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Planar chromatographic direct separation of some aromatic amino acids and aromatic amino alcohols into enantiomers using cyclodextrin mobile phase additives

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Abstract

Some racemic aromatic amino acids and amino alcohols were directly resolved using cellulose phase thin-layer chromatography with mobile phases containing highly concentrated solutions of cyclodextrin (β or α). Enantiomeric resolution is highly dependent on the mobile phase composition and on the structure of the compounds. The common feature of the compounds resolved in this study is that a polar group (amino or hydroxy) is attached at the *para*-position in the aromatic ring. It was found that the retention order of enantiomers of amino acids is opposite to that observed for the amino alcohol series.

Keywords: Enantiomer separation; Mobile phase composition; Cyclodextrin additives; Amino acids; Amino alcohols; Aromatic compounds

1. Introduction

In the last few years, many reports have appeared on chiral separations with the use of cyclodextrins (CDs) and their derivatives. Most of these separations have been carried out using high-performance liquid chromatography (HPLC) with a CD bonded phase or with CD mobile phase additives [1–8]. While a large number of mobile phase additives have been introduced for thin-layer chromatography (TLC) [9–15], the use of chiral stationary phases in TLC is relatively less common; probably because of their shorter lifetime and higher costs compared to HPLC.

Originally, CD was used as a mobile phase additive for TLC to separate a series of structural isomers [9,10]. No racemates have been resolved by TLC using mobile phase additives. One reason for this is the limited solubility of β-CD in water (25°C, 0.017 *M*) although β-CD is the least expensive and most widely used homologue. The concentration of β-CD in water is insufficient for the TLC separation of the enantiomers. However, the solubility of β-CD in water can be increased by adding large amounts of urea. In 1988, Armstrong et al. [11] reported the resolution of 21 racemates by reversed-phase TLC using a mobile phase containing a β-CD saturated solution of urea. Lepri et al. [12] have reported similar results. Another method is to modify the CD synthetically so as to increase its solubility in water. Highly water-soluble CD polymers and derivatives were used as

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Table 1
Comparison of enantiomeric separation of tyrosine and isoprorylepinephrine using mobile phases of various compositions

Mobile phase	DL-Tyrosine				DL-Isopropylepinephrine			
	$R_{F,D}$	$R_{F,L}$	α^*	R_s^{***}	R_{F1}	R_{F2}	α^{**}	Development velocity (min/6 cm)
Methanol–formic acid–β-CD saturated solution of urea (7:1:2)	0.93	0.78	1.19	1.13	0.90	0.75	1.20	22
Acetonitrile–formic acid–β-CD saturated solution of urea (7:1:2)	0.87	0.73	1.19		0.75	0.75	1.00	20
Pyridine–β-CD saturated solution of urea (3:2)	0.87	0.76	1.14					90

$\alpha^*=R_{F,D}/R_{F,L}$, $\alpha^{**}=R_{F1}/R_{F2}$.
$R_s^{***}=2\times$(distance between spots)/(sum of the widths of the two spots). See Ref. [12].

mobile phases in TLC separations of a wide variety of compounds [13]. Duncan and Armstrong [14] reported the enantiomeric separation of amino acid derivatives and alkaloids by TLC on different types of reversed-phase plates with a mobile phase containing maltosyl-β-CD. Partially substituted (hydroxypropyl) and (hydroxyethyl) β-CDs have also been proved to be effective chiral mobile phase additives for the TLC enantiomeric separation of various chiral compounds [15]. In previous reports, more expensive reversed-phase plates were used and the amino acids were separated as derivatives. In this work, based on Ref. [11], we report on the enantiomeric separation of some non-derivatized amino acids and amino alcohols by TLC on cheaper cellulose phase plates with a solution of urea-solubilized β-CD or α-CD as the mobile phase. This separation was needed for a biochemical study.

2. Experimental

2.1. Materials

Cellulose powder was MN 300 TLC (Macherey-Nagel, Germany). β-CD was purchased from Tao Wan Chemical Plant (China) and purified by recrystallization from water. DL, L-tryptophan(Trp), DL, L-tyrosine(Tyr), DL-*p*-aminophenylalanine, DL, L-phenylalanine(Phe), DL-*p*-hydroxyphenylglycine, DL-isopropylepinephrine(Ipepi) were obtained from Shanghai Biochemical Institute (China). L, DL-Epinephrine (Epi), DL-thyronine and DL, L-DOPA were obtained from Sigma (USA). Diethylamine, formic acid, ninhydrin, methanol, acetonitrile, pyridine and urea were all analytical-reagent grade.

2.2. Methods

In this study, all solutions of β-CD and α-CD were prepared with a saturated solution of urea. Stock solutions of amino acids and amino alcohols were prepared with methanol. A little 85% formic acid was added; its concentration is 2 mg/ml. The mobile phase in this study consisted of a β-CD saturated solution of urea and organic modifier as well as formic acid or diethylamine. This mobile phase is transparent and uniform when methanol, acetonitrile or pyridine are used as organic modifier. According to Ref. [16], cellulose plates (7.5 cm×2.5 cm) were prepared. The thickness of the cellulose layer was 0.6–0.7 mm. All developments were carried out at room temperature (25°C) in small glass jars of 250-ml volume. A 1–2-μl volume of the test solute stock solution was applied at 1 cm from the lower edge of the plates and the distance of development was 6 cm.

Table 2
Effect of different ratios between methanol and β-CD solution in the mobile phase on the separation of DL-tyrosine

Mobile phase methanol–formic acid–0.1 *M* β-CD solution of urea (pH 4.5)	$R_{F,D}$	$R_{F,L}$	α	Development velocity (min/6 cm)
7:1:2	0.91	0.77	1.18	18
5:1:4	0.92	0.84	1.10	45
4:1:5	Tailing	Tailing	No separation	98

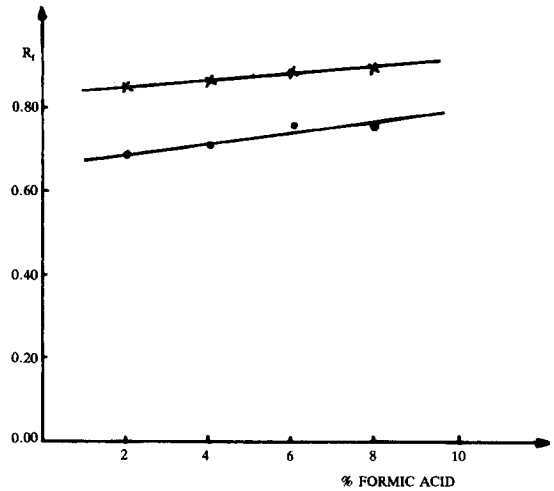

Fig. 1. The dependence of the content of formic acid in mobile phase on the R_F values of DL-tyrosine. ×=R_F values of D-tyrosine; ●=R_F values of L-tyrosine.

The spots were visualized by spraying the developed TLC plates with an ethanol solution of 0.5% ninhydrin, then heating with an infrared lamp to the formation of violet spots for amino acids. A 6-g amount of NH_4CNS and 2 g $CoCl_2$ were added into 40 ml water to prepare a $Co(CNS)_2$ solution. Amino alcohols were detected as blue spots on a light red background after spraying the developed TLC plates with $Co(CNS)_2$ solution.

3. Results and discussion

The separation system was optimized by investigating the effect of different organic modifiers in the mobile phase on the α value ($\alpha=R_{F,D}/R_{,F_L}$ or R_{F1}/R_{F2}) of DL-Tyr and DL-Ipepi. The results are given in Table 1. It appeared that the enantiomeric separation of DL-Tyr and DL-Ipepi could be achieved when methanol, acetonitrile or pyridine were used as the organic modifier in the mobile phase. But when ethanol, propanol, isopropanol or butanol were used as the organic modifier, resolution was not achieved because precipitation occurred in the mobile phase. Among the three organic modifiers in Table 1, it is observed that methanol is the best one; producing spots without tailing, rapid development and a larger separation factor (α). Pyridine and acetonitrile are more poisonous and more expensive. Table 2 shows the dependence of the separation on the different ratios between β-CD saturated solution and metha-

Table 3
Effect of the amount of diethylamine on the enantiomeric separation of three aromatic amino acids

Mobile phase	Compounds	$R_{F,D}$	$R_{F,L}$	α^*	R_s
Methanol–β-CD saturated solution of urea–33% diethylamine (4:1:0.14)	DL-Tyrosine	0.81	0.66	1.23	
	DL-Tryptophan	0.63	0.53	1.19	
	DL-Phenylalanine	0.62	0.62	1.00	
Methanol–β-CD saturated solution of urea–33% diethylamine (4:1:0.28)	DL-Tyrosine	0.81	0.69	1.17	
	DL-Tryptophan	0.76	0.70	1.09	
	DL-Phenylalanine	0.75	0.75	1.00	
Methanol–β-CD saturated solution of urea–33% diethylamine (4:1:0.42)	DL-Tyrosine	0.86	0.77	1.12	1.08
	DL-Tryptophan	0.77	0.72	1.07	
	DL-Phenylalanine	0.80	0.80	1.00	

nol in the mobile phase. It is seen from Table 2 that, when the content of methanol in the mobile phase was reduced, the development velocity was decreased and R_F values of enantiomers were all increased, but a poorer separation was obtained because of a smaller ΔR_F value and tailing spots. When the content of methanol was larger than 70% (v/v) in the mobile phase, the separation also became poorer because the content of β-CD in the mobile phase was reduced. Therefore the content of methanol in the mobile phase was kept at 70% (v/v) in this study.

Fig. 1 shows the influence of the content of formic acid in mobile phase on the separation. As can be seen, the R_F value is slightly reduced and the ΔR_F value is slightly increased with a decrease in the concentration (10–0%, v/v) of formic acid, but in fact when the concentration of formic acid in the mobile phase is decreased, poor resolution occurs due to tailing of the spots. When the content of formic acid in the mobile phase was higher than 10% (v/v), poor resolution also occured due to the decrease in the ΔR_F value and the spots ascended together near the solvent front. In accordance with Ref. [17], the content of formic acid in the mobile phase was kept at 10% (v/v). In this study, the mobile phase consisted of a β-CD saturated solution of urea–methanol–formic acid (2:7:1, v/v) (pH 4.5). CDs are stable in alkaline solution but they are susceptible to acid hydrolysis. However, under normal experimental conditions (pH higher than 3.5 and temperature lower than 60°C), cyclodextrins are fairly stable [18].

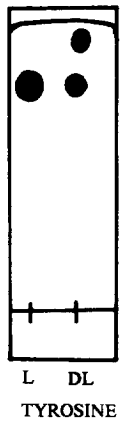

Fig. 2. TLC chromatogram showing the resolution of DL-tyrosine. Stationary phase: cellulose TLC plates. Mobile phase: methanol–formic acid–β-CD saturated solution of urea (7:1:2), pH 4.5.

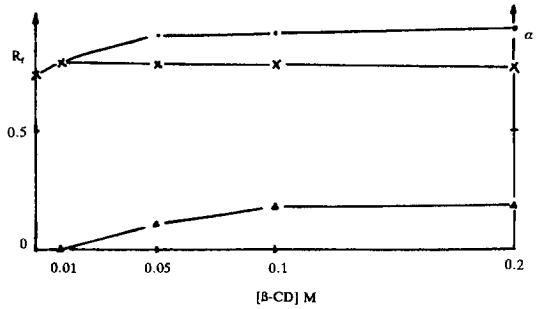

Fig. 3. The effect of the concentration of β-CD in the mobile phase on the R_F values and the α values of tyrosine. •=R_{F_D}–Tyr; ×=R_{F_L}–Tyr; Δ: $\alpha=(R_{F_D}-\text{Tyr})/(R_{F_L}-\text{Tyr})$. Stationary phase: cellulose TLC plates. Mobile phase: methanol–formic acid–β-CD saturated solution of urea (7:1:2). Temperature: 25°C.

Table 3 illustrates the dependence of the concentration of diethylamine (used as acid-base regulator) in the mobile phase on the separation. It was found that the R_F value of three enantiomers increaseed with increasing concentration of diethylamine, but the α value of DL-Tyr and DL-Trp and the tailing of spots were diminished. Fig. 2 gives the cellulose TLC chromatogram of the separation of DL-Tyr. The effect of the concentration of β-CD in the mobile phase on the separation is illustrated in Fig. 3, optimum enantiomeric resolution occurs over a range of 0.1 to 0.2 *M* β-CD. An analogous curve to that shown in Fig. 3 was obtained for DL-Ipepi.

Table 4 and Table 5 give the enantiomeric separation data for some amino acids and amino alcohols using β-CD and α-CD as the mobile phase chiral additive, respectively. These results indicate that larger separation factors α and better resolutions (R_s) are achieved (compared with Tables 1 and 3) because the spot is smaller (or more concentrated) in the case of α-CD. The explanation is simple: the benzene ring is twice less deeply included in α-CD than in β-CD. The solute molecules with a benzene ring are bound more tightly by the steric size of the α-CD cavity [13]. The separation of DL-Phe did not occur under any experimental conditions. The separation of DL-Try only occurred under alkaline conditions using β-CD as the mobile phase chiral additive. The separation of DL-Ipepi is not achieved

Table 4
Enantiomeric separation of aromatic amino acids and aromatic amino alcohols

Compounds	R_{F1}	R_{F2}	α
DL-3,4-Dihydroxyphenyl-alanine(DOPA)	0.75(D)	0.60(L)	1.25
DL-*p*-Hydroxyphenylglycine	0.83	0.61	1.36
DL-Thyronine	0.88	0.81	1.09
DL-*p*-Amino phenylalanine	0.63	0.63	1.00
DL-Epinephrine	0.73(L)	0.68(D)	1.07
DL-Isopropylepinephrine	0.90	0.75	1.20
DL-Phenylalanine	0.83	0.83	1.00
DL-Tryptophan	0.80	0.80	1.00

Stationary phase: cellulose TLC plates. Mobile phase: methanol–formic acid–β-CD saturated solution of urea (7:1:2). Temperature: 25°C, pH 4.5.

with α-CD, but the separation occurs with β-CD. The retention behaviour of DL-*p*-aminophenylalanine, which is not separated with β-CD, is opposite to that observed for DL-Ipepi. The reason is unknown. An analogous curve to that shown in Fig. 3 was generated for the effect of the concentration of α-CD on the separation of DL-Tyr. The optimum separation also occurs over a range of 0.1 to 0.2 *M* α-CD.

From the results above, we noticed that there are two similarities in the structures of the compounds resolved: all compounds resolved contain an aromatic ring and the *para*-position on the aromatic ring has a polar group. In our initial experiment, it was found that DL-Tyr was easily resolved under different conditions but DL-Phe was not resolved. Then the chiral compound which has a structure similar to DL-Tyr was selected. Thereby, it is possible that the solutes resolved with CD formed inclusion complexes, the aromatic ring of the solutes would enter into the cavity of CD. The polar group in the *para*-position of the aromatic ring should be bound to the hydroxy group on the rim of the CD cavity to form a hydrogen bond which results in increased stability of inclusion complex. The two polar groups attached to the chiral carbon atom of the solute are bound to the hydroxy group on the other end of the CD cavity to form hydrogen bonds with different stereoselective stabilities. Therefore the separation of enantiomers is achieved. In addition, another interesting aspect of this technique is that the D-enantiomer always elutes ahead of the L-isomer for amino acids (see Tables 1 and 3), for example, DL-Try and DL-DOPA. The elution order for amino alcohols is opposite to that observed in amino acids, for example for DL-Epi, the L-enantiomer elutes ahead of the D-isomer. This indicates that the stability of the inclusion complexes between the D-isomer and CD is better than that of the inclusion complexes between the L-isomer and CD for the amino acids. However, the inclusion complexes between the L-isomer and CD are more stable than the inclusion complexes between the D-isomer and CD for the amino alcohols because the isomer which exhibits the highest value of the complexation constant should exhibit the highest R_F value. Perhaps this is due to the difference in molecular structure. The only difference in structure between DL-DOPA and DL-Epi is that the chiral carbon of epinephrine is directly attached to the

Table 5
Enantiomeric separation of aromatic amino acids and aromatic amino alcohols

Compounds	R_{F1}	R_{F2}	α	R_s
DL-Tyrosine	0.87(D)	0.71(L)	1.21	3.76
	0.89(D)[a]	0.79[a]	1.13[a]	1.72[a]
DL-3,4-Dihydroxyphenylalanine(DOPA)	0.83(D)	0.72(L)	1.15	
DL-*p*-Hydroxyphenylglycine	0.87	0.62	1.40	
DL-Thyronine	0.88	0.77	1.14	
DL-*p*-Aminophenylalanine	0.82	0.73	1.12	
DL-Epinephrine	0.76(L)	0.76(D)	1.12	
DL-Isopropylepinephrine	0.97	0.97	1.00	
DL-Phenylalanine	0.80	0.80	1.00	
DL-Tryptophan	0.74	0.74	1.00	

Mobile phase: methanol–formic acid–0.2 *M* α-CD solution of urea (7:1:2). Stationary phase: cellulose TLC plates. Temperature: 25°C, pH 4.5.

[a] Mobile phase: methanol–0.2 *M* α-CD solution of urea–33% diethylamine (4:1:0.42)

benzene ring and hydroxy group, but for DOPA, the chiral carbon atom is linked to an amino group and a carboxyl group. There is a spacing of a CH_2 group between the chiral carbon atom and the aromatic ring. The formation of inclusion complexes with differences in steric structure between the DL-Epi and DL-Tyr or DL-DOPA results in the different elution order of the enantiomers.

TLC separations on cellulose plates of the DL-amino acids without derivatives have been reported. Different eluents were used. The dependence of chiral chromatographic separation on the properties of the cellulose plates and the eluents in the chromatographic system has been studied systematically by Yuasa and co-workers [19,20] and Lederer and co-workers [21–23]. The study above indicates that native cellulose gives poorer separations than microcrystalline cellulose. Fig. 3 shows that without CD the separation of the enantiomers did not occur. Therefore CD plays a more important role in these enantiomeric separations. In the experiment, 0.6–0.7 mm thickness of plates was used because it was easier for us to make a uniform layer.

4. Conclusion

At first, this method is particularly simple and attractive. The stationary and mobile phases are all readily available. Some aromatic amino acids and amino alcohols can be resolved without any derivatization. This method is suitable for biochemical studies.

Secondly, under these experimental conditions, only racemic amino acids and amino alcohols with a benzene ring and polar group in the *para*-position of the aromatic ring can be directly separated.

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