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Chiral separation of amino-alcohols using extractant impregnated resins

Katarina Babić *, G.H.M. Driessen, A.G.J. van der Ham, A.B. de Haan

Separation Technology Group, Faculty of Science and Technology, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands

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Abstract

The performance of extractant impregnated resin (EIR) technology for chiral separation of amino-alcohols has been investigated. Phenylglycinol was selected as an archetype model enantiomer and azophenolic crown ether was used as a versatile enantioselective extractant. 1-Phenyloctane was selected as a suitable solvent for this application because of its very low solubility in water. The extraction system was first evaluated by liquid–liquid equilibrium experiments. It was shown that crown ether dissolved in 1-phenyloctane has an intrinsic selectivity of 11.5. However, due to very low solubility of phenylglycinol in 1-phenyloctane, the overall capacity of the crown ether solution in 1-phenyloctane is limited. The extractant solution was immobilized in macroporous polypropylene particles. Competitive sorption isotherms were obtained from batch experiments and successfully described with a predictive model based on the complexation constants and partitioning ratios, either obtained from literature or from independent experiments. The equilibrium selectivity of these EIRs approaches the intrinsic selectivity for low phenylglycinol concentrations. The dynamic behaviour and stability of the system were examined in column experiments. Breakthrough profiles as well as the elution curves of the *R* enantiomer are less sharp than those of the *S* enantiomer proving that the *R* enantiomer is strongly retained on the column. Separation of phenylglycinol enantiomers is favoured by using lower feed flow rates. The column was regenerated by water with only atmospheric carbon dioxide dissolved which proved to be sufficient. After several cycles the breakthrough profiles remain unchanged suggesting that these EIRs will be sufficiently stable.

Keywords: Extractant impregnated resins; Chiral separation; Amino-alcohols; Crown ether

1. Introduction

Optically pure substances, used as raw materials, intermediates and very often as the end products, are very important for the (pharmaceutical and related) life science industries. To obtain optically pure products it is, in most of the cases, necessary to separate racemic mixtures. On industrial scale this is usually done by either crystallisation or by chromatography. Crystallisation is generally considered as inflexible and thus its development for each new racemic mixture is quite time consuming [1]. This process is also relatively slow, difficult to control and requires complicated handling of solids/slurries. Chromatography is more flexible than crystallisation because only a relatively low selectivity is required due to a large number of theoretical stages present, indicating that the same column material can be used to separate several enantiomers. However, existing specific enantioselective adsorbents are very expensive and have a low capacity [2].

In this work we propose extractant impregnated resin (EIR) technology, the concept introduced by Warshawsky [3], as a possible alternative. It is a synergistic combination of adsorption and reactive extraction that combines high selectivity and capacity with simple equipment and operation. This concept is based on the incorporation of an enantioselective reagent into a porous particle by physical impregnation (Fig. 1). When contacted with a racemic mixture the reagent preferentially forms a complex with one of the enantiomers. The formed complex as well as the reagent, remains in the resin phase as they are insoluble in the other phase. The reaction should be reversible but sufficiently strong to increase the enantiomer's affinity for the organic phase by several orders of magnitude to obtain an economically feasible process. In comparison with conventional enantioselective adsorbents, it is expected that a chiral reagent in free solution can be present at a larger concentration than only on a surface, resulting in a higher capacity of the reagent phase. Furthermore, the costs

* Corresponding author. Tel.: +31 53 489 2921; fax: +31 53 489 4821.
E-mail address: k.babic@tnw.utwente.nl (K. Babić).

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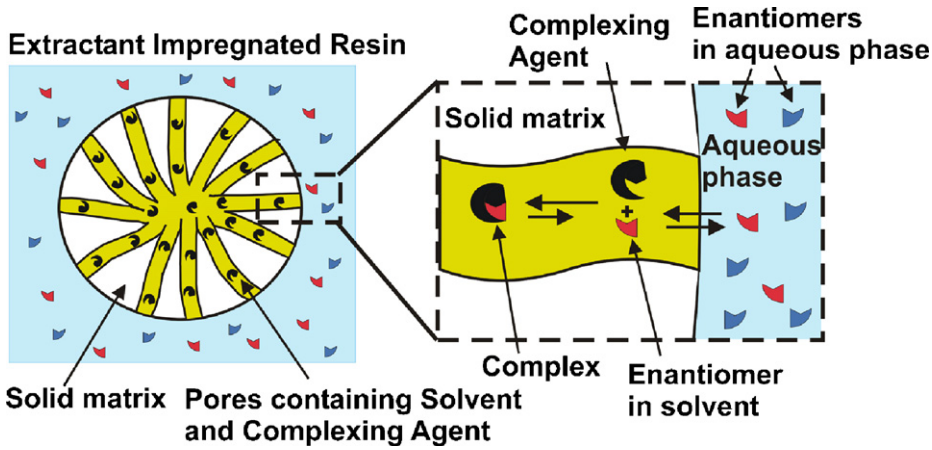

Fig. 1. Extractant impregnated resins.

of this way prepared enantioselective particles are considerably lower.

Extractant impregnated resins have been extensively studied for removal of traces of metals from aqueous solutions [3–5]. There have been reports of using EIRs for recovery of organic compounds like phenols [6], flavonoids [7], carboxylic [8–11] and amino acids [12,13], aldehydes [14] and antibiotics [15]. However, according to our knowledge, this technique has not yet been applied for separation of enantiomers.

The objective of this research was to evaluate the use of EIRs for chiral separations. Phenylglycinol (Fig. 2a) was selected as an archetype model enantiomer. A versatile enantioselective extractant was selected, so the EIR technology can be applied to separate several components from the same chemical class with sufficient enantioselectivity.

In the field of chromatography for enantiomer separation various chiral stationary phases based on different chiral crown ethers have been known to be very effective in resolving racemic components containing primary amino group [16–23]. In these chiral stationary phases, chiral crown ethers are mainly covalently bonded to polystyrene or silica gel. Also, some supported liquid membrane applications of crown ethers have been reported in literature [16–29]. Although this technique can be considered similar to EIR its major drawback is that the selectivity is strongly depending on the permeation rate of the complexes inside the membrane, not on the distribution ratio. As a consequence, the data obtained from liquid–liquid extraction cannot be straightforwardly used in liquid membrane separation processes [5].

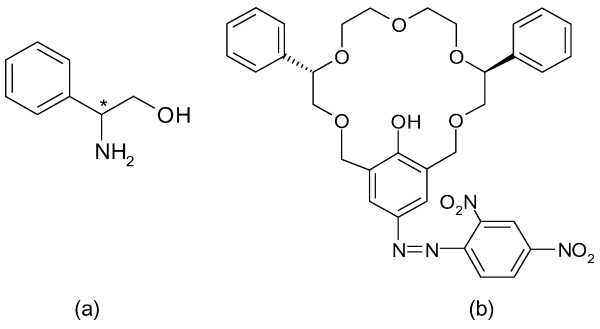

Fig. 2. Stuctures of (a) phenylglycinol (*indicates the chiral center), and (b) azophenolic crown ether.

In the work of Steensma et al. [30,31] an azophenolic crown ether, presented in Fig. 2b (in the rest of text just crown ether), dissolved in toluene was identified as a promising extractant for the chiral separation of amines and amino-alcohols. However, toluene is not an environmentally benign solvent and due to its high volatility and high solubility in water not suitable for this application. Thus, as an alternative for toluene, 1-phenyloctane was selected. The selection of an alternative solvent was made according to the recommendations given by Steensma et al. [31]. Since the solvent has a large influence on the enantioselective performance of the crown ether, a new extraction system had to be evaluated. The extraction system was characterised by liquid–liquid equilibrium experiments. Afterwards, the crown ether dissolved in 1-phenyloctane was immobilized into a porous particle. Competitive sorption equilibrium isotherms were obtained from batch experiments. It was investigated if sorption equilibria can be successfully predicted from the complexation constants and partitioning ratios, which can be obtained from literature or from independent liquid–liquid equilibrium experiments. The predictive model was based on the equilibrium liquid–liquid model already developed [31]. This model was first extended to include the influence of the absorption of atmospheric carbon dioxide, then applied to predict sorption equilibria and experimentally verified. It was also tested if physical impregnation reduces the enantioselectivity like it is the case with covalent bonding [32]. Finally, the dynamic behaviour and stability of the system were examined in column experiments.

2. Experimental

2.1. Reagents

The *R*-phenylglycinol (99%) was purchased from Fluka (Buchs, Switzerland), while the *S*-phenylglycinol (98%) was obtained from Lancaster (Eastgate, England). The racemic mixture of phenylglycinol was made by dissolving equal amounts of

pure enantiomers in MilliQ-water (Millipore, Billerica, USA). The azophenolic crown ether was manufactured by Syncom BV (Groningen, the Netherlands) in a custom synthesis [25]. Toluene (99%) and 1-phenyloctane (>98%) were obtained from Merck (Darmstadt, Germany). NaOH was purchased from Aldrich. All chemicals were used as received. Macroporous polypropylene (MPP) particles were donated by Akzo Nobel, the Netherlands. They are white cylindrically shaped granules with a particle size of 0.8–1.18 mm, and estimated pore size of 500–1000 nm and a porosity of 0.55.

For analytical purpose o-phtalic aldehyde (OPA), methanol, absolute ethanol, mercapto-ethanol (MCE), perchloric acid and boric acid were purchased from Merck (Darmstadt, Germany), potassium hydroxide from Riedel-De Haën and L-norvaline from Sigma. All chemicals were of analytical grade.

2.2. Analytical method

The aqueous enantiomer samples were analysed by HPLC. The HPLC set-up and the analytical method were described in detail in the paper of Steensma et al. [31], with the only difference that in this research the quantification of phenylglycinol was done with the internal standard L-norvaline. The accuracy of the applied analytical method was determined to hold within 3% which was sufficient for most of the experiments.

2.3. Liquid–liquid equilibria

All liquid–liquid experiments were carried out in a jacketed 50 ml glass vessel connected to a Julabo F-32 thermostat bath to enable temperature control. In each experiment, 15 ml of aqueous phase, containing the racemic mixture in a concentration of 2 mM, was mixed with a 15 ml crown ether solution in 1-phenyloctane. The concentration of the crown ether in the organic solvent was varied throughout the experiments. Sufficient contact between the phases was achieved with a magnetic stirrer at a speed of approximately 400 rpm. After, typically 2 h of stirring, when equilibrium is reached, the phases were allowed to settle. Then a sample was taken from the aqueous phase, pH was measured and the sample was analysed by HPLC. The concentration in the organic phase was calculated from the mass balance.

To determine the physical partitioning ratio the same experiment was carried out at a higher pH without the crown ether present. The pH was adjusted to 11.5 by adding NaOH. The higher pH as well as the ratio of aqueous to organic phase of 1:10 was necessary because of the very low partitioning ratio, resulting in a very small difference between the initial and the end concentration of enantiomers in the aqueous phase. Since in that case the experimental error becomes substantial, the measurement was repeated 10 times and a value was given as a range of values obtained from these measurements.

2.4. Determination of complexation constants

Complexation constants between crown ether and *R*- and *S*-phenylglycinol in 1-phenyloctane were determined by UV–vis titration [33]. The experimental procedure follows the one described by Steensma et al. [31]. For each titration experiment a series of samples is prepared containing a constant initial concentration of crown ether of 0.015 mM. The concentration ranges for the *R* and *S* enantiomer were 0.019–0.080 and 0.1–1.3 mM, respectively, in order to assure the complexation range span of 20–80%. Data treatment was done with both the Scot plot [34] and the Rose-Drago plot [35]. For the *R* enantiomer the Scott method could not be applied due to the high complexation constant and the minimum initial concentration of crown ether needed for detection by the UV-vis spectrophotometer. For all the experimental data given, $K\,[CE] < 1$, and therefore these results can be considered as reliable.

2.5. Impregnation procedure

Before impregnation MPP particles were first cleaned with acetone and n-hexane and then dried under vacuum at 60 °C. The dry resin was then contacted with a pre-calculated amount of the crown ether in 1-phenyloctane solution to assure full loading. The saturation point of the resin was previously determined by gradual adding of the solution until the point where the resin becomes adhesive. The exact loading of the resin with extractant was calculated from the increase of mass. Afterwards, EIRs were left typically overnight to assure complete adsorption of the extractant solution. It is assumed that the solution is completely adsorbed when no resin sticks to the glass wall anymore. EIRs were also characterised by density, measured with a helium pycnometer (AccuPyc 1330, Micromeritics).

2.6. Sorption equilibria

Batch adsorption experiments were used to determine the equilibrium isotherms. In each experiment EIRs and an aqueous solution of racemic phenylglycinol (7 ml) were placed in a 50 ml glass-stoppered flask and shaken at 200 rpm and 25 °C for at least 6 h using the thermostated shaking bath (Julabo Shake Temp SW23). Independent experiments showed that 6 h was sufficient to reach equilibrium. The amounts of EIRs (range 0.1–0.75 g) and the concentration of racemic phenylglycinol in water (range 0.5–7 mM) were varied throughout the experiments. Finally, a sample of the aqueous phase was removed using a 10-ml syringe fitted with a filter (Schleicher&Schuel, Spartan 30/0,45 RC), and transferred to a 10-ml amber vial. pH was measured and the concentration of both *R*- and *S*-phenylglycinol were determined by HPLC. Preparation and analysis of the samples were the same as for the liquid–liquid experiments. The amount of *R*- and *S*-phenylglycinol in the EIR particles was calculated from the mass balance for each enantiomer.

2.7. Column experiments

Continuous experiments were performed on a column with a length of 30 cm and internal diameter of 1.595 cm (Superformance 300-16, G"tec, Muehtal, Germany). It was equipped with a water jacket connected to a circulating water bath at 25 °C. Besides the column, the set-up consisted of a solvent organizer

(K-1500, Knauer, Berlin, Germany), degasser (K-5020, Knauer, Berlin, Germany) and a pump (K-1001, Knauer, Berlin, Germany). UV-vis spectrophotometer (K-2600, WellChrom, Germany) was used to monitor the column effluents. The detector acquired data at 254 nm where phenylglycinol shows an absorption maximum. Since the UV detector cannot make a difference between *R* and *S* enantiomer, samples were taken at regular intervals and analysed with HPLC. For the breakthrough experiments column was packed with 24.29 g of fresh EIRs. A 0.5 mM racemic phenylglycinol solution in water was pumped through the column with linear flow rates in range of 75.12–225 cm h^{-1}. Regeneration of the EIRs was done by pumping the MilliQ-water with dissolved atmospheric carbon dioxide through the column until the UV signal was zero.

3. Results and discussion

3.1. Liquid–liquid equilibria

3.1.1. Model description

A schematic representation of the reactive liquid–liquid system studied is given in Fig. 3. As follows from this scheme, the reactive system can be described by a set of physical and chemical equilibrium equations and mass balances.

Physical partitioning of the neutral form of both enantiomers between the phases is the same. It is characterised by the partitioning ratio *P* defined as

$$P = \frac{C_R^{\mathrm{org}}}{C_R^{\mathrm{aq}}} = \frac{C_S^{\mathrm{org}}}{C_S^{\mathrm{aq}}} \tag{1}$$

where *C* stands for concentration in mol/l, while superscripts aq and org denote aqueous and organic phase, respectively. Subscripts *R* and *S* indicate the enantiomer.

The acid–base equilibrium in the aqueous phase is characterised by the dissociation constant K_A which is also the same for both enantiomers.

$$K_A = \frac{C_R^{\mathrm{aq}} C_{H^+}^{\mathrm{aq}}}{C_{HR^+}^{\mathrm{aq}}} = \frac{C_S^{\mathrm{aq}} C_{H^+}^{\mathrm{aq}}}{C_{HS^+}^{\mathrm{aq}}} \tag{2}$$

where the subscript H^+, HR^+ and HS^+ refers to the corresponding species.

Reversible complexation in the organic phase between crown ether and the neutral amine form of the enantiomers is described by equilibrium complexation constants, K_R and K_S for the *R* and *S* enantiomer, respectively.

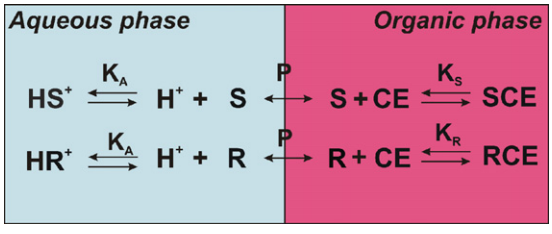

Fig. 3. Schematic representation of the equilibrium reactions in the system.

$$K_R = \frac{C_{RCE}^{\mathrm{org}}}{C_R^{\mathrm{org}} C_{CE}^{\mathrm{org}}} \tag{3}$$

$$K_S = \frac{C_{SCE}^{\mathrm{org}}}{C_S^{\mathrm{org}} C_{CE}^{\mathrm{org}}} \tag{4}$$

CE stands for the crown ether and *R*CE and *S*CE represent the corresponding complexes.

Mass balances for all species present can be written as follows

$$(C_{S,\mathrm{o}}^{\mathrm{aq}} - (C_S^{\mathrm{aq}} + C_{HS^+}^{\mathrm{aq}}))V^{\mathrm{aq}} = (C_S^{\mathrm{org}} + C_{SCE}^{\mathrm{org}})V^{\mathrm{org}} \tag{5}$$

$$(C_{R,\mathrm{o}}^{\mathrm{aq}} - (C_R^{\mathrm{aq}} + C_{HR^+}^{\mathrm{aq}}))V^{\mathrm{aq}} = (C_R^{\mathrm{org}} + C_{RCE}^{\mathrm{org}})V^{\mathrm{org}} \tag{6}$$

$$C_{CE,\mathrm{o}}^{\mathrm{org}} = C_{CE}^{\mathrm{org}} + C_{SCE}^{\mathrm{org}} + C_{RCE}^{\mathrm{org}} \tag{7}$$

With subscript o refers to the initial concentration of *S* and *R*.

The overall distribution ratios D_R and D_S are defined as the ratio of enantiomer concentration in the organic phase in all its forms over its concentration in the aqueous phase in all its forms at equilibrium.

$$D_R = \frac{C_{\mathrm{total}R}^{\mathrm{org}}}{C_{\mathrm{total}R}^{\mathrm{aq}}} = \frac{C_R^{\mathrm{org}} + C_{RCE}^{\mathrm{org}}}{C_R^{\mathrm{aq}} + C_{HR^+}^{\mathrm{aq}}} \tag{8}$$

$$D_S = \frac{C_{\mathrm{total}S}^{\mathrm{org}}}{C_{\mathrm{total}S}^{\mathrm{aq}}} = \frac{C_S^{\mathrm{org}} + C_{SCE}^{\mathrm{org}}}{C_S^{\mathrm{aq}} + C_{HS^+}^{\mathrm{aq}}} \tag{9}$$

The operational selectivity α_{op} is defined as the ratio of distribution ratios (Eq. (10)).

$$\alpha_{\mathrm{op}} = \frac{D_R}{D_S} \tag{10}$$

The selectivity reaches an asymptotic value for high extractant concentrations. This asymptotic value is called the intrinsic selectivity α_{int} and represents the ratio of complexation constants.

$$\alpha_{\mathrm{int}} = \frac{K_R}{K_S} \tag{11}$$

It was already shown by Steensma et al. [31] that this model predicts the extraction equilibria of phenylglycinol with crown ether dissolved in toluene satisfactorily. The model is especially successful if the uptake of CO_2 from the air and the subsequent lowering of the pH is taken into account. To calculate the solubility of CO_2 in water Henry's law can be applied

$$C_{CO_2}^{\mathrm{aq}} = \frac{p_{CO_2}}{He_{CO_2}} \rho_{\mathrm{w}} \tag{12}$$

where p_{CO_2} denotes the partial pressure of CO_2, He_{CO_2} is Henry's coefficient and ρ_{w} is the molar density of water. Dissociation equilibria of carbonic acid are characterised by dissociation constants K_{A1}, K_{A2} and K_{A3} (Eqs. (13)–(15)).

$$K_{A1} = \frac{C_{H_2CO_3}^{\mathrm{aq}}}{C_{CO_2}^{\mathrm{aq}}} \tag{13}$$

$$K_{A2} = \frac{C^{aq}_{HCO_3^-} C^{aq}_{H^+}}{C^{aq}_{H_2CO_3}} \quad (14)$$

$$K_{A3} = \frac{C^{aq}_{CO_3^{2-}} C^{aq}_{H^+}}{C^{aq}_{HCO_3^-}} \quad (15)$$

Electroneutrality condition can now be written as

$$C^{aq}_{OH^-} + 2C^{aq}_{CO_3^{2-}} + C^{aq}_{HCO_3^-} = C^{aq}_{H^+} + C^{aq}_{HS^+} + C^{aq}_{HR^+} \quad (16)$$

All the parameters of the model can be determined from independent measurements or obtained from literature. Complexation equilibrium constants K_R and K_S were determined by UV–vis titration, the physical partitioning ratio was determined by equilibrium experiment without crown ether present, while dissociation constant of phenylglycinol (pK_A of 8.5 [36]) and CO_2 absorption parameters [37,38] were taken from literature.

3.1.2. Partitioning ratio

The partitioning ratio of phenylglycinol between the aqueous and organic phase strongly depends on the pH of the system. At low pH, phenylglycinol is protonated and as such not soluble in the organic phase. Therefore, the physical partitioning ratio can only be measured at high pH. Since the overall partitioning effect is a result of partitioning of phenylglycinol in neutral form (P_{am}) and charged ammonium form (P_{ammo}), it can be represented as a linear combination of mentioned effects

$$P_{exp} = x_{ammo} P_{ammo} + x_{am} P_{am} \quad (17)$$

where mole fractions x depend on the pH of the system and the pK_A of phenylglycinol. The partitioning of the ammonium form can be neglected and therefore the Henderson–Hasselbach equation [39] for amines can be used to describe the resulting effective partitioning P_{eff} as a function of pH.

$$P_{eff} = x_{am} P_{am} \equiv x_{am} P = P\left(1 - \frac{1}{1 + 10^{pH - pK_A}}\right) \quad (18)$$

In this research, P was measured at a pH of 11.5 and an aqueous to organic volume ratio of 1:10. The value of P was found to be in the range of 0.02–0.04. More accurate determination was not possible due to inaccuracy of the applied analytical method. For the modelling of the liquid–liquid equilibrium the average value of 0.03 was used.

3.1.3. Complexation constants

The typical UV–vis titration experiment for determination of complexation constants between crown ether and phenylglycinol is shown in Fig. 4. As the concentration of phenylglycinol increases, the peak at 550 nm, which represents the complex, gets higher, while the peak at 400 nm, characteristic for uncomplexed crown ether, gets lower. The same procedure was repeated for both enantiomers. The calculated complexation constants are $3.3 \pm 0.2 \times 10^3$ and $38.1 \pm 2.5 \times 10^3$ for the S and R enantiomer, respectively, which gives an intrinsic selectivity of 11.5.

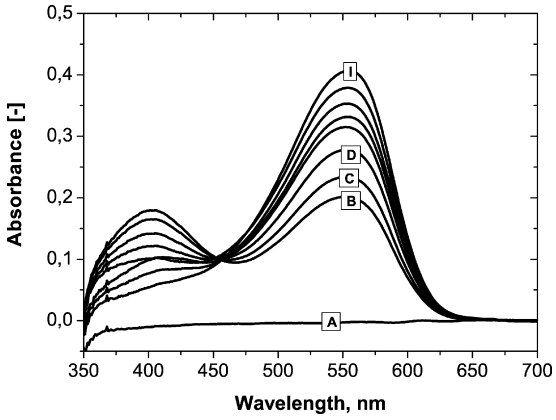

Fig. 4. UV–vis titration experiment for determination of the complexation constants between the crown ether and the *S* enantiomer in 1-phenyloctane at 25 °C; for all samples: 0.015 mM CE, variable concentration of *S* enantiomer. (A) baseline (pure water), (B) 0.10 mM, (C) 0.17 mM, (D) 0.31 mM, (E) 0.42 mM, (F) 0.53 mM, (G) 0.71 mM, (H) 0.90 mM, (I) 1.32 mM.

3.1.4. Influence of the extractant concentration – experimental results and model evaluation

The influence of the crown ether concentration on the extraction performance has been studied at 25 °C with no pH adjustment. The calculated distribution ratios D and operational selectivity α_{op} are presented in Fig. 5. It also shows that

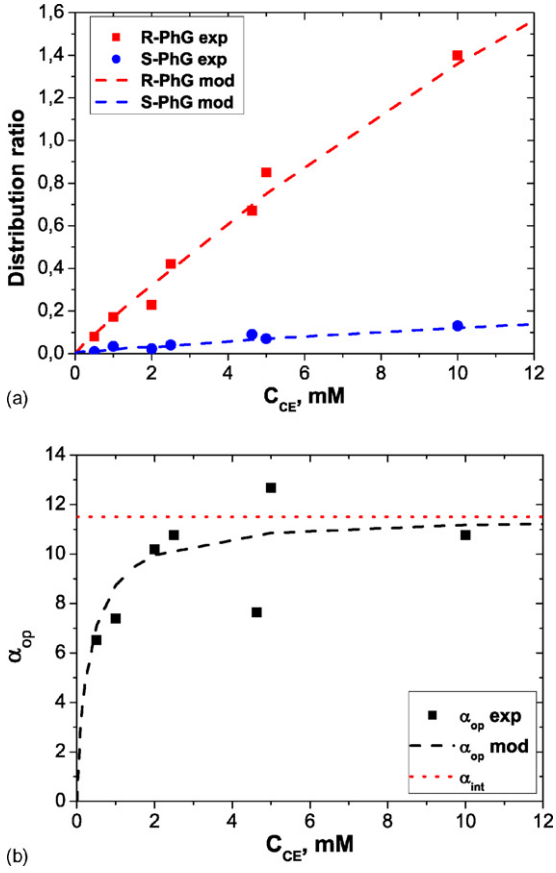

Fig. 5. Influence of the crown ether concentration in 1-phenyloctane on the extraction of phenylglycinol, $[PhG]_{rac} = 2$ mM, $T = 25$ °C. (a) distribution ratios, (b) operational selectivity.

the model given by Eqs. (1)–(18) predicts the experimental results quite good. A very good operational selectivity is already reached at a relatively low excess (2–3) in extractant concentration. Increasing its concentration results in a plateau value for the operational selectivity approaching the intrinsic selectivity α_{int}. On the other hand, distribution ratios increase steadily with increasing crown ether concentration.

Compared to the results obtained by Steensma et al. [31] it can be concluded that the values of the complexation constants of the crown ether with the phenylglycinol enantiomers in 1-phenyloctane are higher and also show a higher intrinsic enantioselectivity compared to the results in the solvents dichloromethane and toluene (α_{int} is 10 for both solvents [31]). However, due to the poor partitioning ($P = 0.03$) of phenylglycinol into 1-phenyloctane (dichloromethane: $P = 0.6$; toluene $P = 0.1$ [31]), the overall distribution ratio is somewhat lower.

3.2. EIR equilibrium performance

MPP particles were fully impregnated with a 10 mM solution of crown ether in 1-phenyloctane. The saturation capacity of MPP resins was determined to be 1.33 cm^3 of 1-phenyloctane solution per gram of MPP; leading to the chiral selector's loading of 6.65 mmol/kgEIR. A concentration of 10 mM was selected to assure a sufficient sorption capacity for the impregnated resins. Competitive sorption isotherms of *R*- and *S*-phenylglycinol were determined and the results are presented in Fig. 6. It shows the sorption capacity q, defined as the total amount of enantiomer, both free and complexed, retained in the particle per unit mass of EIR (Eqs. (19) and (20)) as a function of each enantiomer concentration in the water phase.

$$q_R = \frac{n^{EIR}_{totalR}}{m_{EIR}} \tag{19}$$

$$q_S = \frac{n^{EIR}_{totalS}}{m_{EIR}} \tag{20}$$

The variable n is the amount of enantiomer in mol and m is the mass of EIR in kilogram.

From the presented results, it is clear that the sorption capacity of the *R* enantiomer is much higher than for the *S* enantiomer. In conclusion, this means that the crown ether inside the pores still remains enantioselective. To explain the obtained results, the model based on equilibria presented in Fig. 3 was used. We assumed that the actual complexation happens in the liquid inside the pores and that we can consider the particle backbone as a "dead volume". Therefore, the amount of enantiomer in the organic phase is equal to the amount of enantiomer in the EIR phase (Eqs. (21) and (22)).

$$n^{EIR}_{totalR} = n^{org}_{totalR} = (C^{org}_R + C^{org}_{RCE})V^{org} \tag{21}$$

$$n^{EIR}_{totalS} = n^{org}_{totalS} = (C^{org}_S + C^{org}_{SCE})V^{org} \tag{22}$$

It follows that the model previously described (Eqs. (1)–(16)) can then be used to calculate the unknown equilibrium concentrations.

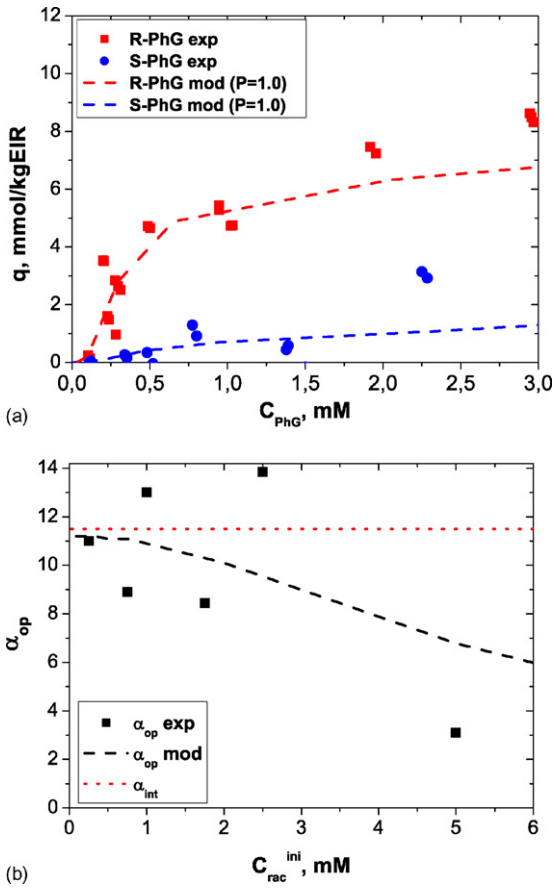

Fig. 6. (a) Competitive sorption isotherms for *R* and *S* phenylglycinol, experimental results and model (with $P = 1.0$) predictions; (b) Operational selectivity as a function of initial concentration of racemic mixture for a given amount of EIR particles (0.5 g).

However, since first model calculations showed that the assumption of an inert particle backbone for the sorption is not realistic, the actual physical partitioning between aqueous and impregnated resin phase had to be determined separately. Therefore, the actual physical sorption was measured with particles impregnated with only 1-phenyloctane. The measurement was performed with two different initial concentrations of phenylglycinol in water, 0.3 and 1.2 mM and different particle to aqueous phase ratios at a pH of 12 by adding NaOH. It was found that the physical partitioning ratio (parameter P of the model) is 1.0 ± 0.2, regardless of the initial concentration or phase (aqueous to organic) ratio, in the concentration range studied. Compared to pure liquid partitioning, this is an increase of about 30 times. It is possible that this effect is caused by the adsorption of phenylglycinol on the polymer surface. Therefore, experiments were conducted with the empty resin to investigate adsorption capacity. However, due to the very low capacity and hence insufficient accuracy of the applied analytical method to determine small differences between the initial and the equilibrium concentration, that adsorption effect cannot be measured with acceptable accuracy. Fig. 6 shows that the EIR-model with a P value of 1.0 can predict the actual sorption isotherm rather well. The prediction of the model is especially good in the lower concentration region (<2 mM phenylglycinol),

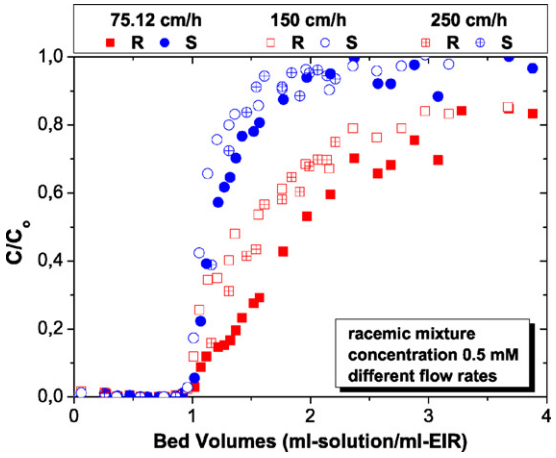

Fig. 7. The influence of the flow rate on breakthrough profiles.

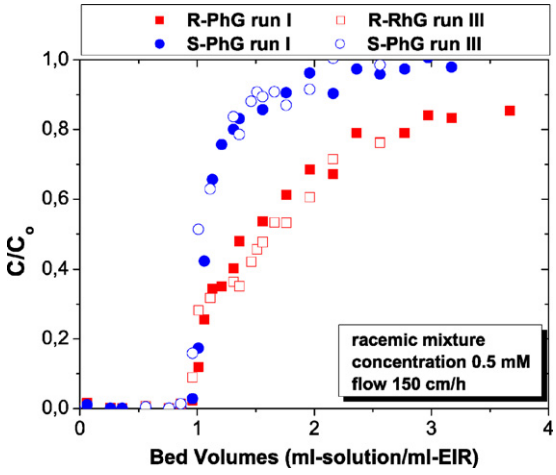

Fig. 8. Column stability.

while in the higher region the prediction is somewhat conservative. It might be that the solubility limit of the complex is exceeded and therefore precipitates, i.e. it is "removed" from the system, which shifts the equilibrium towards the complex formation. However, the higher concentration region was not of interest for our further research and was not investigated in more detail.

Regarding selectivity, Fig. 6 shows that the difference in the slope of the initial part of the two isotherms is rather high resulting in a high selectivity. According to the model, for a given amount of particles the selectivity (defined by Eq. (10)) approaches the value of the intrinsic selectivity in the low concentration region. With the increase of the concentration of enantiomers in the aqueous phase the selectivity decreases. This can also be deducted from the shape of the isotherms.

3.3. Column experiments

The results of the column experiments are presented in Fig. 7. The dimensionless outlet concentration is given as a function of bed volumes defined as volume of the solution fed to the unit volume of EIR particles. It is evident that the breakthrough curve for *R* enantiomer is not so sharp as the one of the *S* enantiomer (it is more gentle). This means that under the same conditions, EIR has a stronger affinity towards *R*-phenylglycinol [40,41]. This agrees with the results of liquid–liquid and sorption equilibrium experiments (Figs. 5 and 6). From the shape of the breakthrough profiles it can clearly be noticed that the phenylglycinol enantiomers can be separated using column packed with crown ether immobilized MPP resins.

The influence of the flow rate can be seen in Fig. 7. As the flow rate decreases, the breakthrough curve of *R* enantiomer becomes less steep. At lower flow rates, the contact time between the liquid solution and the EIR beads is longer leading to the higher amount of *R* enantiomer sorbed. On the other hand, for high enough flow rates (higher than 150 cm/h) its influence on the breakthrough curve diminishes. Opposite to the *R* enantiomer, the flow rates have considerably less influence on the breakthrough curve of *S* enantiomer. Therefore, it can be concluded that the separation is better at lower flow rates.

3.3.1. Column stability

An important feature of a chromatographic column is stability, which is crucial for any application, especially for large scale industrial production. The molecular structure of the crown ether might indicate that its solubility in water cannot be neglected. This might lead to leaching of the extractant and thus gradual loss of capacity. It was already shown [10] that the most of the leaching occurs after the first cycle when extractant adsorbed on the particle outer surface is washed away. Moreover, it was also proven that immobilization decreases the solubility for at least an order of magnitude [14]. In Fig. 8 breakthrough profiles are represented after three cycles. It is evident that the profiles remain unchanged. This means that in this case there was no noticeable leaching of the extractant, opposite to the findings of Traving et al. Therefore, it can be expected that these resins will be rather stable. Nevertheless more detailed investigation regarding stability is needed.

3.3.2. Column regeneration

The scheme of the system equilibria presented in Fig. 3 suggests that the particles can be regenerated by lowering the pH of the aqueous phase which will lead to the back shift of the equilibrium. The pH does not have to be much reduced. In this

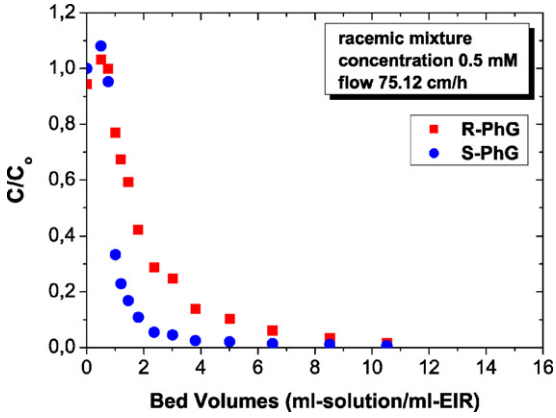

Fig. 9. Elution profiles.

research, for the regeneration of the column, water with only atmospheric carbon dioxide dissolved was used. Fig. 9 shows the elution profiles. It is evident that the column can be regenerated with moderately acidic solution with acceptable solvent consumption. The shape of the elution curves, i.e. larger tailing of the *R* enantiomer, proves once more that it is strongly retained on the column.

4. Conclusions

Extractant impregnated resins are a new, reduced cost technique for chiral separations. It was proven that enantiomers of phenylglycinol can be successfully separated using resins impregnated with a solution of azophenolic crown ether in 1-phenyloctane.

1-phenyloctane is a suitable solvent for this application because of its very low solubility in water. Moreover, crown ether remains enantioselective when dissolved in 1-phenyloctane, with intrinsic selectivity of 11.5. However, due to very low solubility of phenylglycinol in 1-phenyloctane, the overall capacity of the crown ether solution in 1-phenyloctane is limited.

When immobilized in macroporous polypropylene particle this solution keeps its enantioselective complexation properties. The equilibrium selectivity of these EIRs approaches the intrinsic selectivity for low phenylglycinol concentrations. Competitive sorption equilibrium isotherms of *R* and *S* phenylglycinol can be successfully predicted by knowing the complexation constants and physical partitioning ratios, which can all be obtained from independent experiments.

Breakthrough profiles as well as the elution curves of the *R* enantiomer are much less sharp than those of the *S* enantiomer proving that the *R* enantiomer is strongly retained on the column. Separation of phenylglycinol enantiomers is favoured by using lower feed flow rates. The water with only atmospheric carbon dioxide dissolved proved to be sufficient for regeneration of the column. After several cycles breakthrough profiles remain unchanged meaning that there was no leaching of the chiral selector and thus can be expected that these EIRs will be sufficiently stable.

5. Nomenclature

C	concentration (mol/l)
D	distribution ratio (defined by Eqs. (8) and (9))
He_{CO2}	Henry's coefficient
K_R, K_S	reaction equilibrium constant for the *R* and *S* enantiomer (l/mol)
K_A	dissociation constant of phenylglycinol (mol/l)
K_{A1}, K_{A2}, K_{A3}	dissociation constants of carbonic acid
n	amount of substance (mol/l)
p_{CO2}	partial pressure of carbon dioxide
P	physical partitioning ratio (defined by Eq. (1))
q	loading of the particle with enantiomer (defined by Eqs. (19) and (20))
V	volume (l)
x	molar fraction

Greek letters

ρ_w	molar density of water
α_{op}	operational selectivity (defined by Eq. (10))
α_{int}	intrinsic selectivity (defined by Eq. (11))

Subscripts

am	amine form
ammo	ammonium form
CE	crown ether
eff	effective
exp	experimental
HR^+, HS^+	protonated enantiomers
mod	model
o	initial
PhG	phenylglycinol
R, *S*	enantiomers
*R*CE, *S*CE	complex between *R* or *S* enantiomer and crown ether

Superscripts

aq	aqueous
org	organic

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