at all, all drugs giving single peaks. It is interesting that Col A from Wako having the largest AMM gave severely tailing peaks for all basic drugs, so that R_s values decreased drastically. The reason is not clear.

The increase of enantioselectivity with molecular size has been recognized in other oligo- and polysaccharides. Although this phenomenon has been explained as network effect [14] or cluster effect [20,22], more detailed studies will be necessary for complete understanding of this phenomenon.

3.6. Enantioseparation of other basic drugs

The conditions for enantioseparation of other drugs were optimized similarly to PRI. Since the effects of capillary temperature, buffer concentration and Col A concentration were almost the same as

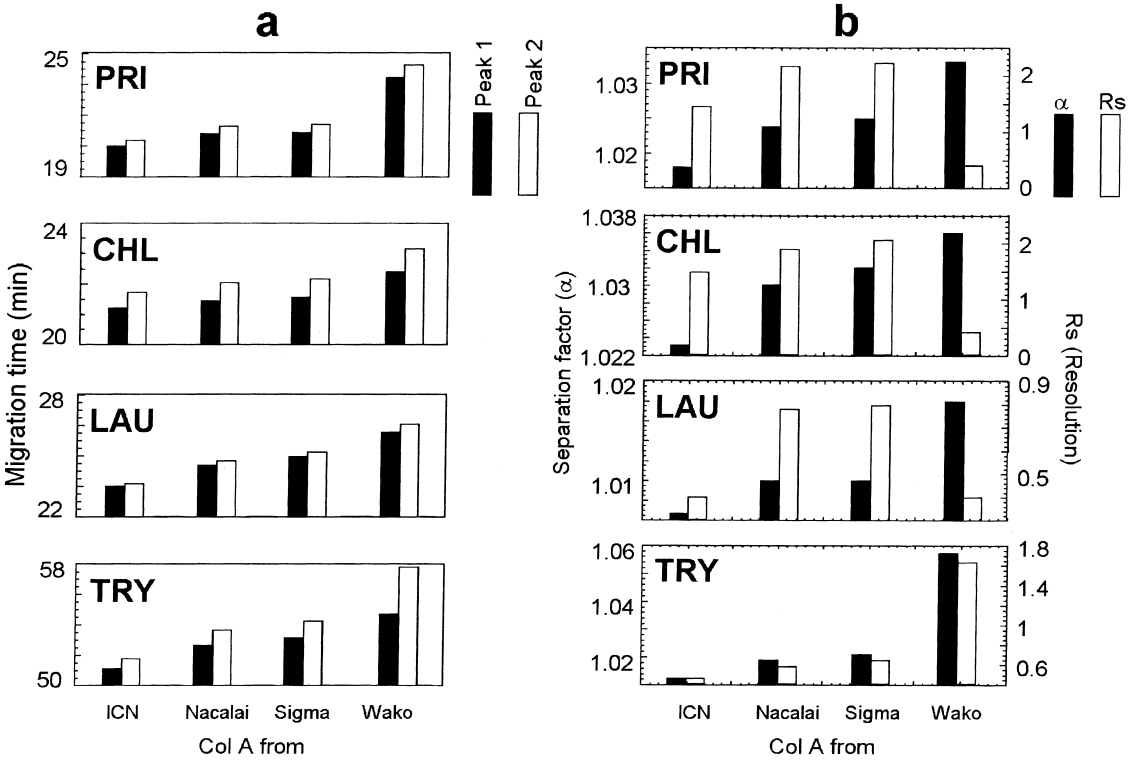

Fig. 7. Effect of AMW of Col A on the migration time and separation of the enantiomers of basic drugs. Capillary, P/Col A double coated (total length 50.0 cm, effective length 37.5 cm); running buffer, 20 m*M* phosphate buffer containing a Col A preparation to a concentration of 9 w/v%; buffer pH, 2.6 (LAU) or 4.0 (other drugs); applied voltage, 10 kV (TRY) or 8 kV (other drugs); capillary temperature, 16 °C; detection, UV absorption at 259 nm (PRI), 256 nm (CHL), 230 nm (LAU), or 280 nm (TRY). Peaks 1 and 2 indicate the first and second peaks.

observed with PRI, these factors were fixed at 16 °C, 20 m*M* and 9 w/v%, respectively, under the established conditions. In contrast, the optimum pH was varied with drugs as shown in Fig. 8. The optimum pH values obtained were as follows. CHL: 4.0, TRY: 4.0, LAU: 2.6, SAL: 3.0.

The variation of the optimum pH for chiral separation among drugs will give important information on the interaction of Col A with these drugs. Since the degree of protonation in basic compounds is dependent on pH, the optimum pH will point to the most favorable state of the amino/imino function in the drugs for binding presumably to the carboxyl group in Col A. However, further studies will be necessary for much more unsophisticated explanation of the mechanism of enantioseparation by this selector.

Table 3 summarizes the α and R_s-values for the enantioseparation of the basic drugs obtained under the conditions individually optimized. PRI, CHL, and TRY were completely enantioseparated, but LAU and SAL were only partially enantioseparated. No separation was observed for PRO, DOX, and TME. Examples of completely and partially resolved electropherograms (for PRI and LAU, respectively) are shown in Fig. 9.

In the present system of the basic drug–Col A combination, it is important to know what kinds of bindings exist between these selector and selectand. One of them can be anticipated to be the hydrogen bonding between the amino/imino group in a drug and the carboxyl group in Col A. The marked enantioselectivity in PRI, CHL and TRY, all of which have a chiral carbon adjacent to the amino/imino group, and no enantioselectivity in PRO and DOX, which do not have such configuration, strong-

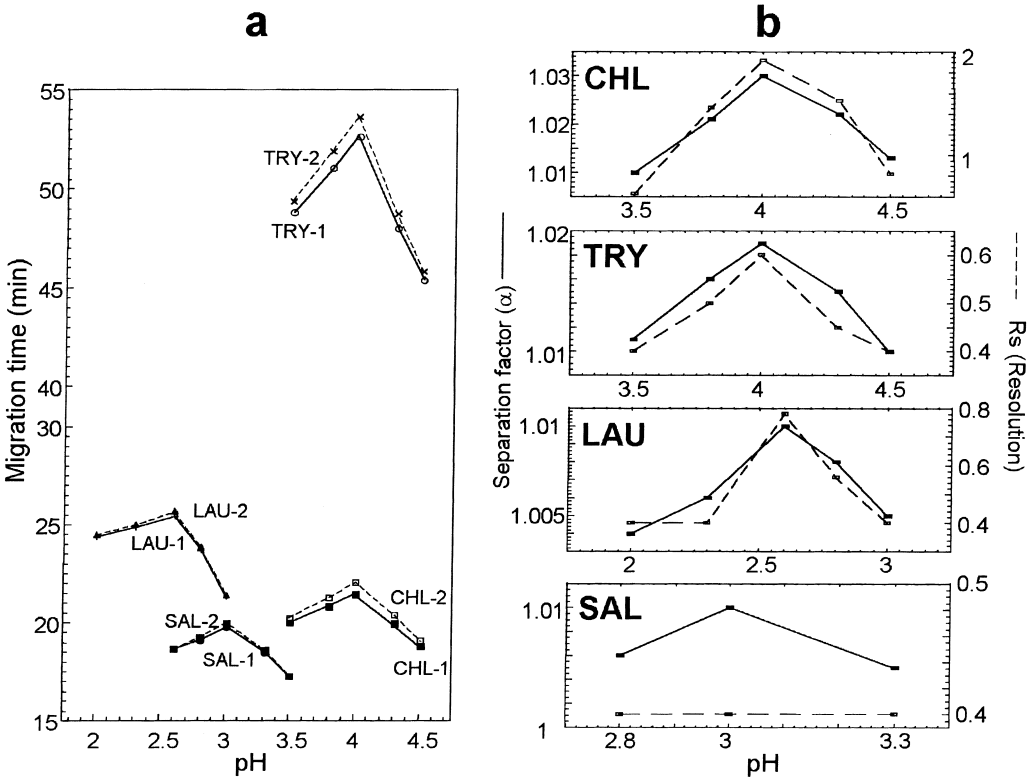

Fig. 8. pH dependence of the enantioseparation of various drugs. Capillary, P/Col A-double coated (total length 50.0 cm, effective length 37.5 cm); running buffer, 20 m*M* phosphate buffer containing Col A (Nacalai) to a concentration of 9 w/v%; applied voltage, 10 kV (TRY) or 8 kV (other drugs); capillary temperature, 16 °C; detection, UV absorption at 280 nm (TRY), 256 nm (CHL), 230 nm(LAU), or 225 nm (SAL). CHL-1 and CHL-2 indicate the first and second peaks, respectively. Such designation is the same for other drugs.

Table 3
The α and R_s values for various drugs under the optimized conditions

Solute	t_1(min)	α	R_s
PRI	20.81	1.024	2.19
CHL	21.71	1.032	2.03
TRY	54.69	1.057	1.65
LAU	25.41	1.010	0.78
SAL	19.77	1.010	<0.4
PRO	20.67	NS	NS
DOX	17.63	NS	NS
TME	18.12	NS	NS

Capillary, P/Col A-double coated (total length 50.0 cm, effective length 37.5 cm); running buffer, 20 m*M* phosphate buffer containing Col A to a concentration of 9 w/v% at pH 2.60 (LAU), pH 3.0 (SAL) or pH 4.0 (the others); Col A preparation, Wako (TRY), Sigma (CHL) or Nacalai (the others); applied voltage, 10 kV (TRY) or 8 kV (the others); capillary temperature, 16 °C; detection, UV absorption at 259 nm (PRI), 256 nm (CHL), 280 nm (TRY), 230 nm (LAU), 225 nm (SAL), 220 nm (PRO), 260 nm (DOX) or 280 nm (TME). NS: not separated. t_1: migration time of the faster moving enantiomer.

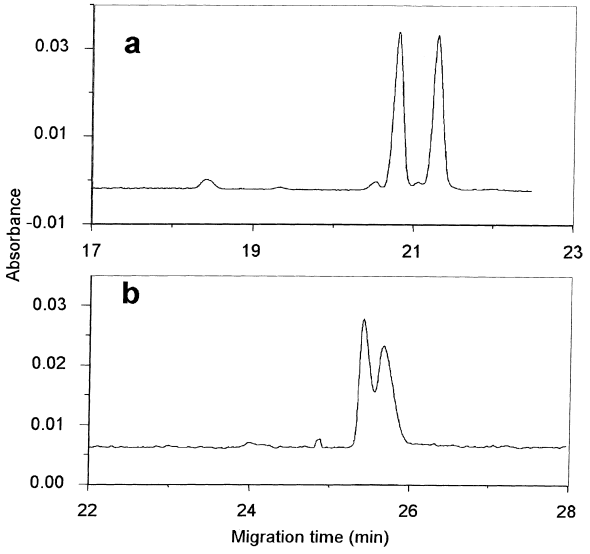

Fig. 9. Examples of enantioseparation of basic drugs with Col A. (a) PRI, (b) LAU. Capillary, P/Col A-double coated (total length 50.0 cm, effective length 37.5 cm); running buffer, 20 m*M* phosphate buffer containing Col A to a concentration of 9 w/v%; buffer pH, 4.0 (PRI) or 2.6 (LAU); Col A source, Nakalai; applied voltage, 8 kV; capillary temperature, 16 °C; detection, UV absorption at 259 nm (PRI) or 230 nm (LAU).

ly suggest the contribution of this amino/imino group. The structure of Col A is unique in that a number of the carboxyl groups are present adjacent to the ketal group. Therefore, the electron density in the carboxyl groups in Col A will be lower than in those in polyuronic acids. This might bring about a different feature in enantioseparation.

For other bindings, however, no direct evidence is provided by the present experimental results alone. In a particular couple of α-amino acid and its derivative (TRY and TME) a great difference of enantioselectivity was observed. This phenomenon points out the importance of the carboxylic acid group in the selectand. The counterpart will probably be the hydroxyl group in Col A. Further study on the mechanism of chiral recognition is going on.

In the present work separation was performed in a Col A-containing buffer filled in a capillary coated with Col A through a Polybrene layer. Therefore, basic drugs are considered to interact with both states of Col A, on the capillary inner wall and in the running buffer. This situation is similar to a certain system of open-tubular capillary electrochromatography (OT-CEC) performed by the electrokinetic chromatography mode, in which a chiral selector was immobilized thick on the capillary inner wall and at the same time dissolved in running buffer. For example, Jakubetz et al. [23] succeeded in the separation of hexobarbital enantiomers in an open tubular capillary covalently bonded with permethylated β-cyclodextrin in a neutral medium. They compared this separation with dual mode separations performed in buffers containing a homologous compound either positively (β-cyclodextin-2-hydroxy-3-trimethyl-ammoniumpropyl ether chloride) or negatively (β-cyclodextrin-sulfo-*n*-propyl ether) charged. Enhancement or suppression of enantioseparation was observed for these systems, respectively, due to concerted or compensated mechanism. In this example of OT-CEC much of the permethylated cyclodextrin was immobilized by a special technique to the capillary inner wall and a long capillary (80 cm) was used in order to ensure sufficient amount of the chiral selector for separation. Contrastively, the amount of the immobilized Col A in our present work was so small, because the aim of immobilization was only to achieve reproducible analysis with symmetric peaks, that enantio-

separation by Col A on the capillary inner wall could hardly be expected. However, the contribution of this capillary coating was considered to be positive, though to a small extent, rather than negative. The enantiomer having stronger affinity to Col A was much more retarded by both states of this selector. The solute was positively charged in contrast to the OT-CEC analysis mentioned above, but the situation was similar to the enhanced enantioseparation with the positively charged selector.

4. Conclusion

We found that CE with Col A, a mixture of poly-NANAs, could well separate the enantiomers of PRI, CHL and TRY, basic drugs having the amino/imino group adjacent to the chiral carbon atom. LAU and SAL gave partial separation of their enantiomers. P/Col A-double coated capillary were stable and analysis in these capillaries at a low temperature (16 °C) with a dilute (20 m*M*) phosphate buffer containing Col A (9 w/v%) as running buffer allowed reasonable retention of these drugs with high reproducibility, and permitted efficient enantioseparation. The optimum pH varied among drugs in the acidic region. As seen in other types of carbohydrates the Col A preparations having higher molecular masses gave better enantioseparation, and NANA, the unit monosaccharide of this polysaccharide, gave no enantioseparation.

The finding of enantioselectivity by this novel polysaccharide will add useful information not only to practical analysis of drug enantiomers but also to studies of chemical and biological functions of carbohydrates.

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