ELSEVIER

Journal of Chromatography A, 694 (1995) 333–345

JOURNAL OF CHROMATOGRAPHY A

Synthesis and characterization of unsaturated bonded phases for high-performance liquid chromatography

Jutta Schmid, Klaus Albert, Ernst Bayer*

Institut für Organische Chemie der Universität Tübingen, Auf der Morgenstelle 18, D-72076 Tübingen, Germany

First received 7 September 1994; revised manuscript received 4 November 1994; accepted 8 November 1994

Abstract

New reversed-phase materials containing unsaturated fatty acids on a silica support were prepared by a two-stage modification of LiChrospher silica and characterized by chromatographic and NMR spectroscopic techniques.

Chromatographic properties of the chemically modified silica were evaluated by separation of two test mixtures, one containing three basic drugs, the other being a homologous series of four *p*-hydroxybenzoic acid esters. The dynamic behaviour of the bonded alkenyl chains was studied by means of NMR relaxation time measurements in the solid and suspended state and correlated with chromatographic properties.

Spectroscopic and chromatographic results were compared to data of the corresponding saturated phases: the unsaturated packings showed higher overall mobility together with better separation characteristics. This is ascribed to different degrees of order in unsaturated and saturated phases and to specific interactions of the double bonds.

1. Introduction

Conventional reversed phases for HPLC are bonded saturated alkyl chains of various length. The interaction between phase and analyte is in general predominantly of hydrophobic nature. Analogue bonding forces in principle occur in biological membranes where molecular self organization of the phospholipid bilayer and of enzymes and receptors in the membrane is also due to Van der Waals interactions [1]. In natural membranes, phospholipids containing unsaturated fatty acid moieties also do occur in addition to those composed exclusively of saturated fatty acyl chains. Although little is known about the influence of such unsaturated lipids upon the mechanisms of self organization of membranes, one can assume certain specific interactions. It was our goal to study the properties and chromatographic behaviour of bonded phases containing alkenyl chains, in expectation that they might show a novel type of selectivity for analytes and that these results could shed insight into the question of why nature has created lipid molecules with unsaturated as well as saturated fatty acids.

A correlation of chromatographic properties and mobility as determined by solid- and suspended-state NMR spectroscopic relaxation time measurements should also reveal relations between structure and chromatographic efficacy of the synthesized packings. For the synthesis of such new reversed-phase materials, in a first reaction step (I) the silica was modified with a functionalized γ-aminopropylsilane (mono- or trifunctional) [2], the reaction creating siloxane

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(94)01097-8

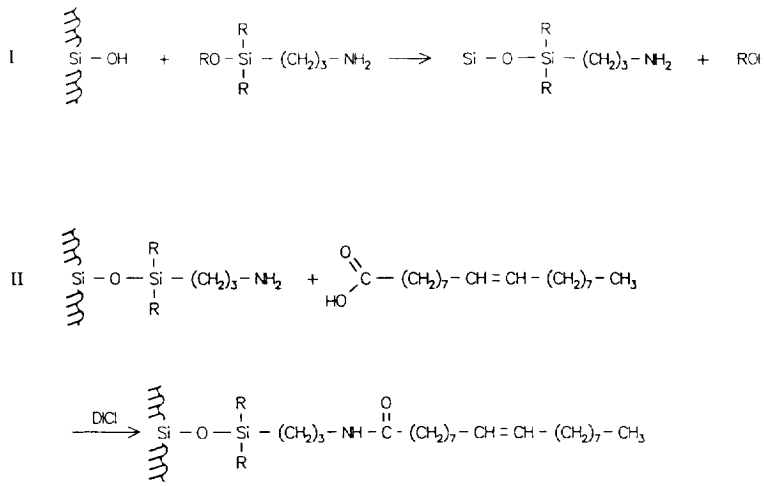

Fig. 1. Synthesis of the unsaturated packings. (I) Silanization of the silica. (II) Carbodiiminide (DICI) coupling with an unsaturated fatty acid.

bonds between the silane and the silica support (Fig. 1, I).

Whereas the reaction of silica with a monofunctional silane results in a well defined monolayer on the silica surface, reaction with a trifunctional silane leads to a more complex surface chemistry [3]. The various possibilities of crosslinking and polymerization as well as the effects of various silane functionalities on the chromatographic properties of the resulting packings have been discussed by Dorsey and Dill [4] and Pfleiderer et al. [5]. In this connection, Dorsey and Dill conclude that partitioning, rather than adsorption, is a dominant mechanism of chromatographic retention. Partitioning, again, depends on chain ordering and, thus, partly on the functionality of the bonded silane, as polymeric phases are more ordered than the corresponding monomeric ones [6,7]. Thus, a distinction between monofunctional and trifunctional packings is also important when discussing mobility and ordering effects.

In a second step (II), the γ-aminopropylsilane-modified silica was treated with an unsaturated fatty acid (oleic acid or elaidic acid) by carbodiimide coupling [8,9] with addition of N-hydroxybenzotriazole, resulting in an amide bond (Fig. 1, II). The saturated analogues were obtained by reaction of the aminopropylsilane-modified silica with stearoyl chloride.

According to elemental analysis, this second reaction step proceeds only to the extent of about 50%. Thus, the resulting packings are "mixed phases" containing both fatty acid amide and free amino groups which might have special characteristics for HPLC separations.

In order to investigate analogies between the synthesized packings and biomembranes the interaction with cholesterol was chosen. Cholesterol decreases the fluidity of biomembranes [10]. Therefore we added cholesterol to the synthesized packings and checked by NMR spectroscopic relaxation time measurements whether it incorporates between the alkyl chains and decreases the mobility of the bonded fatty acyl chains as it does in biomembranes.

2. Experimental

2.1. Materials

LiChrospher silica (batch 5025, particle diameter 5 μm, average pore diameter 60 Å, specific surface area 570 m^2/g) was kindly donated by Merck (Darmstadt, Germany). Dimethyl-

methoxy-γ-aminopropylsilane and triethoxy-γ-aminopropylsilane were obtained from Bayer (Leverkusen, Germany) and Fluka (Neu-Ulm, Germany), respectively, and used without further purification. Diisopropylcarbodiimide (DICI), oleic acid and elaidic acid were obtained from Aldrich (Steinheim, Germany). Morpholine was obtained from Riedel de Haën (Seelze, Germany). N-Hydroxybenzotriazole (HOBT) was prepared by reaction of *o*-chloronitrobenzene and hydrazine hydrate [11]. Stearoyl chloride was prepared by reaction of stearic acid with $SOCl_2$.

Acetonitrile for HPLC was of LiChrosolv grade (Merck), deuterated solvents for NMR spectroscopy were of Uvasol grade (Merck). Test solutes for HPLC were obtained from Merck, Aldrich and Hoffmann-La Roche.

2.2. Solid-state and suspended-state NMR measurements

NMR spectra in solution and in the suspended state were measured in 5-mm sample tubes on a Bruker AC 250 NMR spectrometer. Modified silica samples were suspended in perdeuterated acetonitrile. Solid-state ^{29}Si and ^{13}C magic-angle spinning (MAS) spectra were obtained on a Bruker MSL 200 NMR spectrometer at 4.7 T. Quantities of 200-300 mg of modified silica gel were packed into double bearing rotors of ZrO_2 which were spun at 3.6 kHz by dry air gas drive.

Solid-state NMR experiments were performed by combination of MAS [12], cross polarization (CP) [13] and high-power decoupling [14] in order to obtain high-resolution spectra.

The Hartmann–Hahn condition for CP was calibrated with glycine or Q_8M_8, the trimethylsilyl ester of the octameric form of silicic acid, for ^{13}C and ^{29}Si NMR, respectively. Typically, ^{13}C CP-MAS spectra were recorded with a pulse length of 5 μs, contact times of 1 and 3 ms for monofunctionally and trifunctionally modified packings, respectively, and a repetition time of 2 s. Chemical shifts were externally referenced to liquid tetramethylsilane.

The error limits of relaxation time values are in the order of approximately 5% as was revealed by repeated measurement of the same parameters.

2.3. Chromatography

HPLC separations were performed with an S 1110 HPLC pump (Sykam, Germany), a UV 655 A variable-wavelength detector (Hitachi) and a C-R6A Chromatopac integrator (Sykam).

The modified silica were packed into 60 × 4.6 mm tubes (Bischoff, Leonberg, Germany) by a high-pressure slurry-packing procedure on a Shandon packing pump (Shandon, Frankfurt, Germany).

Chromatographic conditions

Mobile phases: 156 ml acetonitrile + 340 ml phosphate buffer, pH 2.3 (6.66 g of KH_2PO_4 + 4.8 g of 85% H_3PO_4 in 1 l of water for HPLC) for separation of the basic drugs and acetonitrile–water (45:55) for separation of *p*-hydroxybenzoic acid esters; UV detection wavelength: 220 nm for separation of the basic drugs and 254 nm for separation of *p*-hydroxybenzoic acid esters; flow: 0.5 and 0.9 ml/min for basic drugs and esters, respectively; temperature 25°C.

2.4. Procedures

Silanization of the silica

A small quantity of the silica (5 g) was dried under vacuum (10^{-2} Torr; 1 Torr = 133.322 Pa) at 180°C for 12 h. After cooling to 120°C, the flask was aerated, and 1 ml of the γ-aminopropylsilane per g silica was added without any addition of solvent. The flask was covered with a frit (G3) and the mixture held at 120°C for another 12 h. The product was filtered off, washed three times with 25 ml of toluene, methanol and *n*-hexane, respectively and finally air dried (for coverage densities obtained in this first reaction step see Table 1).

Reaction of the γ-aminopropylsilane-modified silica with unsaturated fatty acids

A 5-mmol amount of the unsaturated fatty acid and 6 mmol of HOBT were dissolved in 30 ml of dimethylformamide. After cooling the

Table 1
Characteristics of the synthesized packings

No. of packing	Type of silane	Fatty acid	Coverage				
			P_C(C-3)	P_N(C-3)	α_{C-3}	P_C(C-21)	α_{C-21}
1	M	Stearic acid	7.18	1.59	2.46	15.58	1.15
2	M	Oleic acid	7.18	1.59	2.46	18.66	1.51
3	M	Elaidic acid	7.18	1.59	2.46	19.30	1.60
4	T	Stearic acid	5.33	1.84	3.06	14.33	1.30
5	T	Oleic acid	5.33	1.84	3.06	19.10	1.98
6	T	Elaidic acid	5.33	1.84	3.06	20.51	2.19

Silanes: M = dimethylmethoxy-γ-aminopropylsilane (monofunctional); T = triethoxy-γ-aminopropylsilane (trifunctional). Carbon and nitrogen contents P_C(C-3) and P_N(C-3)/P_C(C-21) (%) and coverage densities $\alpha_{C-3}/\alpha_{C-21}$ (μmol/m^2) of the aminopropylsilane-modified silica and the C_{21} phases, respectively, are given.

solution to 0°C in an ice-bath 5.5 mmol DICI in 3 ml dichloromethane were slowly added. The ice-bath was then removed, the solution stirred for 15 min and a quantity of silica containing 5 mmol of NH_2 groups (2.9 g of the trifunctional or 3.6 g of the monofunctional γ-aminopropylsilane-silica) added. The reaction mixture was then stirred at room temperature for 12 h. The product was filtered off, washed three times with 30 ml of dimethylformamide, methanol and *n*-hexane, respectively and air dried.

Reaction of the γ-aminopropylsilane-modified silica with stearic acid

A 2-g amount of the γ-aminopropylsilane-modified silica was vacuum-dried (10^{-2} Torr) at 100°C for 8 h. After cooling down to 50–60°C, the flask was aerated and 2.6 ml stearoyl chloride and 2.6 ml morpholine were quickly added without any solvent.

The flask was covered with a frit (G3) and the reaction mixture stirred slowly at 100°C overnight. Finally the product was filtered off, washed three times with 30 ml of toluene, methanol and *n*-hexane, respectively and dried under air.

Coverage densities α_{C-3} of the γ-aminopropylsilane-modified silica were calculated according to [15] from carbon and nitrogen contents, as determined with a Model 1104 CHN analyzer (Carlo Erba, Milan, Italy). The degree of reaction of amino to amide groups in the second reaction step and thus the coverage density with the fatty acid moiety α_{C-21} was determined from the carbon content of the C_{21}-amide phase, relative to the theoretical calculated carbon content derived from the known α_{C-3} coverage density.

The specific surface area S_{BET} of LiChrospher was determined by nitrogen sorption using a Model 1800 Sorptomatic instrument (Carlo Erba).

Incorporation of cholesterol into C_{21}-amide phases

A 1-g amount of the C_{21}-amide phase was suspended in a mixture of 20 ml of methanol and 20 ml of water. A 0.5-g amount of cholesterol was added and the mixture stirred slowly overnight. The silica was then filtered off, washed three times with 5 ml of methanol and then air dried.

3. Results

Table 1 contains characteristic data of the synthesized packings. The use of trifunctional γ-aminopropylsilane results in slightly higher coverage densities α_{C-3} than the use of monofunctional silane, the partially cross-linked trifunctional species allowing a closer packing than the monofunctional silanes which contain relatively voluminous methyl groups.

Further on, comparing the coverage densities of oleic acid and elaidic acid packings, it seems that the *trans* double bond allows closer packing of the alkenyl chains than the *cis* double bond. This effect will be correlated with molecular modelling and NMR spectroscopic and chromatographic results.

Coverage densities of the saturated C_{21} phases are clearly lower than those of the unsaturated packings. At present it is unclear whether this is an effect of chain packing or of the different reaction modes (step II) for saturated and unsaturated packings.

3.1. NMR studies

The packings were characterized by ^{29}Si and ^{13}C solid-state CP-MAS-NMR spectra in order to verify that silanes and fatty acids were chemically bonded (Fig. 2).

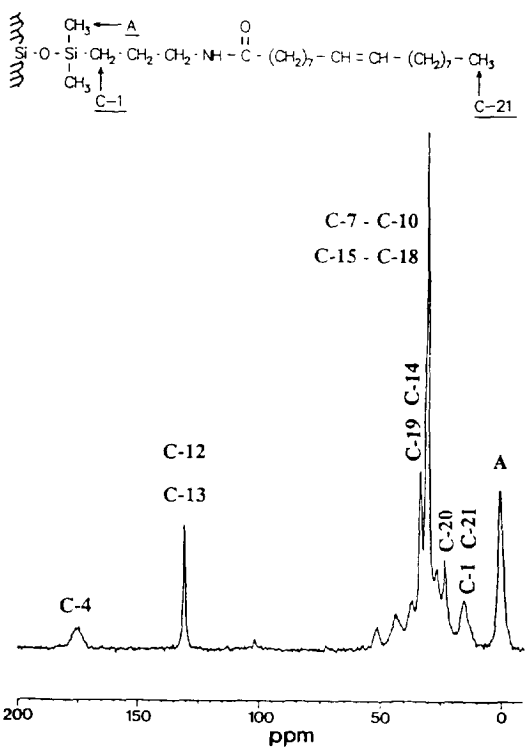

Fig. 2. ^{13}C CP-MAS-NMR spectrum of monofunctionally modified LiChrospher containing elaidic acid.

$T_{1\rho H}$-measurements

NMR relaxation time measurements permit the description of the dynamic behaviour of certain atoms in chemical systems.

$T_{1\rho H}$, the spin lattice relaxation time in the rotating frame, describes motions in the kHz range [16,17]. A systematic determination of $T_{1\rho H}$ values of the various packings according to a pulse sequence developed by Schaefer et al. [18] showed that there is an increase in $T_{1\rho H}$ values from the carbon anchored to the silica to the carbon of the terminal methyl group (Fig. 3a), as has been shown previously for conventional reversed phases [19,20]. Temperature-dependent measurements on comparable systems have shown that increased $T_{1\rho H}$ values indicate higher mobility of the respective carbon atoms [21]. While the increase in mobility towards the terminal methyl group occurs in both conventional and the new C_{21} packings, the absolute $T_{1\rho H}$ values are much lower in the case of our C_{21} packings (up to a factor of about 5), i.e. the aminopropyl spacer together with the planar amide group renders the whole system quite rigid.

The $T_{1\rho H}$ values and thus the mobility of the respective carbon atoms are higher for monofunctionally modified packings than for trifunctionally modified ones. This can be explained by the cross-linking of the trifunctional aminopropylsilane-modified species which renders the system more rigid.

In each of the two groups the packing containing oleic acid (unsaturated *cis*) shows the highest $T_{1\rho H}$ values, followed by the elaidic acid packing (unsaturated *trans*), whereas the corresponding saturated phase (monofunctionally modified) shows relatively low $T_{1\rho H}$ values. This indicates an increase in mobility of the fatty acid chains caused by the C = C double bond, the effect of a *cis* double bond being significantly stronger than that of a *trans* double bond.

The molecular organization of saturated stationary phase chains has been described by Dorsey and Dill [4]: chains which are aligned parallel one to another should be orientated normal to the silica surface resulting in a relatively ordered structure. Pfleiderer et al. [21]

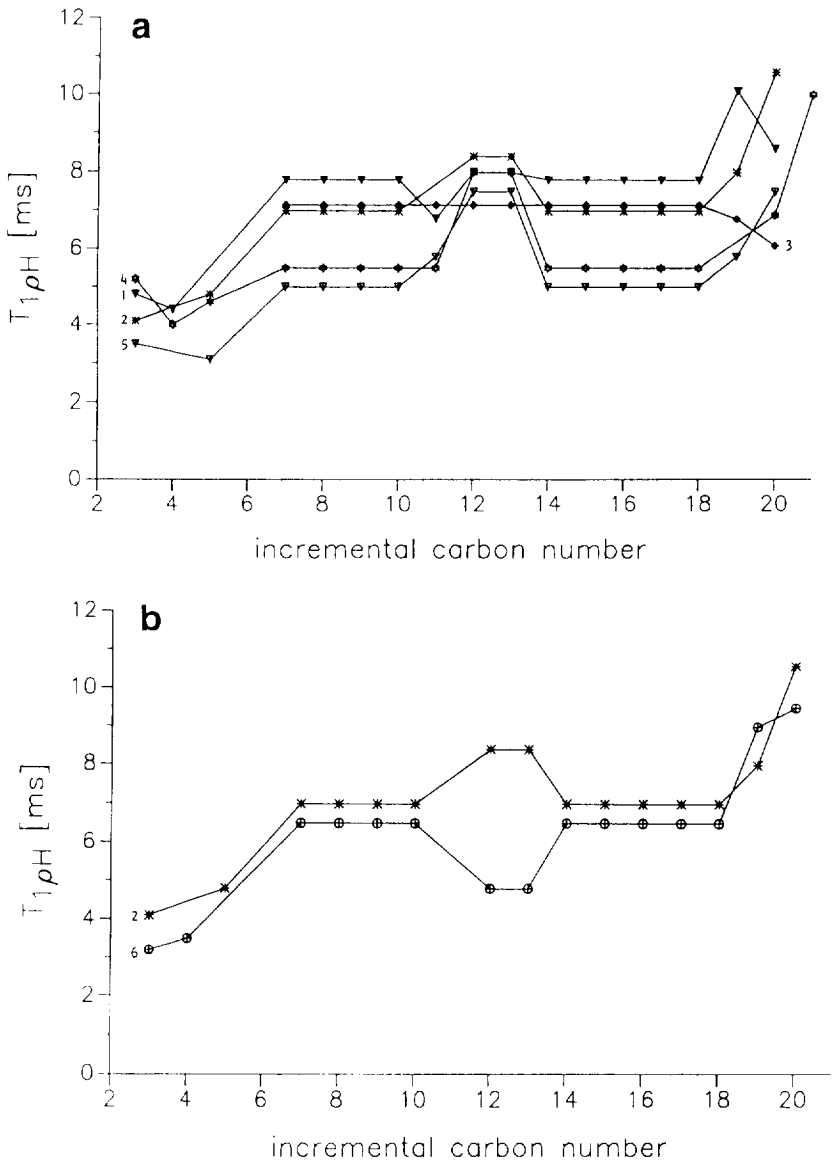

Fig. 3. (a) Dependence of the $T_{1\rho H}$-values on the carbon position within the alkenyl chain. 1 = Monofunctional oleic acid packing; 2 = monofunctional elaidic acid packing; 3 = monofunctional stearic acid packing; 4 = trifunctional oelic acid packing; 5 = trifunctional elaidic acid packing. $T_{1\rho H}$ values of the carbon atoms C_7–C_{10} and C_{14}–C_{18} are not resolved because in the NMR chemical shift range these carbon atoms all result in an overlaped peak at 29.7 ppm. (b) $T_{1\rho H}$ values of the monofunctionally modfied elaidic acid packing before and after addition of the cholesterol. 2 = Monofunctional elaidic acid packing; 6 = monofunctional elaidic acid packing + cholesterol

showed that the alkyl chains of common (saturated) C_{18} phases are indeed almost parallel one to another and much less mobile than the shorter chains e.g. of C_6 or C_8 phases.

An ideally parallel orientation of such alkyl chains is possible in the case of all-*trans* conformation of the carbon atoms only. A double bond always causes a kink in the chain preventing parallel orientation of the chains. The packing thus becomes less ordered and motional freedom increases. This effect is much stronger for *cis* double bonds than for *trans* double bonds.

This result corresponds to the slightly higher coverage densities of the oleic acid packings compared to the elaidic acid packings and has been supported by molecular mechanics calculations of these systems [22].

$T_{1\rho H}$ values decrease significantly after incorporation of cholesterol (Fig. 3b). This influence of cholesterol on relaxation times shows that the cholesterol is really incorporated between the fatty acyl chains of the RP packing and decreases mobility. The lower $T_{1\rho H}$ values are ascribed to lower mobility and thus correspond to the influence of cholesterol on the fluidity of biological membranes which are stabilized by cholesterol [10]. In this respect, the synthesized packings mimic biomembranes.

Contact time experiments

The results evaluated by $T_{1\rho H}$ measurements were verified by contact time experiments.

In these experiments the signal intensity is a function of the contact time. Depending on the carbon atom mobility various contact times are necessary to obtain maximum signal intensity in CP experiments: The higher the mobility, the longer is the contact time to obtain a maximum signal-to-noise ratio ($C_{p\,max}$) of comparable carbon atoms.

The results of the contact time experiments as presented in Fig. 4 clearly confirm the higher mobility of the unsaturated phases compared to the corresponding saturated ones.

^{13}C NMR T_1 measurements in the suspended state

In order to investigate the dynamic behaviour of the packings under actual HPLC conditions, mobility studies in the suspended state were performed. ^{13}C T_1 values can be obtained by inversion recovery experiments [180°–τ–90°–free induction decay (FID)]. Again, higher T_{1C} values can be correlated with higher mobility in the MHz range [23]. Fig. 5 shows a typical set of inversion recovery spectra obtained after various τ values. The dynamic behaviour of the packings in the suspended state is in accordance with solid-state mobilities (Fig. 6). This finding indicates that there is no principal difference between the solid and suspended state.

3.2. Chromatography

DMD test [24]

This test has been developed to determine chromatographic properties of RPLC bonded phases particularly for separation of basic substances. The three compounds of the test mixture are diphenhydramine hydrochloride, diazepam and 5-(*p*-methylphenyl)-5-phenylhydantoin (MPPH).

Daldrup and Kardel [24] described some criteria which a good RPLC column should fulfil with respect to the separation of these three substances:

(1) The elution order should always be diphenhydramine · HCl–MPPH–diazepam. This indeed was found for all our C_{21}-amide-bonded phases (Fig. 7a).

(2) Diphenhydramine is a basic drug which is extremely sensitive to the residual polarity especially that arising from free silanol groups. Ideally it should be eluted in a symmetrical peak without any tailing. Deviations from this situation however can be expressed by an asymmetry factor (which is calculated by a BASIC program on the C-R6A Chromatopac integrator) defined in the usual manner as $f_{as} = (B_{0.1}/A_{0.1})$ [25].

(3) Selectivity: the RRT value (relative retention time) of diazepam which is determined with MPPH as reference substance indicates the resolution; it depends on the degree of effective surface concentration of bonded alkyl phase.

(4) High numbers of theoretical plates/m: theoretical plate numbers (N/m) are calculated based on the retention and elution of MPPH which can be used as a referent substance, because different properties of the bonded phase (according to the variable manufacturing process) have a negligible effect on this component, using Gaussian peak form calculation.

The results obtained are given in Table 2.

The monofunctional as well as the trifunctional unsaturated phases allow very good separation of the basic drugs. Especially, the monofunction-

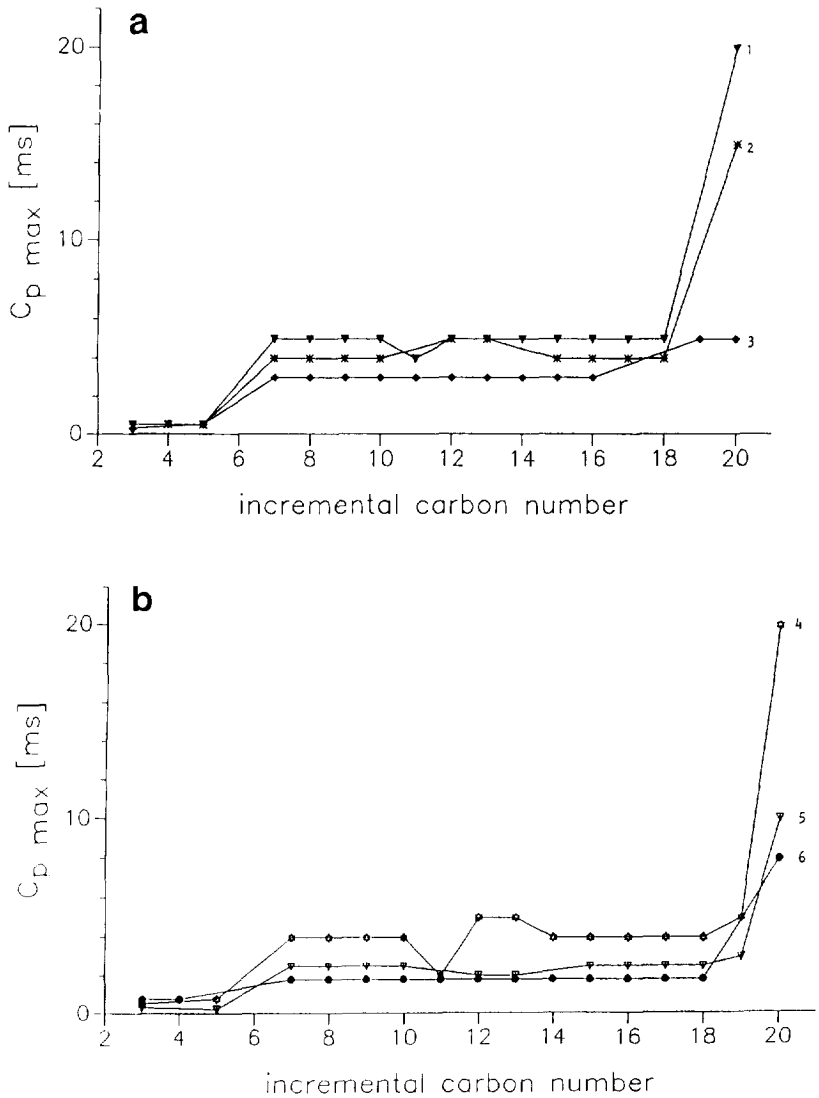

Fig. 4. (a) Contact times for maximum signal-to-noise ratio in dependence on the carbon position within the alkenyl chain of monofunctionally modified packings. 1 = Monofunctional oleic acid packing; 2 = monofunctional elaidic acid packing; 3 = monofunctional stearic acid packing. (b) Contact times for maximum signal-to-noise ratio in dependence on the carbon position witin the alkenyl chain of trifunctionally modified packings. 4 = Trifunctional oleic acid packing; 5 = trifunctional elaidic acid packing; 6 = trifunctional stearic acid packing.

al phases show very high numbers of theoretical plates (for MPPH) even on 60 × 4.6 mm columns, indicating a high quality of the packings. RRT values of all packings and thus the resolution obtained are comparable to commercially available packings [26].

The asymmetry factors for diphenhydramine varying from 1.3 to 2.0 (calculated as aforementioned) indicate a good screening of free silanol groups which results in a small residual polarity of the RP packings. The monofunctionally modified phases show slightly better

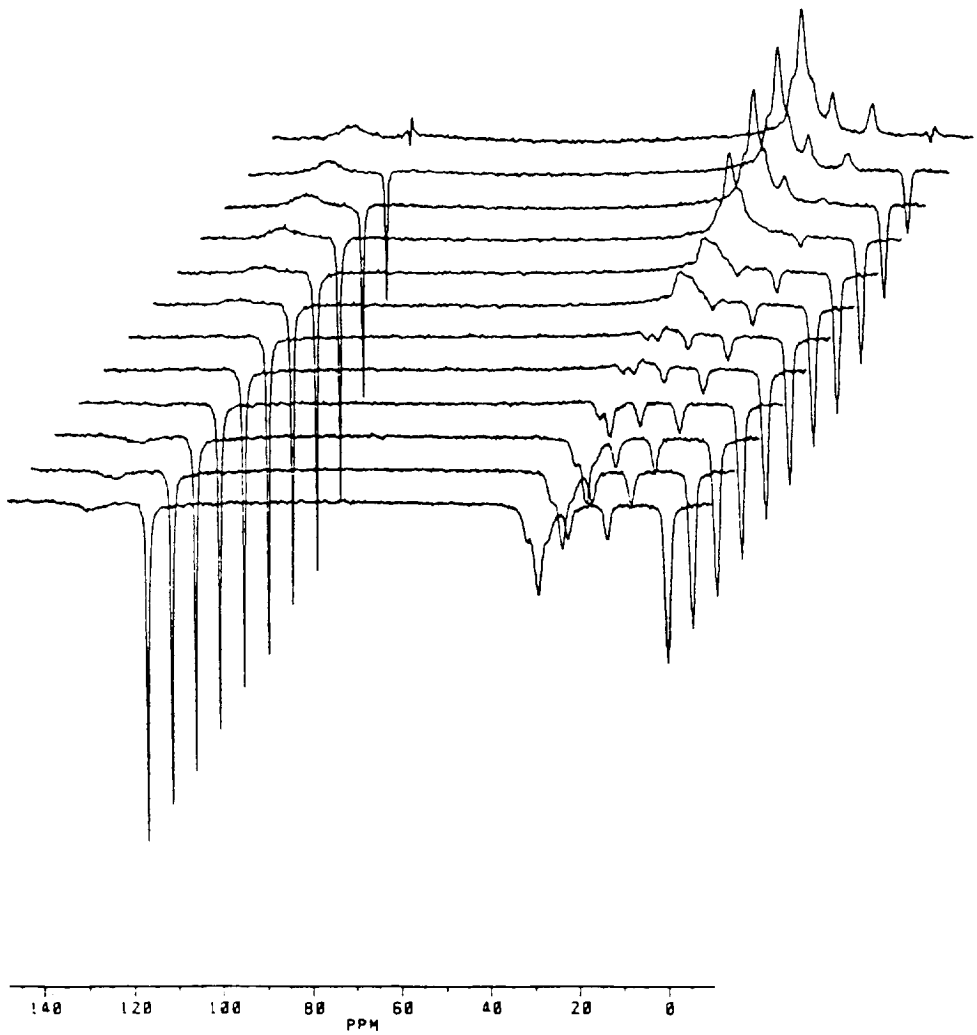

Fig. 5. Inversion recovery spectra of the monofunctional oleic acid packing obtained after different τ values varying from $\tau_{min} = 50$ ms (1st spectrum) to $\tau_{max} = 5$ s (12th spectrum).

asymmetry factors than the corresponding trifunctional phases.

Separation of alkylbenzenes

The homologous series of *p*-hydroxybenzoic acid esters (methyl, ethyl, propyl and butyl ester) was used as a second test mixture: Fig. 7b depicts the chromatogram of *p*-hydroxybenzoic acid esters eluted on the monofunctional modified C_{21}-oleic acid phase. Log k' values obtained on the monofunctional packings are shown in Fig. 8.

All packings showed excellent separation of the esters in very short time on 60×4.6 mm columns. Especially, the selectivity obtained on the unsaturated packings (*cis* and *trans*) is better than that obtained on the corresponding saturated packings.

The stability of the described phases was checked in a three years time period. No remarkable differences could be found in the

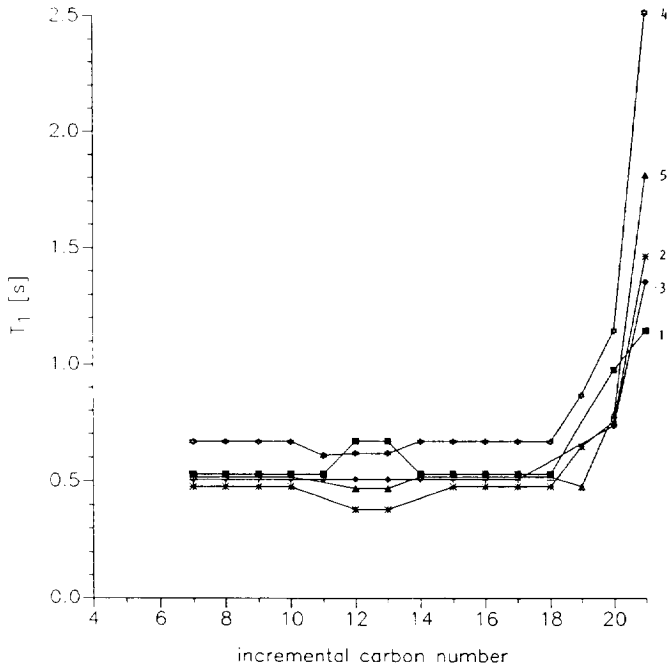

Fig. 6. Dependence of the T_{1H} values in the suspended state on the carbon position within the alkenyl chain. 1 = Monofunctional oleic acid packing; 2 = monofunctional elaidic acid packing; 3 = monofunctional stearic acid packing; 4 = trifunctional oleic acid packing; 5 = trifunctional elaidic acid packing.

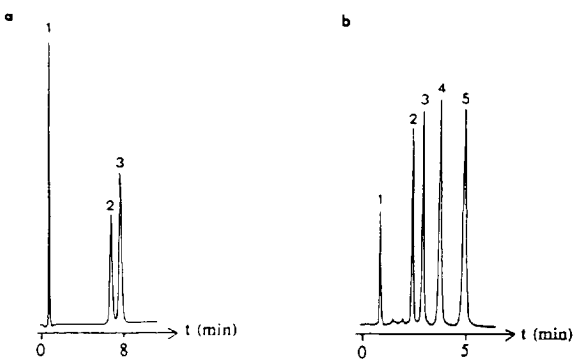

Fig. 7. Separation of two test mixtures on the monofunctional oleic acid packing. (a) 1 = Diphenhydramin hydrochloride, 2 = diazepam, 3 = 5-(*p*-methylphenyl)-5-phenylhydantoin (MPPH); mobile phase: 156 ml acetonitrile + 340 ml phosphate buffer, pH 2.3 (6.66 g of KH_2PO_4 + 4.8 g of 85% H_3PO_4 in 1 l of water for HPLC), flow-rate 0.5 ml/min; UV detection wavelength 254 nm; temperature 25°C. (b) *p*-Hydroxybenzoic acid esters: 1 = KNO_3, 2 = methyl ester, 3 = ethyl ester, 4 = propyl ester, 5 = butyl ester; mobile phase: acetonitrile–water (45:55), flow-rate 0.9 ml/min; UV detection wavelength 254 nm; temperature 25°C.

Table 2
Asymmetry factors f_{as} (diphenhydramine · HCl), RRT values (diazepam/MPPH) and theoretical plate numbers N/m (MPPH) obtained for separation of three basic drugs (diphenhydramine · HCl, diazepam, MPPH) on various packings

Packing	f_{as}	RRT value	N/m
Mono-*cis*	1.7	1.15	74 000
Mono-*trans*	1.8	1.15	69 000
Mono-saturated	1.3	1.06	44 000
Tri-*cis*	2.0	1.24	33 000
Tri-*trans*	1.9	1.19	30 000
Tri-saturated	1.6	1.24	24 000

chromatographic properties and in the solid-state NMR spectra between a newly prepared stationary phase and a stationary phase after three years of use [27].

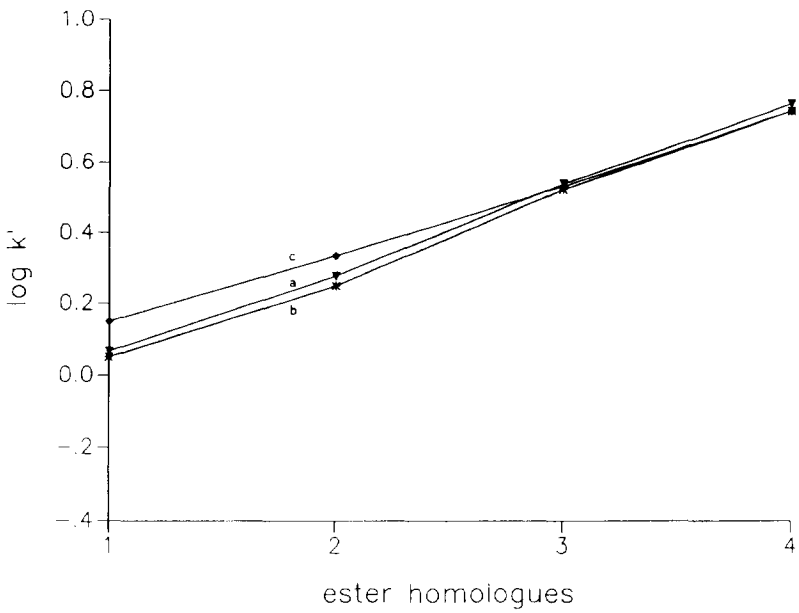

Fig. 8. Log k' values of *p*-hydroxybenzoic acid esters, obtained with acetonitrile–water mixture (45:55). 1 = Methyl ester; 2 = ethyl ester; 3 = propyl ester; 4 = butyl ester. a = Monofunctional oleic acid packing; b = monofunctional elaidic acid packing; c = monofunctional stearic acid packing. Flow-rate 0.9 ml/min. UV detection wavelength 254 nm, temperature 25°C.

4. Discussion

The newly designed packings containing unsaturated fatty acid amides show excellent chromatographic properties as demonstrated on the examples of basic drugs and of *p*-hydroxybenzoic acid esters. Especially, they show better selectivities both for separation of *p*-hydroxybenzoic acid esters and of the basic drugs as well as much higher numbers of theoretical plates than the corresponding saturated phases for both test mixtures.

NMR relaxation time measurements showed increased mobility of the unsaturated packings compared to the saturated ones. This effect proved to be stronger in the case of the naturally occurring *cis* double bonds than in the case of *trans* double bonds. Comparing HPLC and NMR results of oleic and elaidic acid packings with the results obtained on the corresponding saturated ones, it appear that higher mobility of the alkenyl chains grafted to the silica support results in improved HPLC properties of the RP phase for the separation of both basic drugs and *p*-hydroxybenzoic acid esters. This could be explained by a better adjustment of the equilibrium between stationary and mobile phase. The mobility of these phases is, however, lower than that of customary RP phases without aminopropylsilane spacer: $T_{1\rho H}$ values for C-4–C-15 of monofunctional RP-18 materials are in the order of 30 ms (e.g. 28.9 ms for monofunctional LiChrospher Si 100, 10 μm RP-18 material) [19]. This shows that increased mobility cannot be the only reason for the good chromatographic properties of these phases.

Another explanation for the excellent separations on unsaturated packings could be the ability of the double bond to undergo specific Van der Waals interactions with the solutes resulting in an improvement in separation.

We showed earlier [2] that also the saturated C_{21} packings containing amide bonds can be superior (in the sense of better resolution and/or

selectivity) to conventional C_{18} phases e.g. for the separation of basic substances. Therefore we assume a favourable influence of the amide bond and of the unreacted free amino groups on chromatographic properties. Specific interactions of these structural units are more probable the better accessible they are. As shown by the NMR studies, the unsaturated packings show higher mobility than the saturated analogues, which is considered to be an effect of the less ordered structure of the unsaturated materials [22,28]. Here, again, we can assume that a less ordered structure where the fatty acyl chains are not aligned parallel one to another, but having kinks and being somewhat bent around, significantly increases the accessibility of the amide group at least for smaller solute molecules like the described *p*-hydroxybenzoic acid esters and basic drugs.

In addition, such a less ordered structure does not only increase the accessibility of the amide group itself, but also of the whole hydrophobic surface of the fatty acyl chains and thus increases the mass transfer rate.

Comparing the separation characteristics for basic drugs on monofunctionally and trifunctionally modified packings we can assume that theoretical plate heights are much smaller in the case of monomeric packings although coverages are slightly higher for the trifunctional packings. As monomeric phases are less ordered than polymeric ones we suppose that this effect is also due to a higher mass transfer caused by the less ordered structure. This point, again, supports the idea that partitioning (which strongly depends on ordering) is a dominant mechanism of reversed-phase chromatography as proposed by Dorsey and Dill [4].

We can now summarize that incorporation of unsaturated fatty acids into RP packing materials results in higher mobility as compared to the saturated analogues, an effect we ascribe to a less ordered structure. The good chromatographic properties of these unsaturated packings are supposed to be partly due to this less ordered structure which leads to a better accessibility of the hydrophobic region and of the amide bonds. Specific hydrophobic interactions between the double bonds and solute molecules are supposed to improve chromatographic characteristics, too.

Looking at biological systems now, unsaturated fatty acids are known to increase fluidity of biomembranes. This effect as well as the decreased mobility after incorporation of cholesterol absolutely corresponds to our results obtained on the synthesized RP packings. Therefore, we can be sure that the synthesized packings can indeed be used as simple model systems for biological membranes.

We propose now that, together with the increase of mobility itself, ordering and conformation effects as found for the chromatographic packings could play a certain role in biomembranes, too, for example for the build-up of a suitable surrounding for ion channels or peptide binding sites. Additionally, specific hydrophobic interactions of the double bonds probably also have an influence upon self organization of biological membranes.

It is remarkable that *trans* double bonds induce much less differences in mobility as compared to the saturated analogues than *cis* double bonds. This could be a reason why nature has selected the more effective stereochemistry of *cis* double bonds for influencing membrane fluidity.

The transfer of structural characteristics from biomembranes to chromatographic systems has thus resulted in a new class of unsaturated bonded phases with very good separation characteristics which, by correlation of chromatographic and NMR spectroscopic results, could be ascribed to certain structural and ordering features. Analogue influences of unsaturated fatty acids are proposed to be present in biological membranes, too.

References

[1] C. Pidgeon, *Chem. Eng.*, 66 (1988) 23.
[2] B. Buszewski, J. Schmid, K. Albert and E. Bayer, *J. Chromatogr.*, 552 (1991) 415.
[3] D.W. Sindorf and G.E. Maciel, *J. Am. Chem. Soc.*, 105 (1983) 3767.
[4] J.G. Dorsey and K.A. Dill, *Chem. Rev.*, 89 (1989) 331.
[5] B. Pfleiderer, K. Albert and E. Bayer, *J. Chromatogr.*, 506 (1990) 343.

[6] P. Shah, L.B. Rogers and J.C. Fetzer, *J. Chromatogr.*, 388 (1987) 411.
[7] S.A. Wise and L.C. Sander, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 248.
[8] W. König and R. Geiger, *Chem. Ber.*, 103 (1970) 788.
[9] F. Weygand, W. Steglich, J. Bjarnson, R. Akhtar and N. Chytil, *Chem. Ber.*, 101 (1968) 3633.
[10] G. Karp, *Cell Biology*, McGraw-Hill, New York, 1979.
[11] E. Müller and G. Zimmermann, *J. Prakt. Chem.*, 11 (1925) 284.
[12] E.R. Andrew, *Prog. Nucl. Magn. Reson. Spectrosc.*, 8 (1971) 1.
[13] A. Pines, M.G. Gibby and J.S. Waugh, *J. Chem. Phys.*, 59 (1973) 569.
[14] J. Schaefer and E.O. Stejskal, *J. Am. Chem. Soc.*, 98 (1976) 1030.
[15] P. Staszczuk and B. Buszewski, *Chromatographia*, 25 (1988) 881.
[16] G.E. Maciel and D.W. Sindorf, *J. Am. Chem. Soc.*, 102 (1982) 7606.
[17] K. Albert and E. Bayer, *J. Chromatogr.*, 544 (1991) 345.
[18] J. Schaefer, E.O. Stejskal and R. Buchdahl, *Macromolecules*, 10 (1977) 384.
[19] K. Albert, B. Pfleiderer and E. Bayer, in D.E. Leyden and W.T. Collins (Editors), *Chemically Modified Surfaces*, Vol. 3, Gordon & Breach, New York, 1990, p. 233.
[20] D.W. Sindorf and G.E. Maciel, *J. Am. Chem. Soc.*, 105 (1983) 1848.
[21] B. Pfleiderer, K. Albert, K.D. Lork, K.K. Unger, H. Brückner and E. Bayer, *Angew. Chem., Int. Ed. Engl.*, 28 (1989) 327.
[22] J. Schmid, *Diploma Thesis*, University of Tübingen, Tübingen, 1989.
[23] K. Albert, B. Evers and E. Bayer, *J. Magn. Reson.*, 62 (1985) 428.
[24] T. Daldrup and B. Kardel, *Chromatographia*, 18 (1984) 81.
[25] J.P. Foley and J.G. Dorsey, *Anal. Chem.*, 55 (1983) 730.
[26] W. Jost, R. Gasteier, G. Schwinn and M. Tüylü, *GIT Fachz. Lab.*, 32 (1988) 357.
[27] J. Schmid, *Thesis*, University of Tübingen, Tübingen, 1993.
[28] K. Albert, J. Schmid, B. Pfleiderer and E. Bayer, in H.A. Mottola and J.R. Steinmetz (Editors), *Chemically Modified Surfaces*, Vol. 4, Elsevier, Amsterdam, 1992, p. 105.