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ORIGINAL PAPER

Biphasic recognition chiral extraction – novel way of separating pantoprazole enantiomers

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This paper presents a biphasic recognition chiral extraction system developed as a new chiral separation technology for the separation of pantoprazole enantiomers, combining a hydrophilic β-CD derivative in the aqueous phase and a hydrophobic tartaric acid in the organic phase which preferentially recognise the (R)-enantiomer and (S)-enantiomer, respectively. In this study, a number of factors which influence the efficiency of the extraction were investigated including types of organic solvents, β-CD and tartaric acid esters and their concentrations, pH and temperature. As a result, enantioselectivity for pantoprazole enantiomers can be improved up to 1.42 under optimised conditions; in addition, it is clear that the combined action of β-CD and tartaric acid esters leads to formation of the biphasic chiral extraction system with a stronger separation capacity than a monophasic chiral extraction system.

Keywords: pantoprazole, biphasic chiral extraction system, β-cyclodextrin, tartaric acid

Introduction

Drug enantiomers have different effects on pharmacological activity, the metabolism process and toxicity in the human body (Dorey, 2000). Currently more than 50 % of clinical drugs possess chiral elements, and more than 85 % of them exist as racemic mixtures. As a consequence, there is increased demand for the production of enantiomerically pure compounds in the pharmaceutical industry as well as in the agrochemical, flavour and fragrance industries (Horvath et al., 2004; Tombo & Belluš, 1991; Miyako et al., 2004; Zhou et al., 2013). However, since the enantiomers exhibit similarities in terms of physical and chemical properties, their separation is not easy.

Diastereomeric crystallisation, currently the commonest technique used for the commercial production of pure enantiomer compounds, is regarded as inflexible, relatively slow and difficult to control (Babić et al., 2007). More recently, the chiral ligand-exchange concept has been applied to liquid–liquid extraction technology, which is a continuous, linearly scalable and relatively inexpensive separation process (Koska & Haynes, 2001). Steensma et al. (2007) reported the chiral separation of amines, amino acids and amino alcohols by reactive extraction.

A chiral extraction process requires an enantioselective extractant to be dissolved in the extract phase; this binds enantiospecifically and reversibly with a racemic substrate in the feed (Prelog et al., 1989). Several chiral selectors have been reported, such as tartaric acid derivatives (Viegas et al., 2007; Tan et al., 2007), crown ethers (Pietraszkiewicz et al., 1998; Steensma et al., 2007; Colera et al., 2005), cinchona alkaloids (Viegas et al., 2007; Hallett et al., 2009), β-cyclodextrin (β-CD) derivatives (Del Valle, 2004), metal complexes (Koska & Haynes, 2001) and others (Dimitrova & Bart, 2009; Tang et al., 2008; Yoon & Cram, 1997; Ding et al., 1992; Snyder et al., 2005; Kocabas et al., 2006). To obtain information on a chiral

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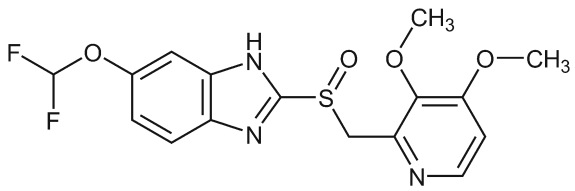

Fig. 1. Chemical structure of pantoprazole.

extraction process, enantioselectivity (α) is used as the most important parameter in extraction to reflect the efficiency. This parameter depends on the difference in free energy $-\Delta(\Delta G)$, hence how to increase $-\Delta(\Delta G)$ has become an issue requiring urgent resolution.

Pantoprazole (PAN) ((*RS*)-6-(difluoromethoxy)-2-[(3,4-dimethoxypyridin-2-yl)methylsulfinyl]-1*H*-benzo[*d*]imidazole) (Fig. 1), a proton pump inhibitor, is administered as a racemic mixture. In the extensive metabolisers, the mean clearance of (*S*)-PAN was only slightly lower than that of (*R*)-PAN and no significant differences were observed between (*R*)- and (*S*)-PAN in the other pharmacokinetic parameters. By contrast, in the poor metabolisers, the clearance values of both enantiomers were significantly lower than those in the extensive metabolisers, and a significant difference in pharmacokinetics between (*R*)- and (*S*)-PAN was observed. The mean elimination half-life for (*R*)-PAN was 3.55 times longer than that of (*S*)-PAN, and the mean maximum concentration and area under the plasma concentration-time curve from 0 to infinity for (*R*)-PAN were respectively 1.31 times and 3.59 times, greater than those for (*S*)-PAN. The metabolism of (*R*)-PAN was impaired to a greater extent than (*S*)-PAN in the poor metabolisers (Tanaka et al., 1997a, 1997b, 2001).

As previously reported, cellulose-based chiral stationary phases in reverse-phase HPLC technology (Tanaka et al., 1995) were developed to separate PAN enantiomers, but with an enantioselectivity α of only 1.26. In preliminary investigations, the chiral separation of PAN by capillary electrophoresis using various cyclodextrins was examined but without success (Eberle et al., 1997). Capillary electrophoresis with protein- and cellulose-based stationary phases was used in HPLC for chiral separations of PAN (Eberle et al., 1997; Haginaka, 2000) as well as supercritical fluid chromatography (Toribio et al., 2005). In this study, in order to improve $-\Delta(\Delta G)$, a new chiral separation technology – biphasic recognition chiral extraction for the separation of PAN enantiomers is presented. As the two chiral extractants are hydrophobic tartrate in the organic phase and hydrophilic β-CD derivative in the aqueous phase, they have the oppositely preferential recognition directions. Biphasic recognition chiral extraction has a much stronger separation capacity than a monophasic system.

Experimental

Pantoprazole (PAN, racemate, purity > 99 %) was purchased from Saen Chemical Co., Ltd. (Shanghai, China). Hydrophilic extractants, hydroxyethyl-β-cyclodextrin (HE-β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD), methyl-β-cyclodextrin (Me-β-CD) and sulphobutyl ether-β-cyclodextrin (SBE-β-CD) were obtained from Shandong Xinda Fine Chemical Co., Ltd. (Shandong, China). Hydrophobic extractants, D- and L-tartaric acid esters ($\geq$ 99 %) were synthesised as described in the literature (Heldin et al., 1991). Octan-1-ol, heptan-1-ol, dichloromethane and 1,2-dichloroethane were purchased from Shanpu & Co., Inc. (Shanghai, China). The solvent used for chromatography was of HPLC grade. All other chemicals were of analytical reagent grade.

The aqueous phase was prepared by dissolving the racemic PAN and β-CD derivatives in a 0.1 M phosphate buffer solution. The organic phase was prepared by dissolving D- and L-tartaric acid derivatives as chiral selectors in organic solvents. Defined volumes of the chiral selector-containing organic phase and PAN/β-CD derivative-containing aqueous phase (each 2 mL) were added to a 10 mL plastic centrifuge tube and shaken sufficiently for 12 h at a constant temperature before being kept in a water bath at 5 ℃ to reach equilibrium. After preliminary phase separation using syringe, the tube was centrifuged to accelerate phase separation. Following the phase separation, the concentrations of PAN enantiomers in the aqueous phase were analysed by HPLC. Since the change in volume is very small, it can be regarded as negligible. The concentrations of PAN enantiomers in the organic phase were calculated from a mass balance.

Quantification of the PAN enantiomers in the aqueous phase was performed by HPLC using a WATERS e2695 apparatus (Waters Co., Ltd.). A UV detector operated at 288 nm was applied. The column was a 5 μm silica gel substrate, 150 mm × 4.6 mm (L × I.D.) coated with diamonsil OJ-RH, cellulose tris (4-methylbenzoate) (Daicel Chemical Industries, Ltd). The mobile phase was a mixture ($\varphi_r = 24 : 76$) of acetonitrile and a 0.1 M aqueous sodium phosphate solution at pH = 5.5. The flow-rate was set at 0.7 mL min^{-1} and the column temperature was set at 35 ℃ (Tang et al., 2006).

Results and discussion

Theory and extraction reaction considerations

To estimate the extraction performance of the extractant, the distribution coefficient (k), enantioselectivity (α) and difference in the Gibbs formation energies ($-\Delta(\Delta G)$ in kJ mol^{-1}) can be calculated by Eq. (1)–(4), where $c_{O,R}$ and $c_{W,R}$ represent the concentration of (*R*)-PAN in the organic phase and aque-

Fig. 2. Diagram showing separation of enantiomers by monophasic recognition chiral extraction with β-CD derivatives in aqueous phase.

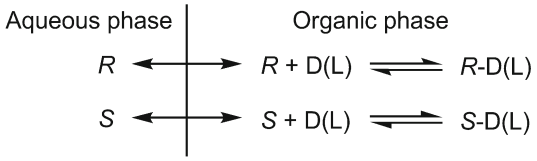

Fig. 3. Diagram showing separation of enantiomers by monophasic recognition chiral extraction with chiral selector in organic phase.

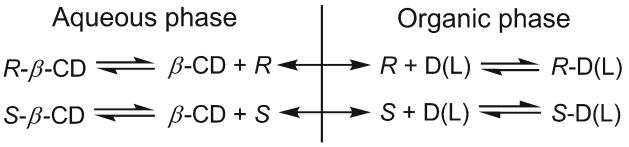

Fig. 4. Diagram showing separation of enantiomers by biphasic recognition chiral extraction.

ous phase, respectively; $c_{O,S}$ and $c_{W,S}$ represent the concentration of (S)-PAN in the organic phase and aqueous phase, respectively; k_R (the distribution coefficient for R-enantiomer; organic/aqueous concentration) and k_S (the distribution coefficient for S-enantiomer; organic/aqueous concentration) represent the distribution ratio of (R)-PAN and (S)-PAN, respectively.

$$k_R = c_{O,R}/c_{W,R} \tag{1}$$

$$k_S = c_{O,S}/c_{W,S} \tag{2}$$

$$\alpha = k_S/k_R \tag{3}$$

$$-\Delta(\Delta G) = RT \ln \alpha \tag{4}$$

In the monophasic recognition chiral extraction system, chiral extraction is carried out by way of the formation of two diastereomeric complexes between (R/S)-enantiomers and chiral selectors existing in either the organic or the aqueous phase, which depends on molecular interactions such as hydrogen-bond, polarisation, induction or electrostatics.

It has been reported that PAN enantiomers can be readily separated in the β-CD stationary phase and β-CD derivatives exhibit a capacity for inclusion with PAN enantiomers. In the monophasic recognition system with only β-CD derivatives as chiral selectors dissolved in the aqueous phase, these derivatives have recognition ability for PAN enantiomers to form with them two diasteromeric complexes (Fig. 2). Furthermore, β-CD derivatives have a better recognition ability for (R)-PAN than for (S)-PAN (Andersson et al., 2007; Redondo et al., 2012; Guan et al., 2008, 2012), which is in accordance with the present results. The driving force for the chiral separation expressed by the difference in $-\Delta(\Delta G)$ between the two diastereomeric complexes in aqueous phase is expressed in Eq. (5). Thus, $-\Delta(\Delta G)_{\beta\text{-CD}} > 0$.

$$-\Delta(\Delta G)_{\beta\text{-CD}} = -\Delta(\Delta G)_{R\text{-}\beta\text{-CD}} - \\ - (-\Delta(\Delta G)_{S\text{-}\beta\text{-CD}}) = RT \ln \alpha_{\beta\text{-CD}} \tag{5}$$

For enantiomers of PAN with the chiral selector dissolved in the organic phase, the separation of (R)-PAN and (S)-PAN is primarily ascribed to the difference in $-\Delta(\Delta G)$ of the two diastereomeric complexes in the organic phase (Fig. 3). It has been reported that D-tartaric acid has a better recognition ability for (S)-PAN than for (R)-PAN (Graul et al., 1999); as a result, $-\Delta(\Delta G)_D > 0$.

$$-\Delta(\Delta G)_D = -\Delta(\Delta G)_{S\text{-}D} - (-\Delta(\Delta G)_{R\text{-}D}) = \\ = RT \ln \alpha_D \tag{6}$$

$$-\Delta(\Delta G)_L = -\Delta(\Delta G)_{S\text{-}L} - (-\Delta(\Delta G)_{R\text{-}L}) = \\ = RT \ln \alpha_L \tag{7}$$

In the biphasic recognition chiral extraction system, the extraction performance is correlated with the recognition of both the β-CD derivative in the aqueous phase and the chiral selector in the organic phase. Clearly, if the β-CD derivative in the aqueous phase and the chiral selector in the organic phase preferentially recognise the (R)- and (S)-enantiomer differently, the separation capability can be greatly improved in the biphasic system (Fig. 4). In this study, both these chiral extractants were added to the aqueous and chiral phases, respectively. Thus, the driving force for the chiral separation of PAN enantiomers in the biphasic recognition chiral extraction system is expressed by Eq. (8) and α for the biphasic system can be calculated using Eq. (9).

$$-\Delta(\Delta G) = -\Delta(\Delta G)_D + (-\Delta(\Delta G)_{\beta\text{-CD}}) = \\ = RT \ln \alpha \tag{8}$$

$$\alpha = \alpha_D \times \alpha_{\beta\text{-CD}} \tag{9}$$

As $-\Delta(\Delta G)_D$ and $-\Delta(\Delta G)_{\beta\text{-CD}}$ are both positive, the driving force for chiral separation $-\Delta(\Delta G)$ is theoretically larger in the biphasic system than in the monophasic system. Therefore, it could be assumed that the biphasic system has a higher separation capability than the monophasic system.

Table 1. Screening of organic solvents[a]

Solvent	k_R	k_S	α	RSD$_\alpha$/%
Octan-1-ol	52.63	55.56	1.06	1.45
Heptan-1-ol	0.085	0.086	1.01	0.99
Dichloromethane	1.98	2.00	1.01	0.98
1,2-Dichloroethane	7.25	7.94	1.10	1.52

a) Conditions: Aqueous phase: [SBE-β-CD derivative] = 0.1 mol L^{-1}, [PAN] = 2 mmol L^{-1}, pH = 5.5; T = 5 °C.

Table 2. Screening of β-CD derivatives[a]

Derivative	k_R	k_S	α	RSD$_\alpha$/%
HE-β-CD	0.0088	0.0093	1.06	1.44
HP-β-CD	5.41	6.12	1.13	1.35
Me-β-CD	0.0016	0.0016	1.00	1.15
SBE-β-CD	7.25	7.94	1.10	1.52

a) Conditions: Aqueous phase: [β-CD derivative] = 0.1 mol L^{-1}, [PAN] = 2 mmol L^{-1}, pH = 5.5; organic phase: 1,2-dichloroethane; T = 5 °C.

Conditions and enantioselectivity

Selecting a proper solvent for chiral extraction could improve the separation capability on account of the solvation. The influence of organic solvents on the distribution behaviour was investigated in various monophasic systems containing 0.1 mol L^{-1} SBE-β-CD in the aqueous phase as the sole extractant (Table 1). This shows that high distribution ratios were obtained but low enantioselectivity was found when octan-1-ol was used as solvent, while, for heptan-1-ol and dichloromethane, SBE-β-CD exhibits the recognition of PAN enantiomers but with poor enantioselectivity. The enantioselectivity and distribution ratios are relatively higher for 1,2-dichloroethane. Therefore, after due consideration, it may be concluded that 1,2-dichloroethane is a proper solvent for the extraction of PAN enantiomers.

β-CD derivatives have a special structure with a hydrophobic cavity and hydrophilic side groups on the cavity rim. The size of the guest determines whether it fits into the cavity, shape and polarity, which influences the possibility of interaction within the cavity or side groups. Whether and to what extent a complex is formed, can be predicted on the basis of size, shape and polarity of the guest molecule and various interactions involving Van der Waals, dispersive forces, dipole-dipole interactions, electrostatic forces and hydrogen bonding. The polarity of β-CD derivatives affects the formation and stability of the complexes of PAN/β-CD derivatives. The difference in polarity eventually leads to the difference in enantioselectivities for PAN enantiomers.

Enantioselectivity and distribution ratios for PAN enantiomers were investigated in several monophasic systems containing four types of β-CD derivatives (HE-β-CD, HP-β-CD, Me-β-CD or SBE-β-CD) in aqueous phase and without chiral selector in 1,2-dichloroethane (Table 2). It can be seen from Table 2 that when HE-β-CD and Me-β-CD were used, both distribution ratios and enantioselectivity were too low to have barely any chiral separation ability for PAN enantiomers which indicates both these two β-CD derivatives have no chiral recognition ability for either of PAN enantiomers. A relatively higher enantioselectivity was obtained for HP-β-CD than for SBE-β-CD and, furthermore, HP-β-CD is more economic

Table 3. Screening of tartaric acid esters[a]

Tartrate	Isomer	k_R (o/w)[b]	k_S (o/w)[b]	α	RSD$_\alpha$/%
Dibutyl	L	31.76	40.10	1.26	1.22
	D	11.42	14.61	1.28	1.19
Diisobutyl	L	13.41	17.27	1.29	1.15
	D	12.89	16.95	1.32	0.90
Dipentyl	L	12.30	15.73	1.28	1.20
	D	13.42	17.41	1.30	0.88
Diisopentyl	L	11.92	14.85	1.25	1.23
	D	11.84	15.29	1.29	1.34
Diheptyl	L	14.98	19.28	1.29	1.18
	D	12.40	16.18	1.30	1.17
Bis(5-methylhexyl)	L	11.93	15.03	1.26	1.84
	D	16.30	20.87	1.28	1.35
Dihexyl	L	12.65	15.75	1.25	1.85
	D	14.73	18.84	1.28	1.19
Cyclohexyl	L	12.44	15.10	1.21	0.83
	D	12.73	15.56	1.22	0.94

a) Conditions: Aqueous phase: [HP-β-CD] = 0.1 mol L^{-1}, [PAN] = 2 mmol L^{-1}, pH = 5.5; organic phase: [tartaric acid ester] = 0.1 mol L^{-1}, 1,2-dichloroethane; T = 5 °C; *b*) o – organic phase, w – aqueous phase.

than SBE-β-CD. Table 2 also shows that k_S are always higher than k_R, which indicates that four β-CD derivatives preferentially recognise (R)-PAN. Therefore, HP-β-CD is attributed to be the most suitable extractant for the extraction experiments with good distribution ratios and enantioselectivity.

The influence of different types of tartaric acid esters was investigated in different extraction systems containing 0.1 mol L^{-1} HP-β-CD in the aqueous phase and 0.1 mol L^{-1} tartaric acid ester in the organic phase to examine the distribution ratios and enantioselectivity for PAN enantiomers (Table 3).

Table 3 shows that enantioselectivity for PAN enantiomers is improved by adding the tartaric acid ester to the organic phase and that the D-tartaric acid derivatives obtained relatively higher enantioselectivity than L-tartaric acid esters, and k_S is higher than k_R, indicating that D-tartaric acid esters have a stronger recognition ability for (S)-PAN than for (R)-PAN. Of the tartaric acid esters investigated, D-diisobutyl tartrate (DIBT) was chosen as the suitable chiral selector in the organic phase. It may also be noted that, by changing the monophasic chiral recognition system into the biphasic system, the enantioselectivity could be increased to 1.32.

pH plays an important part in the biphasic chiral recognition system as it affects the state of PAN enantiomers, which have a pK_a value of 28.91. The distribution ratios and enantioselectivity were examined in the biphasic system with 0.1 mol L^{-1} HP-β-CD in the aqueous phase and 0.1 mol L^{-1} DIBT in 1,2-dichloroethane at different pH values, as is shown in Fig. 5. It is clear that both distribution ratios and enantioselectivity decrease with an increase in pH.

This may be due to the fact that HP-β-CD mainly has a chiral recognition ability and affinity for molecular PAN but not for ionic PAN. At low pH values, PAN enantiomers are not stable in the aqueous phase, and HP-β-CD has scarcely any recognition ability for the ionic PAN. Accordingly, the distribution ratios are relatively high at lower pH values. The increase in pH decreases the dissociation of PAN enantiomers and renders them stable; most PAN enantiomers exist as molecules in the aqueous phase, facilitating their recognition by HP-β-CD so that more enantiomers are able to bind with HP-β-CD. The experimental data indicate that the distribution ratios and enantioselectivity result from the cooperation combined action of HP-β-CD and DIBT. As a result, the low pH values appeared to be correct. However, the distribution ratios at low pH values were too large for this extraction process, and PAN enantiomers are not stable at low pH. After due consideration, it was concluded that the pH value should be maintained at 8.5 to perform the extraction process.

PAN enantiomers can form two diastereomeric complexes with HP-β-CD; this not only enhances their solubility in the buffer solution but also improves the

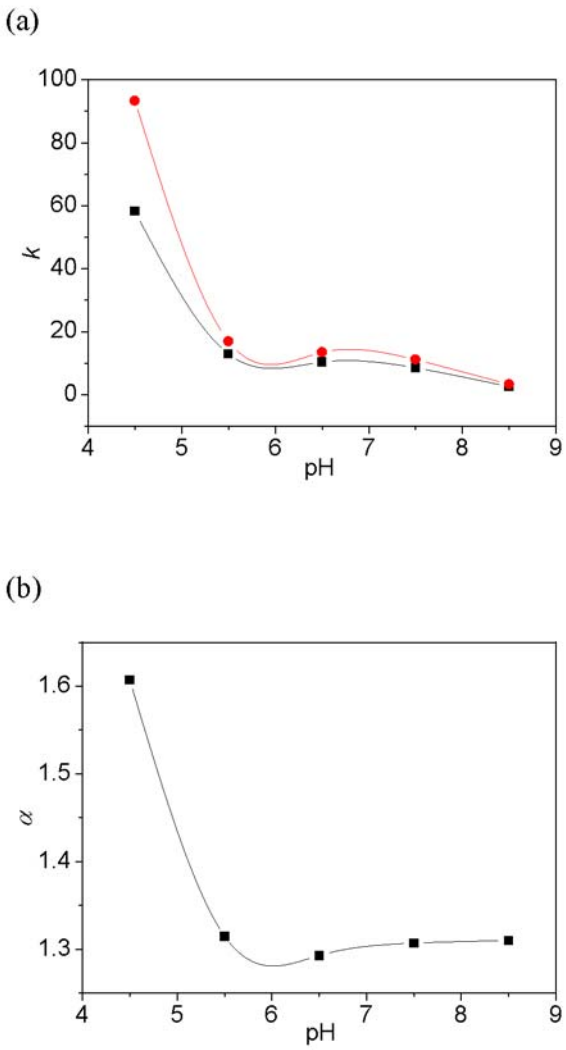

Fig. 5. Influence of pH on distribution ratios k_R (■) and k_S (●) (a) and enantioselectivity α (b). Conditions: Aqueous phase: [HP-β-CD] = 0.1 mol L^{-1}, [PAN] = 2 mmol L^{-1}; organic phase: [DIBT] = 0.1 mol L^{-1}, 1,2-dichloroethane; T = 5 °C.

enantioselectivity for PAN. The change in concentration of HP-β-CD may affect the distribution ratios and enantioselectivity. These two important parameters were investigated and the results are shown in Fig. 6. It may be concluded that distribution ratios and enantioselectivity decrease markedly with the increase in the concentration of HP-β-CD. Enantioselectivity first increases up to a point where the concentration attains 40 mmol L^{-1} and then decreases with any further increase in the concentration of HP-β-CD.

As the chiral selector in the organic phase, the concentration of DIBT has a large influence on the distribution ratios and enantioselectivity. Extraction experiments were performed with varying concentrations of DIBT from 0.025 mol L^{-1} to 0.4 mol L^{-1}, as presented in Fig. 7, showing that distribution ratios and enantioselectivity are greatly enhanced with the increase in

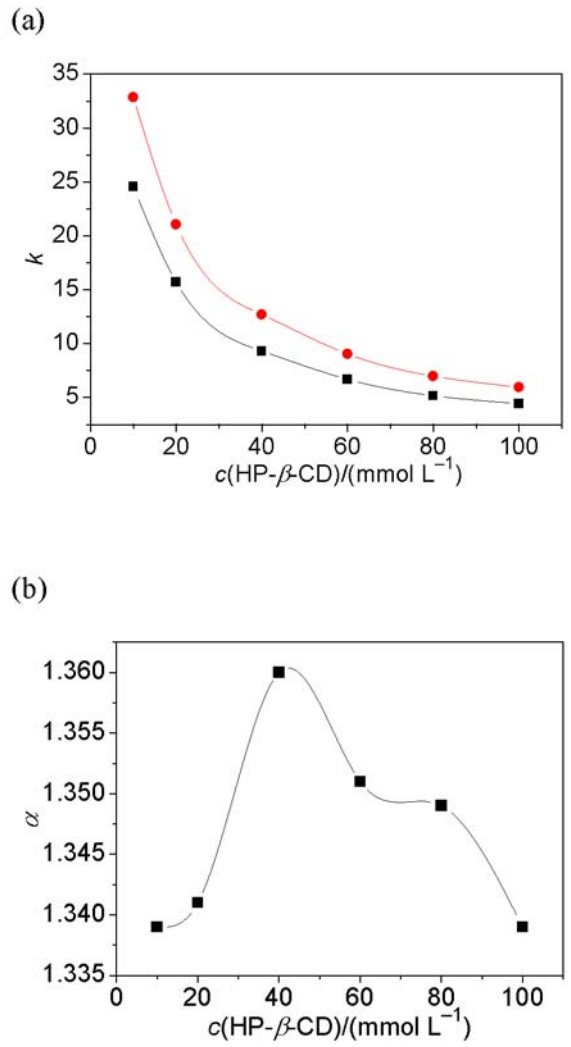

Fig. 6. Influence of concentration of HP-β-CD on distribution ratios k_R (■) and k_S (●) (a) and enantioselectivity α (b). Conditions: Aqueous phase: [PAN] = 2 mmol L^{-1}, pH = 8.5; organic phase: [DIBT] = 0.1 mol L^{-1}, 1,2-dichloroethane; $T = 5$ °C.

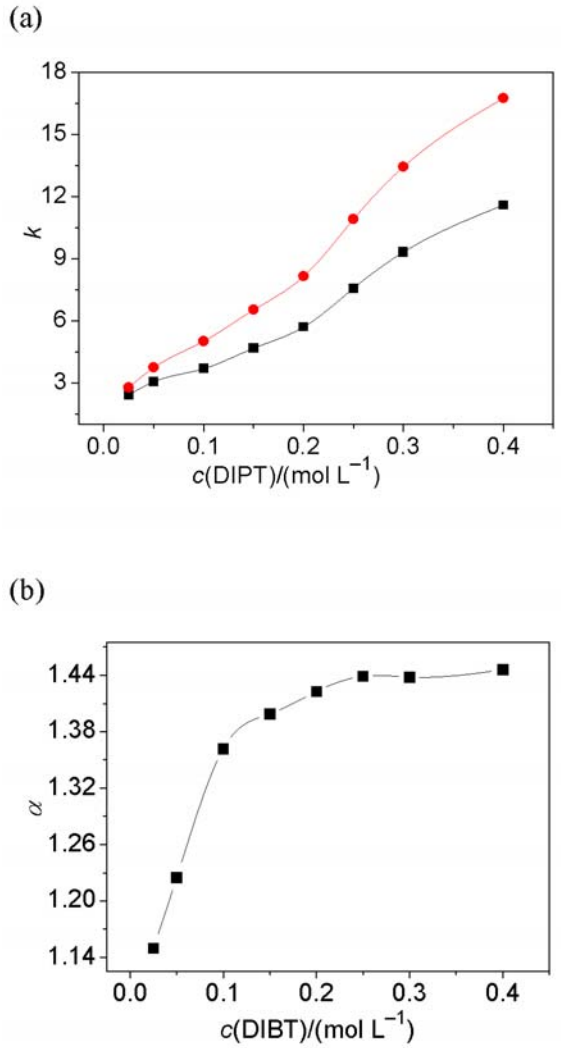

Fig. 7. Influence of concentration of DIBT on distribution ratios k_R (■) and k_S (●) (a) and enantioselectivity α (b). Conditions: Aqueous phase: [HP-β-CD] = 0.1 mol L^{-1}, [PAN] = 2 mmol L^{-1}, pH = 8.5; organic phase: 1,2-dichloroethane; $T = 5$ °C.

DIBT concentration and then attain a plateau at 0.2 mol L^{-1} of the concentration. This can be explained by the larger amount of complexes formed in the organic phase and the combined action of HP-β-CD and DIBT, which results in an increase in the distribution ratios.

A further important factor in enantioselective separation is the concentration of PAN enantiomers. To better understand the influence of PAN concentration on the distribution behaviour of PAN enantiomers, the distribution ratios and enantioselectivity were studied and the results are presented in Fig. 8. It may be concluded that initially the distribution ratios decrease rapidly when the PAN concentration rises to 10 mmol L^{-1}, then the decrease slows until it attains a plateau. On the other hand. enantioselectivity is relatively higher at low concentration then decreases rapidly above the concentration of 12 mmol L^{-1}. This may be due to the fact that, at low concentration, most extraction takes place through enantioselective complexation, while at higher concentrations more non-selective partition occurs.

The distribution ratios and the enantioselectivity were examined in the biphasic system with a range of 5–30 °C to study the influence of temperature on the distribution characteristics of PAN enantiomers. Table 4 shows that a higher temperature leads to an increase in distribution ratios and to a decrease in enantioselectivity. This phenomenon indicates that the non-selective partition increases and the amount of complex formed with HP-β-CD decreases with the effect of the temperature, while a decrease in enantioselectivity can be caused by the selector-enantiomer interaction weakening so that the recognition ability of

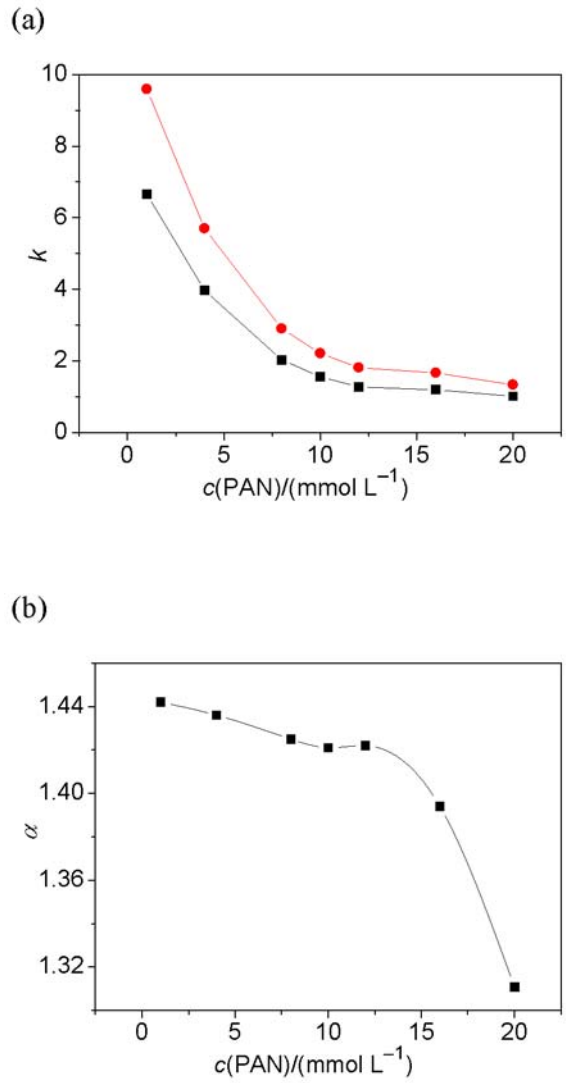

Fig. 8. Influence of concentration of PAN on distribution ratios k_R (■) and k_S (●) (a) and enantioselectivity α (b). Conditions: Aqueous phase: [HP-β-CD] = 0.1 mol L^{-1}, pH = 8.5; organic phase: [DIBT] = 0.2 mol L^{-1}, 1,2-dichloroethane; T = 5 °C.

Table 4. Influence of temperature (T) on distribution ratios (k_R, k_S) and enantioselectivity (α)[a]

T/°C	k_R	k_S	α	RSD$_\alpha$/%
5	1.56	2.22	1.42	0.97
10	1.84	2.56	1.39	1.29
15	2.26	3.06	1.36	1.04
20	2.52	3.36	1.33	1.12
25	3.06	4.04	1.32	0.85
30	4.07	5.18	1.27	0.93

[a]) Aqueous phase: [PAN] = 10 mmol L^{-1}, [HP-β-CD] = 0.1 mol L^{-1}, pH = 8.5; organic phase: [DIBT] = 0.2 mol L^{-1}, 1,2-dichloroethane.

the selectors also weakens with an increased temperature.

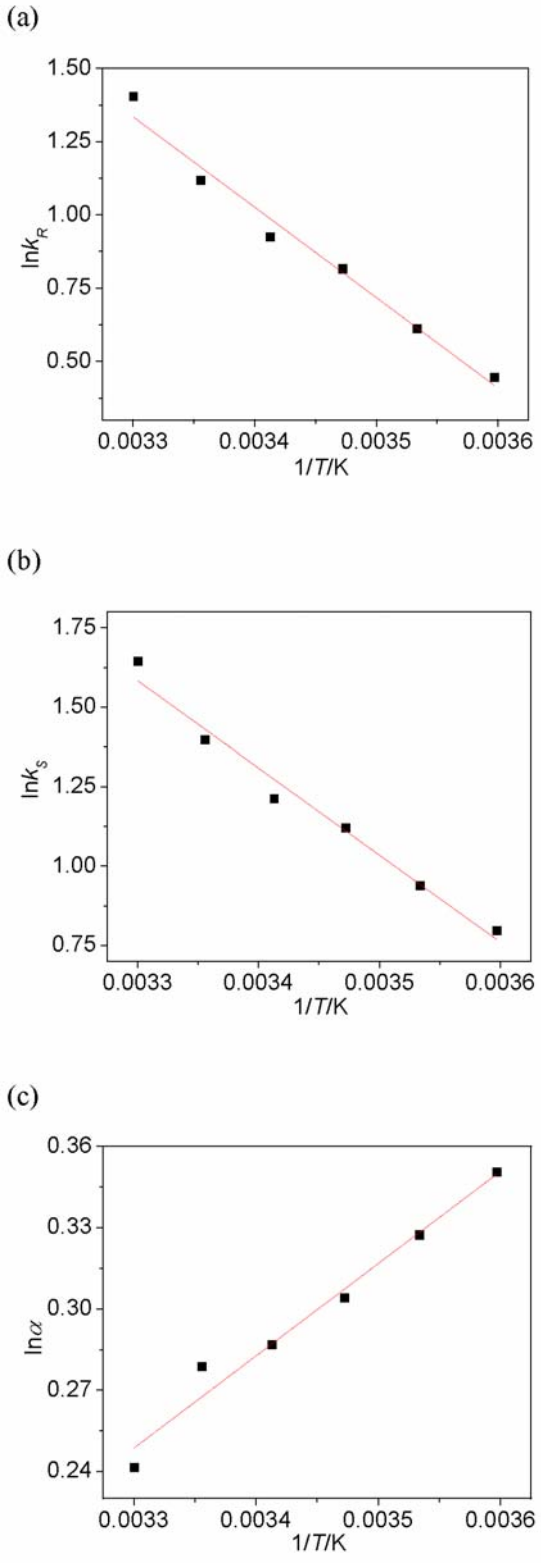

Fig. 9. Influence of temperature on distribution ratios k_R (a) and k_S (b) and enantioselectivityα (c) of PAN; for (a): lnk_R = 11.51576 – 30.85.15271/T, r = 0.990; for (b): lnk_S = 10.63904 – 2744.15886/T, r = 0.990; for (c): lnα = –0.87673 + 340.99385/T, r = 0.987. Conditions: Aqueous phase: [PAN] = 10 mmol L^{-1}, [HP-β-CD] = 0.1 mol L^{-1}, pH = 8.5; organic phase: [DIBT] = 0.2 mol L^{-1}, 1,2-dichloroethane.

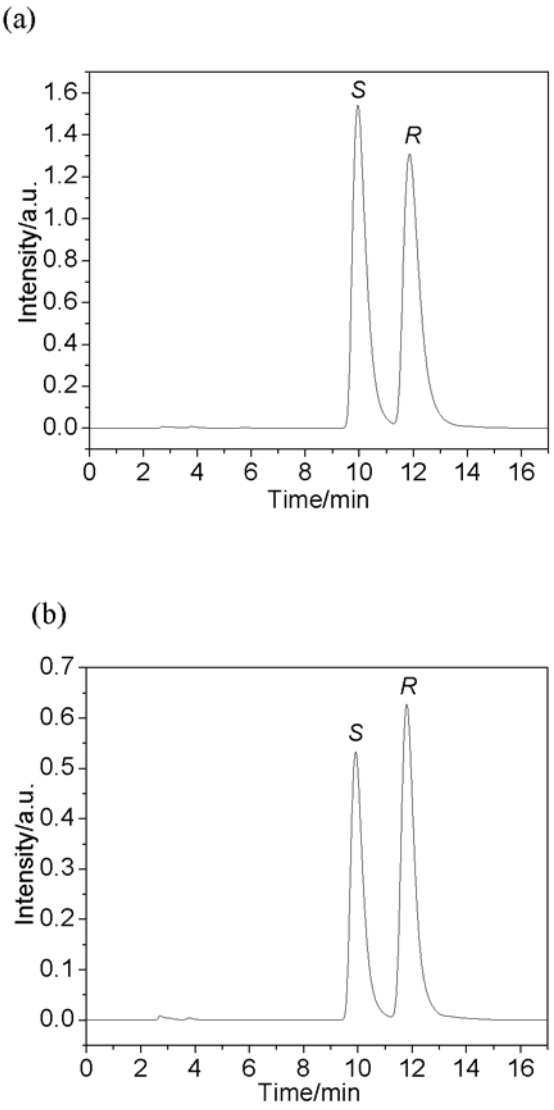

Fig. 10. Chromatograms of PAN enantiomers prior to (a) and post-extraction (b).

Fig. 9 shows the variations of $\ln k$ and $\ln\alpha$ with $1/T$; these are in good agreement with the van't Hoff model, indicating that the complex does not change its conformation over the temperature range studied.

Fig. 10 shows the chromatograms of PAN enantiomers prior to and post-extraction. The extraction conditions are given above. It was found that the enantiomeric excess (*ee*) of (*S*)-PAN in the organic phase attained 17.46 % by one-stage extraction; k_R, k_S, and α are 2.22, 1.56 and 1.42, respectively.

Conclusions

The biphasic recognition chiral extraction for the separation of PAN enantiomers is presented as a new chiral separation technique based on the addition of hydrophilic β-CD and hydrophobic tartrate esters with different preferential recognition ability to the aqueous and organic phase, respectively. The separation ability of the biphasic system is ascribed to the combined action of tartrate esters and the β-CD derivative. Accordingly, the biphasic system has a stronger chiral separation ability than the monophasic system since the enantioselectivity for PAN enantiomers was improved.

Distribution ratios and enantioselectivity were used to evaluate the performance of the reactive extraction. The results reveal that HP-β-CD has the strongest chiral recognition ability towards (*R*)-PAN rather than (*S*)-PAN out of the four β-CDs tested in this paper, while DIBT preferentially recognised (*S*)-PAN over (*R*)-PAN. The most suitable solvent was found to be 1,2-dichloroethane, with the best conditions identified involving the HP-β-CD concentration of 100 mmol L^{-1}, DIBT concentration of 0.2 mol L^{-1}, PAN concentration of 10 mmol L^{-1}, pH value of 8.5 and a temperature of 5 °C. Furthermore, a complete/total separation of PAN enantiomers could be achieved by multi-stage step extraction.

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