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Enantioselective Chromatography: an Essential and Versatile Tool for the Analytical and Preparative Separation of Enantiomers

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Abstract. Chromatography on chiral stationary phases and electrophoresis in the presence of chiral selectors have become essential tools for the determination of enantiomeric purity in connection with synthesis and with biological studies of chiral molecules. Moreover, the chromatographic resolution of racemates on a preparative scale compels increasing recognition as alternative to 'preparing' pure enantiomers. The state-of-the-art of enantioselective chromatography in the analytical and preparative field is reviewed with particular emphasis on the achievements carried out in Switzerland.

1. Introduction

Although the principle of molecular chirality was established by *van't Hoff* and *LeBel* over a century ago, awareness of how this characteristic affects the biological activity of molecules is much more recent. Likewise, systematic investigation of the biological activity (including pharmacology and toxicology) of the individual enantiomers only recently became the rule for all new racemic drugs and agrochemicals. In this context, there has been a rapid development of enantioselective synthetic methodologies, which have now reached a high degree of diversity and complexity. This new trend produced a rapid increase in the demand for stereoselective separation techniques and analytical assays for precise determination of the enantiomeric purity of chiral compounds. For a long time, physical methods, such as optical rotation or nuclear magnetic reso-

nance (NMR) in the presence of chiral solvating agents, were the standard techniques; however, these suffer from a lack of accuracy. Methods based on the indirect determination of enantiomeric purity by chromatographic analysis of the corresponding diastereoisomers obtained by derivatisation of the enantiomers with chiral reagents have also been considered [1]. However, with this approach the accuracy is markedly affected by the optical purity of the chiral reagent. Furthermore, kinetic enrichment during the derivatisation step may lead to erroneous results. Therefore, the development of chiral stationary phases (CSPs) for gas chromatography (GC)

[2] and liquid chromatography (LC) [3] – permitting direct analysis of enantiomeric mixtures without prior derivatisation – rapidly attracted the attention of many scientists, and these two chromatographic techniques soon became the methods of choice for the determination of enantiomeric purity in connection with synthesis and with biological, pharmacological, pharmacokinetic, and clinical studies or field testing. Capillary electrophoresis, applying chiral selectors in the buffer electrolyte, subsequently emerged as a powerful new technique for this kind of analysis [4].

Alongside the analytical utilisation of enantioselective LC, its application on a preparative scale is gaining increasing recognition as an alternative to the 'preparation' of pure enantiomers [5]. In fact, the chromatographic separation of enantiomers on CSPs was originally developed as a preparative tool for the resolution of racemates, long before the method was recognised as being useful for the analytical determination of optical purity. These applications include the pioneering work of *Henderson* and *Rule* as long ago as 1939 [6], *Lecoq* in 1943 [7], and *Prelog* and *Wieland* in Switzerland in 1944 [8] on the use of lactose as a CSP.

The introduction of increasingly elaborate techniques, such as simulated moving-bed chromatography, opens up possibilities which were not conceivable some years ago in this field of separation.

Currently, enantioselective chromatography is applied to a wide variety of separation techniques, such as capillary electrophoresis (CE), micellar electrokinetic chromatography (MEKC), capillary electrochromatography (CEC), gas chromatography (GC), analytical and preparative liquid chromatography (LC), supercriti-

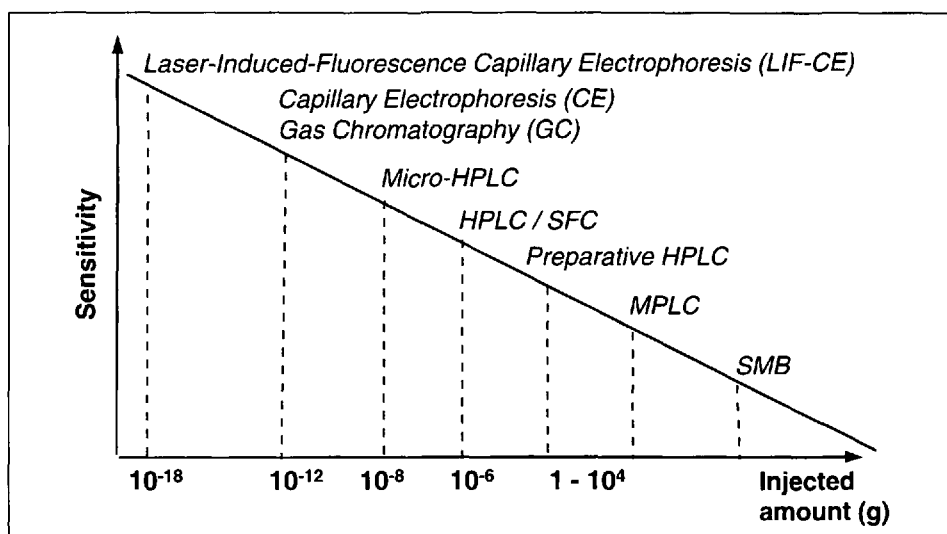


Fig. 1. Application areas of the enantioselective chromatography

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cal fluid chromatography (SFC), simulated moving-bed (SMB) chromatography, and to a lesser extent thin-layer chromatography (TLC). These techniques cover a broad spectrum of applications, from the detection of enantiomers in biological fluids at very low concentrations using CE or GC to the continuous preparative separation of kilogram quantities and in the near future the production of tonnes of enantiomers (Fig. 1).

Even if the basic principle of separation is the same for all these different techniques, the chiral environment used to achieve separation in each case has had to be carefully developed in view of the limitations imposed on the one hand by the technique itself and on the other by the type of application.

In Switzerland, most of the contributions to this field, in terms of both design of new CSPs and chiral selectors and analytical and preparative applications, clearly come from industry.

2. Principle of Enantioselective Separations

In enantioselective chromatography, the separation principle is based on the reversible formation of diastereoisomeric complexes between the enantiomers and the chiral selector. If these complexes have different association constants, separation occurs, the separation factor being defined by the ratio k'_2/k'_1 determined from the retention data in chromatography. These values (k'_1 and k'_2) are related to the thermodynamic association constants. In chromatography, even small differences in

complexation energy are enough to produce a complete separation. In HPLC, e.g., a difference of only 0.24 kcal/mol in interaction energy, corresponding to a separation factor α of 1.5, is sufficient to obtain an excellent separation of the enantiomers, even on a preparative scale. In synthesis, an energy difference of 0.24 kcal/mol gives an enantiomeric excess (ee) of only 25%. In GC, a difference of only 0.03 kcal/mol is sufficient ($\alpha = 1.05$, ee 5%) for a base-line separation to be observed. In LC, separation factors ranging between 1.2 and 3 are generally observed. In this context, it should be mentioned that a separation factor of 7, which is not usual, corresponds to a difference of 1 kcal/mol in the interaction energy. This value, which is considered to be the limit of accuracy for molecular modelling calculations, emphasises the difficulty of applying molecular modelling for the prediction of enantioselective separations.

Obviously, achieving an enantioselective separation requires a chiral environment, which usually consists of a CSP or a chiral selector present in the mobile phase. However, in certain instances, *Dreiding et al.* at the University of Zürich, described the enantiomeric resolution of non-racemic mixtures on achiral stationary phases [9a, b]. This effect was discussed later by *Gil-Av* and *Schurig* [9c].

3. Gas Chromatography

The first commercially available 'chiral column', *Chirasil-L-Val*, was designed for GC in the group of *Bayer* in Germany in 1977 [10]. It was prepared from L-

valine *tert*-butylamide bonded to polysiloxane, and even now it continues to be the standard column for determination of the optical purity of amino acids, which are usually first derivatised as perfluoroalkylamide and isopropylester. This application includes the routine analysis of peptides, which are first cleaved by acidic hydrolysis. Specific applications performed in Switzerland include measurement of the degree of racemisation of amino acids in food by *Liardon* and *Ledermann* at *Nestlé* [11], or in fossils as a method of dating by *Meyer* at the University of Berne [12].

The other most important chiral selectors utilised in GC are based on cyclodextrins, which have been used in the form of native cyclodextrins or homo- or mixed derivatives (alkyl, acyl, benzoyl) diluted in or bonded to polysiloxanes [2]. The chiral discrimination process mainly occurs by formation of stereoselective inclusion complexes in the hydrophobic cavity of cyclodextrin, which consists of cyclic oligo-glucose attached in position 1 and 4 by an alpha bonding. Many columns designed using cyclodextrins have been developed, in particular by the groups of *König* [2a] and *Schurig* [2b] in Germany, and *Armstrong* in the USA [2c]. To date, more than 650 'chiral GC columns' have been described, and of the 120 columns available commercially more than 80 are based on cyclodextrins [13]. Numerous applications have been reported, and these have been listed in several journals [14]. While the polarity and the molecular weight of the substrate restrict the application of GC, the method is particularly useful for the analysis of volatile compounds. This includes flavour compounds investigated at *Firmenich* [15], or lactones added in food as shown by *Grob et al.* at the Cantonal Laboratory in Zürich [16]. GC is also the method of choice for many environmental protection laboratories, e.g., that of *Müller* and *Buser* at the Swiss Federal Research Station [17] and of *Oehme* at the university of Basel [18]. For this application, it is important to be able to detect even traces of components, and only coupling of GC to a mass spectrophotometer (MS) as a detector permits the requisite level of sensitivity to be attained. Applications involving the stereoselective analysis of DDT [17a], chlordane [17b, c] and toxaphene [17d][18] have been reported. The combined GC/MS method is also a powerful quantitative means to determine the stereoselectivity of the pharmacokinetic disposition of chiral drugs in cases where high sensitivity is required. For example, we applied this technique for the

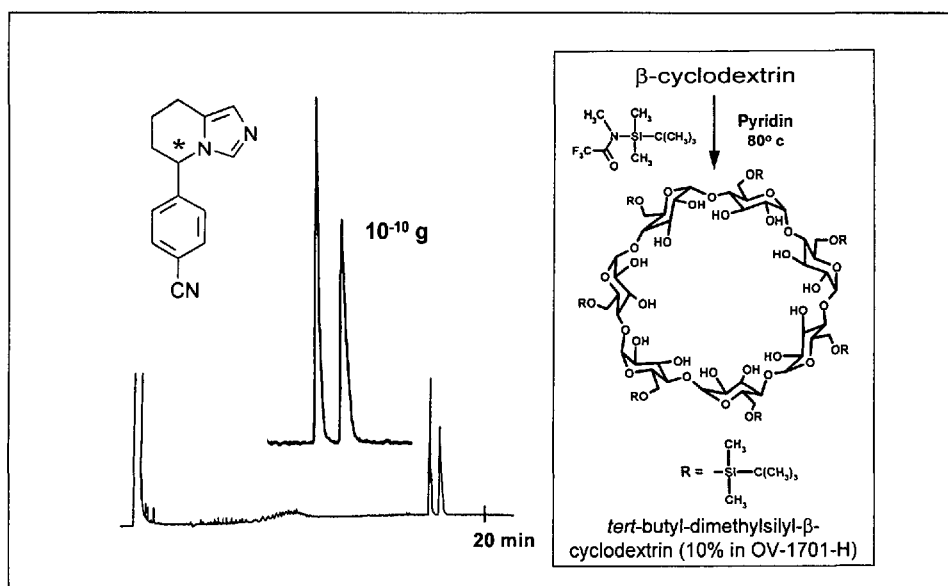


Fig. 2. Separation of the enantiomers of the anticancer agent fadrozole by gas chromatography and preparation of the stationary phase [19]

anticancer agent fadrozole (Fig. 2) [19], which was resolved on a cyclodextrin-based column prepared according to the procedure developed by *Blum and Aichholz* at *Ciba* [20a]. This *O*-[(*tert*-butyl)dimethylsilyl]- β -cyclodextrin phase, which is now available commercially [20b], exhibits unique properties and is increasingly being used in environmental analysis [17][18][21] and for a wide range of volatile compounds [22]. *Tabacchi* and coworkers at the University of Neuchâtel also reported on the elaboration of 'chiral cyclodextrin-based GC columns' exhibiting improved selectivity and consisting of a mixture of cyclodextrin derivatives [23]. In Switzerland, further applications of enantioselective separations on cyclodextrin-based columns have been reported by *Walther and Cereghetti* at *Hoffmann-La Roche* (atropisomers [24]) and by *Küsters et al.* at *Sandoz* (β -hydroxy myristic acid [25a], amino alcohols [25b], and racemic sulfoxides [25c]).

Use of linear polysaccharide derivatives as stationary phases in GC was also attempted by *Francotte et al.* at *Ciba*, but it turned out that when dissolved in polysiloxane or polyethyleneglycol, these chiral selectors were not as powerful as in LC [26].

4. Liquid Chromatography

In the field of enantioselective separations, LC clearly predominates in terms of both applications and the number of chiral selectors investigated. In LC, the chiral selector can either be part of the stationary phase or be dissolved in the mobile phase. In the former case, the chiral material is not consumed and can be regarded as a kind of catalyst, while in the latter case, the chiral selector elutes with the mobile phase and must be continuously regenerated. Furthermore, in this latter case, the molecules detected are diastereoisomeric species which may have widely differing spectroscopic properties, and in preparative applications the presence of the chiral selector, which has to be removed from the mobile phase, complicates isolation of the desired pure enantiomers. For these reasons, the LC approach using CSPs is generally preferred. The accuracy of the method was certainly an important factor in its rapid and successful establishment. This point was critically discussed by *Meyer* in several papers [27].

4.1. Chiral Stationary Phases

Initially, enantioselective LC was mainly focused on the preparative aspect, with

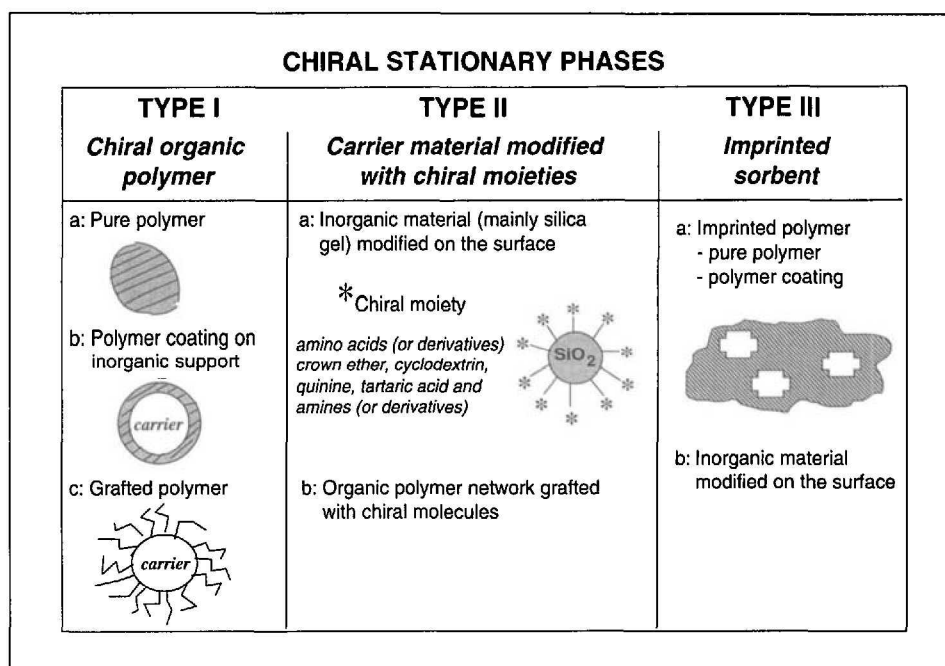


Fig. 3. Classification of chiral stationary phases

the aim of isolating optically active substances not easily accessible by other methods, for synthesis and for biological testing. For example, this was *Prelog's* aim in using lactose as long ago as 1944 [8]. Only much later was the analytical potential of the method fully recognised. Now, more than 850 different CSPs have been reported in the literature, and *ca.* 150 of them are available commercially [13]. This diversity indicates that there is no universal CSP capable of resolving almost all types of racemic molecules, and that selection of an appropriate CSP for a given separation problem is a major issue in enantioselective LC.

The CSPs can be basically classified into three types: chiral polymers (Type I), achiral matrices (mostly silica gel) modified with chiral moieties (Type II) and imprinted matrices (Type III) (Fig. 3). This classification refers to the type of selector (macromolecules or low-molecular-weight compounds) and the mode of preparation of the phase, whereas other classifications are based on the type of interaction. However, as the types of interactions leading to chiral recognition (hydrogen bonding, π - π interactions, ionic or dipolar interactions, hydrophobic interactions) are identical for almost all kinds of CSPs, the former system of classification is more correct, even though in many instances one type of interaction predominates. Moreover, in the polymer-based CSPs not only the molecular structure but also the supramolecular structure may be a determinant of chiral recognition ability. This was clearly demonstrated by the mechanistic investigations carried out by

Francotte and Wolf on cellulose triacetate [28a, b] and by *Francotte and Zhang* on cellulose *meta*-methylbenzoate [28c-e]. The polymer can be in a pure form or in a diluted form when coated or grafted. This type of CSP includes oligo- and polysaccharides and their derivatives, polyacrylamides, polyacrylates, and the protein-based phases. The second-commonest approach employed to prepare chiral sorbents involves the attachment of optically active units to achiral carriers (mainly silica gel) by means of ionic or covalent bonds. A wide range of optically active moieties have already been applied, including amino-acid derivatives, crown ethers, cinchona alkaloids, carbohydrates, amines, tartaric-acid derivatives, cyclodextrins, binaphthol, *etc.* [3]. This type also includes CSPs where chiral discrimination is based on the formation of ligand exchange complexes between a chiral amino acid bonded to the stationary phase and the enantiomers present in the mobile phase (Fig. 4) [29]. This separation principle, which is applicable to almost all amino acids, was simultaneously elaborated by *Bernauer* and his group in Switzerland [30a] and by *Davankov* and his group in Russia [30b]; both applied for a patent in 1968. The work of *Bernauer* led to the preparation of improved CSPs for ligand exchange chromatography (LEC) by *Jeanneret-Gris et al.* [31], and these are available commercially from *JPS Chimie* [32]. An example of enantioselective separation achieved on this kind of CSP is shown in Fig. 4. In enantioselective LC, mention should also be made of the pioneering work of *Mikes and Boshart* at the ETH in

Zürich: in 1978 they published new CSPs based on nitrofluorenone [33a] and on binaphthol [33b] as a chiral selector. These CSPs were designed on the basis of the concept of π - π interactions, an approach similar to that pursued simultaneously by *Pirkle* in the USA [34]. However, these CSPs did not receive as much attention as the phases introduced by *Pirkle*, who indisputably made a major contribution in the field of enantioselective LC. Many other CSPs applying the π - π concept introduced by *Pirkle* were developed around the world. In Switzerland, several CSPs belonging to this class were prepared by the research group of *Arm* in Berne [35]. A

binaphthalene-based CSP was developed at *Sandoz* by *Küsters* and *Dosenbach* for the separation of the enantiomers of racemic benzergoline derivatives [36].

However, the most frequently used CSPs are certainly those derived from polysaccharides (mainly cellulose and amylose). Although native cellulose, which was recently reinvestigated in the group of *Lederer* in Lausanne [37] generally affords poor chiral resolution power, its fully acetylated derivative, introduced more than 20 years ago by *Hesse* and *Hagel* in Germany [38], proved to be a powerful CSP. Several years later, *Okamoto* in Japan showed that other cellulose deriva-

tives also exhibit excellent chromatographic properties as CSPs when coated on an inorganic support such as silica gel [39]. The chiral recognition ability of these CSPs is easily modulated by varying the derivatisation of the hydroxy functions on the glucose units. In our laboratory, we synthesised and examined the chiral recognition ability of mixed esters of cellulose prepared according to this coating process, and we found that for some racemates better separations could be achieved than on the corresponding homosubstituted derivatives [28c, d]. Although coated polysaccharide-based CSPs are considered to be the most versatile ones, they have the drawback of being highly soluble in many organic solvents, which considerably limits the choice of mobile phase. We recently tackled this problem by developing a strategy for immobilising the polysaccharide-based phases by photochemical treatment following the schema shown in *Fig. 5* [40]. Some years ago, we also designed a new process for the preparation of cellulose-based phases consisting of the pure polymer, which proved particularly appropriate for preparative separation owing to their high loading capacity [41]. Many preparative applications for these CSPs have been published (see below) [5].

4.2. Analytical Applications of Enantioselective LC

As mentioned above, enantioselective LC is at present the most widely used method for the stereoselective analysis of enantiomeric mixtures.

With regard to its use as a method of determining the optical purity of chiral compounds from enantioselective synthesis and enzymatic reactions, the major contributions in Switzerland have come from *Ciba* [41a, b][42], *Hoffmann-La Roche* [43], *Sandoz Pharma* [25a, b][36] [44], and the Swiss Federal Research Station [17a-c]. Individual applications have also been published by the groups of *Oppolzer* [45] and *Kündig* [46] at the University of Geneva, and by that of *Seebach* [47] at the ETH-Zürich. Reports relating to pharmacological investigations have come from the University of Lausanne [48], *Ciba* [49], the Institute of Legal Medicine [50] and the Swiss Epilepsy Centre [51] in Zürich, and the University of Geneva [52]. *Fig. 6* illustrates the versatility of enantioselective LC, showing the enantiomeric separation of four different types of chiral molecules, containing a chiral carbon [53a], nitrogen [53b], iron [53c], and sulfur [53a] atom, which we obtained on polysaccharide-based CSPs.

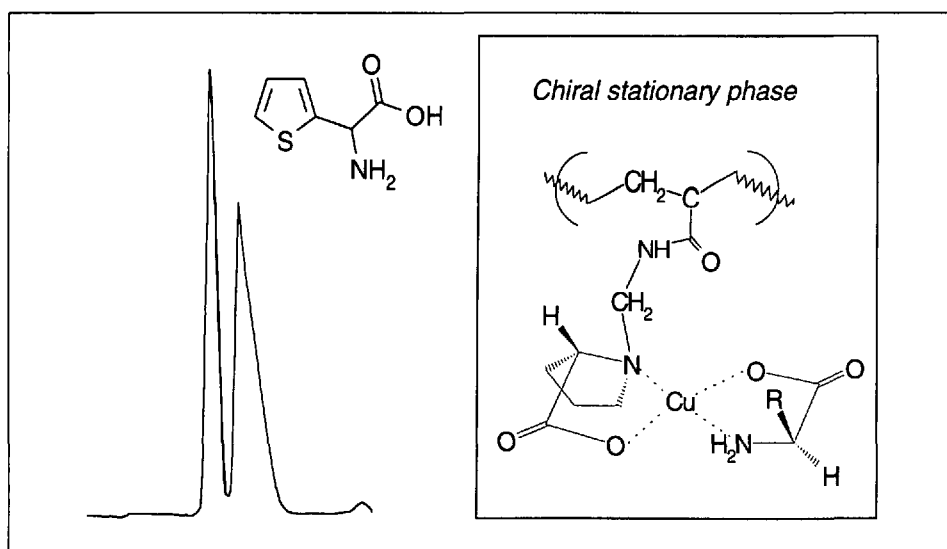


Fig. 4. Separation of the enantiomers of 200 mg of racemic 2-thienylalanine by ligand-exchange chromatography on a L-proline CSP (100 g) and structure of the CSP (from application guide 'Chirosolve', JPS Chimie [32])

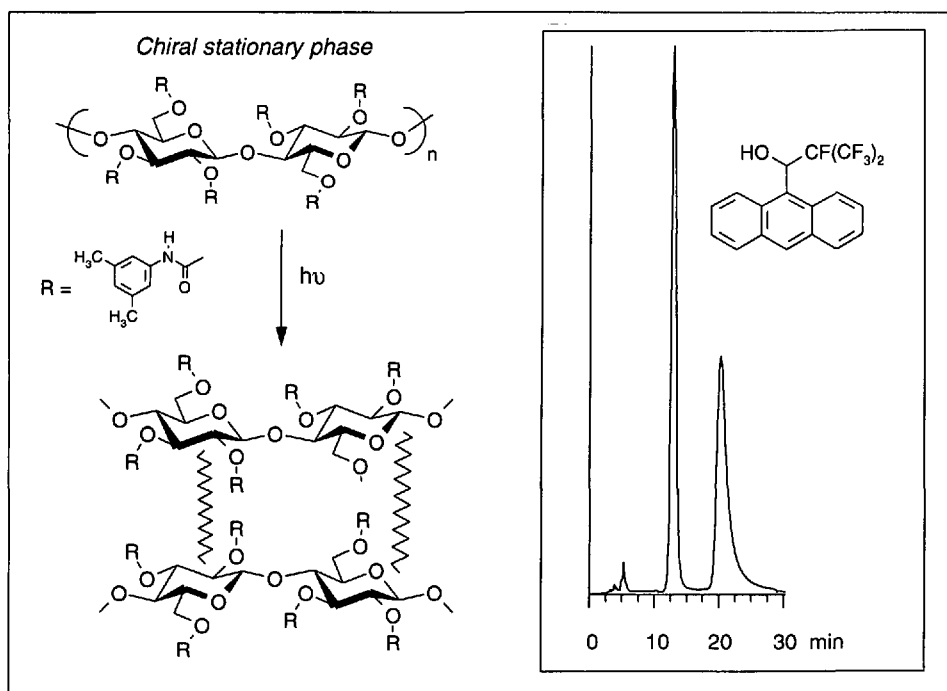


Fig. 5. Process of immobilization of a cellulose-based CSP and separation of the enantiomers of 1-(9-anthryl)-2,2,2-trifluoroethanol (column: 25 x 0.40 cm, mobile phase: heptanol/CHCl₃ 75:25)

4.3. Preparative Applications of Enantioselective LC

Not all CSPs designed for analytical separations can be used for preparative purposes. Among the various factors which are important for preparative separations, *e.g.*, cost factors and the stability and wide availability of the phase, the loading capacity is absolutely essential. The requirements and the strategies for performing preparative enantioselective separation have been discussed in several reviews by *Francotte at Ciba* [5]. Moreover, the contribution of this laboratory in the field of preparative enantioselective separations is widely established and includes several patent applications. The preparative applications include isolation of the enantiomers of lactones [5b][28b][42d][54b], epoxides [5b][42c], drugs [5a-c][54a] and drug intermediates [5b, c][49b][54b-d], fungicides [5b-c][42g][54e-g], herbicides [5b][42g], insecticides [54h-j], chiral synthons [5b, c][41b][42c][54k, l], chiral reagents [5b][53b], and chiral solvating agents for NMR spectroscopy [5b][54m]. Some preparative applications were also achieved in *Küsters's* laboratory at *Sandoz* [36][55]. The method was also used by *Seebach's* group at the ETH in Zürich for the preparation of the enantiomers of 2-phenyl-dioxinones [56a] and of cyclic acetals [56b, c]. As example of preparative application, *Fig. 7* shows the resolution of 52 g of the racemic fungicide clozilacon under recycling conditions.

4.4. Mechanistic Investigations

A limited number of mechanistic investigations have been performed in Switzerland in the field of enantioselective chromatographic separations. Most of these investigations were performed at *Ciba* by *Francotte* and *Wolf* on cellulose triacetate [28a, b][57] and tribenzoate [41b], and by *Francotte* and *Zhang* on other cellulose esters [28c, d]. Mechanistic investigations on a π - π CSP have also been published by *Daepfen et al.* [58].

5. Super- and Subcritical Fluid Chromatography

Super- and subcritical fluid chromatography does not differ fundamentally from the usual LC, except that it uses fluids in the super- or subcritical state as the mobile phase. The main advantage of super-/subcritical fluids is that they have a very low viscosity, which makes it possible to work at a higher flow rate than with conventional LC, thereby reducing analysis time. The same CSPs were used as in

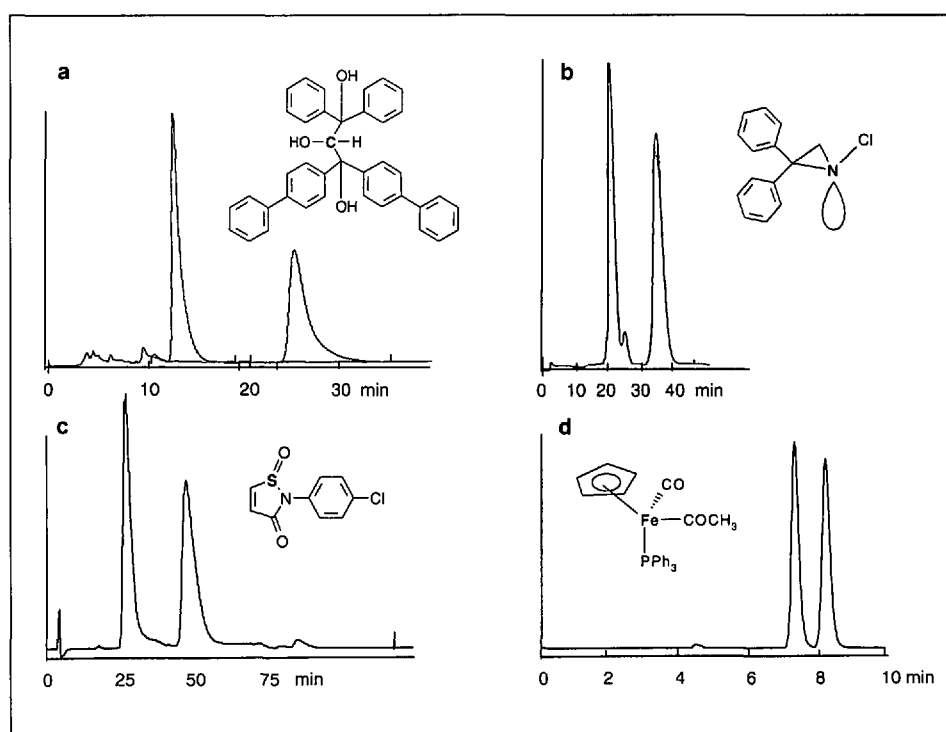


Fig. 6. Separation of the enantiomers of racemates containing four different types of chiral atoms on various polysaccharide-based phases: a) on Chiralpak AD, b) on 3-methylbenzoylcellulose beads, c) on Chiralcel OB-H, and d) on Chiralpak AD

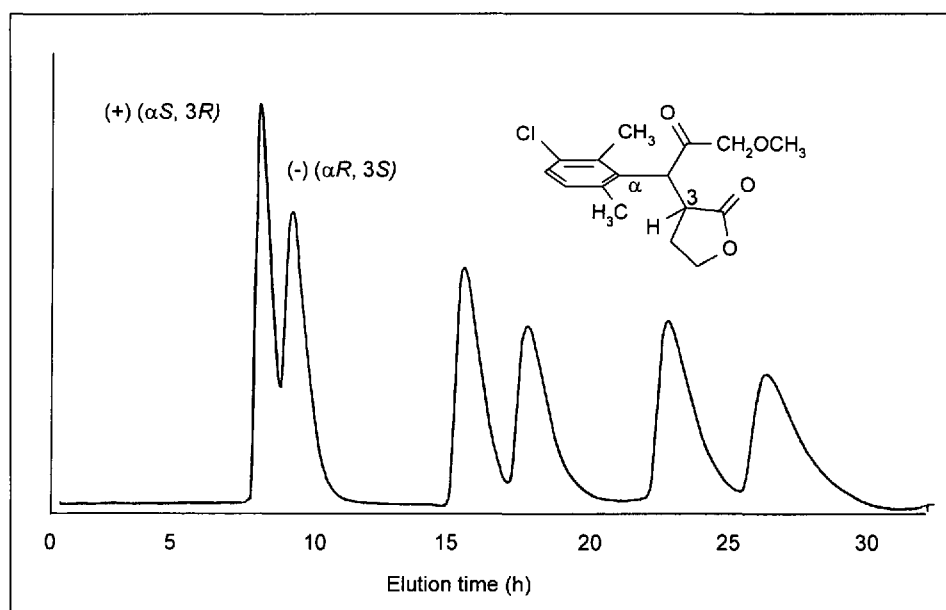


Fig. 7. Preparative chromatographic resolution of 52 g of the racemic fungicide clozilacon on cellulose triacetate, with recycling (column, 20 × 100 cm; mobile phase, EtOH/H₂O 95:5)

LC, and in most applications carbon dioxide was used as a mobile phase. This mode of enantioselective chromatography has been investigated in particular in the groups of *Caude* and of *Tambuté* in France [59a]. In capillaries, most of the investigations have been carried out with immobilised cyclodextrin derivatives by the groups of *Schurig* in Germany [59b], of *Markides* in Sweden [59c] and of *Armstrong* in USA [59d]. In some instances, higher resolution and/or higher selectivity has been reported in SFC for selected racemates on

a given CSP [60], but we have also found examples of compounds for which SFC was less effective than LC [61]. This is not surprising, as it is known that the mobile phase is also a major factor in the chiral discrimination process, and to apply SFC is, precisely, to change the mobile phase. Moreover, a major drawback of SFC is the low solubility of many organic substances in the extremely apolar solvents used, which restricts the possibilities for wider application of the technique on a preparative scale. One preparative application has

been reported by us for the separation of the enantiomers of guaifenesin [62]. In the area of enantioselective separations, excluding separations of diastereoisomers on 'achiral columns', only a limited number of investigations have been performed in Switzerland, although SFC has been a major field of investigation for several years in *Widmer's* group at *Ciba* [63]. *Arm et al.* at the University of Berne reported on the preparation and evaluation of two CSPs under SFC conditions [64], and *Francotte et al.* at *Ciba* investigated the cellulose-based CSPs in open tubular chromatography [26]. A systematic comparison of the performance of enantioselective LC and SFC was also undertaken by *Anton et al.* at *Ciba* for several drugs [61].

6. Simulated Moving-Bed Chromatography

Although most preparative enantioselective separations have been performed in the conventional batch-mode process, there is growing interest in simulated moving-bed (SMB) chromatography. Up to now, elution batch chromatography has clearly predominated in terms of the number of applications, and various approaches have been used for improving throughput, such as close injections, peak-shaving, and recycling [65]. However, large-scale separations require large amounts of CSP and have generally been considered economically unjustifiable in view of the high cost of the CSPs, the high

dilution conditions, the consumption of large amounts of mobile phase, and the difficulties associated with recycling it. But with the recent introduction of SMB technology in this application field [66a], the technical prerequisites for cost-effective performance of large-scale separations can now be attained. This chromatographic mode can save up to 90% of the mobile phase, and a much higher throughput is achieved.

In Europe especially, interest in this technology has been growing rapidly, particularly in industry. The first practical contribution from Switzerland relating to enantioselective separations was reported by *Küsters et al.* at *Sandoz* in 1993 in collaboration with *Nicoud* and his group in France [66b, c], and the first patent application was reported by *Francotte et al.* at *Ciba* also in 1993 for the separation of the enantiomers of the antiasthmatic agent formoterol [66d]. Further applications to chiral drugs and drug intermediates have been developed in our laboratories [66e], and implementation of this technology is progressing rapidly in most European countries. The intensive efforts at the ETH-Zürich, where the groups of *Morbidelli* and *Mazotti* are currently developing mathematical models to predict the optimal conditions for running SMB operations [66f-g], will also certainly promote the further establishment of this technology in Switzerland.

7. Capillary Electrophoresis

Capillary electrophoresis is a relatively young technique. The first applications of this method to the separation of enantiomers were published in 1985 by *Zare* and his group in the USA [67], using cyclodextrin as a chiral selector. By contrast to LC, where the mobile phase is responsible for the displacement of the components to be separated, the driving force leading to separation in electrophoresis is current. Therefore, all compounds which are not charged migrate at the same time with the electroosmotic flow and are not separated. Conversely, charged molecules – including enantiomeric species – will migrate according to their ionic charge. Of course, enantiomers do not differ in their ionic charge, and to achieve separation it is necessary to add a chiral selector capable of interacting preferentially with one enantiomer, thus modifying its ionic charge. Alternatively, the chiral selector can be immobilised in the wall coating of the capillary. This approach has been investigated in particular by *Schurig* and his

group in Germany [68a], and by *Armstrong* in the US [68b]. Cyclodextrins and their derivatives were found to be powerful chiral selectors for this purpose [4]. This technique rapidly attracted the attention of many scientists, and the number of applications has increased considerably over the last five years. Although cyclodextrins and their derivatives are used most frequently, other chiral selectors, such as proteins, polysaccharides, antibiotics, and crown ethers, have also been used [4]. In Switzerland, chiral crown ethers were developed for this purpose by *Kuhn et al.* at *Sandoz* [4e][69]. Examples of separations were reported by *Francotte et al.* at *Ciba* for the enantiomers of various non-steroidal aromatase inhibitors, using cyclodextrins and cyclodextrin derivatives as chiral selectors [70], and at *Sandoz* by *Kang* and *Buck* [71a] and by *Werner et al.* [71b] for amino acids. An example of separation is shown in *Fig. 8* for the enantiomers of aminoglutethimide. Although the technique is now used regularly as an analytical method to determine the optical purity of chiral compounds in drug discovery, it is not yet fully established in development, and applications in pharmacology or toxicology come mainly from academic research. *Blaschke* and his group in Germany were among the first to apply this method to pharmacological investigations [72]. In Switzerland, only two reports have been published, one by *Lanz* and *Thormann* in the Department of Clinical Pharmacology in Berne [73] and one by *Varesio* and *Veuthey* in the Laboratory of Pharmaceutical Analytical Chemistry in Geneva [74].

8. Micellar Electrokinetic Chromatography (MEKC)

As mentioned above, capillary zone electrophoresis (CZE) is only applicable to the separation of charged molecules. However, by adding charged micellar agents, it is possible to affect the migration time of neutral molecules. This can also be applied for the separation of enantiomers, provided that a chiral selector is present in the buffer solution. Once again, in electrophoretic separations of this kind, cyclodextrins and their derivatives have been the most commonly used chiral selectors [4]. *Francotte et al.* at *Ciba* applied this method to the separation of the enantiomers of various drugs [70], including non-steroidal aromatase inhibitors, barbiturates, and the antiasthmatic agent formoterol, which was derivatised with a fluorescence labelling agent to improve

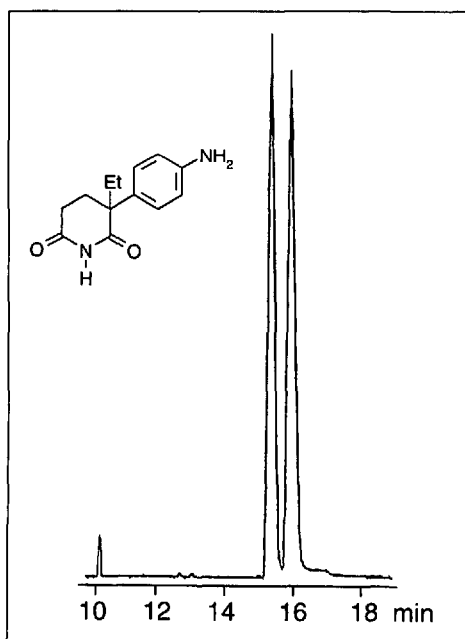


Fig. 8. Electropherogram of the separation of the enantiomers of aminoglutethimide by CE in the presence of β -cyclodextrin [70a]

sensitivity [70b]. In this latter example, use of a laser-induced fluorescence detector allowed zeptomolar amounts of the enantiomers to be detected. *Thormann* and his group also reported on the application of this method to the stereoselective analysis of various phenytoin derivatives in human urine [75].

Instead of using neutral chiral selectors in the presence of charged micellar agents, it is also possible to use charged chiral selectors. This strategy was introduced by *Terabe* in Japan using chiral micellar agents [4], and, more recently, charged cyclodextrin derivatives have also been developed for this purpose [76]. These cyclodextrin derivatives were mainly investigated by *Stobaugh* in the USA [77a] and by *Blaschke* in Germany [77b]. In Switzerland, further derivatives were prepared by *Francotte* and *Jung* [78a]; they also extended this approach to the linear polysaccharide sulfobutylamylose [78b]. One of the major advantages of using negatively charged cyclodextrins is the possibility of reversing the elution order of the enantiomers of ionic solutes compared to CZE, due to the retardant effect of the charged cyclodextrin. Two examples of applications are shown in *Fig. 9* by comparison with the results obtained under CE conditions with native γ -cyclodextrin.

9. Electrochromatography

In view of the increasing complexity of biological and chemical processes, the development and introduction of increasingly efficient analytical tools for research and development is vital. Among these tools, the most exciting and recent development in the field of separation technologies is capillary electrochromatography (CEC) [79]. This unique method combines all the outstanding features and benefits inherent in modern HPLC and advanced capillary electrophoresis (CE). The stationary phase may be either bonded to the walls of the capillary (open tubular CEC) or packed as small particles in the capillary (packed CEC). An electrical field is applied between the two ends of the capillary containing the stationary phase, and the mobile phase is then driven through the capillary by electroosmosis. As in LC, the selectivity arises from partitioning. As the electroosmotic flow shows a flat flow profile, there is no contribution to band broadening from the typical parabolic flow profile observed with pressure-driven systems; high resolutions are achieved as a result. Moreover, the electroosmotic flow

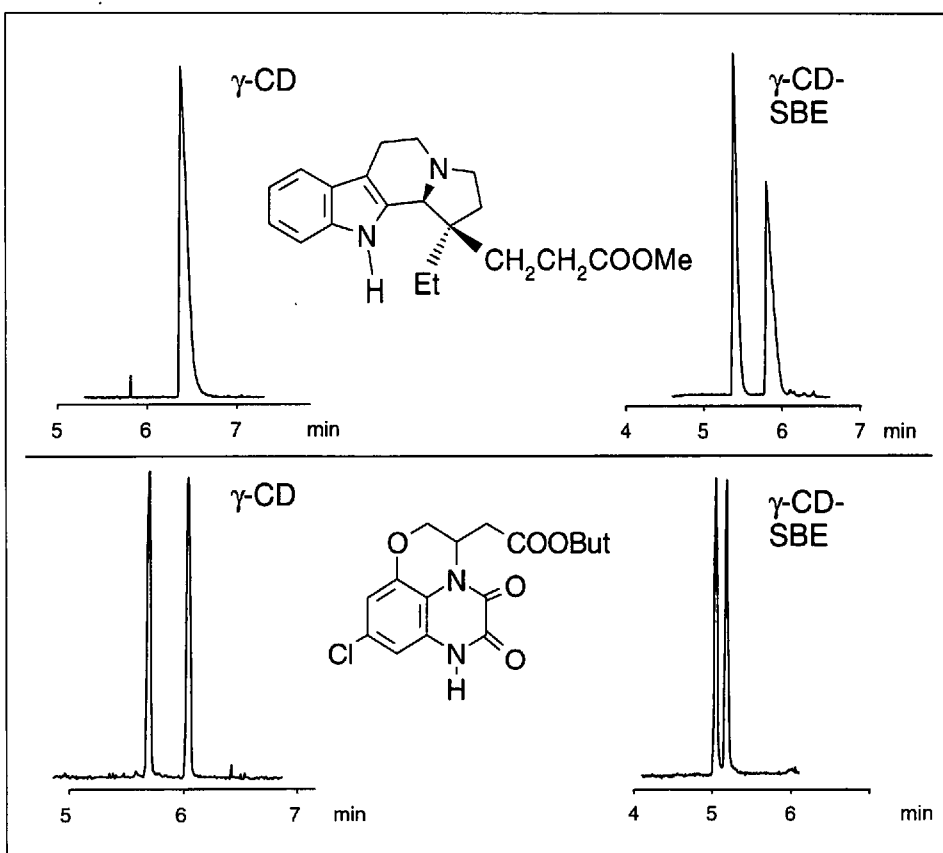


Fig. 9. Separation of the enantiomers of two drug candidates under CE (chiral selector, γ -CD) and MEKC (chiral selector, γ -CD-SBE) conditions [78a]

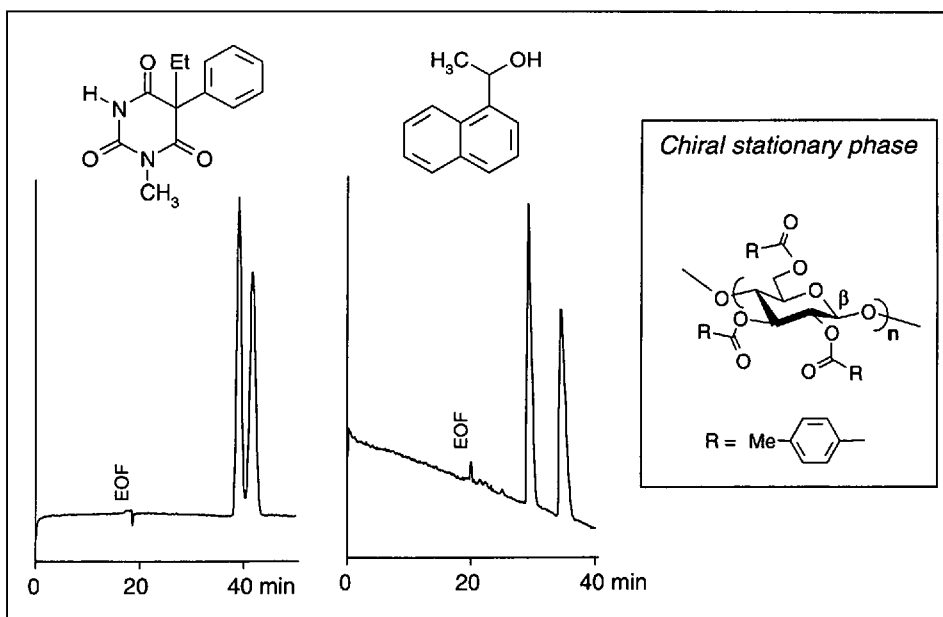


Fig. 10. Separation of the enantiomers of mephobarbital and of 1-naphthylethanol by electrochromatography in a capillary coated with 0.025 μ m of para-methylbenzoylcellulose [81]

eliminates restrictions in the length of the column normally encountered with small particle size packing as a result of excessively high back-pressure in pressure-driven systems. In the field of enantioselective separations, a limited number of applications have been reported to date. Glycoproteins [80a], cyclodextrins [80b–d], and cellulose derivatives [81] have been used

as chiral selectors. In the work with cellulose derivatives – the only application performed in Switzerland – we recently demonstrated the feasibility of enantioselective separations by electrochromatography in open 300 μ m tubes coated with cellulose-based chiral selectors [81]. Examples of CEC separations achieved on this CSP is shown in *Fig. 10*.

Conclusion

Over the past 25 years, the field of enantioselective chromatography has seen intensive research activity, and it is now established as an essential and versatile tool for the analytical and preparative separation of enantiomers. The successful development of this technique was due not only to the elaboration of molecular systems capable of excellent chiral recognition on the basis of chemical interactions but also, crucially, to technological developments.

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