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Immobilization of chiral phases for enantiomer separations in supercritical fluid and gas chromatography

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ABSTRACT

The immobilization of Chirasil-Val on glass and fused-silica capillaries by either radical or thermal reactions and by a combination of both was studied with respect to both the degree of immobilization achieved and the concomitant loss in enantioselectivity of the stationary phase. Thermal immobilization was found to be promoted by the presence of free carboxyl groups in the stationary phase and a tentative mechanism of the immobilization reaction is suggested. Fused-silica capillaries coated with immobilized Chirasil-Val were used to separate amino acid derivatives by supercritical fluid chromatography (SFC). The separations obtained by gas chromatography and SFC are compared.

INTRODUCTION

Extension of the application of chiral stationary phases from gas chromatography (GC) to supercritical fluid chromatography (SFC) would appear to offer potential advantages: the solvating effect of the mobile fluid allows a significant reduction in the temperature of analysis, which in turn should result in a higher enantioselectivity as the difference in the energy of interaction of the enantiomers with the chiral stationary phase is temperature dependent.

A prerequisite for the use of a GC stationary phase in SFC is its amenability to immobilization, and thus the common technique of radical-induced cross-linking, generally accepted in the immobilization of GC stationary phases, has been applied to various chiral GC stationary phases to permit their use in SFC [1–4]. Experience shows that with chiral phases of the amide type (*e.g.*, Chirasil-Val), this reaction is always accompanied by racemization of the chiral centre.

We recently reported the immobilization of Chirasil-L-Val on deactivated glass capillaries [4], investigating the effectiveness of two general methods, radical-induced cross-linking and mild thermal treatment with a reduced carrier gas flow, together with the side-effects of the immobilization reaction on the enantiomeric excess (e.e.) of the phase, the enantioselectivity (α) toward amino acid derivatives and the polarity of the phase. Conditions yielding a degree of immobilization of up to *ca.* 70% but with acceptably low losses in enantioselectivity were established. With increasingly drastic

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reaction conditions, racemization became more serious, but with little increase in the degree of immobilization. In this paper, we report on the possibility of increasing the degree of immobilization by repeated radical reactions and by a combination of thermal and radical reactions. In addition, evidence emerging during the immobilization of numerous batches of Chirasil-Val now allows a tentative explanation of the mechanism of the thermal immobilization.

Finally, fused-silica capillaries (100 μm) coated with immobilized Chirasil-Val were tested under SFC conditions. The elution characteristics of a number of enantiomeric amino acid derivatives were studied and compared with the analogous GC separations.

EXPERIMENTAL

Glass capillaries

Glass capillaries (0.8 mm O.D., 0.1 mm I.D.) were drawn from washed (50% HNO_3–5% HF), rinsed (distilled water) and dried Duran 50 glass tubes on a Hupe/Busch glass capillary drawing machine. Approximately 10-m lengths were leached (6 *M* HCl at 180°C for 18 h), dried (250°C for 4 h) and deactivated with diphenyltetramethyldisilazane (DPTMDS) (Fluka, Buchs, Switzerland) at 200°C for 20 h under vacuum. Approximately 0.3-μm films of the stationary phase were deposited by the static technique from solutions of 10% methylene chloride in *n*-pentane.

Immobilization was carried out by methods described previously [4], both repeatedly or in combination. In corresponding experiments, dicumyl peroxide (DCUP) was coated statically from a solution in *n*-pentane following thermal immobilization. This is possible because after thermal immobilization Chirasil-Val is completely insoluble in *n*-pentane.

Fused-silica capillaries

Fused-silica capillaries (100 μm) (Quartz et Silice, Bad Pyrmont, Germany) were washed with acetone, dynamically coated with a suspension of 0.3% Cab-O-Sil in acetone (prepared by sonication and subsequent centrifugation to remove large particles) and dried at 280°C prior to static coating with the stationary phase.

Gas chromatography

Columns were tested on a Carlo Erba Fractovap 2100 with flame ionization detection and hydrogen as the carrier gas. The α-values of the N-trifluoroacetyl-*n*-propylester of leucine, measured at 100°C, were taken as an indicator of the enantioselectivity of the stationary phases after the various treatments. Determination of the enantiomeric excess of the stationary phase involved hydrolysing the film in the closed capillary with 6 *M* HCl at 110°C for 20 h followed by GC separation of the N-trifluoroacetyl-*n*-propylesters on Chirasil-Val. Determination of the loading of L-valine on batches of Chirasil-Val was performed by the method of enantiomer labelling [5].

Supercritical fluid chromatography (SFC)

Carbon dioxide (99.9985 vol.%) (Messer Griesheim, Griesheim, Germany) as

the mobile fluid was delivered by an Isco μLC micro flow pump, the cylinder of which was cooled to 3°C with a cryostat (Haake K, Haake, Karlsruhe, Germany). The column was thermostated in a Carlo Erba GI gas chromatograph equipped with a flame ionization detector and a Valco C14W valve in place of the injection port. Inlet splitting at a splitting ratio of *ca.* 1:10 was employed. The column was terminated by a glass restrictor fixed to the fused-silica capillary by press fitting, augmented by polyimide glue for additional strength [6,7].

RESULTS AND DISCUSSION

Improvement of the degree of immobilization

Repeated reaction with azo-*tert.*-butane (ATB) has been variously used to increase the degree of immobilization of non-chiral stationary phases for use in SFC [8]. The fact that under the static conditions of the reaction the radical concentration decreases while the concentration of byproducts increases during the course of the reaction indeed suggests that repeated reactions under milder conditions could be advantageous over a single reaction. Multiple reactions can be readily performed with ATB, which is introduced into the incompletely immobilized column via the gas phase. With DCUP, immobilization (at least toward non-polar solvents) in a first step is necessary for repeated reaction.

Combination of both thermal and radical techniques appears promising, as both methods are certainly different in mechanism and could complement each other. Both ATB and DCUP were used in combination with thermal immobilization. The results are shown in Fig. 1, in which the degree of immobilization achieved is plotted against remaining enantioselectivity (measured as α for leucine at 100°C). For comparison, results of the single reactions are also included. All reactions reported here were conducted on a single batch of Chirasil-Val.

It is apparent from Fig. 1 that repetition of reactions offers only marginal advantages: the increase in immobilization is accompanied by racemization of

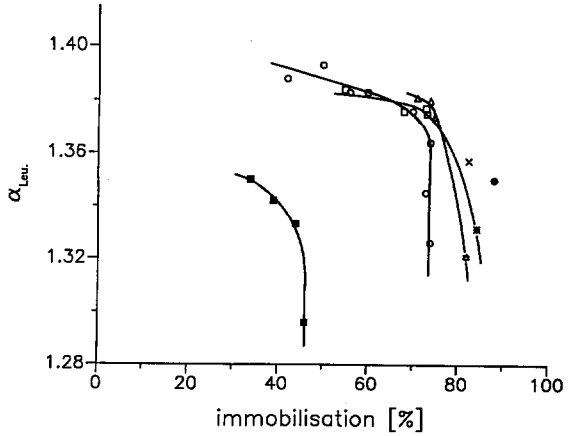

Fig. 1. Improved degree of immobilization (%) *versus* enantioselectivity (α_{Leu}) for various combined methods of immobilization. Single reactions: △ = with water; □ = with ATB; ○ = thermal; ■ = with DCUP. Multiple reactions: ☆ = 2× with water; ✳ = 2× with ATB; × = thermal + ATB; ● = thermal + DCUP.

a magnitude to be expected from extrapolation of the results of the corresponding single reactions under various reaction conditions.

On the other hand, the combination of radical and thermal techniques is more promising: here the enantioselectivity is better sustained at high degrees of immobilization.

Overall, it is still questionable whether the attainment of maximum immobilization at the cost of enantioselectivity is preferable to the decrease in film thickness incurred with moderately immobilized (*ca.* 70–75%) films but with retention of enantioselectivity. The ruggedness of the film of stationary phase under routine SFC conditions could, however, prove an important factor to be considered when deciding how extensive the immobilization of the stationary phase should be.

Mechanism of thermal immobilization

Chirasil-Val is immobilized by heating for 12–24 h between 160 and 220°C under reduced or stopped flow conditions. The same treatment but with normal flow-rates leads to low degrees of immobilization.

If the same experiment is performed on conventional stationary phases, *e.g.*, dimethylpolysiloxane, almost no immobilization is observed. Even with hydroxy-terminated polysiloxanes, considerably higher temperatures (in excess of 300°C) are apparently required [9].

In the course of our experiments, the following observations were made:

(1) the ease of immobilization of Chirasil-Val varied from batch to batch;

(2) for a given copolymer, batches of Chirasil-Val with poorer enantioselectivity were more amenable to immobilization; these batches also carry lower L-valine loadings;

(3) batches of Chirasil-Val synthesized by hydrosilylation reaction of allyl-carboxy-L-valine-*tert.*-butylamide with a methylhydropolysiloxane (Chirasil-Nova) could not be immobilized at all by thermal treatment;

(4) saturation of the carrier gas with water vapour during thermal treatment accelerates the immobilization reaction.

We interpret these observations in the following way. The important difference between batches of Chirasil-Val is the proportion of free carboxyl groups remaining unreacted after the coupling reaction with L-valine-*tert.*-butylamide. Poorer coupling yields are characterized by lower valine loadings as determined by quantitative amino acid analysis and result in lower enantioselectivity of the stationary phase. Chirasil-Val batches which, owing to their synthetic path, cannot contain free carboxyl groups (*e.g.*, Chirasil-Nova) are therefore unable to be immobilized by thermal reaction.

In addition to carboxyl functions, water is a necessary reaction partner and is presumably formed during the immobilization reaction. At low flow-rates or under stopped flow conditions, the water vapour evolved is retained in the capillary rather than being continuously scavenged as occurs under normal flow conditions.

In order to test this hypothesis, we doped two stationary phases which we knew to be non-susceptible to thermal immobilization (PS-255 and Chirasil-Nova) with *ca.* 33% of a polysiloxane containing carboxyl groups (non-coupled Chirasil copolymer). After thermal treatment, these mixed phases were found to be immobilized to the extent of 80 and 85%, respectively. With hydroxy-terminated phases, the effect of free carboxyl groups was more dramatic.

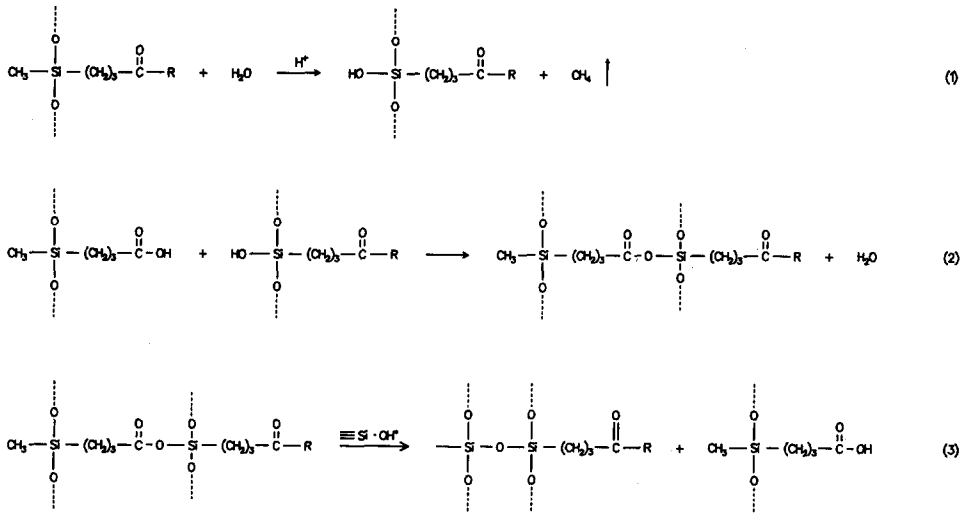

Fig. 2. Tentative mechanism of the immobilization reaction of Chirasil-Val. R = –OH or –Val-*tert.*-butylamide. * = Silanol group may be from either the stationary phase or the vitreous surface.

The following mechanism is therefore proposed (Fig. 2). Silanol groups have been demonstrated to be present in Chirasil-Val by NMR measurements [10]. Additional silanol groups may be formed by hydrolytic cleavage of Si–C groups, probably the Si–CH_3 group adjacent to the carboxy functional moiety (reaction 1). This reaction is known [11]. The silanol condensation is catalysed by the intermediate formation of an acyloxysilyl group through condensation of a free silanol with carboxyl group (reaction 2), followed by further reaction with a free silanol, thus liberating the carboxyl group (reaction 3) [12].

We believe that this technique of immobilization could be of use with other (non-chiral) stationary phases and suspect that at least in some instances it has perhaps been an important factor in immobilizations attributed solely to thermal silanol condensation [13]. In particular with cyanoalkyl silicones, the preparation is such that a certain amount of the cyano groups will be hydrolysed to carboxyl groups [14]. The conditions of immobilization used by David *et al.* [13] [low flow and relatively mild thermal treatment (280°C for 2 h)] are similar to those we used to immobilize Chirasil-Val.

SFC with Chirasil-Val

In order to compare the performance of Chirasil-Val under GC and SFC conditions, N-trifluororacetyl-*n*-propylesters of selected DL-amino acids (Thr, Pro and Leu) were chromatographed at various temperatures on a 100-μm immobilized Chirasil-Val fused-silica capillary and the enantioselectivity was determined via the α values. The results are shown in Fig. 3.

It is apparent that under the conditions employed, the postulated improvement in the α values as a result of the lower temperature of analysis is in fact not realised. The curve of α *vs.* temperature displays a discontinuity on changing from hydrogen as

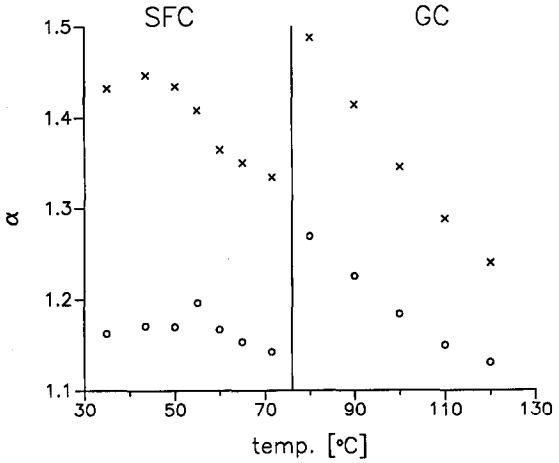

Fig. 3. Dependence of enantioselectivity (α) for D,L-amino acid derivatives on column temperature under both GC and SFC conditions. × = Leu; ○ = Thr.

carrier gas in GC to carbon dioxide in SFC and at corresponding temperatures the enantioselectivity in the SFC mode is lower than that in the GC mode. This must be explained by the possibility of dipole–dipole interaction or hydrogen bonding of carbon dioxide to the optically active region of both Chirasil-Val and the sample. This interaction is competitive with the enantiospecific interaction of substrate with Chirasil-Val.

The resolution of enantiomers by SFC is further hampered by its inherently lower efficiency and longer retention times as a consequence of the higher viscosity of the mobile phase (see Fig. 4).

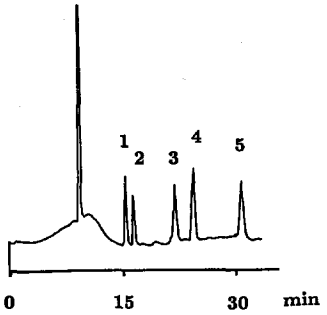

Fig. 4. Enantiomeric separation of amino acid derivatives as N-trifluoroacetyl-*n*-propylesters in capillary SFC. Sample: 1 = D-Thr; 2 = L-Thr; 3 = D,L-Pro; 4 = D-Leu; 5 = L-Leu. Stationary phase, Chirasil-L-Val; capillary column, 10 m × 100 μm fused silica; mobile phase, CO_2, 20 μl/min at 10.0 MPa; temperature, 50°C (isothermal); pressure, 7.7 MPa.

CONCLUSIONS

Improved immobilization of Chirasil-Val (up to almost 90%), but at the cost of increased racemization may be achieved by both radical or combined radical and thermal treatment. The combination of the two techniques is of advantage in that lower decreases in enantioselectivity are incurred for a given degree of immobilization.

Thermal immobilization has been shown to be dependent on the presence of carboxyl groups bound to the polysiloxane and to the presence of both silanol groups and water vapour. It seems probable that silanol groups, if not originally present, may be formed by hydrolytic cleavage of Si–C bonds in the presence of water and carboxyl groups.

Immobilized Chirasil-Val capillaries have been used in SFC. The enantioselectivity in the SFC mode is, however, lower than in the GC mode under comparable temperature conditions. This is presumably the effect of the more polar mobile phase (carbon dioxide in SFC *vs.* hydrogen in GC). Nonetheless, we believe that for some applications, in particular with chiral substances of low volatility, separation via SFC could be advantageous over GC.

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