Enantioseparation of 1-phenyl-1-propanol on Chiralcel OD by supercritical fluid chromatography
I. Linear isotherm

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Abstract

The supercritical fluid chromatography (SFC) separation of the enantiomers of 1-phenyl-1-propanol on the chiral stationary phase Chiralcel OD under linear conditions is studied. Supercritical CO_2 modified with methanol is used as a mobile phase. The effect of modifier concentration, pressure and temperature is studied. An empirical isotherm to account for the effect of density of the mobile phase and modifier concentration has been used to model the experimental results. It was observed that the selectivity and resolution were higher at 30 °C as compared to those at 40 °C.
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1. Introduction

Supercritical fluids, especially CO_2, have found applications in several chemical processes. The fact that supercritical fluids posses properties that are intermediate between those of liquids and gases, and the tunability of these properties by a proper choice of operating conditions have made them good candidates for use as mobile phases in chromatographic applications. The higher diffusivities as compared to those of liquids result in better separation efficiencies and the lower viscosity offers lower pressure drops at preparative conditions where columns are usually operated at higher flow rates. The benign nature of CO_2 also makes it convenient for applications in the food and pharmaceutical industries. Supercritical fluid chromatography (SFC) has been used at both analytical and preparative scales [1]. In the latter case, supercritical fluids have been used as eluents both in single column preparative chromatography and in the supercritical fluid simulated moving bed chromatography (SF-SMB) process [2–5].

Owing to the non-polar nature of CO_2, the solubility of polar solutes is rather modest. Hence, in order to enhance solubility, it is customary to add a polar modifier, usually an alcohol, to the mobile phase. In SFC systems the Henry constant of a solute depends among other parameters on the density of the mobile phase. At higher densities the supercritical solvent may adsorb more, as well known in the case of carbon dioxide [6–9], thus competing with the solute for the adsorption sites, whereas the solute's solubility in the mobile phase increases; both effects lead to a decrease of the Henry constant of the solute. This property can be particularly advantageous in the operation of a simulated moving bed (SMB) unit [10]. This normally consists of four sections, i.e., 1–4. The two middle Sections 2 and 3 perform the separation of the solutes, while Section 1 is used to regenerate the solid phase and Section 4 removes the solute from the solvent, thus cleaning the solvent which can be recycled. One way to enhance productivity is to run the unit under a gradient mode, where a gradient of an intensive operating variable e.g. temperature, or pressure, or modifier concentration, is enforced on the system in such a way that the solvent power of the mobile phase is high in Section 1 and low in Section 4.

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In the case of the SF-SMB, this can be achieved by operating the unit under a pressure gradient mode where the pressure of the mobile phase decreases from Section 1 to Section 4. It has been shown both experimentally and theoretically that this mode of operation can lead to increased productivity and lower solvent consumption as compared to the isobaric mode of operation, where the pressure in all the four sections is equal [4,5].

In the recent past SFC has increasingly been applied to the separation of enantiomers [11]. Several separations of pharmaceutical intermediates feature in the list of reported separations. SF-SMB separations of enantiomers have also been reported [4,12,13]. The design of these processes depends chiefly on the characterization of the separation system. Hence, the first step in the design is to measure the adsorption isotherms at different operating conditions. For a given SFC separation, the degrees of freedom are usually the choice of the stationary phase, the modifier, concentration of the modifier, as well as pressure and temperature. Once the information on the influence of these parameters on the isotherm are available, one can choose the operating conditions of the preparative separation.

In this work, the separation of 1-phenyl-1-propanol on Chiralcel OD is studied. CO_2 modified with methanol is used as a mobile phase. Experiments under linear chromatographic conditions were performed. Four different modifier concentrations namely, 2.4, 4.7, 7.1, and 9.5 wt.%, and four different pressures, namely, 125, 155, 185, and 215 bar were chosen. This set of experimental conditions was investigated at two different temperatures, namely 30 and 40 °C. An isotherm model that combines the effect of the fluid phase density and the modifier concentration on the Henry constant has been used to describe the experimental data.

2. Experimental

2.1. Materials

Carbon dioxide with a purity of 99.995% was obtained from PanGas AG, Luzern, Switzerland. HPLC grade methanol (99.8% purity), racemic mixture of 1-phenyl-1-propanol (97.4% purity) and pure enantiomers of 1-phenyl-1-propanol were obtained from Fluka, Buchs, Switzerland. A Chiralcel OD analytical column obtained from Chiral Technologies Europe, Cedex, France, 250 mm × 46 mm, with an average particle size of 20 μm was used for the experiments.

2.2. Set-up and procedure

The experimental system consists of a SFC system built in-house. The CO_2 flow is provided by an ISCO 260D syringe pump (Lincoln, NE, USA) while an ISCO μLC-500 micro flow pump provides the modifier flow. The CO_2 and the modifier are mixed at a tee, which is followed by a static mixer to ensure single phase flow. The mobile phase is then brought to the experimental temperature through a coil immersed in the thermostated water bath that houses the column and the injection valve Valco C14W (VICI AG, Schenkon, Switzerland) with an external sample loop of 5 μL. The column is followed by a UV detector Jasco UV-1570 (Omnilab, Mettmenstetten, Switzerland) equipped with a high pressure flow cell. A detection wavelength of 210 nm which corresponds to the maximum of the absorption spectra of 1-phenyl-1-propanol was used for all the experiments. The pressure in the system is controlled by a back pressure regulator Jasco BP1580-81 (Omnilab) which is located downstream of the UV detector. Pressure transducers, Trafag 8891 (Trafag, Maennendorf, Switzerland) and PT-100 thermoelements are used to provide the necessary pressure and temperature readings. All the experimental parameters are logged on into a computer using Labview (National Instruments, Austin, TX, USA) and the UV signal response is recorded and the peaks are integrated using a Jasco Borwin (Omnilab) software.

The experiments were performed by setting the back pressure regulator at the desired pressure level and the pumps at the desired flow rate. For all the experiments the CO_2 pump was set at 1 mL/min (with the pump head being cooled at 15 °C), and the modifier pump at a suitable setting to provide the desired concentration of methanol. The flow rate of 1 mL/min was chosen as it provided shorter run times (less than 15 min for a run) with negligible pressure drop across the column (less than 1 bar). Once the flow was started, the system was allowed to reach steady state and then injections of a diluted solution of racemic 1-phenyl-1-propanol in methanol were made using the motor actuated injection valve. The injection was performed in the time-split mode, where the valve was switched from load position to inject position and then back to the load position. The time interval when the valve stays in the injection position was fixed at 0.5 s. This procedure allows one to eliminate the tail that is caused due to laminar flow in the capillary. The fact that this procedure leaves uncertain the exact amount injected is not an issue since the experiments were performed under linear chromatographic conditions. The residence time of the peak was used to calculate the retention parameters. All experiments yielded symmetric peaks which confirmed the linear conditions, and were cross checked by injecting samples at higher and lower concentrations of 1-phenyl-1-propanol in methanol and verifying that the retention times remained unchanged. At every experimental condition, experiments were repeated at least three times in order to ensure reproducibility.

3. Experimental results

There was no separation of the enantiomers when pure CO_2 was used as a mobile phase. The elution order of the enantiomer was tested at 150 bar, 40 °C and a modifier concentration of 5% by injecting a diluted sample of the S-enantiomer and by comparing this with the pulse obtained

upon injection of the racemate. The S enantiomer was the more retained enantiomer under these conditions. In the following, the enantiomer eluting first is denoted as "1" and the one eluting second as "2". The racemic mixture contained an impurity (maximum 2.6%) which always eluted earlier than the two enantiomers.

Under conditions where the adsorption isotherm is linear, i.e. when the concentration of the solute is low enough and the pressure drop in the column is negligible, the retention time $t_{R,i}$ of the species i is given by

$$t_{R,i} = t_0(1 + k_i) \tag{1}$$

where the capacity factor is

$$k_i = \frac{1 - \epsilon}{\epsilon} H_i \tag{2}$$

ϵ being the overall void fraction of the column. In the equations above, t_0 is the retention time of a non-adsorbing compound, i.e. $t_0 = \epsilon V/Q$, with V and Q being the column volume and the volumetric flow rate respectively, and H_i is the Henry constant of species i. However, it is not always possible to find a suitable non-adsorbing species to measure t_0 properly. 1,3,5-Tri-*tert*-butylbenzene (TTBB) is usually suggested as a non-adsorbing species for Chiralcel OD and was used in a few experiments. By calculating the porosity of the column using the retention time of TTBB, and assuming that TTBB did not adsorb, the calculated porosity varied between 0.728 and 0.86. The calculated porosity tended to be larger at lower pressure and lower modifier concentration, and smaller at higher pressure and higher modifier concentration. These results can be explained by considering that TTBB can in fact adsorb on Chiralcel OD. Alternatively, a similar effect, where the calculated porosity decreases with increasing pressure, can occur if the stationary phase swells in the presence of CO_2. In the experiments a clear trend of the measured porosity, i.e. an asymptotic decrease, when increasing either pressure or modifier concentration was observed. The asymptotic value will however be slightly smaller than the minimum value observed in the experiments, i.e., 0.728. This is nicely consistent with the value provided by the vendor, namely 0.722, that has been used in the computations.

The Henry constant of the more retained enantiomer is shown in Fig. 1 as a function of pressure for different modifier concentrations at 30 and 40 °C. At a given temperature it can be seen that for a given modifier concentration, the Henry constant decreases with increasing pressure. This can be rationalized by the fact that under supercritical conditions, the partitioning of the solute between two phases, in the present case between the adsorbent and the fluid phase, is a strong function of the solvent power of the fluid phase. At a given modifier concentration, increasing the pressure of the mobile phase increases its solvent power and thus leads to a decrease in the Henry constant of the solute. It can further be observed that for a given pressure, the Henry constant decreases with increasing modifier concentration. This can be explained by the fact that at a given pressure, the addition of a polar mod-
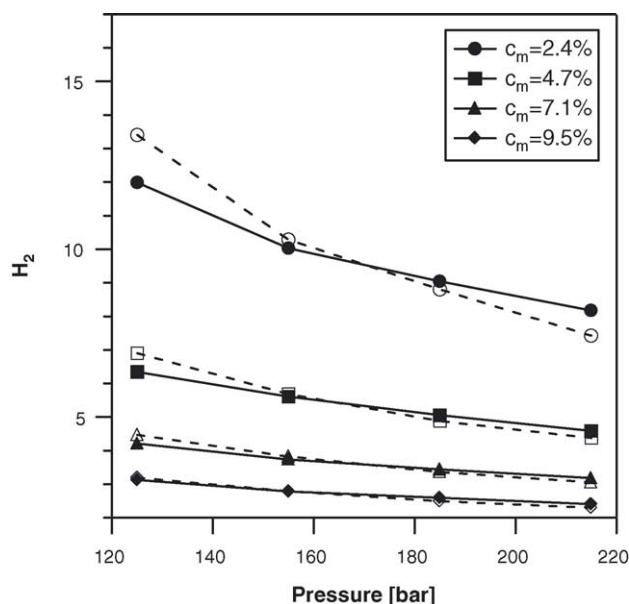

Fig. 1. Dependence of Henry constant of the more-retained enantiomer on pressure at 30 and 40 °C and at different modifier concentrations (c_m, wt.%). Symbols correspond to experimental values, while lines are drawn as a guide to the eye. Symbols: closed, 30 °C; open, 40 °C. Lines: solid, 30 °C; dashed, 40 °C.

ifier, in this case methanol, increases the density and the solvent power of the fluid phase, both of which tend to reduce the Henry constant of the solute. Moreover, the modifier can also adsorb onto the stationary phase, thus creating a competitive effect and further reducing the Henry constant of the solute. By comparing the Henry constants at 30 °C and 40 °C, it can be seen that for certain pressures, the Henry constant at 40 °C is larger than the corresponding one at 30 °C. This effect is usually expected to be the opposite, i.e., at a given pressure, the Henry constant reduces with an increase in temperature since adsorption is an exothermic process. These apparent anomalies in SFC can be clarified by using the density of the fluid as the governing property rather than pressure [4]. Accordingly, the data represented in Fig. 1 are re-plotted, as points, in Fig. 2 as a function of fluid density estimated at the experimental conditions, i.e., temperature, pressure and modifier concentration, using the Peng-Robinson (PR) equation of state with a quadratic mixing rule. It can be seen that at constant density, the Henry constant always decreases with increasing temperature, while its logarithm is linear with density at constant temperature.

Fig. 3 shows the selectivity, $\alpha = H_2/H_1$, as a function of fluid density. In general, density has a minor effect on α, whereas increasing temperature or modifier concentration leads to smaller selectivity values. The effect of modifier concentration becomes less significant at higher values of modifier concentration, c_m. This indicates that an increase in the modifier concentration beyond 5% does not affect the chirally selective sites any further, hence a minor advantage in the selectivity can be achieved by further increasing the modifier concentration. It is worth recalling here that when pure CO_2
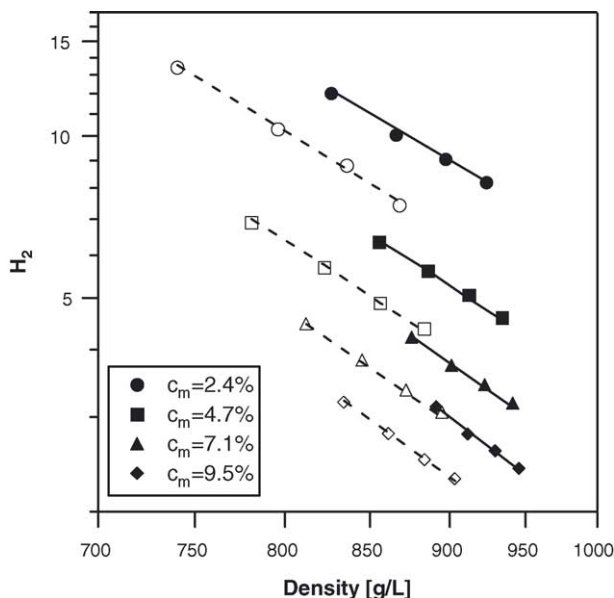
Fig. 2. Dependence of Henry constant of the more-retained enantiomer on density at 30 and 40 °C and at different modifier concentrations (c_m, wt%). The density corresponds to the calculated fluid densities corresponding to the pressures reported in Fig. 1. Symbols correspond to experimental values, while lines are the fit of Eq. (6) to the experimental points. Symbols: closed, 30 °C; open, 40 °C. Lines: solid, 30 °C; dashed, 40°C.

is used, the Henry constants of the enantiomers were much higher compared to the experiments with modified CO_2 and the selectivity was zero. This is caused by the stronger adsorption of the solutes on to the silanol groups which are not chirally selective.
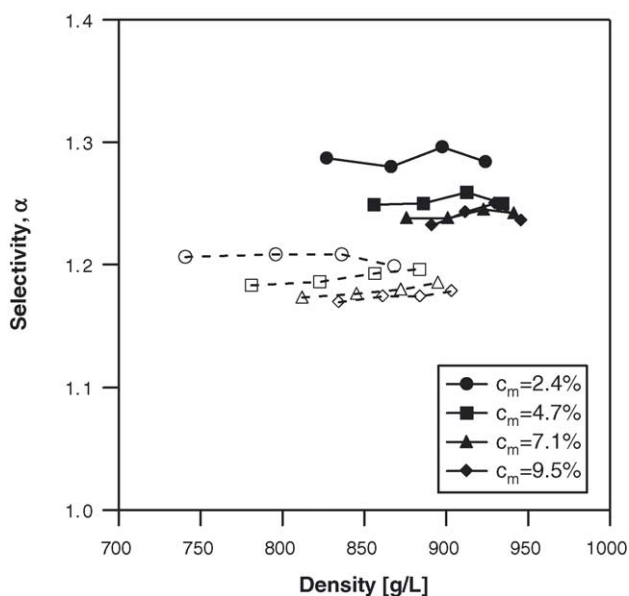

Fig. 3. Dependence of selectivity of Chiralcel OD for the separation of the enantiomers of 1-phenyl-1-propanol on density at 30 and 40 °C at different modifier concentrations (c_m, wt%). Symbols correspond to experimental values, while lines are drawn as a guide to the eye. Symbols: closed, 30 °C; open, 40 °C. Lines: solid, 30 °C; dashed, 40 °C.
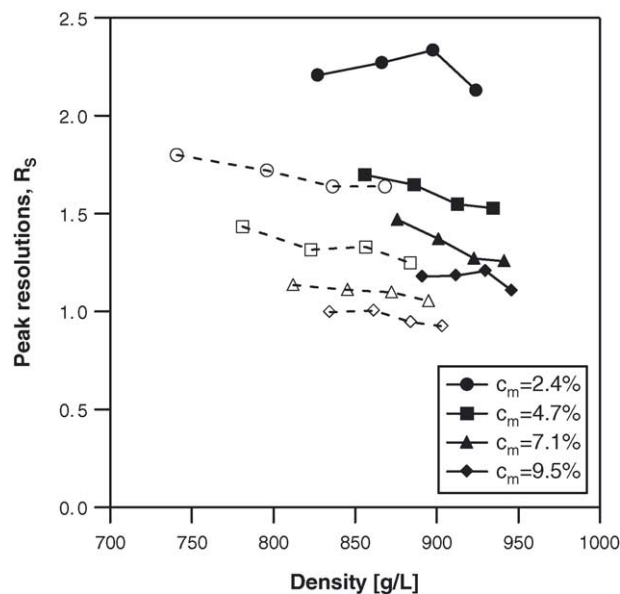

Fig. 4. Dependence of peak resolution of Chiralcel OD for the separation of the enantiomers of 1-phenyl-1-propanol on density at 30 and 40 °C at four different modifier concentrations (c_m, wt%). Symbols correspond to experimental values, while lines are drawn as a guide to the eye. Symbols: closed, 30 °C; open, 40 °C. Lines: solid, 30 °C; dashed, 40 °C.

The peak resolution of the enantiomers, in terms of the number of theoretical plates, N, capacity factor of component 1, k_1, and selectivity, α, is defined as

$$R_S = \frac{\sqrt{N}}{2} \left(\frac{\alpha - 1}{\alpha + 1} \right) \frac{k_1}{1 + k_1} \tag{3}$$

R_S is plotted as a function of the fluid phase density in Fig. 4. The number of theoretical plates, N, has been calculated from the experimental peaks by using the expression

$$N = 5.545 \left(\frac{t_{R,i}}{w} \right)^2 \tag{4}$$

where w is the peak width at half peak height. Qualitatively, the trends are the same as those observed for selectivity. However, the effects of both temperature and modifier concentration are more significant and there is a non-negligible effect of density that tends to reduce the resolution.

At this point it is worth comparing the present results with those of Smith and Ma [14] who studied the same system, but under a limited set of operating conditions. At a temperature of 40 °C and a modifier concentration of 7% the retention factor of enantiomer 1 in the present study was 1.32 which compares well with that of Smith and Ma which is 1.48. The selectivity at these conditions are also comparable. But, when the modifier concentration is reduced to 3% the retention factors are different (3.5 in the present study, compared to 1.98), though selectivities are still comparable. One reason for this could be the way in which t_0, the retention time of a non-retained component, is calculated. It is however worth considering that if the tracer used for the calculation of the retention factor of the enantiomer, does indeed adsorb, the

calculated retention factor of the enantiomer will be smaller than the case when the tracer does not adsorb. It is also important to note, as discussed before, that this effect is expected to be significant at lower modifier concentration and lower density. This possibly could be the reason as to why the comparison of the two results is good at higher modifier concentration and poor at lower modifier concentration. Though this effect of the adsorption of the tracer may affect the retention factors, it may not affect significantly the selectivity. Since Smith and Ma do not report specifically how the evaluation of t_0 was performed, it is not possible to check this explanation.

4. Modelling the adsorption isotherms

Di Giovanni et al. [5] derived an empirical relationship for the adsorption of a solute present in a fluid phase which consists of supercritical CO_2 and a polar modifier. Using this empirical relationship, the design equations for the operation of the SF-SMB under pressure gradient and isocratic modes were derived. A similar strategy will be adopted here after giving a brief description of the approach for the sake of completeness.

When the concentration of the solute is low, the adsorption equilibrium can be represented by a linear isotherm, i.e.

$$n_i = H_i c_i \tag{5}$$

where n_i and c_i are the concentrations (in mass units) of solute i in the adsorbed and the fluid phase respectively. At a given temperature, the relationship between the Henry constant and the fluid phase density can be described as

$$H_i = H_i^0 \left(\frac{\rho^0}{\rho}\right)^{b_i} \tag{6}$$

where H_i and H_i^0 are the Henry constants at the operating and reference densities, ρ and ρ^0, respectively, and b_i is an empirical exponent, which is a function of the molar volume of the solute at infinite dilution in the fluid phase. It has been shown that this equation can be theoretically derived for systems containing low concentrations of the solute [15,16]. Hence a logarithmic plot of H_i versus ρ will be linear as shown in Fig. 2.

The presence of a modifier in the fluid phase can affect the adsorption of the solute in different ways as discussed

before. For the sake of simplicity, but without loss of generality, the effect of the modifier can be accounted for through the following linear relationship:

$$\frac{1}{H_i} = a_i c_{\mathrm{m}} + d_i \tag{7}$$

where c_{m} is the modifier concentration in wt.%, while a_i and d_i are empirical constants [17]. The above expression corresponds to a fixed density of the mobile phase (CO_2 + modifier). In principle, the term $1/d_i$ should correspond to the Henry constant in the case where the modifier concentration is 0, i.e. when pure CO_2 is used as the mobile phase. However, several systems exhibit a behaviour that has no continuity when $c_{\mathrm{m}} = 0$ is approached [17]. This has been attributed to the fact that the interaction of the solute with a modified adsorbent surface and an un-modified adsorbent surface is markedly different due to significant contributions of the silanol groups (that show strong affinity to the solute) which become exposed to the solute at low modifier concentrations.

In order to account for the effects of both density and the modifier concentration, Eqs. (6) and (7) can be combined to give

$$H_i = \frac{1}{a_i c_{\mathrm{m}} + d_i} \left(\frac{\rho^0}{\rho}\right)^{b_i} \tag{8}$$

Now, the values of a_i, d_i and b_i can be obtained from the plot of $\ln(H_i)$ versus $\ln(\rho)$ for different modifier concentrations. The result is illustrated in Fig. 2 where the experimental points along with the best fit lines are shown. From the intercept of the lines, a_i and d_i can be obtained, while b_i can be obtained from the slope. It is found that the value of b_i obtained from the slope, depends linearly on the modifier concentration as shown in Fig. 5. Hence, to account for this effect we write

$$b_i = p_i c_{\mathrm{m}} + q_i \tag{9}$$

The values of p_i and q_i which are obtained by fitting Eq. 9 to the data in Fig. 5 are given in Table 1 along with the values of a_i and d_i. This procedure of obtaining the isotherm parameters is referred to as Method 1 in the table. The final form of the isotherm, describing the influence of the fluid phase density and the modifier concentration on the Henry constant of the solute is obtained by combining

Table 1
Isotherm parameters, corresponding to Eq. (10)

$T(°C)$	Component	Method 1				Method 2			
		a	d	p	q	a	d	p	q
30	1	0.0640	0.0496	0.1401	3.0531	0.0589	0.0652	0.0703	3.1726
	2	0.0519	0.0345	0.1304	3.0608	0.0486	0.0432	0.0716	3.1312
40	1	0.0733	0.0912	0.0896	3.3971	0.0714	0.0916	0.0799	3.3292
	2	0.0621	0.0716	0.0747	3.4223	0.0599	0.0746	0.0580	3.3863

Refer text for the different approaches towards obtaining the parameters namely, Methods 1 and 2.
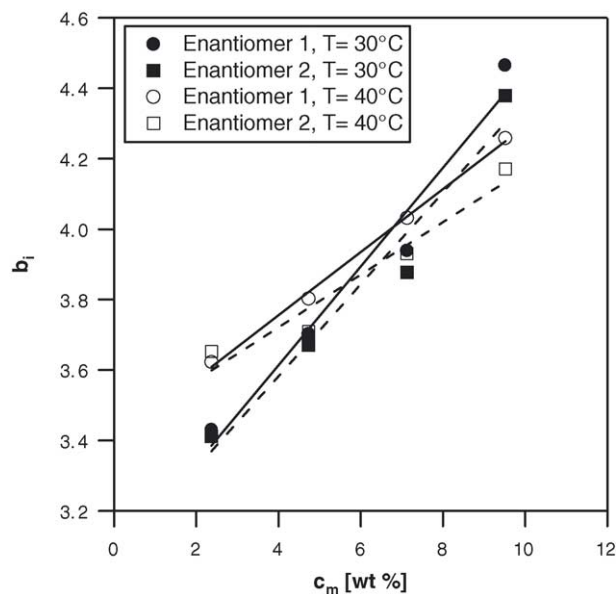
Fig. 5. Dependence of b_i on modifier concentration c_m at 30 and 40 °C. Points correspond to experimental values while the lines correspond to the best fit of Eq. (9) to the experimental points.

Eqs. (8) and (9):

$$H_i = \frac{1}{a_i c_m + d_i} \left(\frac{\rho^0}{\rho} \right)^{p_i c_m + q_i} \tag{10}$$

In order to get the best fit of the above equation to the experimental data, all the parameters were regressed simultaneously by reducing the sum of the residuals at each temperature. The resulting values of the empirical parameters are reported in Table 1 as Method 2.

It can be noted that there are minor differences in the values obtained from the two methods. However, the average error between the predicted Henry constants by either of the methods and the experimental values is less than 1.0%. It has to be emphasized that the extrapolation of Eq. 10, especially to conditions where $c_m \rightarrow 0$ has to be performed with caution.

5. Conclusion

The SFC separation of 1-phenyl-1-propanol on Chiralcel OD has been studied. Henry constants and resolutions were measured. It was found that the separation performance was better at 30 °C as evidenced by better selectivity and better resolution. A mixed theoretical and empirical isotherm has been used to describe the adsorption behaviour of the solutes as a function of temperature, density and modifier concentration at the same time. These results constitute a firm basis for the design of the SF-SMB separation of the two enantiomers.

Acknowledgements

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