CHIMIA 50 (1996) Nr. 6 (Juni)

CONFERENCE REPORTS

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organized by the Section of Analytical Chemistry (SACh)

University of Fribourg, Institute of Chemistry

'Chiral Separations'

Why did we choose this topic: 'Chiral Separations'?

The technological and methodological progress in analytical chemistry is growing at such a fast rate that there is a need for all scientists working in this field to keep abreast with the latest development. This rapid and constant progress in the development of analytical sciences stems directly from the stringent demands for rationalisation of quality control, the need to solve new problems and demands for the development of new technologies.

So the aim of this Symposium was to inform all scientists from academic as well as industries and research institutions about the recent progress in the area of chiral separations.

We know that chirality is essential to all living organisms at the molecular level. Consequently, the preparation and study of pure enantiomers is of paramount importance in a great many disciplines lying at the interface between chemistry and biology. In addition to the well recognized significance of stereochemistry in the pharmaceutical, agrochemical, environmental, flavor and fragrance fields, the study of enantiomers is of a crucial importance in many disciplines in particular biosynthetic studies, quality control of products of natural origin and biotechnology. But this cannot be done without analytical chemistry interested in the two fundamental steps: separation of chiral compounds and enantiomer ratio determination.

So, for this purpose we have invited seven outstanding specialists in this topic. Three of them came from overseas. They were Prof. *Wainer* coming from Canada, Prof. *Pirkle* and Prof. *Armstrong* coming from USA. All of them are internationally reknowned in this field. Our invitees from Europe are also eminent in this field. They were Prof. *Lindner* from Austria, Dr. *Francotte*, Prof. *Veuthey* and, last but not least, a women, Dr. *Meyer* from Switzerland.

Prof. W. Haerdi

The Use of Computational Chemistry and Molecular Modelling to Describe and Predict Enantioselective Separations

Irving W. Wainer, McGill University, Montreal, Quebec

Quantitative structure-retention relationships (QSRR) is an approach to the analysis of chromatographic data which can be used to predict and describe solute-stationary phase interactions. These studies require a set of quantitatively comparable retention data (dependent variable) for a sufficiently large set of solutes and a set of quantities (independent variables) reflecting structural features of these solutes. Through the use of chemometric computational techniques, retention parameters are characterized in terms of various combinations of solute descriptors or in terms of systematic knowledge extracted from these descriptors. The QSRR analysis can then be used to guide molecular modelling studies to give a comprehensive view of solute-stationary phase interactions.

When the stationary phase contains a chiral selector and the solutes are enantiomeric, the QSRR/molecular modelling can provide an insight into the chiral recognition mechanism as well as predict enantioselective separations. This experimental strategy has been illustrated by the results from a study which investigated the enantioselective separation of a series of 45 α-alkyl arylcarboxylic acids on a chiral sta-

tionary phase composed of amylose tris(3,5-dimethylphenylcarbamate) (AD-CSP). The results indicate that unlike the standard 'three-point interaction' model of chiral recognition, enantioselectivity was due to a 'conformationally driven' chiral recognition process.

T.D. Booth, I.W. Wainer, 'Investigation of the enantioselective separations of α-alkyl arylcarboxylic acids on an amylose tris(3,5-dimethylphenylcarbamate) chiral stationary phase using quantitative structure-enantioselective retention relationships (QSERR): Identification of a conformationally driven chiral recognition mechanism', *J. Chromatogr. A*, in press.
T.D. Booth, I.W.Wainer, 'Mechanistic investigation into the enantioselective separation of mexiletine and related compounds chromatographed on an amylose tris(3,5-dimethylphenylcarbamate) chiral stationary phase', *J. Chromatogr. A*, in press.

(Abstract by the author)

Advances in the Mechanistic Aspects of Chiral Stationary Phase Design; Improvements and Applications

William H. Pirkle, University of Illinois, Urbana-Champaign

An understanding of how a chiral selector actually differentiates between enantiomers allows one to design a selector which functions in a known manner. This, in many instances, permits one to predict the occurrence and sense of chiral recognition on a chiral stationary phase derived from the selector. While the understanding of chiral recognition mechanisms is far from complete, progress has been made and selectors intended to recognize certain structural features of enantiomers can be designed. Such a selector, its mode of action, and its scope will be described. Numerous HPLC and SFC separations of enantiomers will be presented.

W.H. Pirkle, C.J. Welch, *J. Chromatogr.* **1996**, *683*, 347, and ref. cit. therein. Most of the reports of the use of this chiral phase are still in press.

(Abstract by the author)

Some Examples of Applications to Pharmaceutical Compounds: Separation of Amphetamines by HPCE and of Methadone by HPLC

Jean-Luc Veuthey, Université de Genève

Two examples of a chiral separation of pharmaceutical compounds in biological matrices have been presented. For both applications due to the complexity of the tested matrices (urine and blood), an extraction step is necessary. We chose the solid-phase extraction on a mixed-mode bonded phase, because it offers a good selectivity and the procedure is easily automated and coupled to a chromatographic or an electrophoretic method. The separation of methadone enantiomers was carried out on chiral stationary phases by liquid chromatography. The results of the analyses of 36 patients receiving a racemic mixture of methadone daily have been discussed.

For the separation of amphetamines' enantiomers, we chose the capillary electrophoresis with a derivative β-cyclodextrin added as a chiral selector in the buffer. With this optimised method, it is possible to analyse, in less than 30 min, the five amphetamines most commonly consumed in Europe, among them the very popular Ecstasy or MDMA.

(Abstract by the author)

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Enantioselective Interactions and Separations with Macrocyclic Antibiotics

Daniel W. Armstrong, University of Missouri-Rolla, Department of Chemistry, Rolla, Missouri

There are a large number of macrocyclic antibiotics which encompass a variety of different structures. Many of these compounds have the type of functional groups and the spacial arrangement needed for enantioselective interactions. We have worked with over a score of these macrocycles. The efficacy of four of them (in terms of enantiomeric separations) have been discussed. Their usefulness as chiral selectors in LC and particularly in CE appears to exceed that of many other common selectors. For example, CE resolution factors (Rs) for many enantiomers are in the 5–20 range. The chiral selectors discussed were rifamycin B (an ansa compound), vancomycin, ristocetin A, teicoplanin, and thiostreptin (a cyclic peptide). Each of these compounds are ionizable but have different isoelectric points and differ in size and selectivity. Although these macrocycles are among the newest chiral selectors, they have been successfully utilized in hundreds of enantiomeric separations.

Some mechanistic aspects of separations involving macrocyclic antibiotics were discussed. As expected, when these macrocycles are used as mobile phase additives in chromatography or as chiral selectors dissolved in solution for CE, they are treated using the pseudophase model that we developed for micelles and cyclodextrins over fourteen years ago. Several research groups have 'derived' and re-expressed (an euphemism for rearranging a known equation and using different symbols so that it looks original) equations that use electrophoretic parameters to calculate association constants to chiral selectors and/or micelles. As has been shown, these basic equations have been reported previously many times dating back at least to 1951. Despite this some prominent groups in the CE field continue to report incorrect numbers using incorrect equations and/or assumptions.

(Abstract by the author)

Peak Overlap and Quantitative Enantioselective Analysis

Veronika R. Meyer, University of Bern, Organic Chemistry, Bern

Overlapped peaks give rise to inaccurate integration due to geometrical effects. In a pair of *Gauss* peaks the area of the small peak is too small, irrespective of elution order. In a pair of tailed peaks the area of the first peak ist too small, irrespective of size ratio. The effect for the large or the second peak, respectively, is *vice versa*, and in the case of fronting the first peak is too large.

Rider peaks (small peaks sitting on the tail of a much larger one) cannot be integrated accurately, neither by the vertical drop or tangent method nor by area or height determination.

A small, *Gauss* or tailed peak which is eluted before its large neighbour can be quantitated almost accurately by its height even in cases of severe overlap.

If quantitation in the 1:100 mass ratio range is necessary it is strongly recommended to use the technique of 'high-low-chromatography', *i.e.*, injection of a concentrated *and* of a diluted sample solution.

Practical examples include (±) menthol, (*R,S*)-1-(phenylethyl)-1-naphthoic acid amide, and (D,L)-proline. For the latter compound it is not yet clear which derivative is suited best for the enantioselective GC analysis on *Chirasil-Val*.

V.R. Meyer, *LC GC Int.* **1994**, *7*, 94; *J. Chromatogr. Sci.* **1995**, *33*, 26; *Chromatographia* **1995**, *40*, 15; *Chirality* **1995**, *7*, 567.

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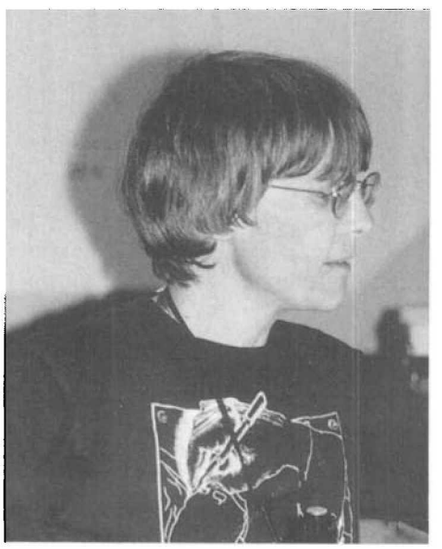

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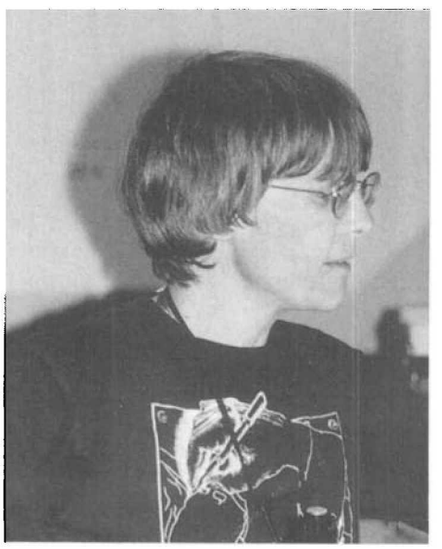

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Optimisation and Application of Quinine and Quinidine Derivatives as Highly Efficient Selectors in Liquid Chromatography

Wolfgang Lindner and Michael Lämmerhofer, Institute of Pharmaceutical Chemistry, Karl-Franzens-University, Graz

Chiral selectors commonly used for liquid chromatographic enantioseparation employing aqueous mobile phases and adopted for the resolution of ionic selectands are either based on: *a*) protein-type compounds, *b*) amino acids or derivatives thereof for ligand exchange type systems and *c*) macrocycles of the crown ether type, cyclodextrin type or of the various antibiotics type compounds. The underlying stereoselective selector-selectand interaction principles (particularly for the high molecular weight selectors) seem to be difficult to deconvolute due to their complex chemical and spatial structure.

In the presented study we investigated a relatively simple but rigid structured set of chiral compounds and chiral selectors, respectively, based on derivatives of quinine and its pseudo-enantiomer quinidine and their capability to be used as chiral anion exchange systems in HPLC [1]. It turned out that carbamates of the cinchona alkaloids express unique stereoselectivity for *N*-protected amino acids. *Via* spectroscopic investigations an optimisation strategy and QSAR studies with respect to the size and shape of the substituent of the carbamoyl group we were able to tune the stereoselectivity and to reach α-values up to 20 for 2,4-dinitrobenzoyl amino acids. These new CSPs allow the efficient chiral resolution of virtually all the various *N*-protected proteinogenic α- but also of many β- and γ-amino acids. Enantiomeric purity determinations of these compounds, which means also of free amino acids after simple non-chiral derivatization, down to 99.98 level are feasible [2]. These results have some impact in the field of peptide synthesis and on the investigation phenomena in the course of immobillisation, protection and deprotection protocols.

[1] M. Lämmerhofer, W. Lindner, *J. Chromatogr. A* **1996**, in press.
[2] O. Kleidernigg, M. Lämmerhofer, W. Lindner, *Enantiomer* **1996**, submitted.

(Abstract by the author)

Preparative Chiral Separations by Chromatography: a Powerful Approach for the Isolation of Optically Pure Compounds

Eric R. Francotte, *Ciba*, Basel

Thanks to the concomitant development of a wide range of chiral stationary phases (CSPs) and the introduction of new chromatographic instrumentation, the chromatographic separation of enantiomers on a preparative scale is gaining increasing acceptance as a simple, rapid, and generally applicable method for supplying pure enantiomers of bioactive compounds and chiral synthons. The method is especially useful as a relatively inexpensive means of supplying the pure enantiomers of new drugs or pesticides in the amounts needed for preliminary biological, pharmacological or toxicological testing, and for field trials. Among the different CSPs used for the preparative separation of enantiomers, the most widely applied have been derived from polysaccharides and, in particular, cellulose derivatives. The wide range of application and the relatively high loading capacity of these CSPs have certainly contributed much to their prevalence. Concerning the strategy to be adopted for preparative chiral resolutions, both options of selecting an appropriate CSP for a defined racemate or adapting the structure of the racemate to a defined CSP have to be considered. Especially the latter strategy has been shown to lead to successful resolutions of racemates that otherwise could not be separated on a defined CSP. Nevertheless, the preparation of a new CSP specially designed for the resolution of a particular racemate could also become a useful approach for compounds due to be produced in large amounts.

Up to now, elution batch chromatography clearly dominates in terms of the number of applications, and various approaches were used for improving throughput, such as close injections, peak-shaving, and recycling, but large-scale separations would require large amounts of CSP and have been considered economically unjustifiable because 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. However, with the recent introduction of the simulated moving bed technology in this application field, the technical prerequisites for the performance of such large-scale separations under cost-effective conditions can now be met. This chromatographic mode can save up to 90% of the mobile phase and achieves a much higher throughput. It can be expected that this chromatographic technique will soon be considered a suitable alternative to the more classical approach for producing optically pure compounds in large amounts.

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