INSERTION OF VARIOUS LONG ALKYL CHAIN MOLECULES IN BRUSH-TYPE GRAFTED MONOLAYERS

CHROMATOGRAPHIC STUDY OF THE RESULTING MATERIALS

C. GONNET, D. MOREL, E. RAMAMONJINIRINA and J. SERPINET*
Laboratoire de Chimie Analytique III, Université Claude Bernard, Lyon I, 69622 Villeurbanne (France)
and
P. CLAUDY and J. M. LETOFFÉ
Laboratoire de Thermochimie Minérale, I.N.S.A., 69621 Villeurbanne (France)
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SUMMARY

A monolayer was prepared on the surface of macroporous silica gels by the insertion of various long alkyl chain molecules in a densely grafted C_{22} alkyl-silica. The original properties of these layers are briefly described, together with inverse gas chromatographic and some differential scanning calorimetric and NMR spectroscopic experiments. The feasibility of using these materials in reversed-phase high-performance liquid chromatography is exemplified by the separation of three classical mixtures. The advantages of these layers are discussed.

INTRODUCTION

Most reversed-phase high-performance liquid chromatography (RP-HPLC) are effected with bonded silicas, particularly with apolar octadecyl-bonded materials. Although these are very satisfactory packings for a great many analyses, there is undoubtedly room for improvement.

The principal defect of alkyl-bonded silicas as chromatographic packings is probably the lack of reproducibility of the retentions and selectivities from one commercial product to another and even from one batch to another of the same product[1]. This situation makes it impossible to identify significant compounds (*e.g.*, pollutants) on the basis of the published chromatographic data.

The origin of this difficulty is clear, but it can not be completely overcome. There are two causes of irreproducibility: the first originates from the structure of the layer and is a consequence of the extensive use of polyfunctional bonding reagents, which lead to ill-defined structures and even to polymers; the second (even for monofunctional bonding) lies in the nature of the methylalkyl or dimethylalkyl graft and the influence of the silica texture. The limited density attainable with such grafts (about 4.0–4.2 μmole/m^2) originates from the steric hindrance[2] of the weakly
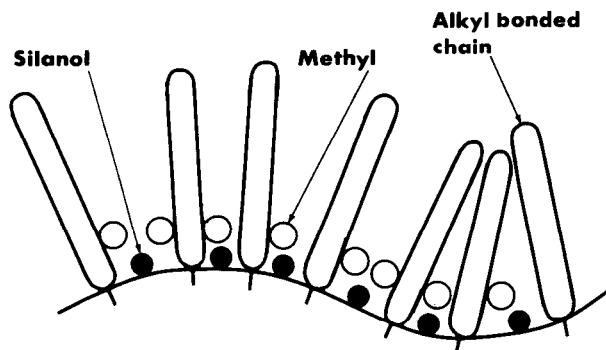
Fig. 1. Dense bonding by an alkyldimethylsiloxy radical of the strongly curved surface of a microporous silica.

interacting methyl groups bonded to the silicon atom. This phenomenon is strongly influenced by the texture of the narrow-pore silicas used in HPLC (Fig. 1). Consequently, the accessibility of the residual silanol groups may vary.

The use of siliceous materials in RP-HPLC is not essential. For instance, various carbonaceous products have been used. However, bonded silica has some distinct advantages. It is a firm material, commercially available and characterized by a favourable porous texture, and it exists with different particle sizes and pore diameters.

Nevertheless, it is desirable for a bonded silica for chromatography to fulfil two kinds of requirements: (1) Silicas have to be completely deactivated so as to remove the influence of the residual silanol groups, because this influence is far from always being useful and because the accessibility of these silanol groups is hardly reproducible. (2) The packing must be available in a number of varieties in which the surface is covered by various chemical groups in a stable form. Alkyl chains are not suitable for all kinds of analysis.

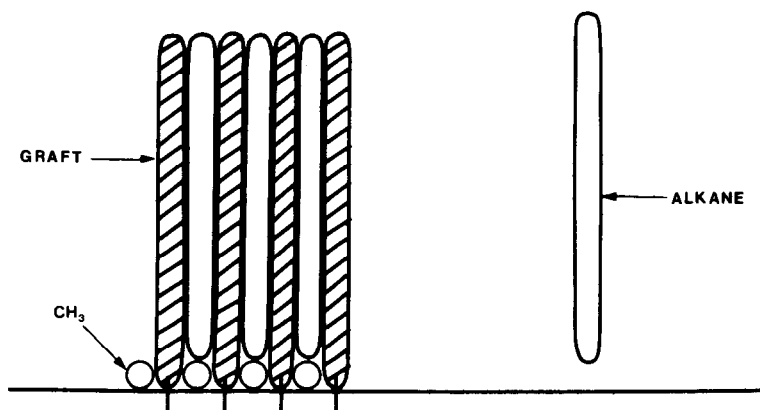Our work on the properties of mixed monolayers of bonded alkyl chains and

Fig. 2. Alkylbonded silica densified after insertion of an *n*-alkane, in its solid surface state at low temperature.
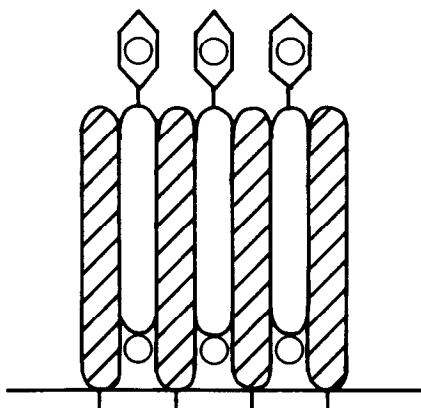
Fig. 3. Insertion of molecules of a compound containing a long alkyl chain such as an alkylbenzene between the bonded chains at the surface of the silica.

alkyl compounds has led us to new kinds of materials that fulfil these requirements very well and have some other advantages pertaining to their flexibility over the existing products.

Coating a densely grafted (4.0 μmole/m^2) C_{18} or C_{22} alkyl-bonded silica with an n-alkane with a comparable chain length leads to a very dense (8 μmole/m^2) mixed monolayer, both chemisorbed and physisorbed, in which the alkane molecules are inserted between the bonded radicals (Fig. 2). By contact angle measurements in grafted capillaries, Riedo et al.[3] were the first to obtain evidence of such layers, which on heating undergo a "melting-like" transition 10–20°C above the transition temperature of the non-coated macroporous silica.

The corresponding surface phases have been studied thoroughly in this laboratory using "inverse gas chromatography"[4,5] and recently by us[5] and by André[6] using differential scanning calorimetry (DSC).

Recently we generalized the preparation of these alkyl–alkane layers by inserting many molecules consisting of a long alkyl chain and various atom groups and chemical functions. Fig. 3 exemplifies the structure of such a layer in the case of a long-chain alkyl-bonded silica and octadecylbenzene molecules.

In this paper, we describe the preparation and some significant physical properties of these layers, which represent a generalization of the alkyl–alkane films. We demonstrate the feasibility of using these densified bonded phases in RP-HPLC with water–methanol mobile phases and we emphasize some distinctive advantages of these new stationary phases.

EXPERIMENTAL

Apparatus

Inverse gas chromatographic experiments were carried out with a classical laboratory-made gas chromatograph provided with the necessary liquid bath thermostats and pressure and flow measuring devices[7,8]. Liquid chromatographic experiments were effected with a simple device consisting of a Chromatem 380 pump, a Rheodyne six-port injection valve and a Shimadzu spectrophotometric detector.

DSC curves were obtained with a Mettler TA 2000 B calorimeter, which allows a quantitative exploitation of the signal after suitable treatment[9].

NMR experiments were carried out at the Centre d'Etudes Nucleaires de Grenoble (France) with a Bruker CXP200 spectrometer by observing the proton resonance at 200 MHz. Fourrier transform was effected after accumulation of twenty successive precession signals. Variable temperature was obtained by nitrogen circulation and temperature regulation.

Materials and reagents

Taking into account the criticisms made in the Introduction about the use of ill-defined layers, in the study of the physical properties of these layers we used a very macroporous silica (Spherosil XOB 015; Rhône Poulenc, France) of specific surface area 27 m²/g and mean pore diameter 125 nm, and a monofunctional reagent, docosyldimethyl(dimethylamino)silane, prepared in the laboratory. Dimethylaminosilanes, first prepared by Szabo *et al.*[10], lead to particularly dense and regular layers.

The bonding method has been described previously[4].

The choice of the C_{22} graft instead of the usual C_{18} was made mainly because of a more favourable temperature range for the observation of the described phenomena. However, some experiments on octadecyl-bonded silica give rise to similar results.

For gas chromatographic experiments, 1/4-in. I.D. tubes of length 10–30 cm were used as columns.

Bonding density of the silicas

Bonding density has been determined both by carbon analysis (Laboratoire d'Analyse du CNRS, Solaise, France) and by hydrofluoric acid (40%) attack and gas chromatography of the resulting fluorosilane[11]. The latter method is more precise for low-surface-area substrates.

Measuring the molecular area in monolayers by inverse gas chromatography

A volatile test solute (for example, *n*-heptane) can be used for the measurement of the molecular area of the chains in the film. This determination is based on plotting $V_s/\tau = f(1/\tau)$ at a determined temperature for a number of packings containing
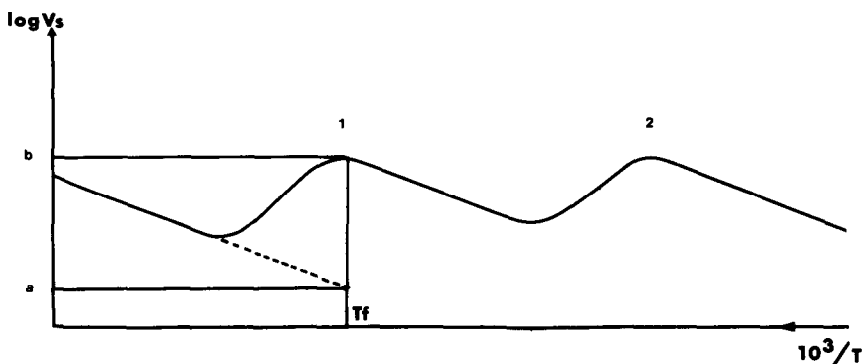

Fig. 4. Measurement of ΔV_s, the retention volume corresponding to the bulk material in excess. $\Delta V_s =$ antilog b − antilog a. 1, Melting transition of the bulk material; 2, transition of the mixed film.

τ % of stationary phase [weight by weight of substrate (grafted silica)], where V_s is the absolute retention volume per unit mass of solid substrate. Coating percentage(s) corresponding to compact (complete) monolayer(s) correspond to breaks in this linear relationship. This method was first applied to brush-like physisorbed films of fatty compounds[7] and extended recently[5] to dense monolayers of alkyl radicals and n-alkane molecules. The percentage of bonded chains has to be added in order to calculate the global molecular area. A faster and more straightforward method may be applied for the physisorbed (or mixed) molecules at the melting point in bulk or around this melting point (Fig. 4).

The method is based on the gas chromatographic determination of the (small) three-dimensional excess of the coating substance that has been deliberately deposited. With this aim, the amplitude of the corresponding melting transition, ΔV_s, is observed in (log V_s, $1/T$) coordinates in order to evaluate the percentage of stationary phase in the bulk after its specific retention volume V_g (determined by separate experiments on silanized Chromosorb). By subtracting the percentage of bulk compound from the percentage coating, the percentage of stationary phase in the film is obtained. This leads to the molecular area of the film if the surface area of the substrate has been measured, for instance, by nitrogen adsorption. Conversely, the surface area may be determined for films of well known molecular area, as explained previously[12].

The first application of these techniques to alkyl-bonded silicas densified by n-alkanes has been described previously[4].

The results of these two inverse gas chromatographic methods of molecular area determinations are generally in very good agreement.

RESULTS AND DISCUSSION

Preparation of mixed layers: gas chromatographic characterization
    Most of the mixed layers were obtained by classical coating of the grafted silica
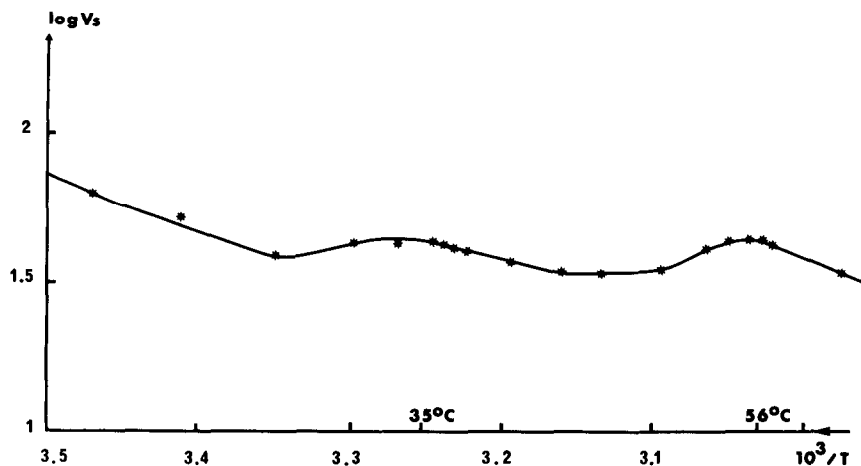

Fig. 5. Variation of log V_s with $1/T$ for n-heptane as solute on C_{22}- bonded Spherosil XOB 015 coated with 3,54% (w/w) of octadecylbenzene.

TABLE I

SOME COMPOUNDS INTRODUCED IN ALKYL LAYERS

Compound	End transition temperature (°C)	Compound	End transition temperature (°C)
$C_6H_5C_{18}H_{37}$	56.8	$BrC_{22}H_{45}$	62.8
$C_6H_{11}C_{18}H_{37}$	61.9	$CH_2=CH(CH_2)_{18}CH=CH_2$	62.7
$C_8F_{17}C_{12}H_{25}$	55.1	$C_{21}H_{43}COOCH_3$	66.7
$Si(CH_3)_3C_{18}H_{37}$	55.5	$(C_{10}H_{21})_2O$	57.0
$(C_9H_{19})_2CO$	67.6	$(C_8H_{17})_2NH$	57.0

by means of dichloromethane. For every new alkyl compound, the coating mass was chosen in order to double the alkyl chain density at the surface of the silica (from 4 to about 8 μmole/m^2) plus about 10%, which leads to perfect completion of the densified layer and results in a small three-dimensional excess in the pores. As explained under Experimental, this excess may be determined by "inverse" gas chromatography. A second method of preparation of these layers, connected with liquid chromatographic applications, will be described later.

The insertion is revealed by the disappearance of the transition for the silica that is merely bonded (which ends at 48°C for docosyl-bonded chains) and by the appearance of a new transition at higher temperature. In (log V_s, $1/T$) coordinates, Fig. 5 exemplifies such a diagram for octadecylbenzene. The transition at 35°C cor-
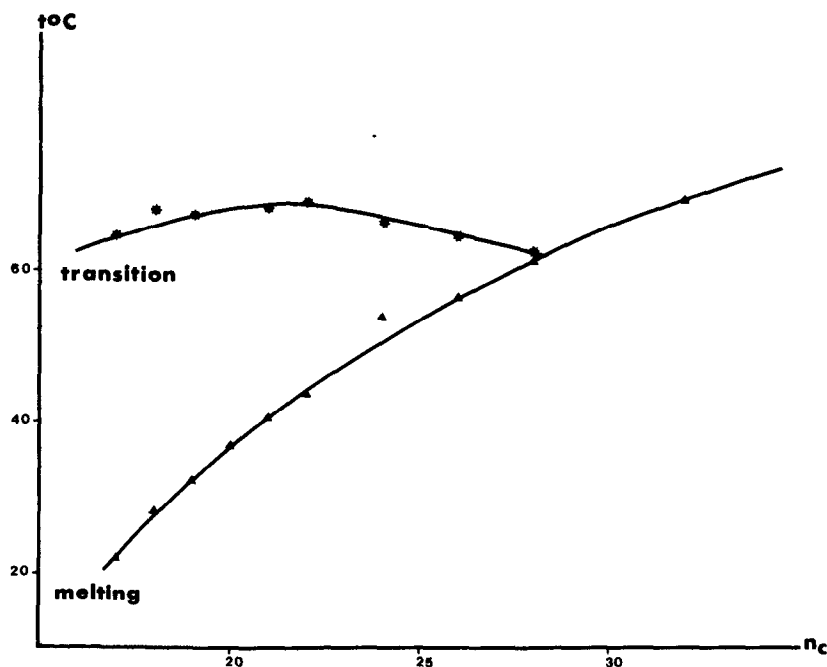

Fig. 6. Variation of the end transition temperature with the number of carbon atoms in the *n*-alkane in a mixed layer with a C_{22}-bonded alkyl chain.

responds to the melting of the small three-dimensional excess. The phenomenon observed at 56°C is the phase transition of the film.

Table I lists various alkyl compounds for which such a phenomenon has been observed. As can be seen, the phenomenon is far from being limited to pure methylenic chains. The alkyl part of the molecule may include some heteroatoms. The best "fit" corresponds to an *n*-alkane of about the same chain length. With docosyl-bonded silica (C_{21}- or C_{22}-coated), this mixed layer undergoes a phase transition at about 69°C.

The longer the chain of the molecule is beyond 22 carbon atoms, or the more cumbersome the non-alkyl group is, the lower is the transition temperature. It is as if the poor "fit" of the coating molecule were to result in a decrease in the stability of the layer (Fig. 6).

The insertion of very polar fatty compounds (primary alcohols and fatty acids) is particularly difficult and requires special techniques, as will be explained elsewhere[13]. This situation comes from the high stability of the usual dimeric form of these compounds in the bulk state.

The global "molecular area" of chains (physisorbed and chemisorbed), measured as explained under Experimental, is 0.21–0.24 nm² below the transition temperature.

As for the alkyl–alkane mixed layers, the low-temperature form of the film is characterized by about the same molecular area as for solid two-dimensional phases of polar fatty compounds at the surface of the trough of the Langmuir film balance. This corresponds to the maximal compactness of the fatty chains. Such a surface density is completely impossible with pure bonded films for steric reasons.
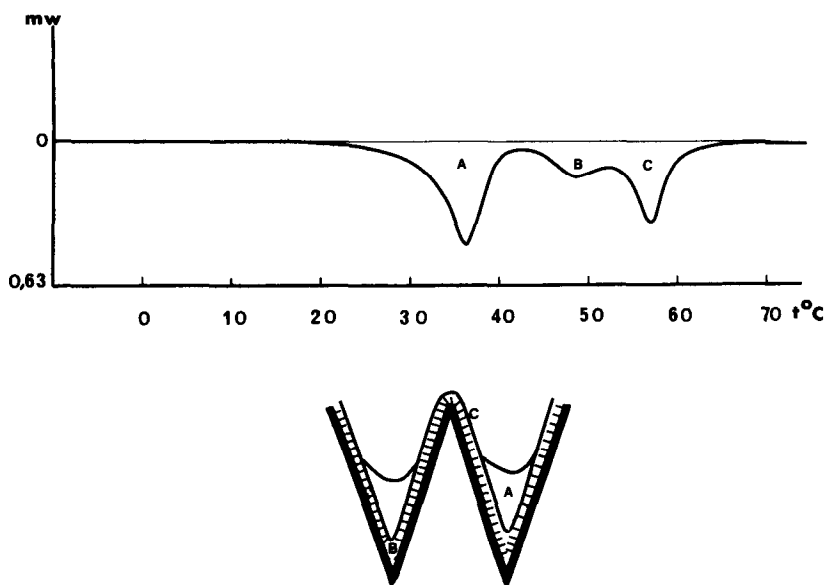

Fig. 7. DSC diagram for the mixed layer prepared with a C_{22} alkyl graft and the $C_{12}H_{25}$–C_8F_{17} compound. The different transitions are ascribed to the phases reported in a schematic representation of a pore.

However, above the transition temperature, the molecular area increases to 0.28–0.30 nm². Consequently, as for purely physisorbed layers of fatty alcohols on silica[7], the phase transition results in the ejection of part of the adsorbed molecules in the pores as bulk material. In addition, the absence of the transition observed at 48°C for the (non-coated) docosylsilica, which is merely bonded, demonstrates the participation of the grafted chains in the transition of the mixed layer at higher temperature. In conclusion, this "melting" of the film appears to be a complex phenomenon.

Calorimetric measurements

The densified film transition is clearly illustrated by DSC curves (Fig. 7, peak C). If there is an excess of the alkyl compound (here $C_{12}H_{25}$–C_8F_{17}), two supplementary peaks are observed. The first peak (A), at the melting point of the compound, corresponds to the normal fusion of the bulk material in excess. The second peak (B) corresponds to the transition of the part of the film that is covered by the capillary condensate in the pores, as indicated by experiments with increasing percentages of coating compound[13]. Nevertheless, if the percolation process described for liquid chromatographic applications is used, there is no longer any three-dimensional excess and, therefore, peak C is the only peak observed.
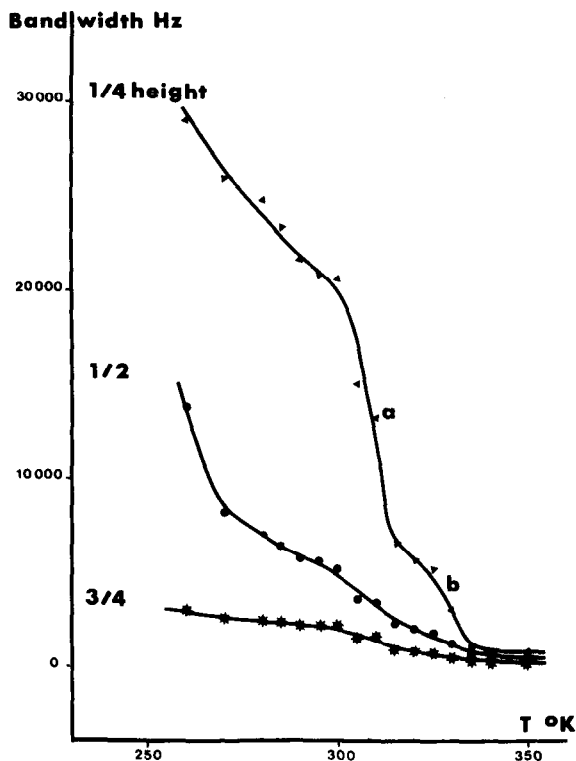

Fig. 8. Variation of the NMR band width *versus* temperature for a C_{22} alkylbonded silica coated with heneicosane, according to a dynamic method in methanol. The three-dimensional excess of *n*-alkane is very limited. (a) Bulk material melting; (b) transition of the mixed film. ▲, Width at one quarter of the peak height; ●, at half of the peak height; *, at three quarters of the peak height.

Enthalpy measurements shed some light on the nature of the mixed film. If the alkyl molecule is an *n*-alkane the enthalpy of transition may be compared with the enthalpy of melting of the compound, according to the results of André[6], in agreement with our own experiments[5]. However, for other compounds the situation is very different.

For example, the enthalpy of transition per gram of inserted compound is 98 J/g for $C_{12}H_{25}$–C_8F_{17}, compared with 47.4 J/g for the (very low) enthalpy of melting of the fluorinated compound. This observation may be interpreted by the participation of the bonded chains.

In contrast, for octadecylbenzene, the enthalpy of transition, 135 J/g, is much lower than the enthalpy of melting, 198 J/g. The anomaly may conceivably be interpreted by the (exothermic) increase in the interaction between the aromatic rings, which results from the partial desorption of octadecylbenzene during the transition (see above).

Wide band NMR measurements

As Fig. 8 shows for a C_{22} bonded silica densified by heneicosane, the interpretation of the transition by an increase in the mobility of the chains is supported by the narrowing of the NMR band (b), in the temperature range of the phenomenon revealed by chromatography and DSC. The first decrease (a) observed in NMR may be ascribed to the fusion of a very small three-dimensional excess, which is very easily revealed by NMR.
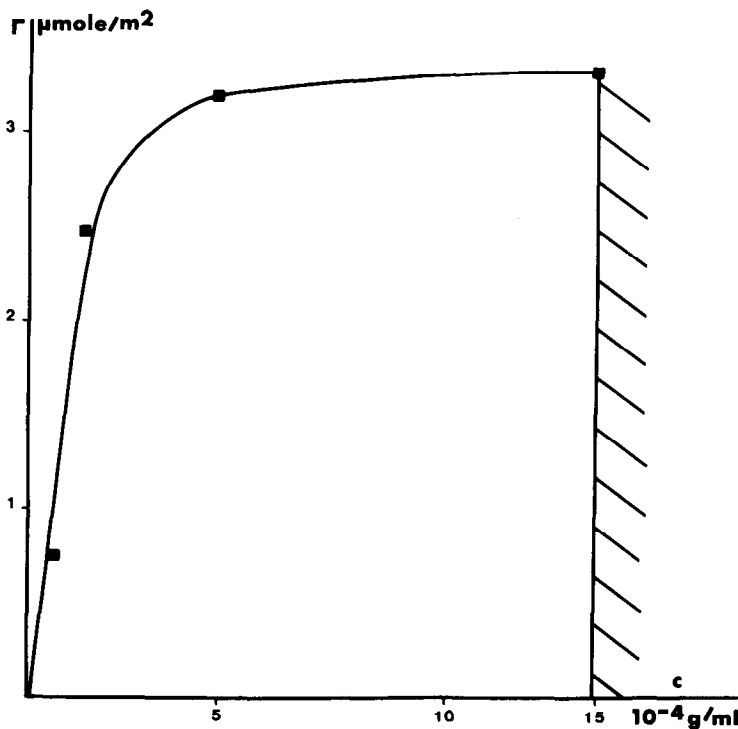

Fig. 9. Variation of the surface density of heneicosane chains with the concentration of this *n*-alkane in methanol at equilibrium up to saturation.

The increase in the mobility of the chains corroborates the observations in gas chromatography where, with increasing temperature, the transition gives the solute access to new retention sites in the inner part of the layer.

Adsorption isotherms in water–methanol media. The percolation coating process

By means of frontal chromatographic experiments, we obtained the sorption isotherms of heneicosane on C_{22} bonded silica in methanol (Fig. 9) and water–methanol solutions at 25°C (not shown). These isotherms indicate the strong affinity of the bonded chains for the *n*-alkane.

From a practical point of view, the isotherms suggest the possibility of preparing mixed layers by simple percolation of a saturated solution of the *n*-alkane in methanol. The final break point can easily be observed by means of a refractometric
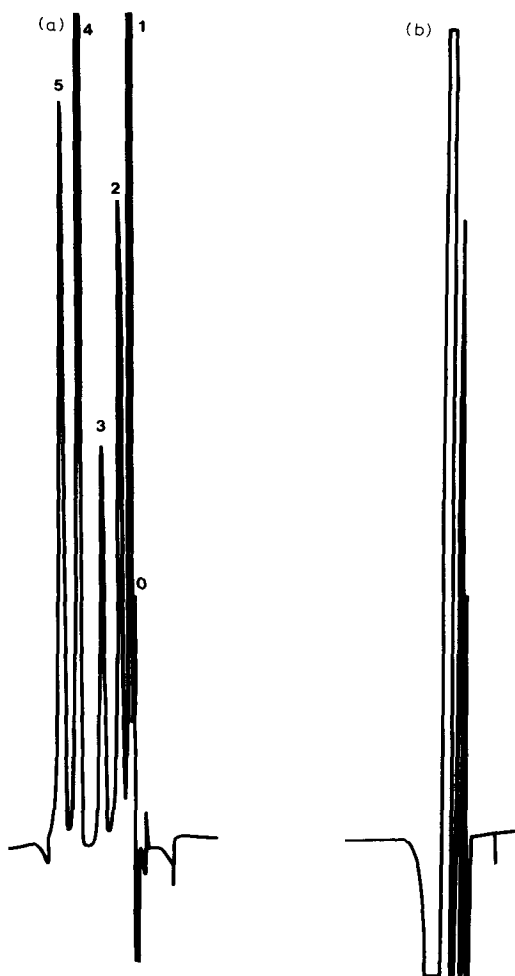

Fig. 10. Separation of the amines listed in Table II. Mobile phase: acetonitrile (22 volumes) + acetic acid + potassium chloride (3 g/l) + sodium heptanesulphonate (1.5 g/l) (78 volumes). Stationary phase: (a) pure C_{22} graft; (b) heneicosane-densified graft.
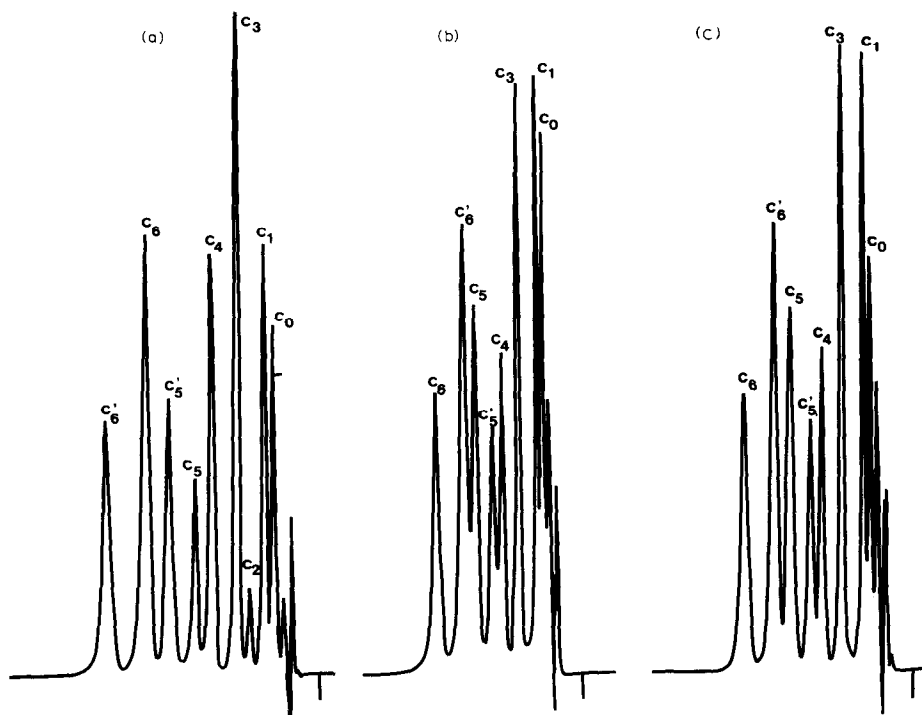
Fig. 11. Separation of monoalkylbenzenes with 0–6 carbon atoms in the alkyl group (Table II). Mobile phase: methanol–water (80:20, v/v); flow-rate, 1 ml/min. Stationary phase: (a) pure C_{22} graft; (b) heneicosane-densified graft; (c) octadecylbenzene-densified graft.

detector after the column or by gas chromatographic analysis of the mobile phase.

A small column of coated Chromosorb can be conveniently used as saturator. Preparation of the layer is made easier by the shape of the sorption isotherm and by the very low saturating concentration in most instances (about $1.5 \cdot 10^{-3}$ g/ml for heneicosane in pure methanol). In addition, the use of the saturator during the chromatographic experiments is unnecessary if there is more than 10–20% of water in the mobile phase. Finally, the layer may be prepared *in situ* in the liquid chromatographic column and the coating process is perfectly reversible.

These experiments allow a very straightforward evaluation of the percentage of *n*-alkane in the film by measurement of the mobile phase volume used before the break point. The molecular area of the layer can be deduced from this percentage.

Finally, similar experiments were carried out in the gas phase with a slightly more volatile *n*-alkane, pentadecane. After this experiment, the column is simply weighed. All of these measurements give excellent confirmation of the molecular area of the film under and above the transition temperature, namely, 0.21* and 0.28 nm², respectively.

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* As far as *n*-alkanes are concerned. For other compounds, this area may be higher (0.21–0.24 nm²).

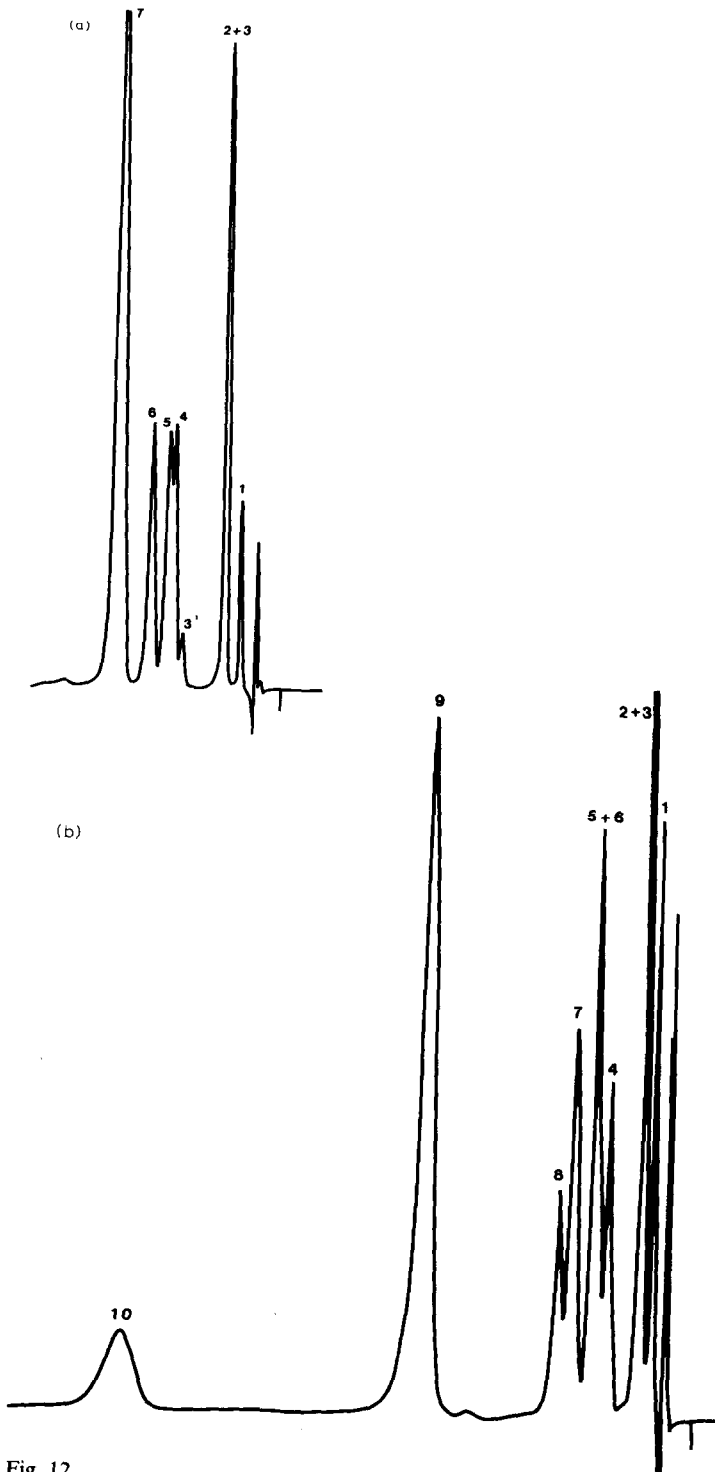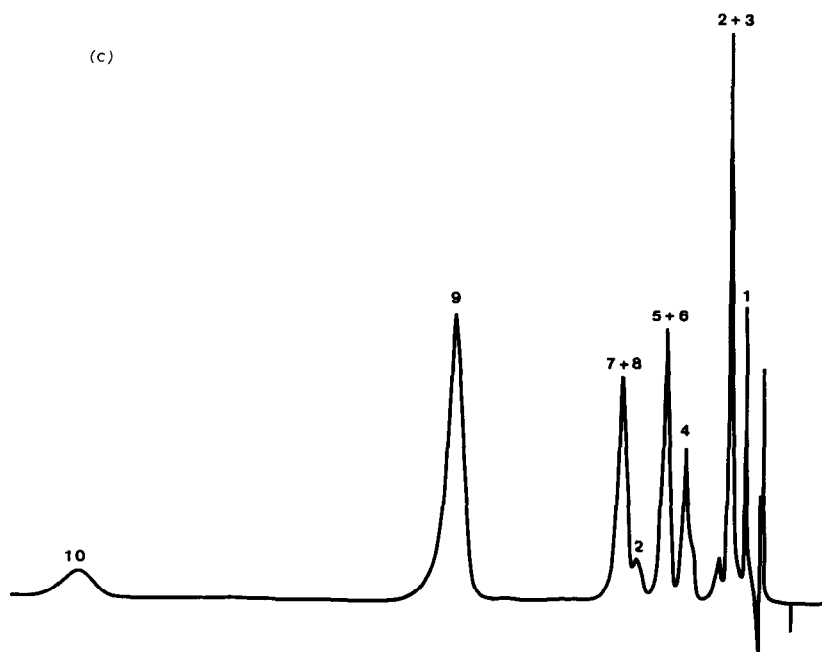Fig. 12.

Fig. 12. Separation of polynuclear aromatic hydrocarbons listed in Table II. Mobile and stationary phases as in Fig. 11.

HPLC separations on mixed layers

The feasibility of using the mixed films in their low-temperature state for RP HPLC separations was demonstrated by a few experiments.

We used a very classical silica packing, LiChrosorb Si 100, with a particle diameter of 10 μm and a surface area of 300 m^2/g. It was bonded by means of docosyldimethyl(dimethylamino)silane as explained under Experimental. The 10 cm × 4.6 mm I.D. column was prepared as usual.

In the first stage, the column was used in its original state with a pure C$_{22}$ graft for the separation of amines of biological interest (Fig. 10a), alkylbenzenes (Fig. 11a) and polycyclic aromatic hydrocarbons (Fig. 12a) with the polar mobile phases indicated in the captions. The compounds are listed in Table II. In the second stage, the methanol mobile phase was saturated in heneicosane by means of a coated Chromosorb saturator, as explained in the preceding section, until completion of the mixed layer. Subsequently, the same mixtures were analysed with the same mobile phases (Figs. 10b, 11b and 12b). In the third stage, heneicosane was swept away by percolating a pure methanol mobile phase until the disappearance of the n-alkane from the effluent after gas chromatographic analyses. Subsequently, the pure methanol was replaced with a saturated solution of octadecylbenzene in water–methanol (10:90, w/w), until the new mixed layer was completed. Alkylbenzenes and polynuclear aromatic hydrocarbon mixtures were analysed again (Figs. 11c and 12c).

Some conclusions can readily be drawn, as follows. (1) The separation of the amines is completely suppressed by the insertion of heneicosane (Fig. 10a and b). If, according to Papp and Vigh[14], the separation of such compounds on bonded silicas

TABLE II

COMPOUNDS SEPARATED USING THE PROPOSED STATIONARY PHASES

Type	No.*	Compounds
Amines of biological interest	0	Dopa
	1	Methyldopa
	2	Benzylamine
	3	Epinephrine
	4	Dopamine
	5	Isoprenaline
Monoalkylbenzenes	C_0	Benzene
	C_1	Methylbenzene
	C_2	Ethylbenzene
	C_3	Propylbenzene
	C_4	Butylbenzene
	C_5	Pentylbenzene
	C_5'	Impurity (probably branched isomer)
	C_6	Hexylbenzene
	C_6'	Impurity (probably branched isomer)
Polycyclic aromatic hydrocarbons	1	Benzene
	2	Fluoranthene
	3	Naphthalene
	3'	Impurity
	4	Phenanthrene
	5	o-Terphenyl
	6	Anthracene
	7	m-Terphenyl
	8	Pyrene
	9	Chrysene
	10	3,4-Benzopyrene

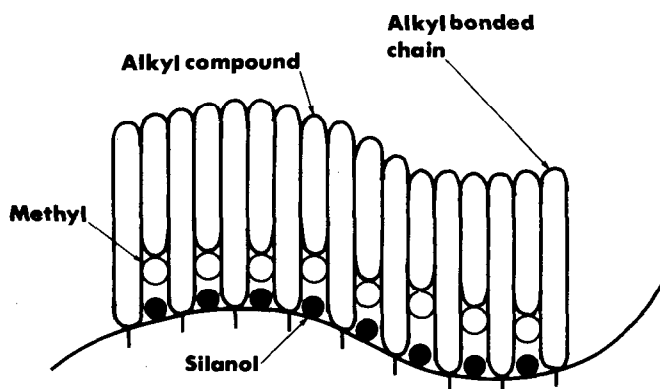* These numbers refer to the peaks in Figs. 10–12.

Fig. 13. Mixed layer at the surface of a strongly curved silica (compare with Fig. 1).

takes place on the residual silanols by ion exchange, the disappearance of the retention merely denotes the complete masking of the residual silanols by the compact solid thick monolayer. (2) The efficiency of the column is not impaired by the insertion of heneicosane or octadecylbenzene. (3) Insertion modifies the retention of the solutes.

CONCLUSION

The macroporous substrates used in the above-described physico-chemical study of the new mixed layers are not essential for the preparation of these films. Fortunately, genuine HPLC packings can be used. The numerous interactions between the long chains in the densified layer force the construction of a solid, well defined film, in spite of the surface irregularities (Fig. 13). This situation is completely different in the pure bonded layer, in which the surface density is limited by two slightly interacting methyl groups bonded to the silane's silicon atom. Consequently, the mixed films can be readily used in classical RP-HPLC. Their main originality lies in the perfect dissimulation of the silanols to the solute behind an extremely dense (7.9 μmole/m^2) and thick solid monolayer of long alkyl chains.

The possibility of introducing varied functions on the silica surface leads to perfect control of the interacting atom groups for selective separations. As the insertion of the alkyl groups in the chains is perfectly reversible, this advantage will be particularly useful for microbore or grafted capillary columns, for which dismantling of the apparatus may be undesirable.

This kind of column is not the first in which the stationary phase has been modified by a reagent introduced in the mobile phase. However, this modification is not critically dependent on the mobile phase and on the concentration of the inserted compound in the mobile phase. It leads to a well-defined new compact monolayer.

The preparation of various stationary phases of this type is in progress in our laboratory.

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