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Resolution of (±)-2-aminoheptane enantiomers by inductive adsorption chromatography

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ABSTRACT

The use of inductive adsorption chromatography for enantiomer resolution involves a chiral additive to the mobile phase, the so-called inductive agent, which has the same chemical nature as the enantiomers to be resolved. In this way, (±)-2-aminoheptane enantiomers were partially resolved on a silica column by using (−)-α-methylbenzylamine as the mobile phase additive; the first-eluted fraction was composed of optically pure (−)-2-aminoheptane and represented up to 81% of the injected amount. The experimental conditions were varied in order to enhance the separation. Optically pure fractions were obtained at low loadings per unit cross-section and high capacity factors, but exceptions were observed. The inductive agent concentration has a large effect on retention, by displacement action, but not on the resolution.

INTRODUCTION

Inductive adsorption is a separation method in which the selectivity is due to the coadsorption of an inductive agent together with the isomers to be separated, which acts by steric interactions among neighbouring chains of adsorbed molecules. This phenomenon takes place when the adsorbent surface is saturated with a monolayer dense enough to allow such interactions and thus requires strong adsorption of the species. Inductive adsorption is typically exemplified by adsorption of amines on silica [1].

The steric nature of the selectivity has been demonstrated by adsorption equilibrium experiments using monofunctional compounds, the chemical group of which is bonded to the adsorption site and where the carbonaceous structure alone is accessible to interactions [1]. The repulsive character of such interactions has been confirmed by means of statistical thermodynamics [2].

Equilibrium experiments related to coadsorption of an optically pure inductive agent together with a racemate on an achiral adsorbent have shown that such steric interactions in the monolayer are sufficient to create enantioselectivity [3]. A close-packed organization is formed on the adsorbent surface; it exhibits chirality owing to the presence of the chiral inductive agent and it favours the adsorption of one of the enantiomers, resulting in the enrichment of the liquid phase with the other enantiomer. The inductive agent and the racemate must have the same chemical nature, and thus

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comparable heats of adsorption, in order to be distributed between the liquid and solid phase to comparable extents. For example, the enantioselectivity of (±)-2-aminoheptane coadsorbed with (−)-α-methylbenzylamine on silica reaches 1.18. This study has also shown that, when the ratio of inductive agent to racemate is varied, maximum enantioselectivity is attained; when the amount of inductive agent is too low or too high, the enantioselectivity vanishes.

In this paper we report the resolution of enantiomers of (±)-2-aminoheptane by chromatography on a silica column with (−)-α-methylbenzylamine as inductive agent. It is worth noting that, in all previously reported chromatographic resolutions of (±)-2-aminoheptane on chiral stationary phases, preliminary derivatization was required. For example, enantiomeric amides derived from 2-aminoheptane have been resolved on cellulose tribenzoate coated on macroporous silica [4]. The selectivity values were 1.22, 1.69 and 2.06 for N-acetyl, N-benzoyl and N-*p*-totuoyl derivatives, respectively. For N-benzoyl or N-trifluoroacetyl derivatives, lower separation factors (1.05) were obtained on N-(3,5-dinitrobenzoyl)phenylglycine silica and another π-acceptor phase [5]. However, the 3,5-dinitrobenzoyl derivative of 2-aminoheptane was resolved with a separation factor of 1.21 on a chiral stationary π-donor phase [6].

In inductive adsorption chromatography, as the creation of enantioselectivity requires the enantiomers to be surrounded by a chiral inductive agent during their migration within the column, the inductive agent is diluted in a solvent and fed continuously into the column; the operation is exactly the same as in chromatographic resolutions with a chiral additive in the mobile phase, such as dynamic ligand exchange, the ion-pairing technique or inclusion complexation by cyclodextrins and modified cyclodextrins. Armstrong and Han [7], Pettersson [8] and Allenmark [9] have recently reviewed this mode of enantiomer resolution. The difference between a conventional mobile phase additive and an inductive agent comes from the mechanism of the creation of the enantioselectivity: the former involves the formation of definite labile diastereoisomeric complexes with the enantiomers to be separated, whereas an inductive agent acts only by steric interaction at the adsorbent surface.

This mode of creation of enantioselectivity imposes particular operating conditions. A particularity arises from the necessity to saturate the adsorbent surface. Experiments have shown that the inductive adsorption selectivity vanishes when a solvent or a non-chiral agent is adsorbed [1]; this species intercalates among the adsorbates and destroys the chiral organization of the monolayer. The adsorbent saturation implies strong adsorption of the racemate and of the inductive agent. Hence the inductive agent acts as a displacement agent and pushes the enantiomers out of the column while discriminating them. Thus the process operates in the displacement mode.

EXPERIMENTAL

Ordinary high-performance liquid chromatographic equipment was used to carry out the separation. The eluent consisted of (−)-α-methylbenzylamine in isooctane at a concentration high enough for saturation of the silica column (typically 0.1 *M*). The enantiomers were injected with a 20-μl sample loop and were indirectly detected by UV absorption. Methylbenzylamine, the inductive agent, has a strong UV response whereas 2-aminoheptane has none. When 2-aminoheptane is eluted from the

column, because of conservation of the adsorbent coverage there will be less of the methylbenzylamine present at the detector and a smaller signal will be observed.

The elution profiles are badly resolved and do not give quantitative information on the extent of the enantiomer resolution. Therefore, in some experiments, the elution profile was fractionated and each fraction derivatized according to the König's procedure [10] and analysed by gas chromatography (GC) on a 50-m chiral capillary column coated with XE-60–*S*-valine–*S*-α-pea (Chrompack). By this means the enantiomer peaks were reconstituted.

RESULTS

Shape of the elution profile

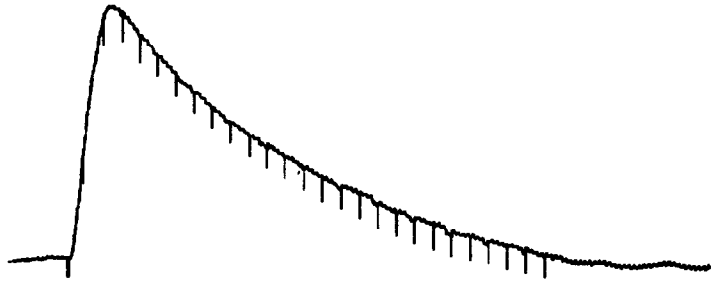
A number of experiments were performed using silica columns of different sizes and under different operating conditions in order to obtain the best separation of the (±)-2-aminoheptane enantiomers. In every run, the elution profile of the enantiomers was broad and did not look like a classical elution profile. Two main shapes could be identified: one triangular peak, with a sharp front and a slanted tail (Fig. 1a), or two broad and badly resolved peaks (Fig. 2a).

The chiral GC analysis of the triangular peak fractions indicated that the enantiomer resolution is beginning, although it does not appear on the chromatogram (Fig. 1b); the first-eluted fraction is enriched in (−)-2-aminoheptane when (−)-α-methylbenzylamine is used as the inductive agent, indicating preferential adsorption of the (+)-2-aminoheptane enantiomer. This finding is in accordance with the adsorption equilibrium of racemic 2-aminoheptane on silica in the presence of (−)-α-methylbenzylamine [3], where preferential adsorption of (+)-2-aminoheptane was observed. The elution order was reversed when the inductive agent was replaced by its antipode, (+)-α-methylbenzylamine.

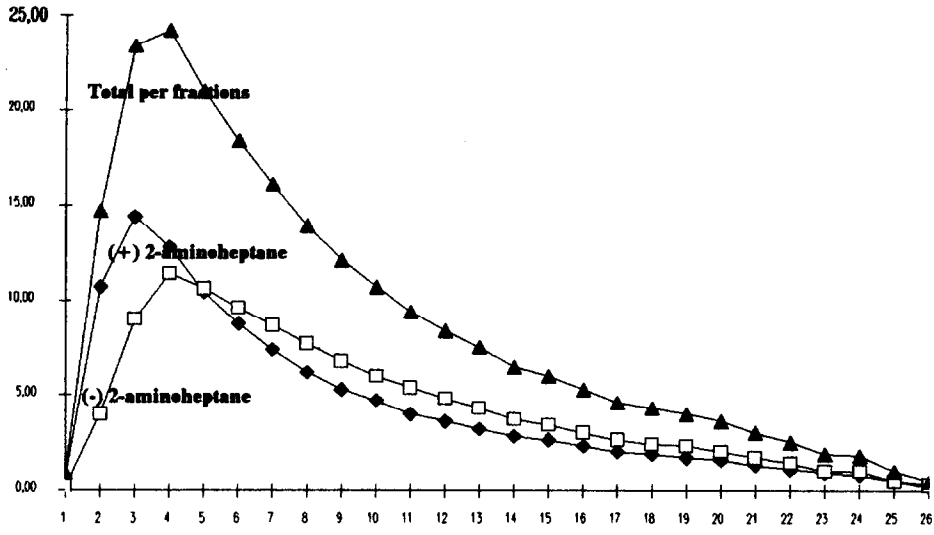
For the two distinct peaks, the reconstitution of the chromatogram by chiral GC analysis showed that the first-eluted fraction is composed of optically pure (−)-2-aminoheptane (Fig. 2b) and the pure fraction represents 75% of the loaded enantiomer. Other examples are reported in Table I; in all these experiments, the first fraction, collected until the valley between the two peaks, is optically pure. The experiment related to the 50 cm × 0.46 cm I.D. column needs a special mention, because it corresponds to an intermediate elution profile (Fig. 3); the peak is unique but exhibits a slight valley and the fraction collected from the beginning to the valley is composed of optically pure (−)-2-aminoheptane.

Concerning the efficiency of the separation, the results are better when the two peaks can be distinguished. We have not been able to relate the occurrence of one or another shape to the experimental conditions. The following parameters were examined, without indicating any absolute correlation: flow-rate, linear velocity (flow-rate divided by the cross-sectional area of the column), sample loading, sample loading per unit cross-sectional area, sample loading per column saturation capacity, inductive agent concentration and capacity factor. The peak shape and operating conditions ranges are indicated in Table II; the flow-rate was preferably expressed per unit cross-sectional area and the loading per unit column volume.

The shape seems to depend mainly on the column used: a triangular shape was obtained with columns 1, 2, 5 and sometimes 3. This finding may be rationalized with



(a)



(b)

Fig. 1. Elution profile of the so-called "triangular peak": reconstitution of the enantiomer peaks by GC analysis of collected fractions. (a) Chromatogram; (b) reconstitution. Conditions: sample, 6.9 mg of (±)-2-aminoheptane; mobile phase, 0.1 *M* (−)-α-methylbenzylamine in isooctane; flow-rate, 0.3 ml/min; column, 5-μm silica (25 cm × 0.46 cm I.D.); UV detection at 272 nm.

columns 1 and 2. On the one hand these columns are short and do not give an opportunity for peaks to develop, and the capacity factors associated with these columns are lower than the others. On the other hand, triangular peak profiles are usually associated with a Langmuir isotherm under overloaded conditions [11], and overloading can be involved. A rough correlation of the peak shape with loading and retention can be drawn (Fig. 4): triangular peaks appear at heavy loading and low retention. It is noticeable that experiments related to column 3 occupy an intermediate position on the graph and have effectively an intermediate shape. However, the correlation is not absolute and fails with column 5 (50 cm × 1.0 cm I.D.): the peaks are clearly triangular, but the loading is low and the retention is high, so another explanation must be invoked, *e.g.*, poor packing.

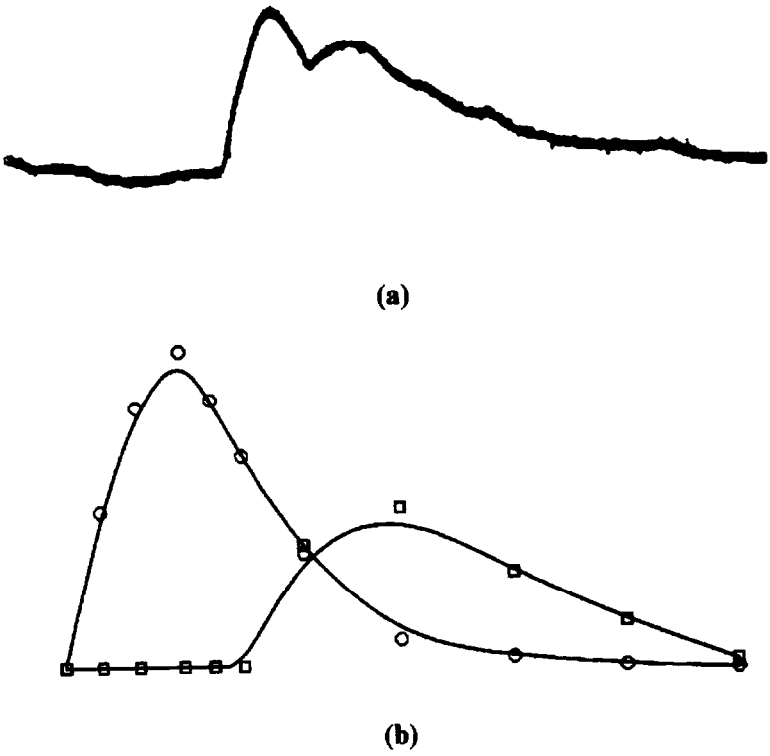


Fig. 2. Elution profile of the so-called "distinct peaks": reconstitution of the enantiomer peaks by GC analysis of collected fractions. (a) Chromatogram; (b) reconstitution. Conditions: sample, 1.15 mg of (±)-2-aminoheptane; mobile phase, 0.12 *M* (−)-α-methylbenzylamine in isooctane; flow-rate, 1.3 ml/min; column, 5-μm silica (50 cm × 0.71 cm I.D.); UV detection at 272 nm.

TABLE I

EXPERIMENTAL CONDITIONS FOR RESOLUTION OF (±)-2-AMINOHEPTANE YIELDING A FIRST-ELUTED FRACTION COMPOSED OF OPTICALLY PURE (−)-2-AMINOHEPTANE

Inductive agent, (−)-α-methylbenzylamine; columns, 5-μm silica.

Column (cm × cm I.D.)	Flow-rate (ml/min)	Loading (mg)	Inductive agent concentration (*M*)	Capacity factor, *k'*	Separation factor, α	Resolution factor, R_s	Pure fraction (%)
50 × 0.46	0.5	2.3	0.10	5.8	—	—	28
50 × 0.71	1.2	2.3	0.12	5.3	1.02	0.44	55
50 × 0.71	1.3	1.15	0.12	5.3	1.04	0.57	75
50 × 0.71	1.5	1.15	0.14	4.9	1.03	0.60	81

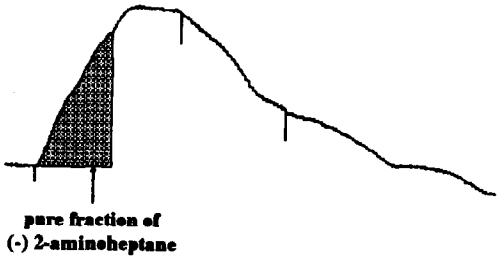


Fig. 3. Elution profile of the so-called "intermediate" and GC analysis of the hatched fraction. Conditions: sample, 2.3 mg of (±)-2-aminoheptane; mobile phase, 0.1 *M* (−)-α-methylbenzylamine in isooctane; flow-rate, 0.5 ml/min; column, 5-μm silica (50 cm × 0.46 cm I.D.); UV detection at 272 nm.

Influence of operating parameters on the separation

For triangular peaks, the extent of the enantiomer resolution is assessed from the enantiomeric ratio, *E*, defined as the ratio of (−)-2-aminoheptane to (+)-2-aminoheptane in the fraction corresponding to the upward part of the peak and limited by the maximum. For distinct peaks, conventional chromatographic parameters, such as the separation factor α and the resolution factor R_s, are measured on the chromatogram. It should be noted that the determination is not very precise because of the tailing shape of the elution profile and the low separation factors. The enantiomeric ratio *E* is the preferred quantity for triangular peaks because the corresponding α values fall in a very narrow range (1.00–1.02).

The flow-rate has little influence on the enantiomeric ratio *E* for triangular peaks (Table III): there is a linear velocity range, around 3–6 cm/min, in which the enrichment is better. This behaviour calls to mind the characteristic plot of the height equivalent to a theoretical plate *versus* the mobile phase velocity, the so-called Van Deemter curve [12]: the loss of separation at high linear velocity may be attributed to

TABLE II

COMPARISON OF THE SHAPE OF ELUTION PROFILES AND THE RANGES OF OPERATING CONDITIONS

Silica column, 5 μm				Range of operating conditions			Shape[a]
No.	Dimensions (cm × cm I.D.)	Packing	Specific surface area (m^2/g)	Mobile phase velocity (cm/min)	Loading per unit column volume (mg/cm^3)	Inductive agent concentration (*M*)	
1	15 × 0.46	Spherisorb	250	1.5–9.0	0.05–1.38	0.10	1
2	25 × 0.46	Hypersil	170	1.2–3.6	0.28–2.77	0.10–0.20	1
3	50 × 0.46	Intersil	540	3.0–6.0	0.07–0.28	0.10	1*
4	50 × 0.71	Intersil	540	2.0–6.3	0.03–0.12	0.08–0.17	2
5	50 × 1.00	Intersil	540	1.9–4.5	0.03–0.12	0.12	1
6	50 × 0.71 + 50 × 0.46	Intersil	540	4.9–5.7	0.02–0.08	0.12–0.30	2

[a] 1, One triangular peak; 2, two distinct peaks; 1*, intermediate shape.

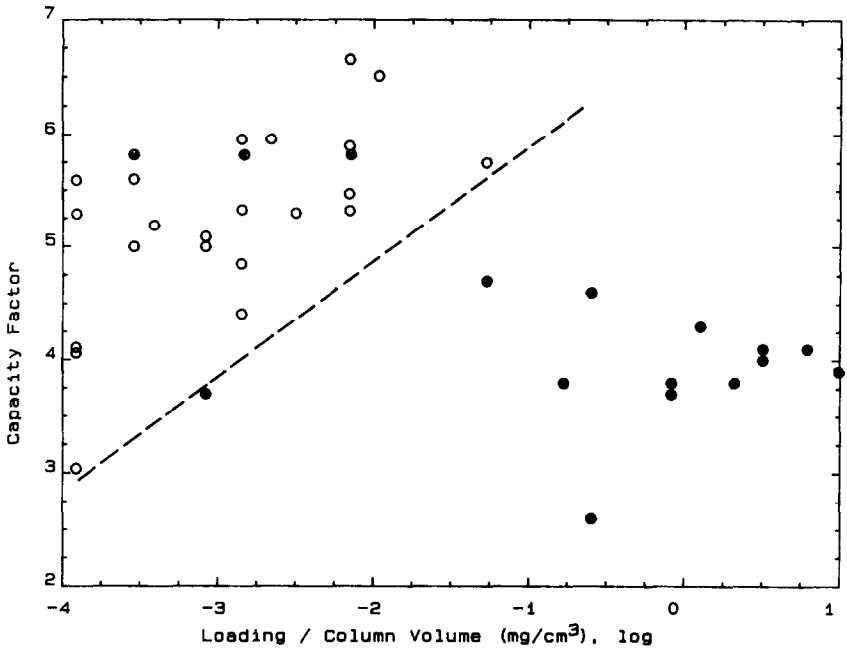


Fig. 4. Correlation between the shape of the elution profile and the sample loading and the capacity factor. (●) One triangular peak; (⊗) one triangular peak, column 5; (○) two distinct peaks.

the contribution of poor mass transfer. In the 3–6 cm/min range, the flow-rate does not affect the separation factor α of partially resolved peaks (Table III). The flow-rate has no influence on the capacity factor, k' (Table III).

The influence of the sample loading on the separation efficiency depends on the shape of the elution profile: for triangular peaks, an increase in loading seems to enhance slightly the enrichment of the first-eluted fraction (Fig. 5). However, this result cannot be extrapolated, as one must consider that when peaks are resolved, the

TABLE III

INFLUENCE OF FLOW-RATE ON ENANTIOMER SEPARATION

No.	Column (cm × cm I.D.)	Flow-rate (ml/min)	Velocity (cm/min)	Loading (mg)	Inductive agent concentration (M)	Capacity factor, k'	Enantio. ratio, E	Separation factor, α
1	15 × 0.46	0.25	1.5	2.3	0.10	3.8	1.34	–
1	15 × 0.46	0.5	3.0	2.3	0.10	3.8	1.50	–
1	15 × 0.46	1.0	6.0	2.3	0.10	3.8	1.35	–
1	15 × 0.46	1.5	9.0	2.3	0.10	3.8	1.05	–
4	50 × 0.71	1.5	3.8	0.92	0.15	5.1	–	1.03
4	50 × 0.71	2.0	5.1	0.92	0.15	5.0	–	1.03
4	50 × 0.71	2.4	6.1	0.92	0.15	5.1	–	1.03

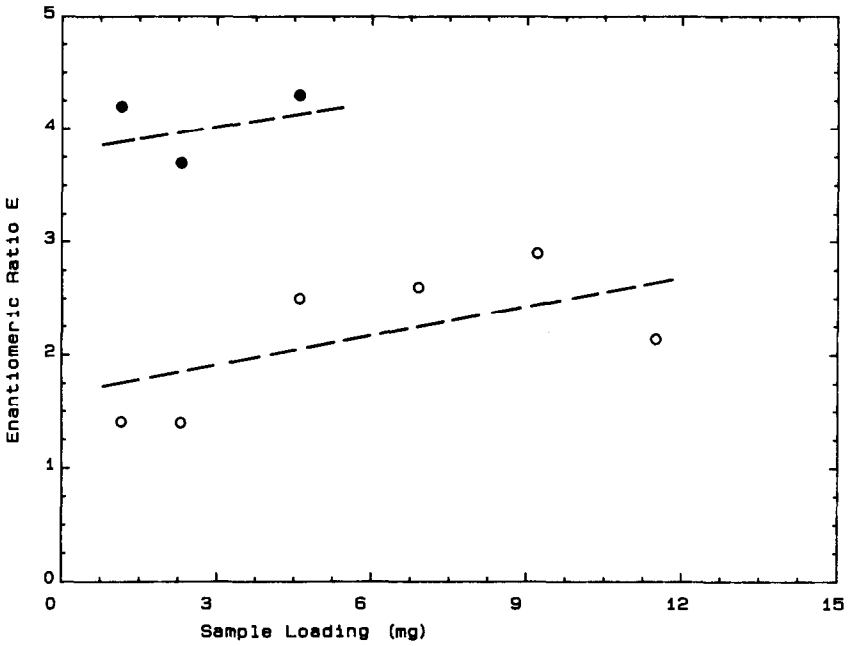


Fig. 5. Influence of sample loading on the enantiomeric ratio E. Conditions: (○) C_{IA}, 0.1 M; column, 25 cm × 0.46 cm I.D.; (●) C_{IA}, 0.12 M; column, 50 cm × 1.0 cm I.D.

first-eluted fraction is optically pure (the enantiomeric ratio E tends to infinity) and, as mentioned above, distinct peaks appear at low loadings. Experiments yielding two distinct peaks indicate that, for a given column, the loading does not have a real influence on the separation factor (Table IV).

The inductive agent concentration, C_{IA}, has no influence on the separation factor α in the experimental concentration range (0.08–0.30 M) (Table V). At first sight, this finding is surprising; actually, adsorption equilibria of racemic 2-aminoheptane on

TABLE IV

INFLUENCE OF SAMPLE LOADING ON ENANTIOMER SEPARATION

No.	Column (cm × cm I.D.)	Flow-rate (ml/min)	Loading (mg)	Inductive agent concentration (M)	Capacity factor, k'	Separation factor, α	Resolution factor, R_s
4	50 × 0.71	2.0	0.575	0.12	5.6	1.04	
4	50 × 0.71	1.3	1.15	0.12	5.3	1.04	0.57
4	50 × 0.71	1.2	2.30	0.12	5.3	1.03	0.44
4	50 × 0.71	1.5	2.30	0.12	5.5	1.03	0.60
6	2 columns	1.5	0.575	0.15	5.3	1.02	
6	2 columns	1.5	0.92	0.15	5.2	1.02	
6	2 columns	1.5	2.30	0.15	5.3	1.01	

TABLE V

INFLUENCE OF INDUCTIVE AGENT CONCENTRATION ON ENANTIOMER SEPARATION

No.	Column (cm × cm I.D.)	Flow-rate (ml/min)	Loading (mg)	Inductive agent concentration (M)	Capacity factor, k'	Separation factor, α	Resolution factor, R_s
4	50 × 0.71	1.5	1.15	0.08	6.0	1.03	0.76
4	50 × 0.71	1.3	1.15	0.12	5.3	1.04	0.57
4	50 × 0.71	1.5	1.15	0.14	4.8	1.03	0.57
4	50 × 0.71	1.2	1.15	0.17	4.4	1.03	0.60
6	2 columns	1.5	0.575	0.12	5.6	1.03	1.0
6	2 columns	1.5	0.575	0.15	5.3	1.02	0.65
6	2 columns	1.5	0.575	0.20	4.1	1.02	0.70
6	2 columns	1.5	0.575	0.30	3.0	1.03	0.50

silica and other acidic adsorbents in the presence of (−)-α-methylbenzylamine have shown that there is an optimum (−)-α-methylbenzylamine/(±)-2-aminoheptane ratio r_c; when too small an amount of inductive agent is adsorbed, the chiral environment is not sufficient to create selectivity, whereas too large an amount of inductive agent causes the enantioselectivity to disappear [3]. We therefore expected an (−)-α-methylbenzylamine concentration value yielding to an optimum enantiomer separation. In

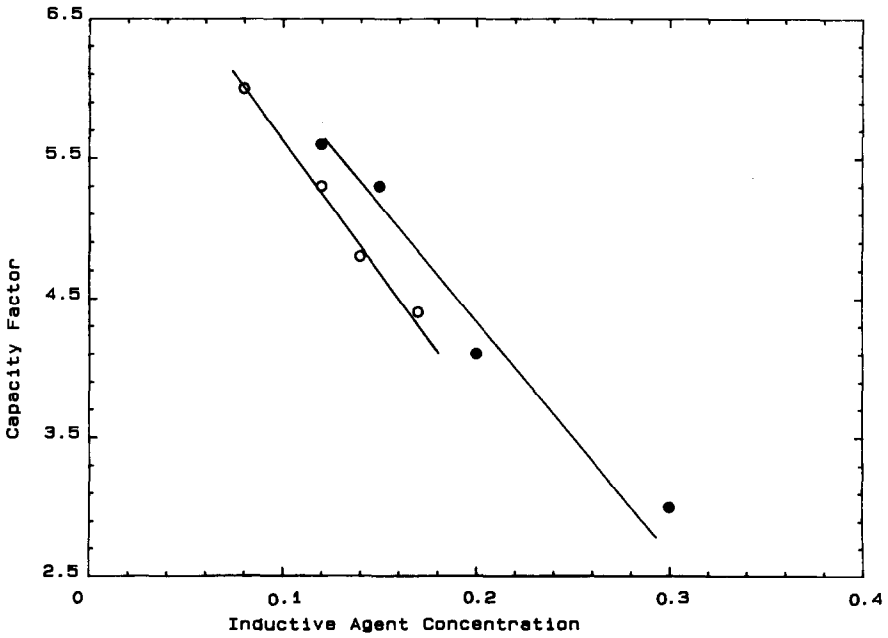


Fig. 6. Influence of the inductive agent concentration on the capacity factor. Conditions: (○) sample, 1.15 mg of (±)-2-aminoheptane; column, 50 cm × 0.71 cm I.D.; (●) sample, 0.575 mg of (±)-2-aminoheptane; columns, 50 cm × 0.71 cm I.D. and 50 cm × 0.46 cm I.D. in series.

fact, the finding may be rationalized by considering the progressive dilution of the enantiomer bands during their travel into the column. The dilution is a basic concept of chromatography and results from the distribution of the compounds between the mobile and stationary phases. The enantiomer concentration decreases all along the column and the concentration ratio r_c increases. The variation of the equilibrium enantioselectivity with r_c means that only a certain range of r_c values is effective in the separation. Thus, as the enantiomer peak becomes too small because of its broadening, there is no further enrichment in the column and, on the contrary, the axial dispersion contributes to remixing the peaks. As a consequence, only a portion of the column is effective for the separation, and the active portion is displaced inside the column according to the concentration of the inductive agent. This could explain the

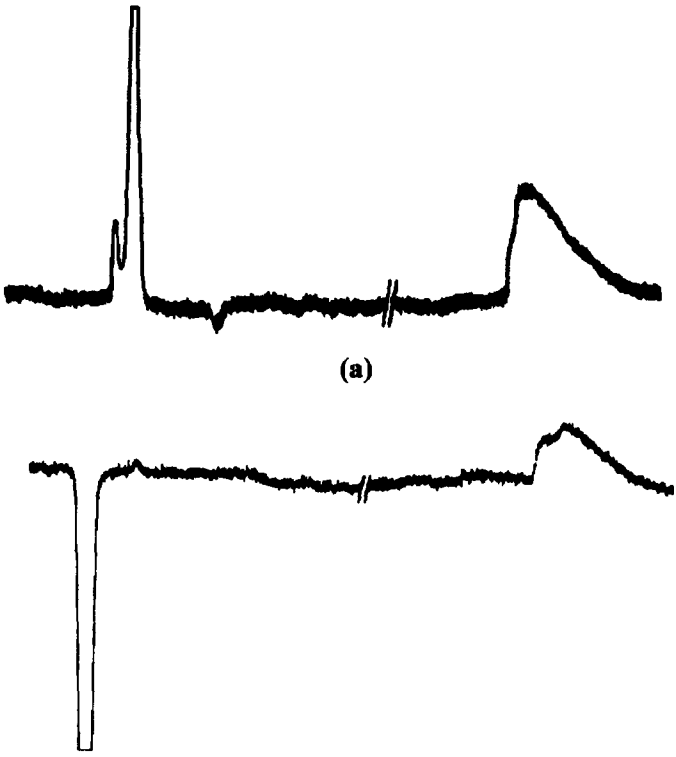


Fig. 7. Evolution of the system peaks with the concentration difference between mobile phase and sample. Conditions: (a) sample, 10 μl of 0.5 *M* (0.575 mg) (±)-2-aminoheptane; mobile phase, 0.3 *M* (−)-α-methylbenzylamine in isooctane; flow-rate, 1.5 ml/min; columns, 5-μm silica (50 cm × 0.46 cm I.D. + 50 cm × 0.71 cm I.D. in series); UV detection, 272 nm; (b) sample, 20 μl of 0.1 *M* (0.230 mg) (±)-2-aminoheptane; mobile phase, 0.15 *M* (−)-α-methylbenzylamine in isooctane; flow-rate, 1.0 ml/min; column, 5-μm silica (50 cm × 0.46 cm I.D.); UV detection, 272 nm.

experimental results obtained with the 1 cm I.D. column (column 5), with a large diffusion contribution leading to poor separation efficiency.

The inductive agent concentration C_{IA} has a strong effect on the capacity factor k', which decreases linearly as C_{IA} increases (Fig. 6). This variation can be interpreted as a displacement effect and expresses competition between the inductive agent and enantiomers for adsorption sites: the higher is C_{IA}, the fewer are the adsorption sites available for enantiomer adsorption, *i.e.*, the lower is the column capacity for enantiomers.

The variation of the operating conditions has shown that the main peak in the system peaks is composed of inductive agent. In fact, the magnitude of this peak is related to the concentration difference between the mobile phase and the sample (Fig. 7): when the racemate concentration is higher than the inductive agent concentration, the adsorption of the racemate in the column will expel the inductive agent from the stationary phase and, because of saturation of the column, this displaced inductive agent cannot adsorb elsewhere and is eluted very rapidly. On other hand, when the racemate concentration is lower than the inductive agent concentration, it is a lack of inductive agent, relative to the mobile phase concentration, that leads to a negative peak.

CONCLUSIONS

Partial enantiomer resolution has been achieved by inductive adsorption chromatography and, under the optimum conditions, 81% of the first-eluted enantiomer was recovered with an optical purity of 100%. The tailing is strong, and the second eluted enantiomer cannot be recovered with a good optical purity with the same inductive agent. It should be possible by using the inductive agent antipode.

In inductive adsorption chromatography, the same compound, the inductive agent, creates the enantioselectivity and displaces the enantiomers. This double role makes the behaviour of the chromatographic column uncommon. It gives rise to a broad and unusual elution profile and to a wide range of possible operating conditions. The concentration of the inductive agent plays a decisive role in the retention of enantiomers but has little influence on the resolution. Short columns are probably preferable.

From a fundamental point of view, inductive adsorption chromatography has demonstrated for the first time that a chiral additive in the mobile phase that has the same chemical nature as enantiomers and cannot bond with them nevertheless allows the resolution of the enantiomers. Moreover, this is the first reported chromatographic resolution of (±)-2-aminoheptane enantiomers without derivatization.

REFERENCES

1 F. Duprat, R. Gassend and G. Gau, *Ind. Eng. Chem. Res.*, 27 (1988) 831.
2 F. Duprat and G. Gau, *Ind. Eng. Chem. Res.*, 29 (1990) 1424.
3 R. Gassend, F. Duprat and G. Gau, *J. Chromatogr.*, 404 (1987) 87.
4 I. W. Wainer and M. C. Alembik, *J. Chromatogr.*, 358 (1986) 85.
5 R. R. Brügger, A. R. Marti, V. R. Meyer and H. Arm, *J. Chromatogr.*, 440 (1980) 197.
6 R. Däppen, V. R. Meyer and H. Arm, *J. Chromatogr.*, 361 (1986) 93.
7 D. W. Armstrong and S. M. Han, *CRC Crit. Rev. Anal. Chem.*, 19 (1988) 175.

8 C. Pettersson, *Trends Anal. Chem.*, 7 (1988) 209.
9 S. G. Allenmark, *Chromatographic Enantioseparation. Methods and Applications*, Wiley, New York, 1988, pp. 132–145.
10 W. A. König, *The Practice of Enantiomer Separation by Capillary Gas Chromatography*, Hüthig, Heidelberg, 1987.
11 G. Guiochon, S. Golshan-Shirazi and A. Jaulmes, *Anal. Chem.*, 60 (1988) 1856.
12 V. R. Meyer, *Practical High-Performance Liquid Chromatography*, Wiley, Chichester, 1988.