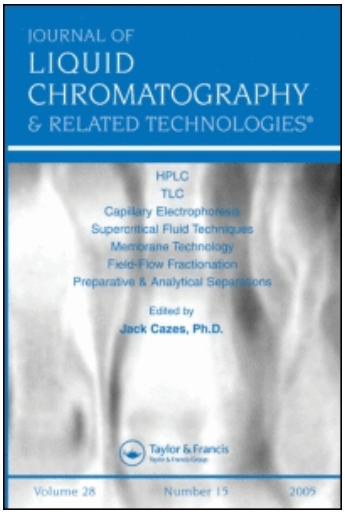

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Direct Resolution of Enantiomers in Column Liquid Chromatography

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To cite this Article Audebert, R.(1979) 'Direct Resolution of Enantiomers in Column Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 2: 8, 1063 — 1095
To link to this Article: DOI: 10.1080/01483917908060125
URL: http://dx.doi.org/10.1080/01483917908060125

JOURNAL OF LIQUID CHROMATOGRAPHY, 2(8), 1063-1095 (1979)

DIRECT RESOLUTION OF ENANTIOMERS IN COLUMN LIQUID CHROMATOGRAPHY

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ABSTRACT

Important progress has been performed during the ten past years, in the direct resolution (without derivatization) of enantiomers by column liquid chromatography. Generally chiral packings proceeding from natural or synthetic optically active polymers are used. Complete resolutions of hole families of chiral solutes is obtained in systems involving a steric locking interaction (charge transfer between aromatic substituents, crown ether cavity, crowded complexes with metal ions). For analytical purposes an other promising way is the use of a classical packing and a chiral mobile phase.

INTRODUCTION

Resolution of enantiomers has a theoretical interest in the study of interaction mechanism between chiral molecules but also an industrial one for chemists, biologists and pharmacologists.

Various methods of enantiomers separation were intiated at the end of the XIXth century ; the most used now is the selective crystallization of diastereoisomers. It needs three steps : the enantiomer mixture first reacts with a pure optically active compound leading to two derivatives (diastereoisomers), then these species are separated by crystallization and finally pure enantiomers recovered by inverse derivatization.

Chromatography, which is an efficient method of fractionation , may replace crystallization in the previous separation process : as it exists special derivatives for an easy recrystallization of diastereoisomers, various chiral reageants are proposed to lead to their easy chromatographic separation. Drawbacks and benefits are the same for the two methods. They need an optically pure reagent and, above all, direct and inverse derivatizations are long and expensive. As a benefit, it can be noticed that the chiral reagents are generally operative not only for a given mixture of enantiomers but for all optically active parent compounds. Besides, chromatography presents some typical advantages : it can be used even with a mixture of more than two chiral compounds, analytical tests can be run with very small sample amounts but, in liquid chromatography, preparative experiments may also be expected.

In spite of its defects, chromatographic separation of diastereoisomers is a very convenient and largely used technique. Its defects could be avoided by direct chromatographic resolution (without derivatization) of enantiomers.

As depicted in different reviews [1-12] partly devoted to this technique, it is in constant progress during the ten past years. And this review is thus limitated to this period and to this field. Neither resolution by reactive chromatography (stereoselective chemical reaction on a packed chiral reagent) nor separation of diastereoisomers is considered here.

MECHANISM OF DIRECT CHROMATOGRAPHIC RESOLUTION OF ENANTIOMERS

For an optically active molecule, a chiral recognition can be effective only by an other chiral molecule : an asymetric substrate, schematized in Fig.1 can be unequivocally associated by three interactions (respectively AA', BB' and CC') with a chiral solute, whereas the association AA", BB", CC" is prevented by the bulkiness (R") of the other optical isomer.

If only two interaction points are involved (i.e. AA', BB') the spatial position of the solute molecule is not definite and,

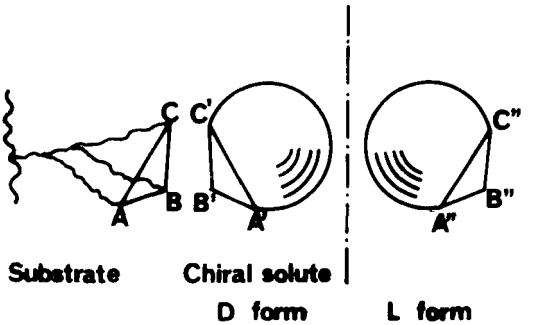


Figure 1 : The three point rule : the only D chiral solute may lead to a stable complex through AA', BB' and CC' interactions.

as a result, there is no stereoselectivity. This mechanism, suggested by Dalgliesh [13] is known as the "three points" rule. In fact, each "point" (binding site) is not always clearly defined ; all kinds of molecular interaction (or repulsion) may participate (possibly more than three for each system). The only important matter is that one of the solute isomers must be spatially locked in a position which is instable for the other one. By taking into account the above model, the resolution of enantiomers on a chiral packing appears as possible but according to the following conditions :

1) specific binding sites are currently obtained through hydrogen bonding or dipolar interactions and efficient substrates and well separated enantiomers have to be polar compounds. The resolution of chiral alkanes by liquid chromatography is thus a very difficult problem. In fact, most of the optical isomers of practical interest are polar compounds and this first condition is not drastically restrictive.

2) the stereoselection occurs only during the time when all the binding sites are effectively interacting. A substrate with a strong interaction point and two other very weak binding sites, leads to high chromatographic retention ... but to poor resolution.

3) as a consequence of the indispensable and successive "adsorption" and "desorption" in triple points of the solute, the kinetic of the equilibrium

solute in the mobile phase $\Leftrightarrow$ solute in the stationary phase

runs the risk to be slow and consequently chromatographic efficiency may be low : the abnormal peak broadening is a real risk in this way of separation.

4) some types of interactions lead to a decrease of the adsorbed solute freedom degrees of more than one unit. For instance, only one charge transfer interaction between two aromatic molecules may lock the two species in close parallel planes (Fig.2a). The situation is the same for chiral molecules when a substituant R of an asymetric carbon atom (Fig.2b) is blocked in an asymetric host (i.e. chiral crown ether or cyclodextrin) : an interaction site does not define a point but an axis. In the same way, the complexation of the chiral solute in a planar metallic complex (Fig.2c) permits to define the position of the chiral center with only two binding sites.

These three types of complexations are of special interest and will be examined separately.

5) the deductions proceeding from the "three points model" suppose that the involved complexes are rigid (i.e. assertions in note 4 are wrong if there is a long flexible chain between asymetric

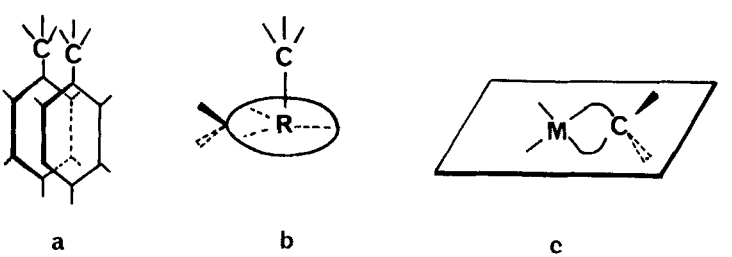


Figure 2 : Three steric blocking interaction systems.

center and binding site). A good efficiency supposes that the binding sites are close to the chiral center.

6) as suggested in Fig.1 and independently of the binding points, the bulkiness of substituents (R', R") are involved in the stereoselection.As a result, a substrate leading to a good resolution for a given solute pair in not necessarily efficient for other solutes of the same family (same binding functions but different R substituents). The lack of generality of chiral packings used in direct resolution of enantiomers is a problem not yet completely solved and rather a disadvantage is compared with separation through diastereoisomers.

7) the "three points" rule suggested that taylor-made chiral cavities can be built and used as chiral packings. Let us imagine a polymerizable system (Fig.3a) where a polymerizable substrate is covalently bound to one isomer of the compounds to be resolved. A convenient polymerization (more precisely a copolymerization with an inert cross-linking agent) yield an inert matrix with inclusions of active species (Fig.3b). By appropriate chemical reactions, covalent bounds are broken, evolving the sites (A, B, C Fig.3c) of molecular interactions and a cavity convenient for the only isomer is thus created. Some results with such very selective packings (enzyme analogous structure) will be described here.

8) there is a completely different way to succeed in direct resolution : a chiral packing may not be used if eluents are optically active. Interactions between appropriate eluent and solute molecules give complexed species which can be considered as labile diastereoisomeric compounds. This method avoids some disadvantages of the technique based upon chiral packings. As the retention of the pseudodiastereoisomers may be brought by a part of the molecule far from the chiral centers, a peak broadening due to slow formation or dissociation kinetics of the pseudodiastereoisomer complexes is irrelevant here. (see remark 3). For the same reason the chiral eluent is " a priori" convenient for the resolution of

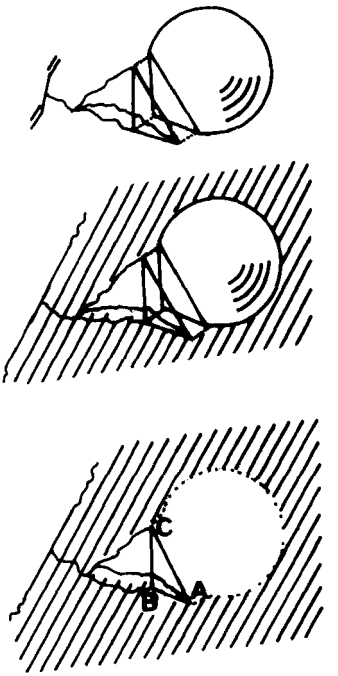


Figure 3 : The building of a taylor-made chiral cavity.

a large number of chiral solutes of the same family (see remark 6). In counterpart, the optical purity of the eluent is preponderant for the purity of the separated compound. At last, though no chemical operation is needed, a separation of the isolated isomer of the chiral eluent (generally an optically active molecule in solution in an inert solvent) is necessary.

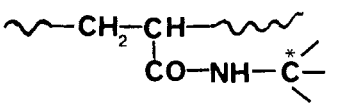
USE OF CHIRAL PACKINGS

Very early chromatographic attempts here performed with chiral packings. First of them were optically active natural substrates such as quartz or polyglucosidic substances and later synthetic chiral polymers. The first results were disappointing

but refinements of the technique lead to partial resolutions and some rare total separations. Mandelic acid, a compound of high specific rotation and good solvent properties is often tested as a solute.

Most of the synthetic polymers used are derivatives of acrylamide (and methacrylamide). The monomer is generally obtained by reaction between acryloyl chloride and an amine type compound.

Subsequent copolymerization with a cross-linking agent gives a packing with general formula :



The chiral carbon atom is in the vicinity of the amide fonction which may be a potential interaction point. With a chiral packing proceeding from pyroglutamic acid [14], a rather good separation is observed for basic α aminoacids but it is poor for other compounds of this family. With other chiral substituents based upon aminoacids derivatives[15-16], only partial resolution of α aminoacids and derivatives are observed.

The most complete work about such packings was performed by Blaschke and al[17-23]. They used a large number of substituents: amine[18-20,22], amidoamide[17], α aminoacid derivatives[21-23] (specially phenylalanine). In some experiments, they substituted the amide function by an ester group. Some aromatic amines and amides are completely resolved (Fig.4), and numerous other solutes partly. As the nature of the stereospecific complex packing/solute is not elucidated, the effect of the polymerization procedure is pointed out[17-18]. Thus, according to the synthesis of the packing (i.e. the texture of the beads obtained) the optical yield, in the resolution of mandelamide is between I and 43 %.

Styrenic packings were also tested[24-31]. They are prepared in a classical way, by reaction of a chloromethylated (or sulfochloride) derivative of the polymeric chain with an amine[24]

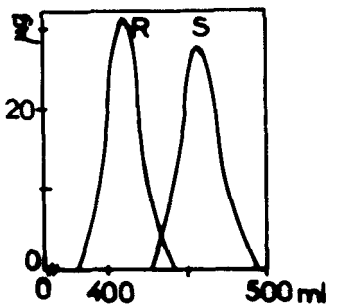
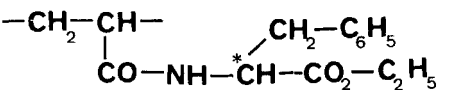


Figure 4 : Resolution of mandelamid on a packing of

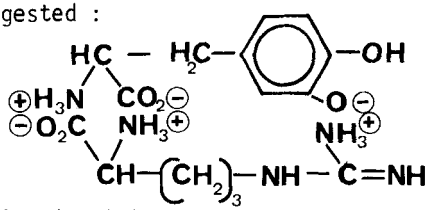


eluent benzene/dioxan. In [21].

aminoalcohol[31] amidoalcohol[28] and α aminoacid derivatives [25-27,21,30]. Only partial resolution is observed. Related supports with styrenic macromolecular network may be obtained from ion exchangers. Thus Gaal[32] saturating ion exchanger by D tartrate ion completely resolved the D and L threo-1-(p nitrophenyl)-2-amino-propanediol-1,3.

Il we except a chiral polyvinylic packing[33] used in the resolution of optically active polyolefins, other simple supports used natural compounds. Non macromolecular species may be used (for instance D lactose laid down on Al_2O_3, leads to partial resolution of trisacetylacetonato complexes of Al (III) and Fe (III) [34]) but most of them are polymeric[35-44]. Harworth[35] using D-quartz and microcrystalline cellulose and combining various resolution processes succeeded in separating the optical isomers of tris(ethylenediamine)-cobalt (III) iodide. Cellulose was used in thin layer chromatography[42,44] (separation of tryptophan and related compounds[44]) and also in column chromatography[37]. By this way, Handes et al. resolved kynurenine derivatives and tryptophan. Hess[43] obtained complete separation of chiral phenols on

potato starch. Baczuk et al[35] succeeds in good resolution of β -3,4, dihydroxyphenylalanine (DOPA) using sephadex G -25 grafted with L arginine via cyanuric chloride. The separation is explained in terms of three points rule and a structure of the stereoselective complex is suggested :



This mechanism justifies the observed partial resolution of tyrosine and the absence of separation of phenylalanine. It is also noticed that other macromolecular network (cellulose, styrenic polymers) with the same chiral graft, give packings not so efficient as those proceeding from sephadex.

Other natural polymers such as polygalacturonic or alginic acid also display resolution possibilities towards β aminoacids (or ester)[38-40]. A systematic investigation of this kind of supports shows that their efficiency is maximum for an optimum value of their swelling[39]. They appear as more operative than packings obtained with dextran base ion exchangers (CM, DEAE and QAE sephadex)[41].

The behaviour of cellulose acetate is peculiar : this polymer exhibits resolution power towards various solute enamides and diaziridines[46,49-50], mandelic acid, phenylnorbornen and cholesterol derivatives[48]. Hesse et al, which especially resolved the Trögel's base[47] and 2 phenylcyclohexanone[48], have proved that the secondary structure of cellulose triacetate is involved in the separation. If cellulose is dissolved, before its acetylation, the non crystalline material obtained loses its efficiency and even shows a reversed residual stereoselectivity[47,48]. They conclude that the solute (especially phenyl substituent one) interacts not simply with one glucose ester moiety but is inserted between two such moieties which are in appropriate position in the macromolecular chain arrangement. Crystalline cellulose triacetate acts, in these experiments, as a kind of chiral cavity.

PACKINGS WITH "CHIRAL CAVITIES"

The principle of separation of enantiomers through a selective adsorption in a "taylor made" cavity, has been presented previously. This idea is supported by the behaviour of inclusion compounds in asymetric ureas[51] and by the very high selectivity of chiral recognition in enzymatic systems which suggests a key-lock system. This last aspect is for instance illustrated by the work of Stewart and al[52] : as bovine serum albumine is known for an antipodal specificity in the binding of tryptophan, they linked it to agarose beads and completely resolved tryptophan by "affinity chromatography" (Fig.5).

The idea of having packings with "chiral cavities" was developped by Wolff et al[53-57] and procceeds from this kind of affinity chromatography on synthetic enzyme model. For instance the copolymerization of 4-nitrophenyl α-D mannopyranoside-2,3,4,6 di-O-(4 vinylphenylborate) (A) with ethylene dimethacrylate and methylmethacrylate leads to a macroporous polymer (B) in which, after hydrolysis of the template (4 nitrophenyl α-D mannopyranoside), chiral cavities with two boronic acid group are present. With this packing, a resolution with a yield of 87% is obtained for D,L nitrophenylmannopyranoside (Fig.6). As expected, this packing is inoperative if it is synthetized with low content of cross linking agent or used in a swelling solvent.

PACKINGS WITH CROWN ETHERS OR RELATED COMPOUNDS

Crown ethers are well known for their strong and specific interactions with metal ions which is a function of their size. If their bore fits well with ammonium ion they can complex organic molecules which possess this substituent[59].

Such crown ethers, if asymetric, have a chiral recognition for optically active amino compounds[58-60,66] and have been successfully used in chromatographic experiments for the resolution of amines and aminoacids, specially by Cram et al[61-63,65].

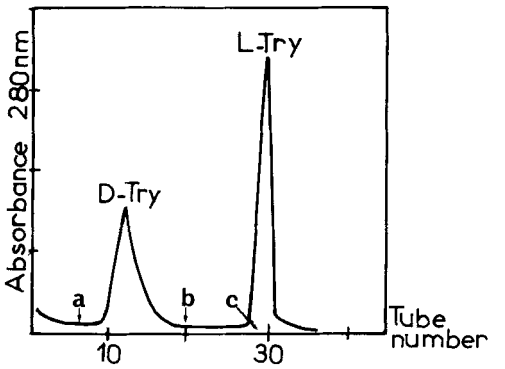


Figure 5 : Chromatography of DL-tryptophan on defatted bovine-serum albumin-succinoylaminoethyl-agarose. DL-tryptophan (500 nmol.) dissolved in 0.1 ml of 0.1 M borate buffer (pH 9.2) containing 1% (v/v) $(CH_3)_2SO$, was applied to a 0.9 X 25cm column of defatted bovine-serum albumin-succinoylaminoethyl-agarose. The column contained a total of 630 nmol. of bovine-serum albumin. The column was eluted at 30 ml/hr with the borate buffer (no$(CH_3)_2SO$) for 20 tubes then with 0.1 N acetic acid. The void volume was determined from the elution volume of $(CH_3)_2SO$. In[52]

a : void volume

b : 0.1 M acetic acid applied

c : acetic acid breakthrough

The crown host is generally a substituted binaphtyl derivative (Fig.7) but other models may be used (see[63] and ref therein). It may be graft on silica[64] or bonded to a styrenic polymer[61,65].

Numerous aminoacids are completely resolved by this way (Fig.8). The incidence of the structure of the crown ether and the solute has been studied using chromatographic and spectroscopic experiments, X rays analysis and examination of molecular models.

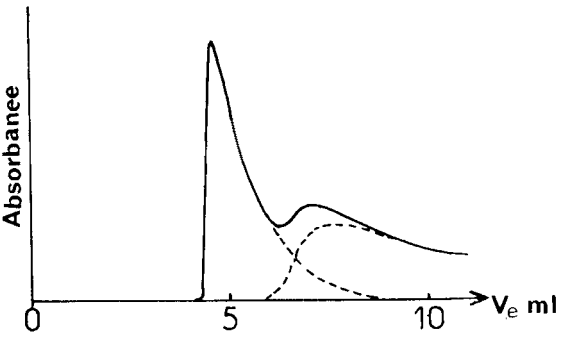
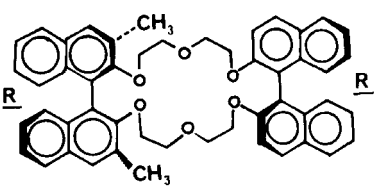


Figure 6 : Chromatography of the racemate of 4-nitrophenyl mannopyranoside. Flow rate: 0,0204 ml/min ; solvent : methanol/piperidine (98 : 2) ; sample : 100µg D,L-4-nitrophenyl mannopyranoside ; temp. 65°C, α-value : 1,85, optical yield : 87%. In [57].



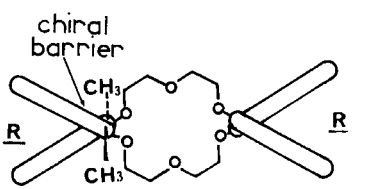


Figure 7 : A substituted dinaphtyl crown ether. The lower view shows the chiral walls of the dinaphtyl units, practically normal to the macroring. In [65].

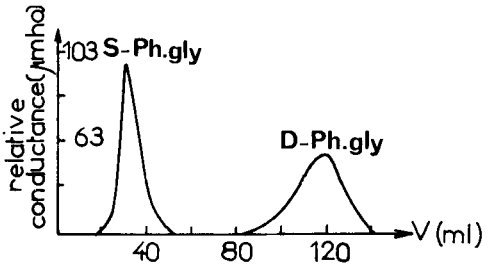


Figure 8 : Elution of phenylglycine perchlorate on a styrenic packing (formula in Fig.9). In[65].

The basicity of the crown heteroatoms plays a part in the intensity of the complexation. There are three hydrogen bonds between heteroatoms of the crown and the three atoms of the ammonium ion (Fig.9). The substituents of the nitrogen atom of NH_3^+ - R are arranged like a tripod, the base of which is the mean plane of the crown and the $\geq N$ - R axis (that is the N — C axis for aminoacids) roughly normal to this plane. The dinaphtyl units are about normal to the macroring and form chiral walls which hinder free rotation arond the N - C axis. The efficiency of the chiral recognition is governed by $\pi - \pi$ interaction between the naphtalene wall and the substituent ester (or acid) of the guest (they are always close together) and, the size ant the shape of asymetric atom substituent and its electronic interaction with the aromatic part of the host.

Cyclodextrins are sometimes considered as natural counterpats of crown ethers. The mechanism of inclusion of molecules in cyclodextrins is not so clearly elucidated as for crown but they are also able to chiral recognition[67-68] and cross-linked α and β cyclodextrins lead to partial resolution of mandelic acid and its derivatives[69].

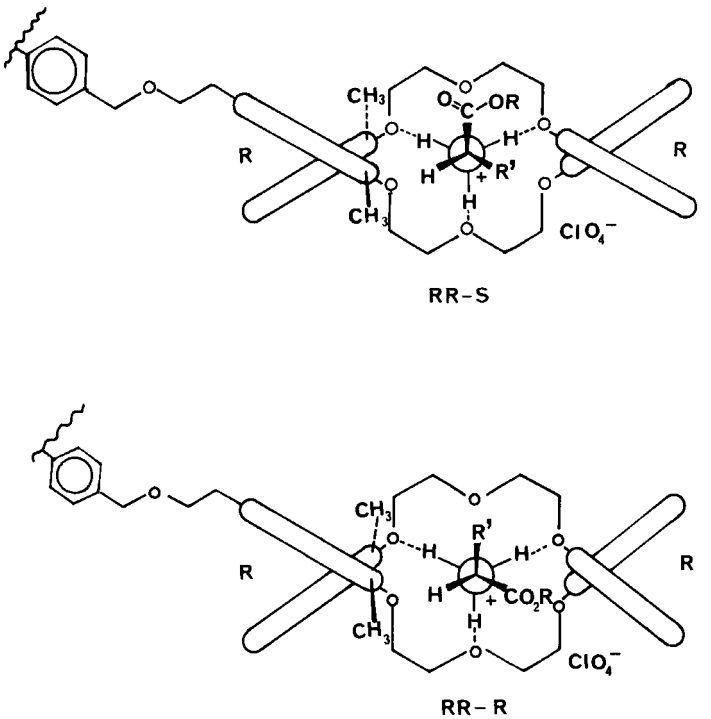


Figure 9 : The stereoselectivity in aminoacid (ester)/dinaphtyl-crown ether complex. The complex RR-R is the more stable. In[65].

PACKINGS INVOLVING CHARGE TRANSFERT INTERACTIONS

The idea of using donor or acceptor stationary phases for charge transfer interaction was put forward, in the begining of the sixtres. By this way, Klemm et al[70] succeded in a partial resolution of helicenes. But the complete resolution of twelve of these compounds was only presented in 1976 at the Bermingham meeting, by Mikes, Boshart and Gil-Av[71] (Fig.10). Independently and almost at the same time, Numan, Helder and Wynberg[72], using the same chiral selector (2(2,4,5,7-tetranitro-9-fluorenylidene-

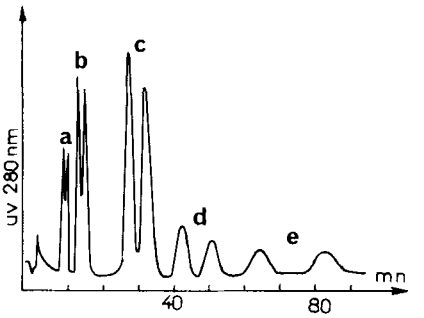


Figure 10 : Resolution of carbohelicenes : mixture of the racemates of (6)-,(8)-,(10)-,(12)- and (14)-helicenes. The more strongly retained enantiomer was the (+)-helicenes in all instances. Column : 25% (-)-TAPA mobile phase 25% dichloromethane-cyclohexane, u = 0.26 cm/sec. In[71].

aminoxy) propionic acid or tapa) described the resolution of helicenes and heterohelicenes. Except in a patent[78], the solutes resolved by this way are helicenes[71-77] or parent compounds like non planar aromatic derivatives[79]. Chiral selectors other than TAPA were tried[71,73-75], they can only be deposited on a mineral support (SiO_2, Al_2O_3)[72-73,79] or covalently bonded to silica[71-74,76].

The mechanism of chiral recognition was early proposed[71] (Fig.11).

The aromatic part of TAPA has to be close and oriented parallel to the solute to give strong charge transfer interaction. The molecular model shows that hydrogen and methyl group attached to the asymetric carbon are readily enclosed by the solute in its (+) form, whereas these substituents tend to lift the - enantiomer off the surface of the selector, diminishing the intensity of the complexation. As expected the - isomer is first eluted.

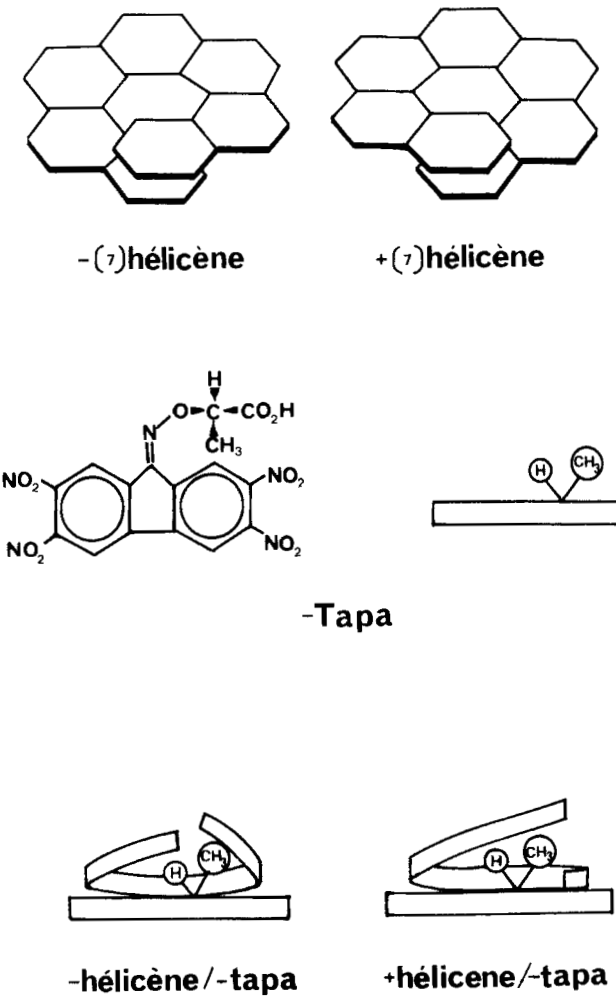


Figure 11 : The bulkiness of H and CH_3 moiety of -TAPA prevents strong complexation in -helicene/-TAPA system. In[71].

PACKINGS INVOLVING LIGAND EXCHANGE

If the asymetric sorbent give a multidentate complex with a metal ion which is also a complexant for the solute, a process of ligand exchange may be put forward. Then, the use of packings

previously complexed by metal ions, greatly enhances the resolution : let us compare, for instance, the results of polystyrenic supports with a chiral graft described in § III (Ref. 24-31) or in the sixties[2], with parents compounds used here in ligand exchange chromatography.

The first results in this new technique were obtained about ten years ago by Bernauer[80-82], Snyder and Angelici[83] and the group of Rogozhin and Davankov[84-85]. For the first time, a large number of parent compounds (α aminoacids) were at least partly resolved with the same packing. Russian workers made a thorough study of the question and published more than forty papers in this field. They used almost exclusively polystyrenic derivatives on which is grafted a chiral substituent through appropriate chloromethylation of the support. Various complexing ions were tested, especially Co (II), Ni (II) and Cu (II). The last is generally the most efficient.

First experiments were run with L-proline as chiral graft [84-85]. Various α aminoacids, bi or triodentates, were tested for this use : valine [86-87], histidine[86,90-91], aspartic acid [86,93], sufur aminoacids[86,89,91], alamine[87], leucine and derivatives[100], isoleucine[87], serine[88], threonine[88], phenylalanine[94p. Non aminoacid molecules were also used as graft [95] but cyclic aminoacids [86,96-99] provide the best resolution of solutes (aminoacids and some ion complexing organic molecules).

Constant improvements were made during the ten past years. Whereas in 1974 a given packing was only able to completely resolve two aminoacids in runs of about ten hours[86], in recent publications[96-99] the separation is complete for 13 aminoacids, mandelic acid, β phenylβalanine, 2 aminopropanol 1 and N benzylpropylenediamine 1-2, in each case for experiment times no longer than 2 hours(Fig.12). In the best experiments, the HEPT is about 1mm[98] and classically in the scale 3-12mm[96]. This amelioration was not due to the choice of a new graft (proline and hydroxyproline was used from the very beginning of the experiments) but by an increase of the ligand exchange kinetics obtained by an appropriate

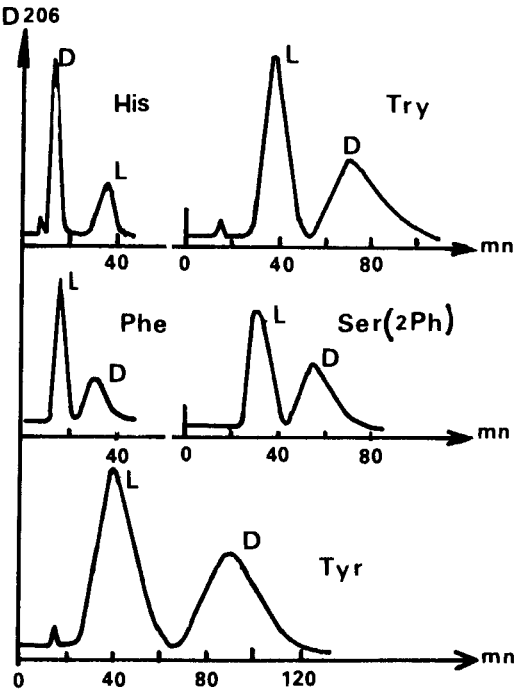


Figure 12 : Separation of several aminoacids on a strenic packing grafted by L hydroxyproline and complexed by Cu (II). For each run are given the level of copper saturation (%), the NH_4OH con. (mole) in eluent and flow rate (ml/h).
Histidine (30 ; 0.5 ; 25), tryptophan (30 ; 0.4 ; 20), phenylalanine (45 ; 0.1 ; 20), 2-phenylserine (45 ; 0.5 ; 14) and tyrosine (65 ; 0.1 ; 16). In[98].

choice of a cross-linking system for the macromolecular network ("isoporous gel").

Analogous results were observed in other works using polystyrenic supports with an L α aminoacid as chiral graft and aminoacids as solutes[101-105].

The choice of the best parameters for the resolution and its mechanism were largly discussed[104,106-114]. Studies involving chromatographic techniques, potentiometric titrations, X rays measurements, CD and UV spectroscopy, not only with polymers but also with models of low molecular weight (N benzylproline or N-benzylvaline for instance) were published.

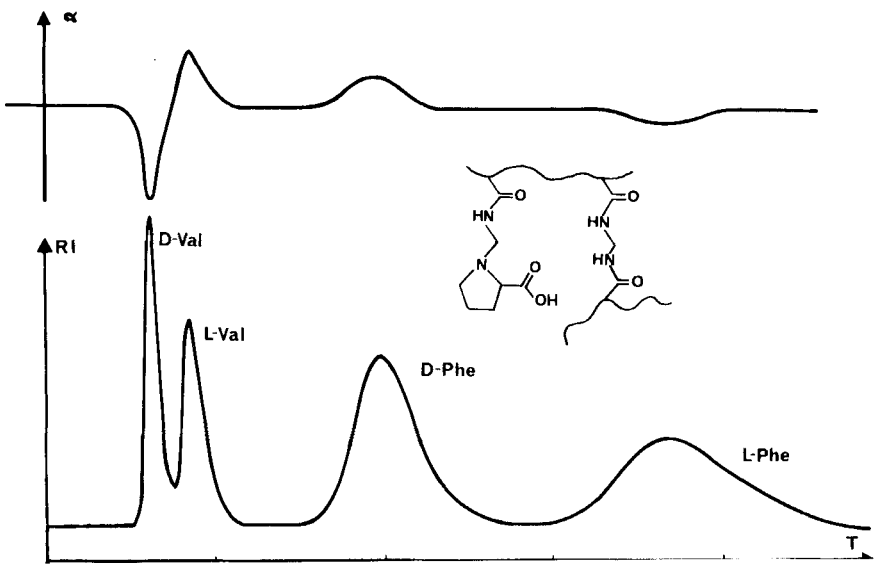
Classical chromatographic conditions use a gradient elution with aqueous ammonia solution. Lowering the temperature increase the difference between the elution volumes of two isomers. For a given capacity an increase of the content of the metal fixed on the support enhances both the retention volumes and the resolution this effect is explained in terms of steric hindrance in the complexes involving two grafts.

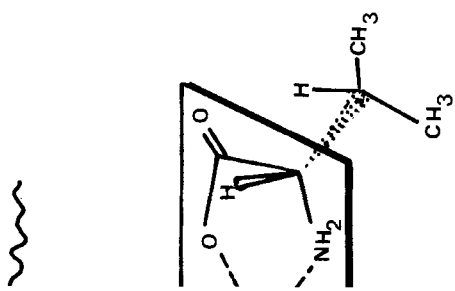
Let us consider two complexes G/M/S where G is a N substituted aminoacid (like the graft), M a metal ion and S (solute), an aminoacid either D or L form.

The stereoselectivity in the complexation can be estimated by the difference ΔG in the free enthalpy of formation of these two complexes. The stereoselectivity is difficult to predict because there is a balance between enthalpic and entropic contrituions[110,113]. Enthalpic effect may be due to different states of hydration and entropic effect to differences in symetry of the complexes. For instance, with a graft L proline supported by a styrenic structure, copper complexes are generally more stable with D aminoacid than with L form (L isomer first eluted). The corresponding values of ΔG are about 100-600 cal/mole. (that is rather less than the ΔG values observed in chiral crown ether complexation (as much as 1900 cal/mole [66]).

Owing to the poor chromatographic performances (HEPT) originally described for ligand exchange chromatography on styrenic support, we prepared and studied in our Laboratory very hydrophilic packings[115-117]. By this way, we hoped to increase the rate of ligand exchange and by using an acrylamide derivative network to improve the possible interaction sites with solutes. In fact, efficient packings were promptly obtained : with L proline as graft

except for leucine and methionine, all the α aminoacids tested are at least partly resolved. With a 30cm long column (or shorter) twelve of them are completely resolved and also malic acid and phenylalanine amide. The HEPT is low enough (about 1mm for beads of 20μ in the dry state) to lead to complete resolution in about 1 hour (Fig. 13).



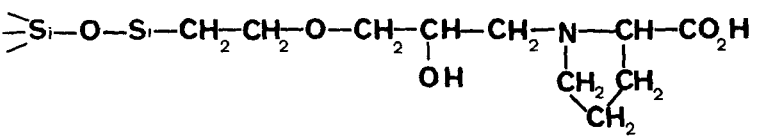


L-Valine

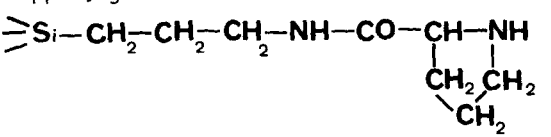
hindrance between the
ine) in a D form

Nevertheless, the slowness of the ligand exchange is probably still a limitating step of the resolution as pointed out by the influence of the temperature : for valine solute, retention time is practically not modified by increasing the temperature in the range 0-50°C but the retention factor increases from 0.5 to 2. All experiments were performed in pure water (inert salt solution for aminoacid solutes with acidic or basic substituent). About a dozen of complexing ions and eleven L α aminoacids grafts were tested. As for parent compounds with styrenic packings, cyclic aminoacids are the best grafts and cupric ions generally the most efficient (this last result is in connection with general kinetic behaviour of the metal ions in their complexes[10]. However, the chiral recognition mechanism is quite different in the two types of packings. In acrylamide derivatives, the carbonyl group of the amide function is involved in the complex (Fig.14). The lower stability of the L graft/Cu/D solute complex is explained in terms of steric hindrance in the rotation of the aminoacid substituent due to the carbonyl group, bonded in apical position in the complex[118].

An improved efficiency may be expected by the use of complexing chiral grafts directly bonded on silica packings. Gubitz et al[119] with a proline derivative as graft (with copper) :



succeed in the complete resolution of tryptophan and tyrosine, relative capacity factors as high but Hept rather low. Recently, Foucault, Caude and Oliveros[120] with 7 µm spherosil, bonded with copper, graft :



and as eluent ammonia solution, completely resolved tryptophan and partly tyrosine and phenylalanine with HEPT of about 0.07 mm.

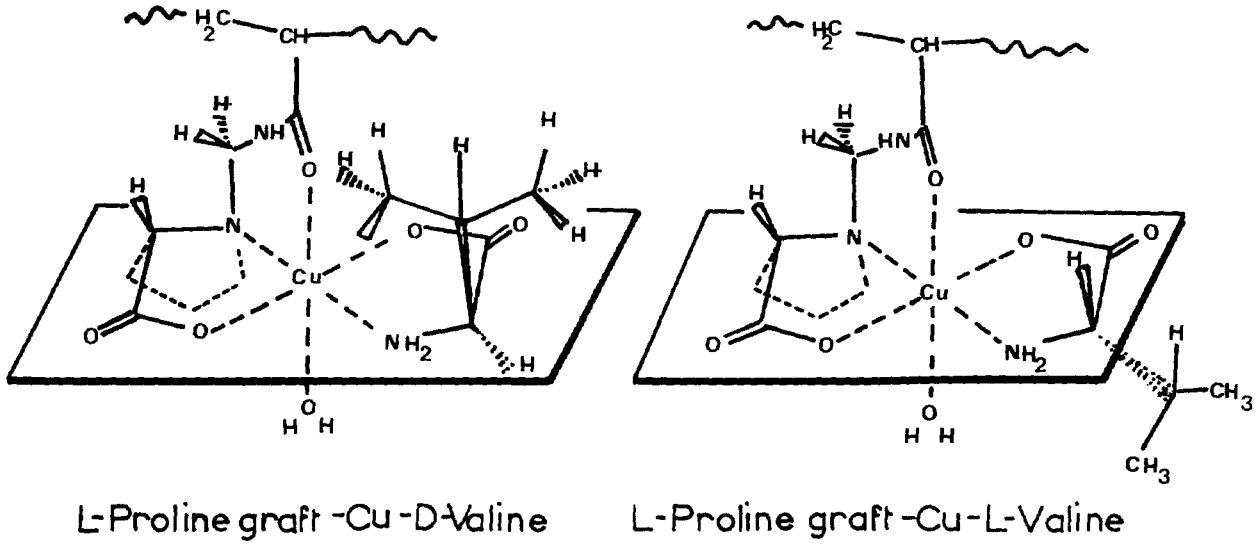


Figure 14: Stereoselectivity in acrylamide L-proline packing: the steric hindrance between the carbonyl group of the amide function and the substituent of the solute (Valine) in a D form prevents strong complexation.

Ligand exchange chromatography has been also proposed with natural chiral polymers[121-122] by previous fixation of optically active complex Co (III)/ethylene diamine on a cation exchange resin (Bio Rex 70). Gaal and Inczedy succeeded in the complete resolution of aspartic and mandelic acid[123].

USE OF CHIRAL ELUENTS

Instead of using a chiral species grafted on the packing, it is, theoretically, always possible to perform chromatographic resolution with a convenient a chiral stationary phase, but by incorporation in the mobilephase an optically active molecule, model of the graft.

This was illustrated by Cram et al who completely resolved hexafluorophosphate salts of aminoacids (ester form) on celite or alumina with a chiral crown ether in the mobile phase[62-124].

Systems putting forward charge transfer interactions between solute. (nitrophenylsulfoxydes) and eluent (solution ofanthrylcarbinol) were also proposed[125].

Cheap chiral reagents such as tartric acid derivatives which are besides complexants for metal ions, are used in ligand exchange chromatography. Yoshikawa et al[126-127,132-133] and later Yoneda et al[128-129] and Searle[134] resolved various optically active complexes of Co (III), or other metal complexes[136].

The more spectacular result obtained recently in this way is a very efficient fractionation of aminoacids by Karger[130-131] (Fig.15). The addition of (A) L-2 alkyl-octylethylenetriamine-M (Zn (II) or Cd (III) to the mobile phase of a bonded reversed phase column, leads to complete resolutions of the dansyl derivatives of D,L aminoacids (B). Whereas the capacity factors of the two optical isomers are not very different : k" ratio values (α) minus than 1,2), the high efficiency of the packing may be used thanks to the large alkyl part of the labile diastereoisomer complexes involved and without any constraint of the rate of ligand exchange. Resolutionsare complete.

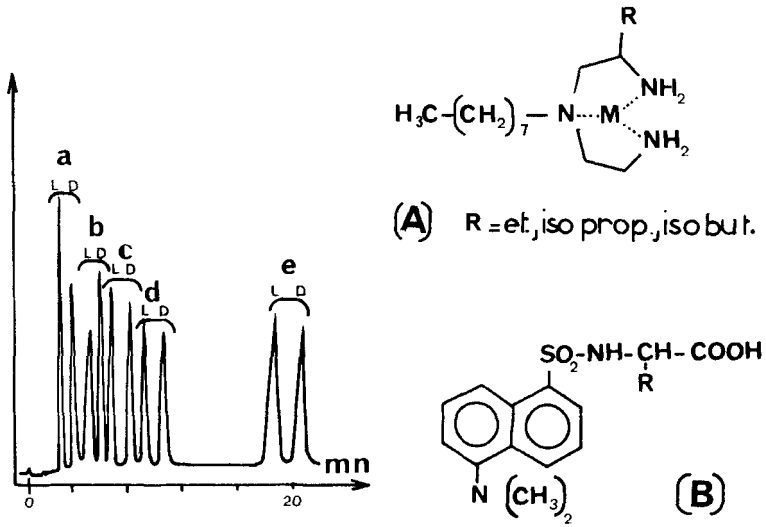


Figure 15 : Separation of D-L-dansyl amino acids. Conditions : 0.65 mM L-2-isopropyl-dien-Zn (II) ; 0.17 M NH_4 Ac to pH 9.0 with aqueous NH_3 ; 35/65 AN/H_2O ; T = 30°C flow rate 2mL/min ; column 15 cm by 4.6 mm i.d. Hypersil 5µm C_2 :
solutes : a = threonine, b = norvaline, c = leucine, d = norleucine, e = phenylalanine. $In^{(131)}$.

CONCLUSION

Very important progress has been performed during the past ten years, in the direct resolution of enantiomers. If originally complete resolution were exceptions, the use of steric locking interaction system (charge transfer between aromatic substituents, crown ether cavity, crowded complexes with metal ions) leads to complete resolution of whole families of chiral compounds. Results were sufficiently promising to justify the registration of about a dozen of patents[16,19,26-27,53,61,78,80-81,84,115].

Table A reviews the publications and shows the increasing number of papers in this field.

TABLE A

year / Field	1969 - 70	1971 -72	1972 - 74	1975 - 76	1977 - 78
Classical chiral polymer or natural polymer	8	6	7	7	5
Chiral cavities packings	-	2	1	-	2
Crown ether or parent compounds packings	-	-	-	4	1
Packings involving charge transfer interactions	-	-	-	3	5
Packings involving ligand exchange	5	12	6	5	18
Chiral eluents	1	2	3	2	5
Total number of publications (including patent)	14 (4)	22	17 (2)	21 (1)	36 (3)

This Table may not be exhaustive, particularly, chiral recognition mechanisms and systems potentially usable in chromatography but not involving this technique (i.e. preferential solvent extraction) are generally not taken in account.

The mechanisms of chiral recognition are now beginning to be known and a new generation of more improved systems may be expected.

Nevertheless, limitations of present systems must be kept in mind. The built of chiral cavities is probably important for the comprehension of enzymatic activity but highly specific for the

solutes to be separated and its efficiency is not yet sufficiently tested. Chiral crown ethers give high stereoselectivity but their use is presently limited to amino compounds. Moreover, they are expensive,consequently,not convenient for large scale preparative chromatography or for the use as chiral eluent. Systems with charge transfer interactions give spectacular results for the helicene resolution, they are potentially interesting for a large class of chiral species but, practically, studies have scarcely begun. Packings involving ligand exchange are of course limited to metal ion complexing solute, they are generally not very expensive and preparative chromatography may be expected. However, in spite of constant progress in the quality of the matrix (the reduce HEPT was divided by more than ten in five years) their efficiency is partly limitated by the slow kinetics of ligand exchange. The recent trend to use an optically active eluent and an achiral stationary phase is very promising for excellent results are observed with resolution factors largely lower than those obtained when the chiral species is grafted on the packing. Nevertheless, if this method is remarkable for analytical examinations, it is difficult to use it in a preparative way because of the inevitable separation of the mixture obtained by solvent evaporation of the isolated fractions.

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