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Displacement chromatography on cyclodextrin silicas

IV. Separation of the enantiomers of ibuprofen

Gyula Farkas[☆], Leif H. Irgens[☆☆], Gilberto Quintero[☆☆☆], Michelle D. Beeson, Arif Al-Saeed and Gyula Vigh*

Department of Chemistry, Texas A and M University, College Station, TX 77843-3255 (USA)

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ABSTRACT

A displacement chromatographic method has been developed for the preparative separation of the enantiomers of ibuprofen using a β-cyclodextrin silica stationary phase. The retention behavior of ibuprofen was studied in detail: the log k' *vs.* polar organic modifier concentration, the log k' *vs.* pH, the log k' *vs.* buffer concentration and the log k' *vs.* $1/T$ relationships; also, the α *vs.* polar organic modifier concentration, the α *vs.* pH, the α *vs.* buffer concentration and the log α *vs.* $1/T$ relationships have been determined in order to find the carrier solution composition which results in maximum chiral selectivity and sufficient, but not excessive solute retention ($1 < k' < 30$). 4-*tert.*-Butylcyclohexanol, a structurally similar but more retained compound than ibuprofen, was selected as displacer for the separation. Even with an α value as small as 1.08, good preparative chiral separations were observed both in the displacement mode and in the overloaded elution mode, up to a sample load of 0.5 mg.

INTRODUCTION

In previous parts of this series [1–3] and a related paper [4], we demonstrated that preparative liquid chromatographic separations of geometrical isomers, positional isomers and enantiomers can be achieved in the displacement mode of operation using columns packed with commercially available β-cyclodextrin silica stationary phase [5,6]. Because these papers and the references cited therein present a detailed review of the characteristics and use of the β-cyclodextrin silica stationary phase and the displacement chromatographic technique, the reader is referred to them for an introductory discussion. Recent work with the displacement chromatographic separation of chiral compounds led us to a method development scheme which will be discussed in this paper using the non-steroidal anti-inflammatory agent α-methyl-4-[2-methylpropyl]benzeneacetic acid, ibuprofen, as a model substance. The major steps of the method development scheme are (a) selectivity maximization, (b) retention optimization, (c) displacer selection, (d) completion of the displacement chromatographic separation, and (e) evaluation of the purity of the pooled material by fraction analysis.

Because the production rate in preparative chromatography increases dramatically with the value of the selectivity factor, α [7], and because the α values for enantiomer separations on native β-cyclodextrin silica are generally low (1.03 to 1.15) [8], selectivity optimization is the first and the most crucial step of any separation

* Corresponding author.
☆ Present address: Chinoin Pharmaceutical Company, Budapest, Hungary.
☆☆ Present address: DuPont de Nemours, Belle, WV, USA.
☆☆☆ Present address: Tennessee Valley Authority, Chattanooga, TN, USA.

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method development scheme. In the reversed-phase mode, chiral separations on cyclodextrin silicas are believed to occur due to the balanced concerted action of inclusion complex formation, hydrogen bond formation, and steric hindrance between the bonded cyclodextrin moiety and the solute [9]. These interactions —and consequently, solute retention and separation selectivity— are influenced by the type and the concentration of the polar organic modifier in the eluent, the type, the concentration and the pH of the buffer, and the temperature of the eluent. Therefore, these parameters have to be investigated in detail in order to select chromatographic conditions which lead to maximized chiral selectivity and appropriate solute retention ($1 < k' < 30$). Then a suitable displacer can be selected based on its relative retention vis-a-vis the more retained ibuprofen enantiomer. This is followed by the completion of the displacement chromatographic run, the collection and the analysis of the enantiomer fractions, and the calculation of the yield and the production rate as a function of the enantiomeric purity of the pooled fraction.

EXPERIMENTAL

The equipment used for these studies was built from commercially available components as described in refs. 1–3. 5-μm β-cyclodextrin silica, Cyclobond I (ASTEC, Whippany, NJ), was slurry-packed into 250 mm × 4.6 mm I.D. stainless-steel columns (BST, Budapest, Hungary). The column temperature was controlled within ± 0.5°C using a water jacket, by a Type UF3 circulating water bath (Science/Electronics, Dayton, OH, USA).

Racemic and *S*(+)-ibuprofen standards were obtained from Aldrich (Milwaukee, WI, USA), along with the displacers and buffer components. HPLC grade methanol, acetonitrile and tetrahydrofuran were purchased from J.T. Baker (Philipsburg, NJ, USA). Aqueous pH standards (pH 4 and 7) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Water was obtained from a Milli-Q unit (Millipore, Bedford, MA, USA). All chemicals were used as received, without further purification. The carrier and displacer solutions were freshly prepared using the weighing method described in Part 1 [1].

RESULTS

Retention studies of ibuprofen

Because ibuprofen is a weak acid with pK_a = 5.39 [10], the retention of the ibuprofen enantiomers —and the value of the chiral selectivity factor— are influenced by the type and the concentration of the polar organic modifier, the pH, the buffer concentration, the concentration of the tailing-reducing additive (triethylamine), and the temperature of the eluent. Therefore, the effects of all these factors were individually studied as described below.

Preliminary work indicated that acetonitrile was a good eluent modifier for the analytical separation of the enantiomers of ibuprofen on the native β-cyclodextrin silica stationary phase [3,4,19]. Therefore, acetonitrile was used in the first phase of the selectivity studies and the retention studies. In order to reduce peak tailing, triethylamine (TEA) was added to every eluent, as usual [6]. First, the effects of the acetonitrile concentration were studied in 70 m*M* citrate–acetonitrile–water eluents. The apparent pH of the eluents was adjusted to 4.0 and 6.0, respectively, by adding a few μl of concentrated sodium hydroxide solution to the hydroorganic eluent and monitoring the pH with a combined glass electrode, standardized against pH 4 and 7 aqueous pH standards [11].

The k' values of (*S*)-(+)-ibuprofen, the more strongly retained enantiomer, are shown as solid lines plotted against the left axis in Fig. 1. The retention behavior of ibuprofen is unexpected: as long as there is more than 20% (v/v) acetonitrile in the eluent, the anionic form of ibuprofen is more retained (in pH 6 eluents) than the free acid form (in pH 4 eluents); the opposite of what is observed on octadecyl silica stationary phases [12] operated in the reversed-phase mode. On octadecyl silicas, weak acids are more retained when the pH of the eluent is below the pK_a value of the solute (*i.e.*, the acids are not dissociated). However, extrapolation of the k' *vs.* acetonitrile concentration curves to zero

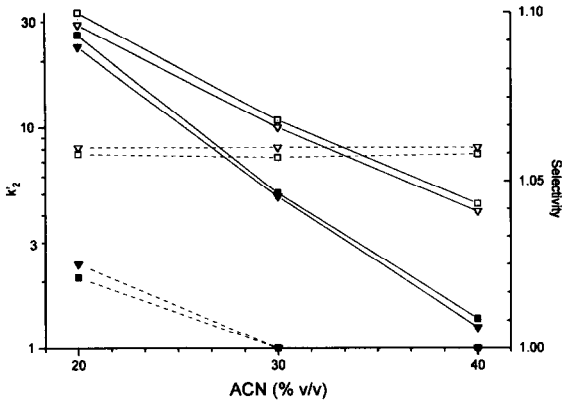

Fig. 1. The capacity factor of (*S*)-(+)-ibuprofen (solid lines plotted against the left axis) and the chiral selectivity factor for the separation of the (*S*)-(+)- and (*R*)-(−)-enantiomers of ibuprofen (dotted lines plotted against the right axis) as a function of the acetonitrile concentration (%, v/v), TEA concentration (m*M*), and pH of the eluent. Total citrate concentration: 70 m*M*. Full symbols = pH 4, open symbols = pH 6, ▽ = 0 m*M* TEA, □ = 30 m*M* TEA. Column temperature: 30°C, eluent flow rate: 1 ml/min.

acetonitrile concentration shows that there the neutral form of the weak acid solute is more retained than its dissociated form, in agreement with our previous findings in HPLC [13] and capillary electrophoresis [14]. The slope of the log k' *vs.* % (v/v) acetonitrile curve is lower at pH 6 than at pH 4, indicating that the intermolecular interactions between the anion and the β-cyclodextrin are weakened by the polar organic modifier to a lesser degree than those which exist between the non-dissociated weak acid and the β-cyclodextrin. Therefore, ibuprofen separations which utilize a higher pH eluent are more rugged and less sensitive to slight variations in the concentration of the polar organic modifier of the eluent than those which use a low pH eluent. This behavior is clearly an asset for preparative separations.

The chiral selectivity (α) values which were observed for the ibuprofen enantiomers are shown as dotted lines plotted against the right axis of Fig. 1. At pH 6, the chiral selectivity values are much higher than at pH 4, and they do not vary with the acetonitrile concentration in the range tested, as they do at pH 4. This behavior permits the control of solute retention over a broad range of k', without a concomitant loss of chiral selectivity, by changing the acetonitrile concentration of the eluent.

Triethylamine (TEA) is a widely used masking agent in β-cyclodextrin silica-based separations [6,15,16,19]. Therefore, the influence of the TEA concentration on the separation of ibuprofen enantiomers was studied as well. Increasing amounts of TEA were added to the 70 m*M* citrate–acetonitrile–water eluents to produce 5, 10 and 30 m*M* TEA concentrations. The final pH of the eluents was adjusted by adding a few μl of a concentrated sodium hydroxide solution, as before. It can be seen in Fig. 1 that as the TEA concentration is increased from 0 m*M* (triangle symbols) to 30 m*M* (square symbols), the k' values of the solutes, whether almost neutral (ibuprofen at pH 4), or anionic (ibuprofen at pH 6), remain more or less unchanged, both at high and low pH values, and in both the acetonitrile rich and the acetonitrile poor eluents. However, the peak shape improves as the TEA concentration is increased from 0 through 5 m*M* to 10 m*M*. No further improvement is seen when the concentration is raised to 30 m*M*. Selectivity remains the same, both at low pH and at high pH, as the TEA concentration is changed from 0 m*M* (triangle symbols) to 30 m*M* (square symbols). However, as discussed above, peak shape is better when the TEA concentration of the eluent is 10 m*M* or higher. Therefore, unless stated otherwise, the eluents always contained 10 m*M* TEA in the rest of the experiments.

In order to investigate briefly the effects of the type of buffer anion on the selectivity, citrate was replaced with acetate. The shape of the α *vs.* % (v/v) acetonitrile concentration curve obtained was similar to the one in Fig. 1 [4]. However, the pH of the eluent had to be increased to pH 6.8 to obtain selectivities comparable to those shown in Fig. 1. As neither the buffer capacity of the eluent nor the long-term stability of the column are as good at this higher pH with acetate as the buffer anion as at pH 6 with citrate as the buffer anion, all further studies were carried out with the citrate buffer.

The observations summarized in Fig. 1 for the weakly acidic ibuprofen solute are very impor-

tant, because they indicate that (i) when the eluent pH is sufficiently higher than the pK_a value of the chiral weak acid (*e.g.* one or more pH units), k' can be varied over almost two orders of magnitude, without sacrificing the chiral selectivity of the system, by simply varying the concentration of the organic modifier, and (ii) higher α values can be achieved at the higher pH values where the solubilities (a prime consideration in preparative separations) of the weak acids are higher due to their ionic nature.

Because ibuprofen is mostly anionic at pH 6, it could be expected that solute retention, and perhaps chiral selectivity, would be influenced by the concentration of the buffer anion. Therefore, the analytical citrate concentration was systematically varied using 30% (v/v) acetonitrile: water eluents, while the apparent pH was maintained at 6.0. The log k' values of the more retained enantiomer, (*S*)-(+)-ibuprofen (solid line), are plotted on the left axis in Fig. 2, as a function of the logarithm of the analytical citrate concentration.

Over the range studied, the retention of non-charged solutes, *e.g.* that of the 4-*tert.*-butylphenol increases very slightly (results not shown), reminiscent of a weak salting-out effect in ordinary reversed-phase HPLC [17], but that of the anionic ibuprofen decreases by almost an order of magnitude as the citrate concentration is varied from 0.5 m*M* to 10 m*M*. Chiral selectivity (dotted line), plotted on the right axis in Fig. 2, on the other hand, does not decrease with the citrate concentration. Thus, the concentration of the buffer anion is a powerful variable for the control of the separation of anionic chiral solutes: it can be used to greatly vary solute retention without compromising chiral selectivity.

It could be expected that on native β-cyclodextrin silicas —as in ordinary reversed-phase chromatography— the type and the concentration of the polar organic modifier in the eluent would also influence solute retention and chiral selectivity. Therefore, a series of common water-miscible organic solvents were tested as eluent components [19]. The concentration ranges studied were selected such that the k' values for the more retained (*S*)-(+)-ibuprofen enantiomer fell between 1 and 30. Once again, the observed k' and α values are plotted against the left and right axes of Fig. 3, respectively.

It can be seen in Fig. 3 that the elution strengths of the various organic solvents parallel their usual reversed-phase behavior: the slopes of the log k' *vs.* % (v/v) modifier concentration

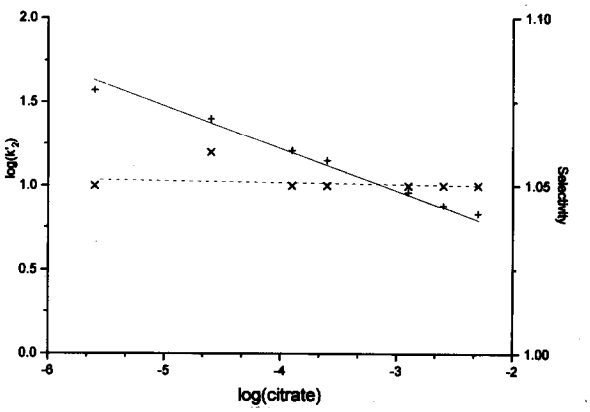

Fig. 2. The capacity factor of (*S*)-(+)-ibuprofen (solid line plotted against the left axis) and the chiral selectivity factor for the separation of the (*S*)-(+)- and (*R*)-(−)-enantiomers of ibuprofen (dotted line plotted against the right axis) as a function of the analytical citrate concentration (*M*) of the eluent. Eluent: 30 % (v/v) acetonitrile, 10 m*M* TEA, pH 6. Symbols: + = k' of (*S*)-(+)-ibuprofen, × = α values. Other conditions as in Fig. 1.

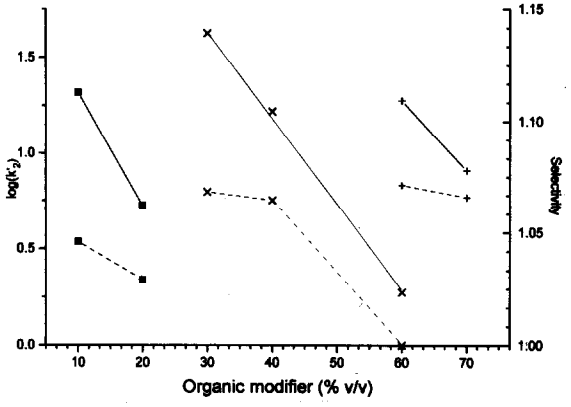

Fig. 3. The capacity factor of (*S*)-(+)-ibuprofen (solid lines plotted against the left axis) and the chiral selectivity factor for the separation of the (*S*)-(+)- and (*R*)-(−)-enantiomers of ibuprofen (dotted lines plotted against the right axis) as a function of the organic modifier concentration (%, v/v) of the eluent. Total citrate concentration: 5 m*M*, TEA concentration 5 m*M*, pH 6.8, temperature: 25°C. Symbols: + = methanol, × = acetonitrile, ■ = tetrahydrofuran.

lines of the various solvents are similar, indicating that similar retention can be achieved with any of these solvents when used at the appropriate concentration. However, the selectivity plot in Fig. 3 shows that chiral resolvation strongly varies with the concentration of the different organic modifiers; selectivity is improved and a limiting maximum value is approached as the water concentration of the eluent is increased. The more hydrophobic the polar organic solvent, the lower its concentration must be in order to secure the limiting α value. Therefore, the behavior shown in Fig. 3 allows one to consider other solvent-related factors as well, such as the solubility of the analyte in the chosen polar modifier, the volatility of the modifier, its purity, price, etc., in selecting the type of the organic modifier to be used for a given preparative chiral separation.

Finally, the effects of the eluent temperature on both solute retention and chiral selectivity were investigated. The log k' *vs.* $1/T$ and the log α *vs.* $1/T$ curves are plotted against the left and right axes in Fig. 4, respectively. There is a significant increase in the k' values (approximately half an order of magnitude) and in the chiral selectivity (approximately 0.07 α units) as the column temperature is decreased from 37°C to 0°C.

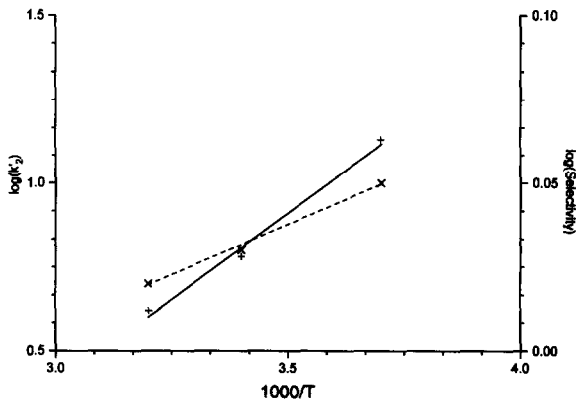

Fig. 4. The capacity factor of (*S*)-(+)-ibuprofen (solid line plotted against the left axis) and the chiral selectivity factor for the separation of the (*S*)-(+)- and (*R*)-(−)-enantiomers of ibuprofen (dotted line plotted against the right axis) as a function of the inverse absolute temperature of the eluent. Conditions as in Fig. 2. Symbols: + = k' of (*S*)-(+)-ibuprofen, × = α values.

It can be concluded from the retention studies that there are two types of eluent parameters which can be changed to optimize the separation of weak acid chiral solutes on native β-cyclodextrin silica columns. The first type of parameter (the organic modifier and its concentration, the pH and the temperature of the eluent) will change both the k' and the α values; the second type of parameter (the buffer concentration of the eluent) will only change k', without compromising the value of α. Therefore, one can, and should, maximize the value of α using the eluent parameters which belong to the first group, then adjust the retention of the solute to the desired level using the parameters which belong to the second group. These observations can also be utilized for the selection of the displacer; after maximizing chiral selectivity for the weak acid solute, its retention can be decreased by increasing the buffer concentration to a value that is lower than the capacity factor of an otherwise suitable non-charged displacer.

Preparative chromatographic separation of the ibuprofen enantiomers in the overloaded elution mode and in the displacement mode of operation

The retention and the selectivity studies discussed above allowed us to select separation conditions which represent a reasonable compromise between separation selectivity, the length of time required to complete the separation and the achievable yield and production rate values. The carrier solution selected contained 10 m*M* TEA, 10 m*M* citrate and 35% (v/v) acetonitrile in a pH 6.5 solution. The separations were completed at 4°C, at a flow-rate of 0.2 ml/min, using two Cyclobond-I columns connected in series (2 × 250 mm × 4.6 mm I.D.), yielding a chiral selectivity, α value of 1.08. Because it was known from previous studies [4,19] that 4-*tert.*-butylcyclohexanol has favorable retention and adsorption characteristics on the Cyclobond I column, it was selected as a possible non-charged displacer for the separation of the ibuprofen enantiomers.

Several displacement mode and overloaded elution mode separations were completed by doubling the ibuprofen sample load from 62.5

μg/injection upwards, until the amount of the (*R*)-(−)ibuprofen enantiomer (at 95% enantiomeric purity) produced in the separation began to decrease. As discussed in [4], the concentration of the 4-*tert.*-butylcyclohexanol displacer was kept at 2 m*M* for all of these separations; this concentration falls onto the strongly nonlinear region of the Langmuirian adsorption isotherm. Fractions measuring 20 to 90 μl were collected throughout the separations and analyzed for enantiomeric purity using another Cyclobond-I column.

The reconstructed displacement chromatogram of the last sample where production still improved with the load (a nominal 500-μg sample) is shown in Fig. 5. With the 2 m*M* 4-*tert.*-butylcyclohexanol displacer, the bands of the two enantiomers reach the 0.4 m*M* (for the (*R*)-(−)-enantiomer) and the 0.6 m*M* (for the (*S*)-(+)-enantiomer) concentration levels. The reconstructed chromatogram for the overloaded elution mode separation of a similar sample —obtained under the same conditions as the displacement chromatogram— is shown in Fig. 6 (nominal 500-μg sample). The peak concentrations of the enantiomers are much lower than in the displacement mode: 0.24 m*M* and 0.14 m*M*,

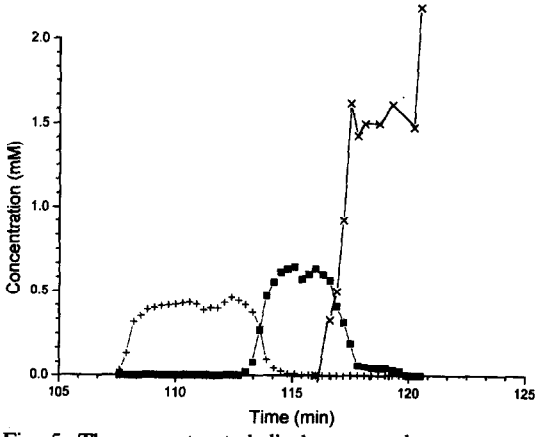

Fig. 5. The reconstructed displacement chromatogram of a nominal 500 μg ibuprofen sample using two Cyclobond I columns, connected in series. The displacer is a 2 m*M* solution of 4-*tert.*-butylcyclohexanol, which is dissolved in a carrier solution of 10 m*M* TEA, 10 m*M* citrate, 35 % (v/v) acetonitrile, pH 6.5, 4°C, 0.2 ml/min. Symbols: + = (*R*)-(−)-ibuprofen, ■ = (*S*)-(+)-ibuprofen, × = 4-*tert.*-butylcyclohexanol displacer.

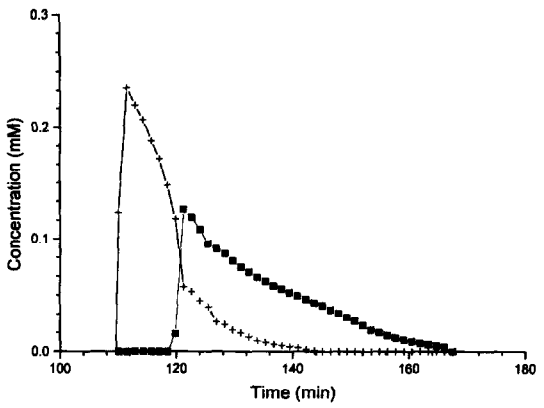

Fig. 6. The reconstructed overloaded elution mode chromatogram of a nominal 500 μg ibuprofen sample using two Cyclobond I columns connected in series. The eluent is the same as the carrier solution in the displacement mode separation (10 m*M* TEA, 10 m*M* citrate, 35 % (v/v) acetonitrile, pH 6.5, 4°C, 0.2 ml/min). Symbols: + = (*R*)-(−)-ibuprofen, ■ = (*S*)-(+)-ibuprofen.

respectively. There is a strong decrease in the concentration of the less retained enantiomer as soon as the elution of the more retained enantiomer begins, in agreement with the predictions of the ideal model of overloaded elution mode chromatography [18].

The reconstructed displacement chromatogram permits the calculation of the enantiomer productions (mg) and the % recoveries as a function of the % enantiomeric purity of the

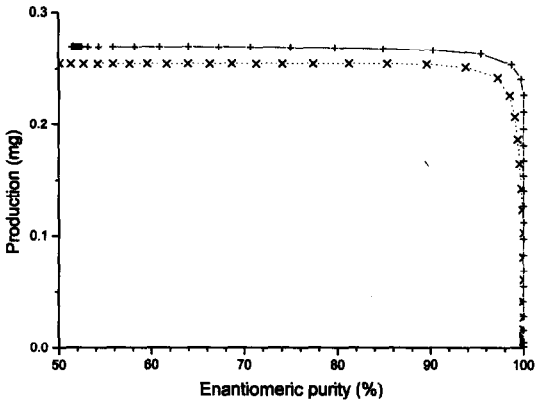

Fig. 7. Production (mg) of the (*R*)-(−)- and the (*S*)-(+)-ibuprofen enantiomers as a function of the % enantiomeric purity of the pooled fractions obtained from the displacement chromatographic separation. Conditions as in Fig. 5. Symbols: + = (*R*)-(−)-enantiomer, × = (*S*)-(+)-enantiomer.

collected fractions. The productions are shown in Fig. 7 for the less retained enantiomer (solid line) and the more retained enantiomer (dotted line), respectively. The % recoveries are shown in Fig. 8, again for the less retained enantiomer (solid line) and the more retained enantiomer (dotted line), respectively. Since the purpose of this separation is chiral resolution, the enantiomeric purities rather than the chemical purities are used in the figures. For the less retained enantiomer, the enantiomeric purity and the chemical purity are identical. For the more retained enantiomer, the chemical purity is less than the enantiomeric purity, because the tail-end of the band of the more retained enantiomer is contaminated by the displacer. However, because the 4-*tert*.-butylcyclohexanol displacer is non-ionic, its traces can be easily removed from the anionic second enantiomer by an additional ion exchange step.

For the corresponding overloaded elution mode separation the calculated enantiomer productions (mg) and % recoveries are shown in Figs. 9 and 10, respectively, for the less retained enantiomer (solid line) and the more retained enantiomer (dotted line).

It can be seen from Fig. 7 that in the displacement mode a little more material can be produced at the 95% enantiomeric purity level from the less retained enantiomer (0.27 mg), than from the more retained enantiomer (0.25 mg). This difference in production is more pronounced in the overloaded elution mode: 0.22 mg can be produced from the less retained enantiomer (solid line), 0.10 mg from the more retained enantiomer (dotted line in Fig. 9). This means that while under the given conditions both the displacement mode and the overloaded elution mode separations yield quite commensurable amounts of pure material for the first enantiomer, the overloaded elution mode yields less of the second enantiomer than does the

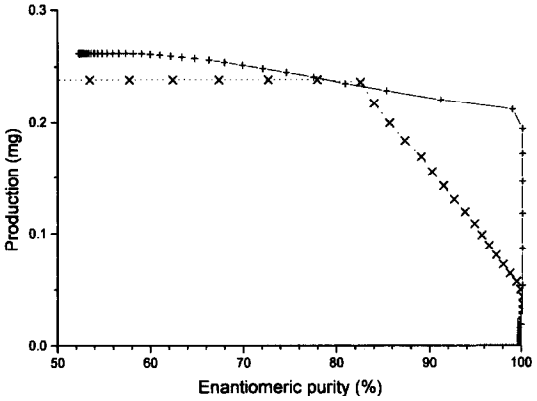

Fig. 9. Production (mg) of the (*R*)-(−)- and the (*S*)-(+)-ibuprofen enantiomers as a function of the % enantiomeric purity of the pooled fractions obtained from the overloaded elution mode separation. Conditions as in Fig. 6. Symbols: + = (*R*)-(−)-enantiomer, × = (*S*)-(+)-enantiomer.

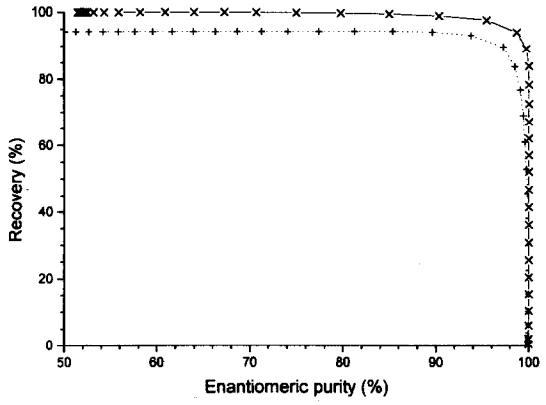

Fig. 8. Recovery (%) of the (*R*)-(−)- and the (*S*)-(+)-ibuprofen enantiomers as a function of the % enantiomeric purity of the pooled fractions obtained from the displacement chromatographic separations. Conditions as in Fig. 5. Symbols: × = (*R*)-(−)-enantiomer, + = (*S*)-(+)-enantiomer.

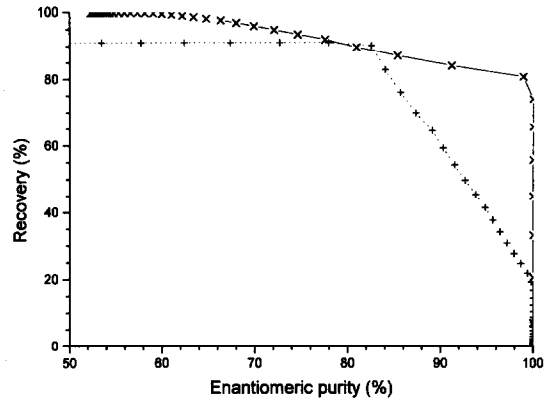

Fig. 10. Recovery (%) of the (*R*)-(−)- and (*S*)-(+)-ibuprofen enantiomers as a function of the % enantiomeric purity of the pooled fractions obtained from the overloaded elution mode separation. Conditions as in Fig. 6. Symbols: × = (*R*)-(−)-enantiomer, + = (*S*)-(−)-enantiomer.

displacement mode. These observations are fully supported by the % recovery *vs.* enantiomeric purity curves (Figs. 8 and 10): at the 95% enantiomeric purity level less material is recovered in the elution mode than in the displacement mode, but the difference is more pronounced for the more retained enantiomer than for the less retained enantiomer. When it comes to production rates, under the given conditions, the displacement mode fares worse in the case of the less retained enantiomer (due to column regeneration time), but the two techniques have more comparable production rates for the second enantiomer (due to low recovery in the overloaded elution mode).

CONCLUSIONS

A displacement chromatographic method has been developed for the separation of the enantiomers of ibuprofen by a native β-cyclodextrin silica stationary phase on the basis of independent selectivity maximization and retention control. The log k' *vs.* polar organic modifier concentration, the log k' *vs.* pH, the log k' *vs.* buffer concentration and the log k' *vs.* $1/T$ relationships have been determined, along with the α *vs.* polar organic modifier concentration, the α *vs.* pH, the α *vs.* buffer concentration and the log α *vs.* $1/T$ relationships in order to select the carrier solution composition which results in maximum chiral selectivity and sufficient, but not excessive retention for the less retained enantiomer ($1 < k' < 30$). 4-*tert.*-Butylcyclohexanol, which is a non-charged substance with a structure similar to that of the analyte, is more retained than the more strongly adsorbed enantiomer of ibuprofen, and has a suitable, Langmuirian adsorption isotherm [4,19], could be used as displacer for the separation. A comparison of the productions (mg) and % recoveries as a function of the % enantiomeric purity of the pooled fractions indicates that under the given conditions the displacement mode and the overloaded elution mode separations perform quite comparably for the less retained enantiomer, but the displacement mode performs better for the more retained enantiomer.

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