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CHROMATOGRAPHIC STUDIES OF THE INFLUENCE OF WATER AND THERMAL TREATMENT ON THE ACTIVITY OF SILICA GEL

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SUMMARY

Two deactivation modes for silica gels commonly used in high-performance liquid chromatography are compared : deactivation of silica by introducing a known amount of water and deactivation by thermal treatment. In both instances, water adsorption isotherms from vapour phase and liquid phase give information about the heterogeneity in activity of adsoption sites. In the first instance, silica gel activity depends on two factors: with small amounts of water on the chemical nature of silica surface and with larger amounts of water on the porosity of the silica. Thermal treatment leads to faster equilibrium kinetics than water deactivation. Separations of herbicides, barbiturates and pyrimidines are shown, using these two deactivation modes.

INTRODUCTION

In liquid–solid chromatography, the water content of the adsorbent plays a critical role, and numerous studies on this subject have been published. Water is usually added to the adsorbent in order to optimize the linear capacity and column efficiency[1,2]. The water composition significantly affects the capacity factor values (k'), and the amount of water must therefore be held constant in the stationary and mobile phases, *i.e.*, in a controlled activation state of the column in order to give reproducible separations.

With a mobile phase modified with water, it is important to know the amount of water that is adsorbed on the support and how much of the water-modified mobile phase has to be passed through the column before true equilibrium is attained. The first condition is fulfilled by the establishment of water adsorption isotherms from either the vapour or liquid phase.

These relationships between the water content in the eluent (or the water vapour pressure) and the equilibrium amount of water on the active solid support give interesting indications of the behaviour of silica gel. They show that silica columns can be adjusted to defined activity levels simply by changing the water content of the eluent.

A recent study[3] reported the deactivation of silica gel by thermal treatment and the use of such material in the production of bonded phases.

EXPERIMENTAL

Equipment

Two chromatographs were used: a Chromatronix Model N3520 (Chromatronix, Berkeley, Calif., U.S.A.) and a Siemens S100 (Siemens, Karlsruhe, G.F.R.).

The column effluent was monitored with either a Varian 2-1428-3 photometer (Varian, Palo Alto, Calif., U.S.A.) or a Zeiss PM2 DLC spectrophotometer (Carl Zeiss, Oberkochen/Wurttemburg, G.F.R.).

Columns

The column tubes used were 50 cm × 4.6 mm I.D. for particle diameters $< 40\ \mu m$ and 10 cm × 4.6 mm I.D. for microparticles ($5\ \mu m$). They were all packed using a technique described previously[4].

Table I summarizes various physical characteristics of the silica gels used in this study.

LiChrosorb SI 100 and LiChrosorb SI 60 were obtained from Merck (Darmstadt, G.F.R.), Partisil 5 and Partisil 20 from Whatman–Reeve Angel (Ferrières, France) and the different Spherosils from Rhone-Poulenc (Paris, France).

TABLE I

PROPERTIES OF SILICA GEL PACKINGS

Silica gel	*Particle size (μm)*	*Weight of silica gel in column (g)*	*Surface area (m^2/g)*	*Mean pore diameter (Å)*	*Particle shape*	*Pore volume (cm^3/g)*
Spherosil XOA 1000, batch EM 50	<40	5.48	1096	18*	Spherical	0.50**
Spherosil XOA 600, batch A 45	<40	3.40	600	70*	Spherical	1.06**
Spherosil XOA 400, batch 226	<40	1.95	465	108*, 94**	Spherical	1.26**
Spherosil XOA 200, batch 174	<40	3.00	170	224*, 160**	Spherical	1.04**
LiChrosorb SI 60, batch EF 30	5	1.14	500	60**	Irregular	0.75**
LiChrosorb SI 100, batch EF 27	5	1.04	400	100**	Irregular	1.00**
Partisil 5, batch A 270	5	1.22	400	55**, 60**	Irregular	0.55*, 0.60*

* Values calculated using the equation $d_p = 4\ V_P/S$.

** Values provided by suppliers.

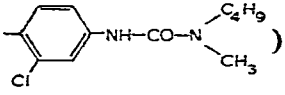
Reagents

Dry dichloromethane was obtained by passing the commercial product (Merck, zur analyse, Cat. No. 6050) through a column (60 × 3 cm) of activated silica (Merck silica gel 60, 0.063–0.200 mm). Dry isooctane (Merck, zur analyse, Cat. No. 4727) was obtained in the same way.

Water-saturated dichloromethane was prepared by vigorously stirring dichloromethane with an excess of water. Berry and Engelhardt[5] showed that saturation is achieved in less than 15 min. The water content of dichloromethane was determined with a Metrohm E547 automatic Karl Fischer titrator (Metrohm, Herisau, Switzerland).

Adsorption–desorption isotherms of water on silica

Water adsorption isotherms from the vapour phase. Samples of 3 g of various silica gels were submitted during 1 week to different water vapour pressures (relative humidity) by storing them over sulphuric acid solutions. The water content by weight was then determined for each relative humidity[6].

Water adsorption isotherms from the liquid phase. In this study, the *in situ* equilibration technique was used to establish an equilibrium amount of water on the adsorbent. First, in order to ensure that the column was at true equilibrium, a test-solute (linuron, ) was injected until a constant capacity factor value (k') was attained. Then, the equilibrium amount of water on the silica gel was calculated as the difference between the water content of the initial mobile phase (inlet of the column) and the water content of the mobile phase collected at the outlet of the column until equilibrium was achieved.

Thermal modifications

The modifications were carried out by heating 3-g portions of silica gel contained in a silica crucible for 5 h at the appropriate temperature in the range 350–900°. The silica gel used was LiChrosorb SI 60 (5 μm). The properties of thermally deactivated silica gels are shown in Table II. The same batch of silica gel (LiChrosorb SI 60, batch No. EF 54) was used for this thermal treatment. No particular care was taken to exclude atmospheric moisture during storage of the modified silica gels.

TABLE II

PROPERTIES OF DEACTIVATED SILICA GEL

Property	*Temperature of modification (°C)*										
	Untreated	*350*	*400*	*450*	*500*	*550*	*600*	*650*	*700*	*800*	*900*
Weight of silica gel in column (mg)	757	774	773	770	786	779	742	808	823	1016	1415
Column dead volume (ml)	1.3_0	1.2_2	1.2_3	1.2_3	1.2_3	1.2_5	1.2_5	1.2_4	1.2_4	1.1_6	0.9_6
Surface area (m^2/g)	463		441		455		472	435	367	186	39

RESULTS AND DISCUSSION

Water adsorption on various silica gels: water adsorption isotherms

Water adsorption isotherms from the vapour phase. The activity of the adsorbent decreased when the amount of physisorbed water increased. This water content depends on atmospheric moisture and the hydrophilic character of the silica. Therefore, it was interesting to study the dependence of the activity of silica gel on the relative humidity of the atmosphere.

As the silica gels usually employed in chromatographic studies have different surface characteristics (chemical nature, number of hydroxyl groups per surface unit, pore structure and specific area), water adsorption isotherms from the vapour phase were studied for various adsorbents, as shown in Table I.

Fig. 1 shows the percentage of water adsorbed on different silica gels against the relative humidity of the atmosphere. It appears that LiChrosorb SI 60 (surface area, $S = 500$ m^2/g; particle diameter, $d_p = 60$ Å) and Spherosil XOA 600 ($S = 600$ m^2/g; $d_p = 70$ Å), which have approximately the same specific areas and pore volumes, show similar behaviour up to 90% relative humidity. This result would indicate that these two silica surfaces have a similar chemical nature. However, the isotherms of the ratio of the number of water-adsorbed molecules per 100 Å^2 (n_{H_2O}/

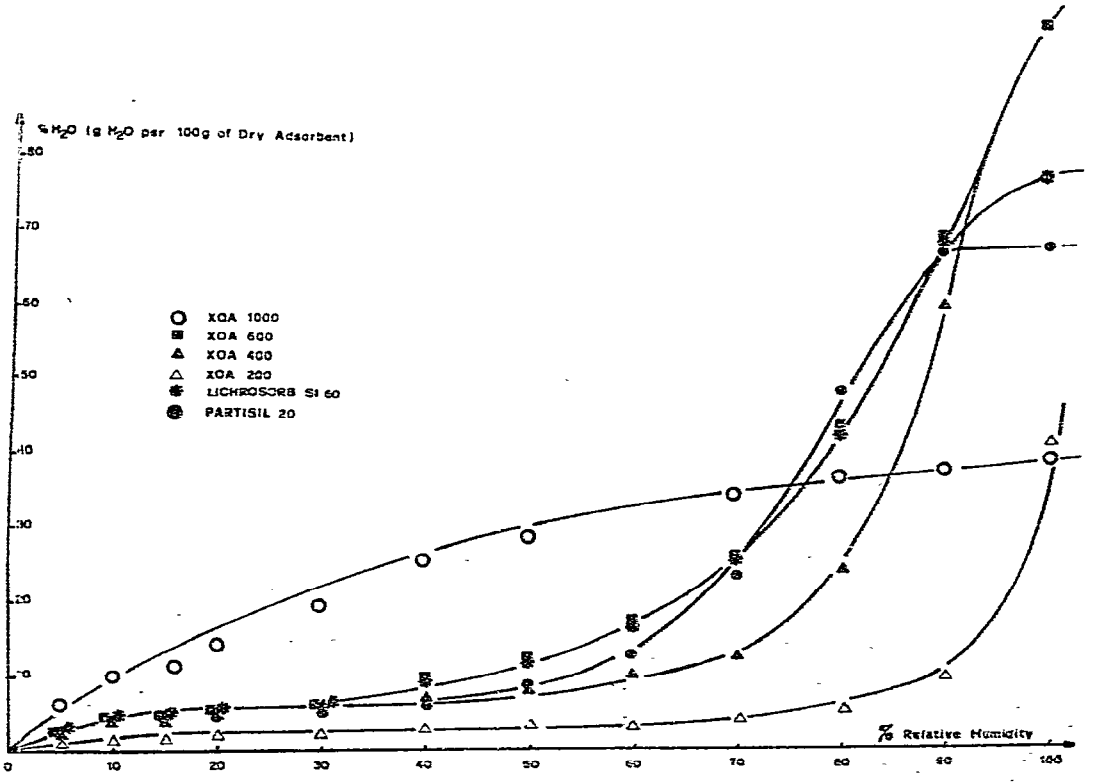


Fig. 1. Water adsorption isotherms from the vapour phase.

100 Å²) against the relative humidity (Fig. 2) show a more important affinity for LiChrosorb SI 60 than for Spherosil XOA 600 up to 90% relative humidity. Above this value, the latter takes up more water, in accordance with its greater pore volume.

For the same reasons, Spherosil XOA 1000 ($S = 1096$ m²/g; $d_p = 18$ Å) takes up more water than any other silica gel up to 50% relative humidity, but at

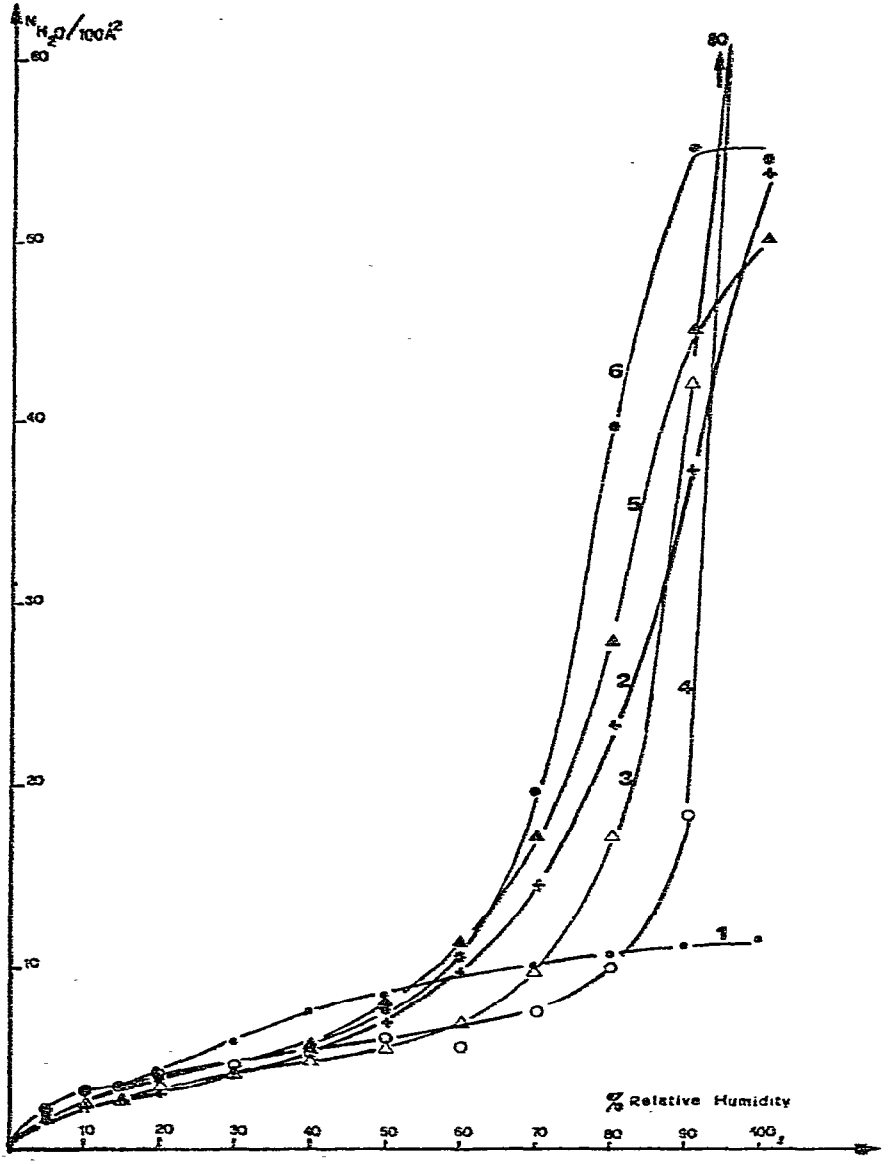


Fig. 2. Water adsorption isotherms from the vapour phase. Curves: 1, Spherosil XOA 1000; 2, Spherosil XOA 600; 3, Spherosil XOA 400; 4, Spherosil XOA 200; 5, LiChrosorb SI 60; 6, Partisil 20.

higher relative humidities it takes up less water, in accordance with its smaller pore volume.

The general behaviour of these silica gels depends on the relative humidity. For relative humidities below 60–70%, the smaller the pore diameter, the greater is the activity of silica gel. According to Snyder[2], wide-pore silica gels possess a majority of free hydroxyl groups, while the surface of microporous silica gels consists of reactive and bound hydroxyl groups; above 70% relative humidity, the amount of water adsorbed on the silica gel surface depends mainly on the pore volume of the adsorbent[7].

Water adsorption isotherms from the liquid phase. The experiments were carried out with dichloromethane containing various amounts of water as mobile phases. As before, the *in situ* equilibration technique was used to establish an equilibrium amount of water on the adsorbent. The water adsorption isotherms from such mobile phases are shown in Fig. 3 for different silica gels. The difference observed between various types of silica gels will be discussed later from the chromatographic point of view.

Kinetic aspects of activation (water desorption) and deactivation (water adsorption). Fig. 4 shows that, for a given silica gel (Spherosil XOA 1000), the volume of dry mobile phase required to achieve equilibrium between the dry solvent and the adsorbent increases when the initial water content of the adsorbent increases. Water adsorption is a reversible process: starting from silica gels with different water con-

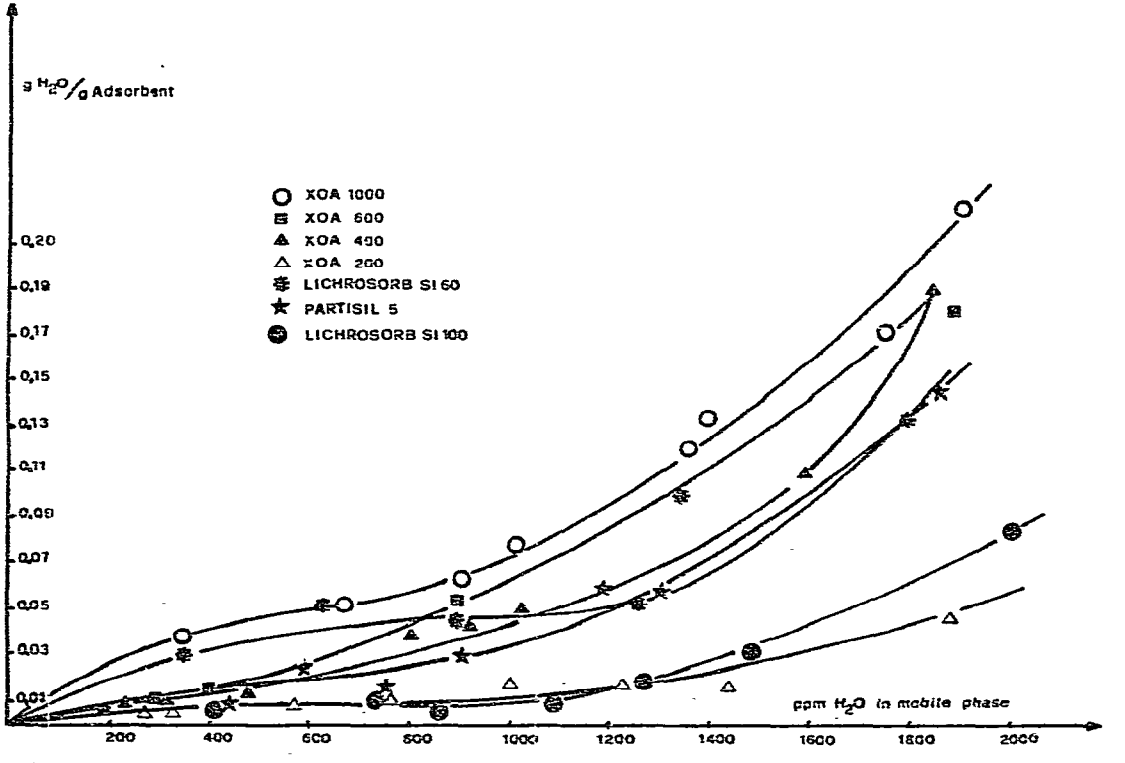


Fig. 3. Water adsorption isotherms from the liquid phase.

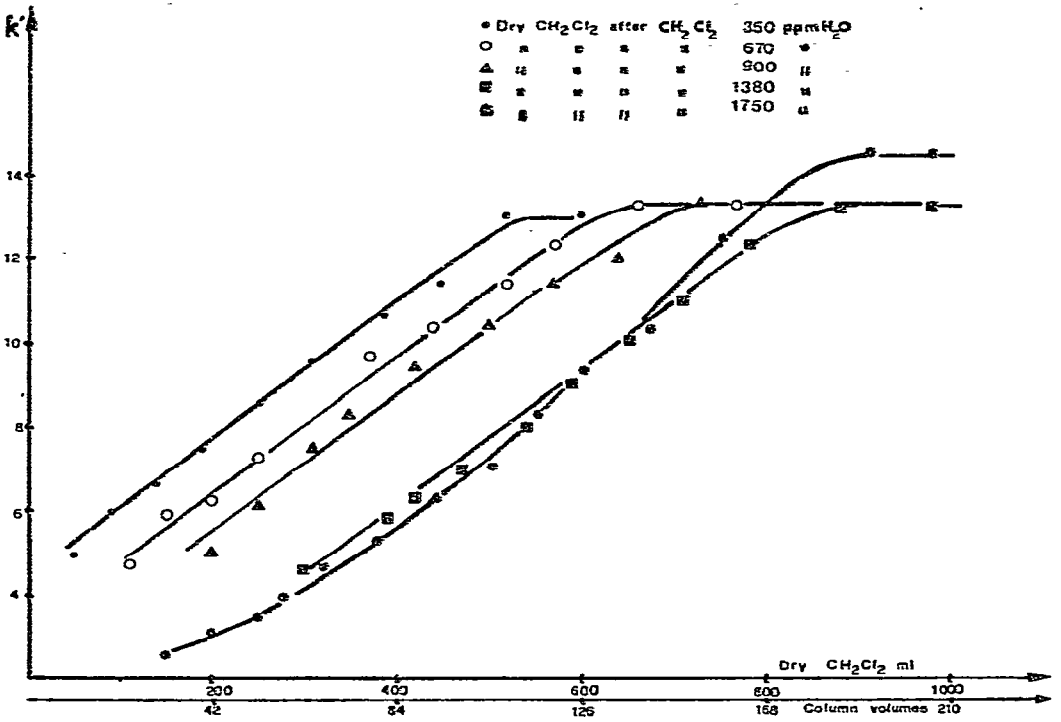


Fig. 4. Volumes of dichloromethane required in order to achieve activation equilibrium. The volumes of the eluent are given in millilitres and in terms of column dead volumes. Adsorbent, Spherosil XOA 1000; solute, acetophenone.

tents, the same activation equilibrium is reached after passage of "dry" dichloromethane. The same results are observed in the deactivation process (Fig. 5).

The time required for column equilibration depends on the water solubility in the mobile phase and the geometric structure of the silica, as shown in Fig. 6.

These curves show a minimum in all instances. Using low water-saturated dichloromethane, it took more time to reach equilibrium because of the slow diffusion of water molecules. Using water-saturated dichloromethane, the amount of water on the adsorbent increases until all of the pores are filled with water; access to the pores then becomes increasingly difficult.

Influence of water on chromatographic parameters

Water content and retentions (capacity ratio). The curves illustrating the change in capacity ratio, k', for the test solute (standard) with the water content of the mobile phase are shown in Fig. 7.

These curves reveal three steps. The first step is characteristic and there is a significant decrease in the k' values corresponding to a small increase in the water content of the mobile phase. This effect has been observed by other workers[8,9]. The larger the specific area of silica gel (the smaller the pore volume), the greater is the decrease in k' values. After this significant decrease (in accordance with the neutralization of the most active sites), the k' values are less affected by further deactivation of

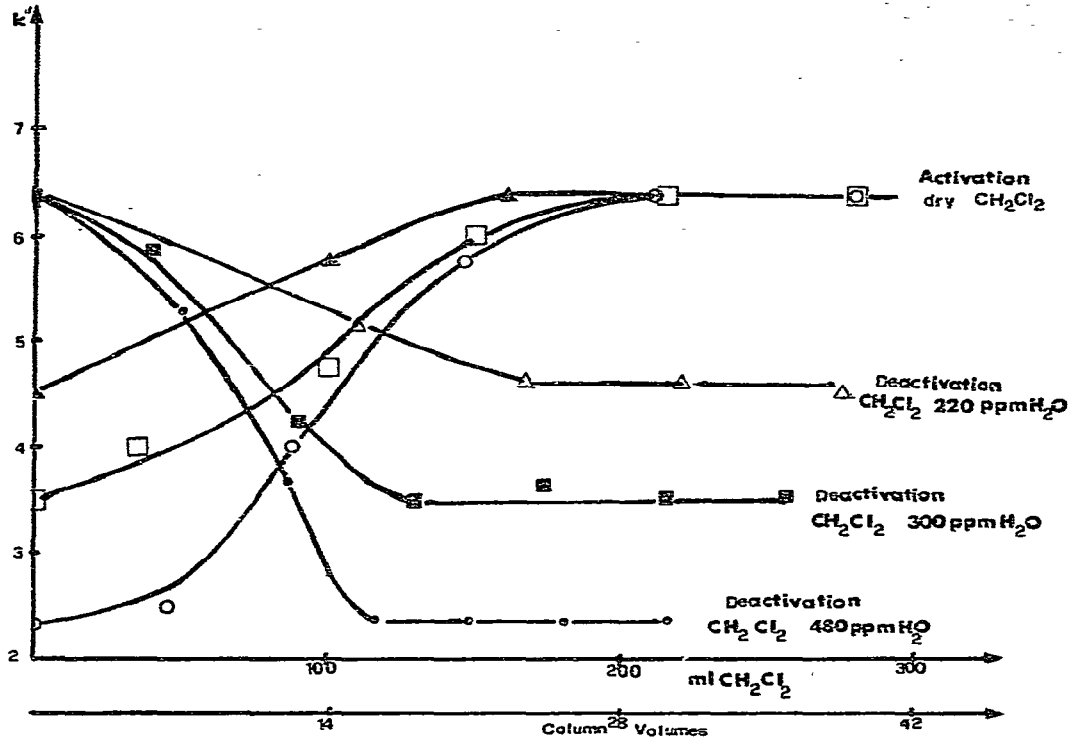


Fig. 5. Activation and deactivation kinetics. The volumes of the eluent are given in millilitres and in terms of column dead volumes. Adsorbent, Spherosil XOA 400 (d_p = 10–40 μm); solute, linuron.

silica gel (from 1 to 3 water molecules adsorbed per 100 Å^2). Finally, for water contents above 1500 ppm, a further decrease in k' values is observed, probably resulting from capillary condensation in the pores.

This description is only qualitative. In order to illustrate the influence of water on retention, it is better to plot the number of water molecules adsorbed per 100 Å^2 against the water content of the mobile phase. The influence of the silica gel surface is then eliminated. Such isotherms are shown in Fig. 8.

It can be seen that certain silica gels, such as Partisil 5, have a normal behaviour while some others, such as LiChrosorb SI 60, show a very different behaviour. The latter seems to be most active of the silica gels studied. Both the isotherms obtained from vapour phase and the curves of k' values against the water content of the mobile phase seem to indicate a greater activity of LiChrosorb SI 60.

It appears that each silica gel has its own characteristics and it is very difficult to derive its behaviour from its geometric properties: although these properties are nearly identical for LiChrosorb SI 60 and Partisil 5, the former shows a greater activity and hence needs more water to neutralize its most active sites.

It would therefore be of particular interest if each silica gel manufacturer would provide the water adsorption isotherms and more details concerning the methods of preparation.

Water content and efficiency. The experiments for studying the influence of

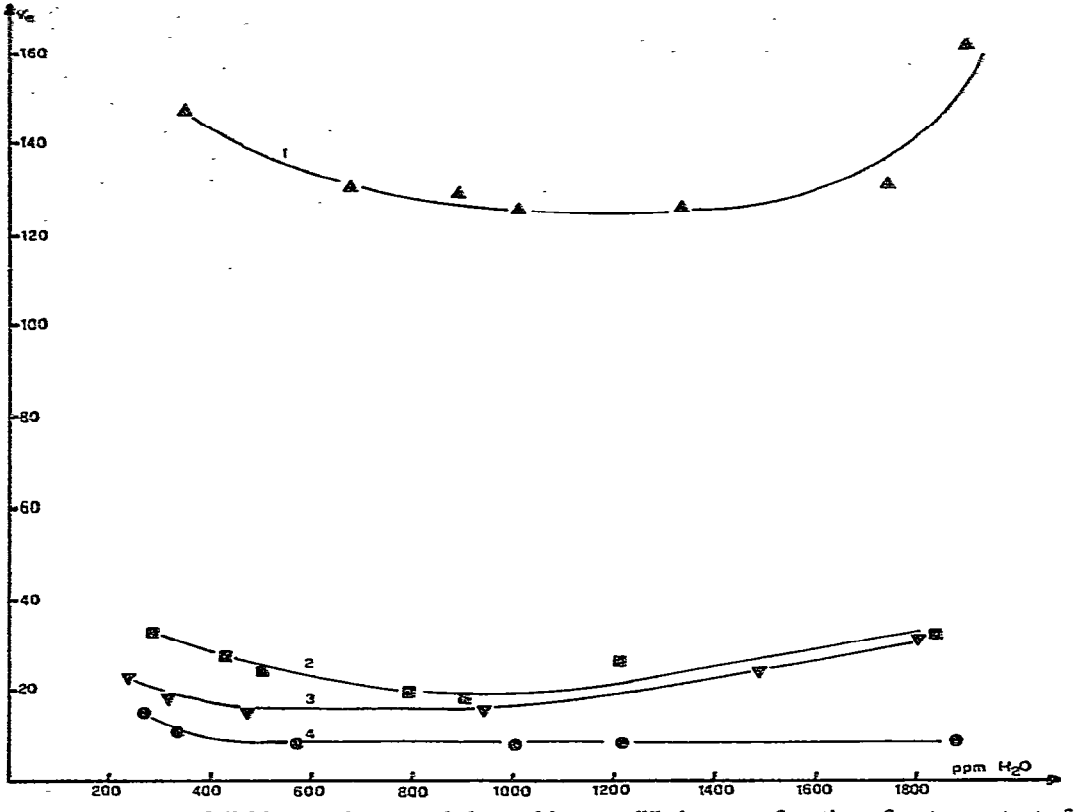


Fig. 6. Volumes of dichloromethane needed to achieve equilibrium as a function of water content of the mobile phase. V_e = number of column dead volumes. Curves: 1, Spherosil XOA 1000; 2, Spherosil XOA 600; 3, Spherosil XOA 400; 4, Spherosil XOA 200.

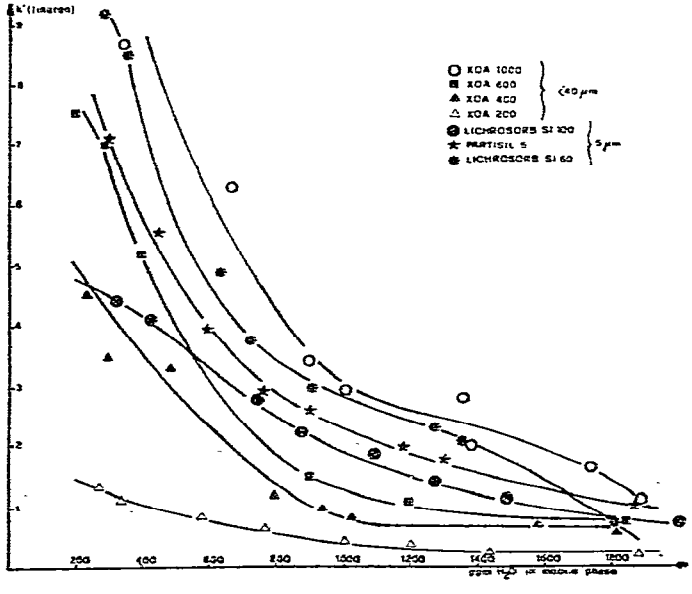


Fig. 7. Changes in k' values (linuron) against water content of the mobile phase (ppm).

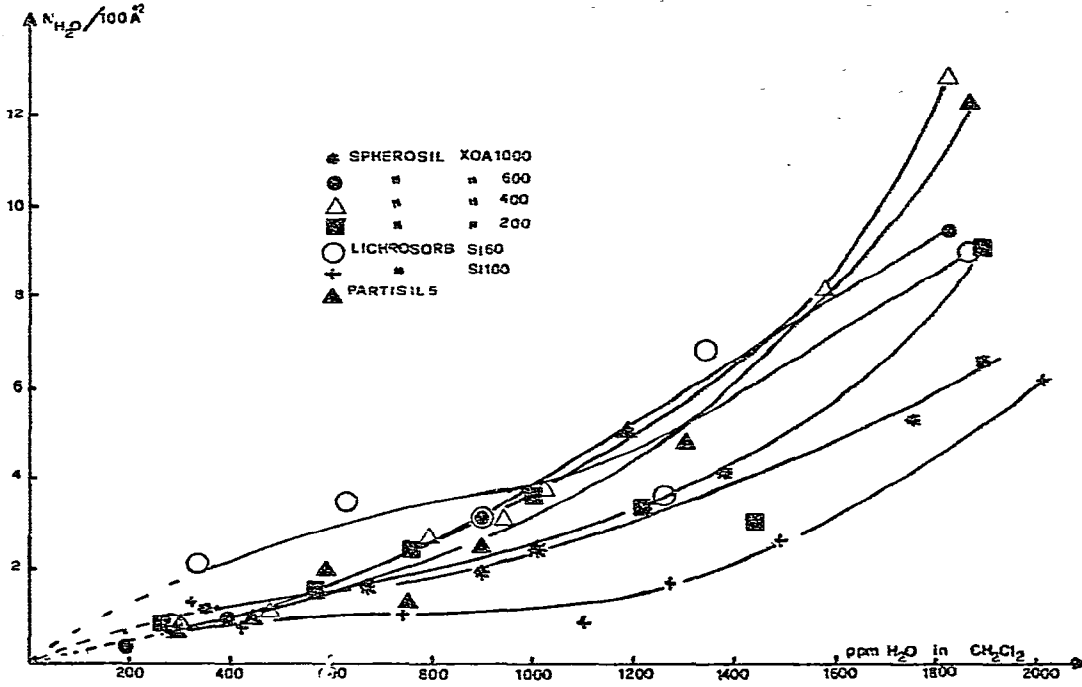


Fig. 8. Number of water molecules adsorbed per 100 Å² as a function of water content of the mobile phase.

water on column efficiency were carried out with microporous silica gel (5 μm). Each HETP value is the mean value of several injections. Table III gives HETP values for different water contents of the mobile phase.

With LiChrosorb SI 60, the best efficiency is obtained for water contents of dichloromethane between 900 and 1260 ppm (4.7–5.4% of adsorbed water on silica). Above the higher value, a sharp decrease in efficiency occurs. LiChrosorb SI 100 shows more regular behaviour with maximal efficiency in the range 0.9–1.2% of adsorbed water. With Partisil 5, the lowest HETP values are found in the range of about 3–6% of adsorbed water. More water decreases the efficiency but gives better results than those obtained with under 3% of adsorbed water.

In agreement with Snyder[10], we found that the low efficiencies obtained with a dry mobile phase can be related to the strong activity sites of the silica surface, leading to slow adsorption–desorption kinetics. The neutralization of these sites by water molecules gives a more homogeneous surface activity, and therefore a better efficiency. When the amount of water increases, the affinity of the solute for water and the pore volume must have a major influence on efficiency.

Water content and peak symmetry. The symmetry of the peaks decreases as the water content of the eluent increases. This effect is demonstrated in Fig. 9, which shows chromatograms obtained with water-saturated dichloromethane (curve 1), dichloromethane with different water contents (2, 3) and dry dichloromethane (4). The solute is acetophenone and the adsorbent is Spherosil XOA 1000. The reason for the change in the peak shapes is not clear; perhaps the phenomena can be explain-

TABLE III

HETP VALUES AS A FUNCTION OF THE WATER CONTENT OF THE MOBILE PHASE

System A: column packing, LiChrosorb SI 60 (5 μm); solute, linuron; linear velocity, 0.20 cm/sec; mobile phase, dichloromethane. System B: column packing, LiChrosorb SI 100 (5 μm); solute, linuron; linear velocity, 0.14 cm/sec; mobile phase, dichloromethane. System C: column packing, Partisil 5 (5 μm); solute, linuron; linear velocity, 0.14 cm/sec; mobile phase, dichloromethane.

System	*Water in mobile phase (ppm)*	*HETP (μm)*
A	—*	34
	280	28
	340	30
	630	21
	900	19
	1260	18
	1340	45
	1800	38
B	—*	22
	320	16
	420	16
	740	17
	1100	20
	1270	21
	1490	22
	2010	21
C	—*	21
	290	22
	440	19
	556	20
	900	15
	1180	14
	1300	17
	1860	18

* "Dry" dichloromethane.

ed by strong adsorption when the silica gel is completely reactivated, resulting in slow kinetics. When the water content increases, the silica gel surface becomes more homogeneous and then peak asymmetry is significantly reduced or even disappears. Two peaks are particularly apparent with dry dichloromethane (Fig. 9).

Changes in peak shape have already been observed by Kirkland[11], with organic alcohol modifiers. In this work, the asymmetry is almost observed when using silica particles with diameters ranging between 10 and 40 μm. This modification in peak shape is not apparent with microporous silica particles (5 μm), but the effect is reproducible in the first instance.

Effect of thermal treatment on the chromatographic properties of silica gel

The silica gel used was LiChrosorb SI 60 (5 μm). An infrared spectroscopic study of the modified silica surfaces was also carried out and the results will be published elsewhere.

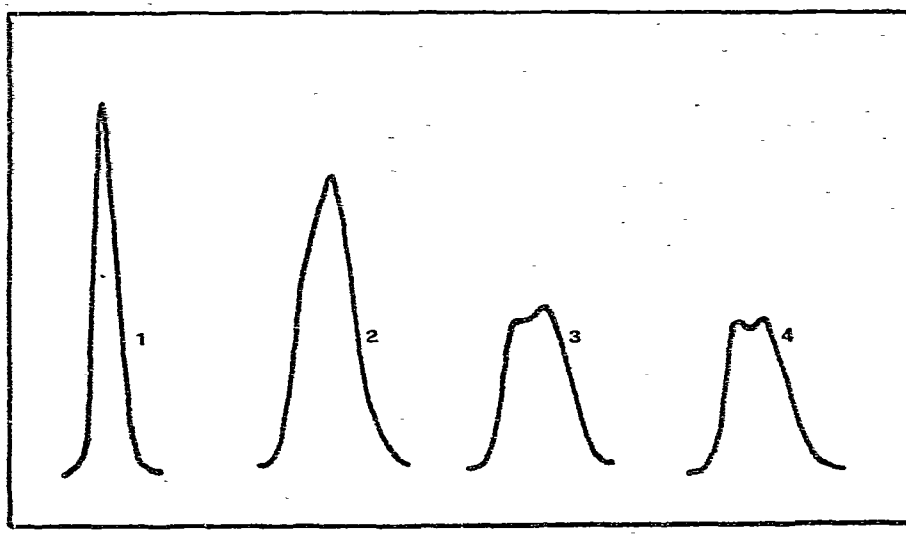


Fig. 9. Peak shapes of acetophenone. 1, Deactivated Spherosil XOA 1000; 2, partially deactivated Spherosil XOA 1000; 3 and 4, activated Spherosil XOA 1000.

Rehydration of thermally modified silica gel. Fig. 10 shows the amount of water adsorbed on thermally dehydrated silica gel (from the same mobile phase) against the temperature of treatment.

Up to 350°, the amount of water adsorbed on modified silica is of the same order as on unmodified silica. Between 350° and 450°, there is a rapid decrease in the

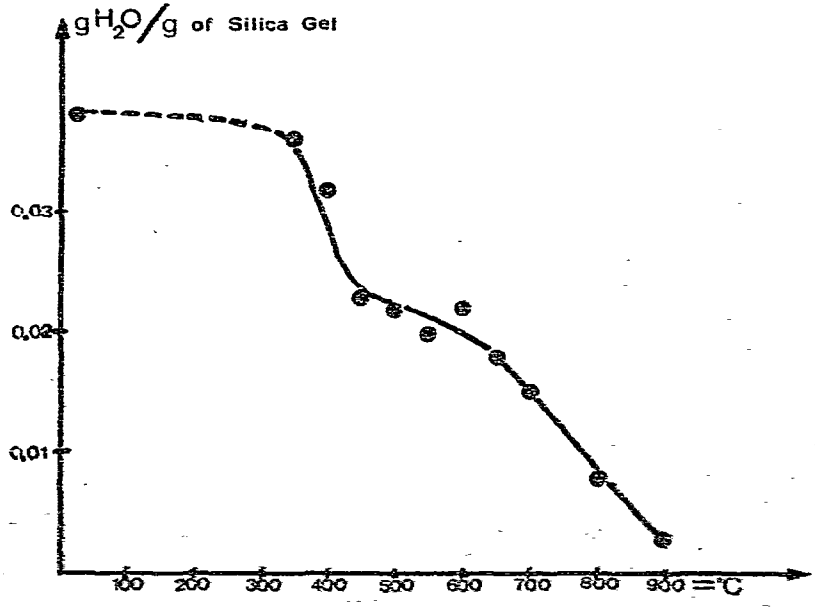


Fig. 10. Water adsorption of modified silica gel against deactivation temperature.

amount of water; the water take-up becomes less and less because of the condensation of surface hydroxyl groups[10,12–14], which are responsible for the molecular water adsorption. From 450° to 600°, there is no significant change. Above the last value, the amount of adsorbed water decreases considerably as the temperature continues to increase owing to the significant decrease in the surface area of silica, which occurs above 700°.

Kinetic aspects of hydration and dehydration with thermally modified silica gels. Volumes of mobile phase needed for reactivating or deactivating a column plotted against the thermal treatment temperature are shown in Fig. 11. In both instances (water adsorption and water desorption), the volume required decreases as the treatment temperature increases. At 350°, this volume is the same as that required with an untreated silica gel. It can be stated that up to this temperature there is no significant modification of the silica.

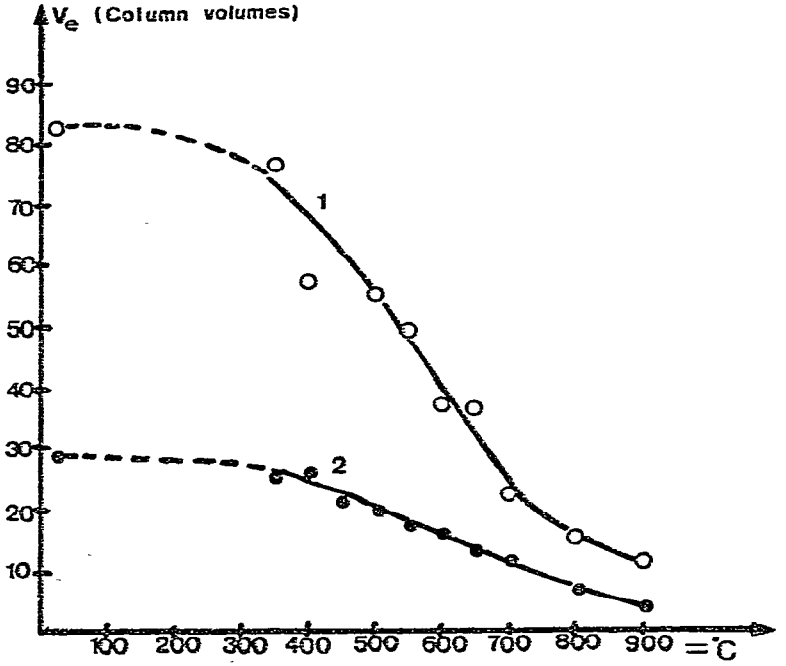


Fig. 11. Volumes needed to achieve activation (1) and deactivation (2) equilibrium. V_e = number of column dead volumes.

It should be noted that the volume needed to dehydrate a column is greater than that needed to rehydrate the same column while reaching the same equilibrium state as before.

Influence of thermal treatment on chromatographic parameters. The changes in retention for two classes of solutes (benzene, naphthalene, anthracene and linuron, neburon, phenobenzuron) chromatographed with dry isooctane and dry dichloromethane are shown in Figs. 12 and 13, respectively.

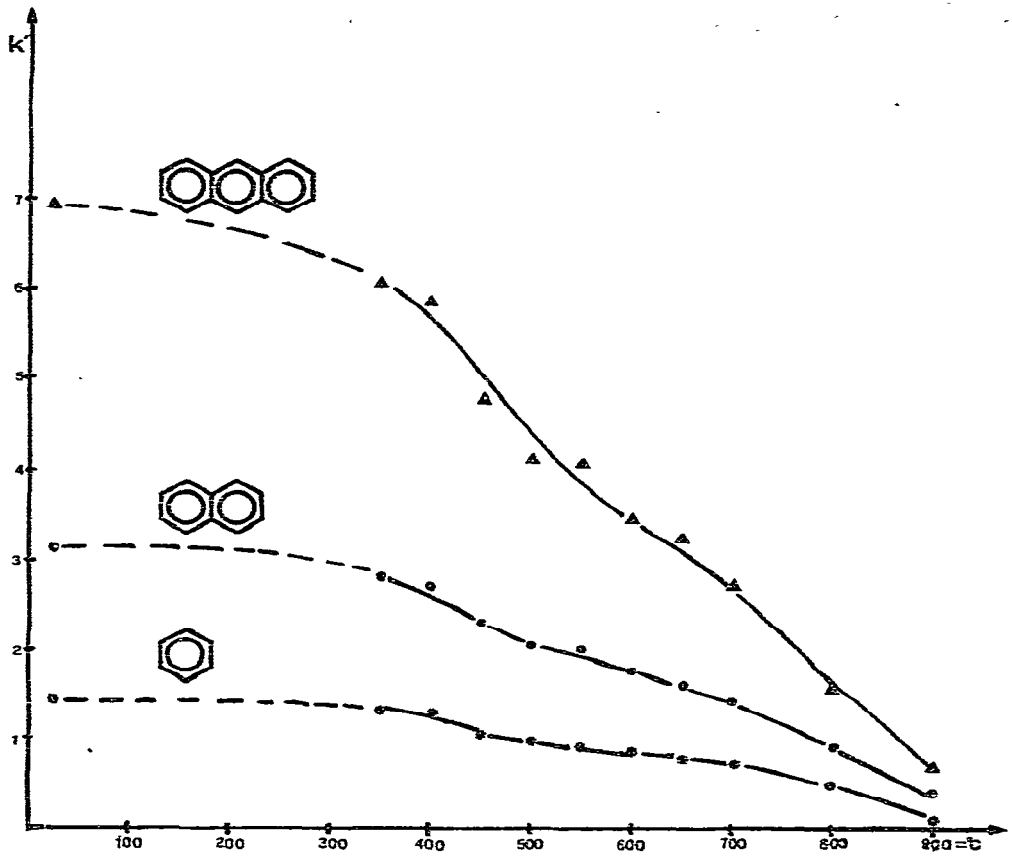


Fig. 12. Changes in k' values (benzene, naphthalene, anthracene) against deactivation temperature. Mobile phase: dry isooctane.

When working with dry mobile phases, the k' values decrease with the increase in treatment temperature. Using water-modified dichloromethane, the k' values increase from 350° and are at a maximum between 500° and 650°. Above the latter temperature, they decrease rapidly to the same k' values as those obtained with untreated silica gel.

With dry mobile phases (issoctane or dichloromethane), the silica is in its highest "activation state". It has been shown that thermal treatment causes the number of surface hydroxyl groups to decrease[10,12–14]. At higher temperatures, the specific surface area decreases considerably while the mean pore diameter becomes greater[14–16]. For LiChrosorb SI 60, the decrease in specific surface area is observed at about 650–700° (Table III).

On the basis of these results, it can be argued that, in a first step (below 650°), the decrease in retention observed is caused by a decrease in the total number of hydroxyl groups condensed thermally. In a second step (above 650°), an additional factor in this decrease is played by the geometric modification of silica.

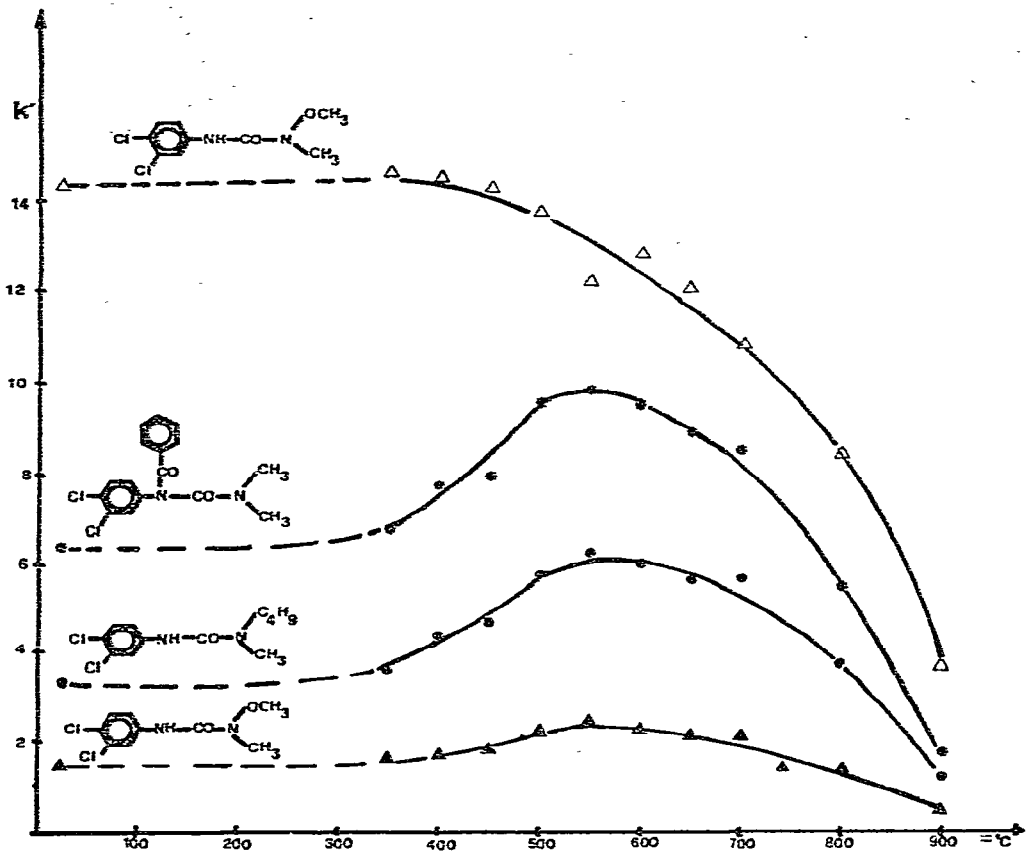
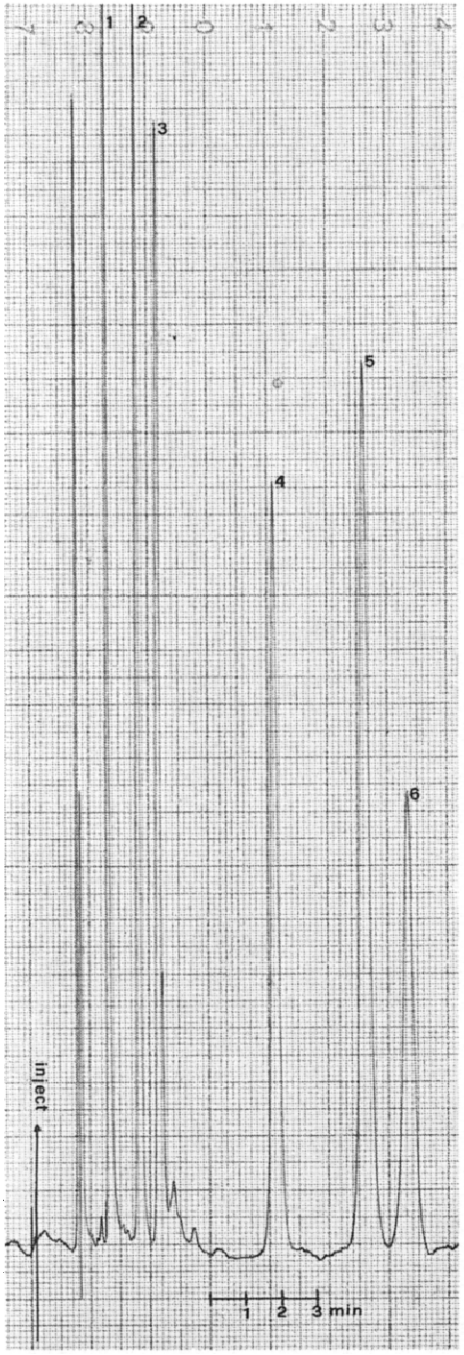


Fig. 13. Changes in k' values of herbicides (linuron, neburon, phenobenzuron) against deactivation temperature. Mobile phase: dichloromethane.

The curves obtained with water-modified dichloromethane (Fig. 13) are very different from those obtained with dry dichloromethane. Below 350°, the retentions are identical with those obtained with untreated silica. Between 350° and 650° the retentions increase to a maximum. Karl Fischer titration shows that this maximum corresponds to the decrease in the amount of water on silica; meanwhile, the specific area does not change. In the previous step, the strong site neutralization led to a weaker retention. In this step, the progressive elimination of these sites in favour of new free hydroxyl groups leads to a better retention (this was observed by IR measurements). The decrease in retention starting at 650° corresponds to the decrease in the specific surface area.

Figs. 14–19 show some examples of separations obtained using both modes of silica gel deactivation.

With water-containing mobile phases, Fig. 14 shows the separation of some herbicides on LiChrosorb SI 60 silica gel[17]. The mobile phase is water-saturated dichloromethane. Fig. 15 shows the separation of barbiturates with dichloromethane–isopropanol–water as the mobile phase[18]. With an identical type of mobile phase, pyrimidines have been separated on LiChrosorb SI 60 (Fig. 16).



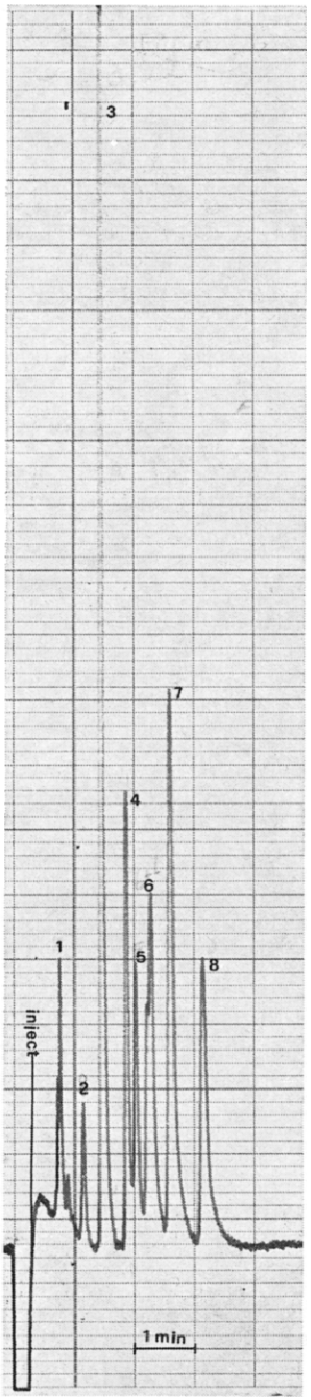


Fig. 14. Separation of herbicides. Column, 14.5 cm × 4.6 mm I.D. LiChrosorb SI 60 (5 μm). Isocratic elution; eluent, saturated dichloromethane (20°). UV detector, $\lambda = 254$ nm. Linear velocity, 0.21 cm/sec; $\Delta P = 70$ bar. Peaks: 1, linuron; 2, neburon; 3, phenobenzuron; 4, diuron; 5, monuron; 6, isoproturon.

Fig. 15. Separation of barbiturates. Column, 15 cm × 4.6 mm I.D. Partisil 5. Isocratic elution, room temperature; mobile phase, dichloromethane–isopropanol (97.5:2.5), saturated with water. UV detector, $\lambda = 254$ nm. Linear velocity, 0.62 cm/sec. Peaks: 1, allobarbital; 2, prominal; 3, hexobarbital; 4, secobarbital; 5, tetrallobarbital; 6, amobarbital; 7, phenobarbital; 8, barbital.

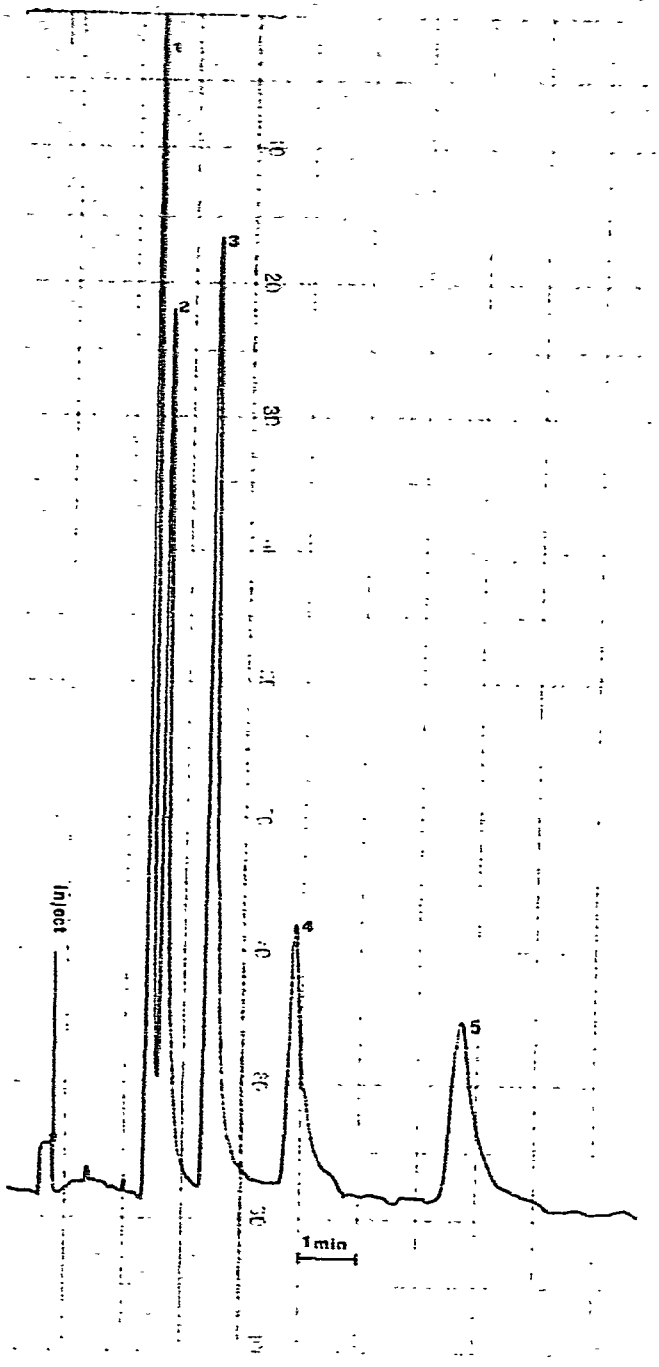


Fig. 16. Separation of pyrimidines. Column, 15 cm × 4.6 mm I.D. LiChrosorb SI 100. Isocratic elution, room temperature; mobile phase, dichloromethane–isopropanol (75:25), saturated with water. UV detector, $\lambda = 254$ nm. Linear velocity, 0.36 cm/sec; $\Delta P = 30$ bar. Peaks: 1, thymine; 2, 5-fluorouracil; 3, uracil; 4, thymidine; 5, 2'-desoxyuridine.

With thermally deactivated silica gels, Fig. 17 represents the separation of the same herbicides as shown above for water-modified silica gel; the treatment temperature is 550°. Barbiturates have also been separated on heated silica gels. The in-

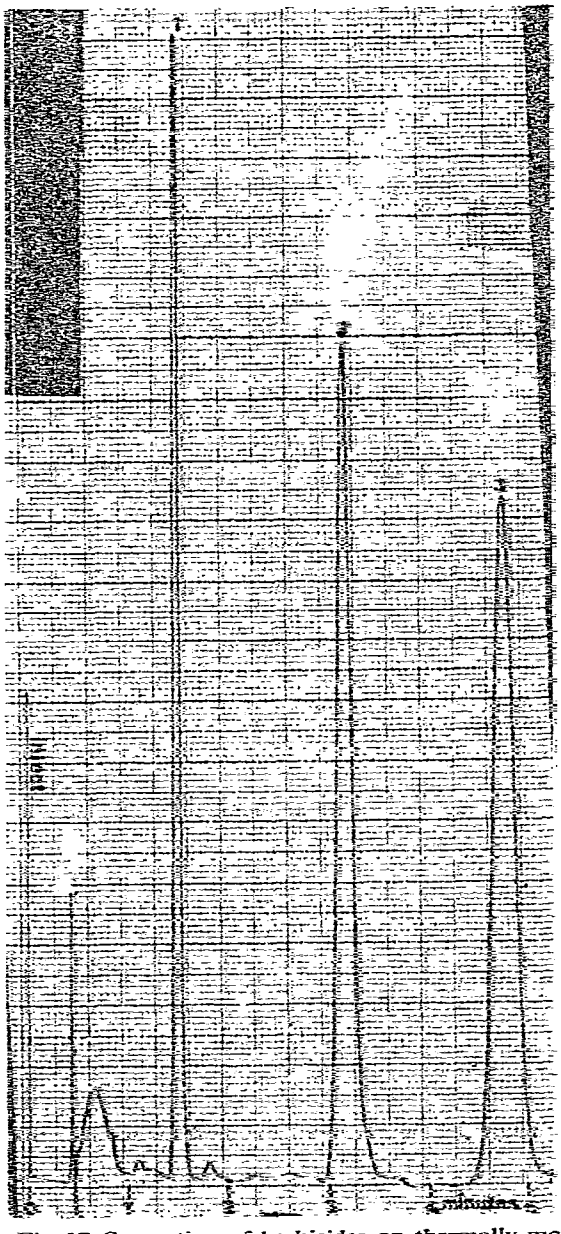


Fig. 17. Separation of herbicides on thermally modified silica gel. Column, 10 cm × 4.6 mm I.D. LiChrosorb SI 60. Modification temperature, 550°; mobile phase, dichloromethane + 1100 ppm of water. Isocratic elution, room temperature. UV detector, $\lambda = 254$ nm. Linear velocity, 0.37 cm/sec; $\Delta P = 48$ bar. Peaks: 1, linuron; 2, neburon; 3, phenobenzuron.

fluence of the treatment temperature is shown: on untreated silica gels, the first two solutes are separated but the second and third are not. With treated silica gels, the retentions do not change significantly, but the same does not hold true for the relative retentions (Fig. 18).

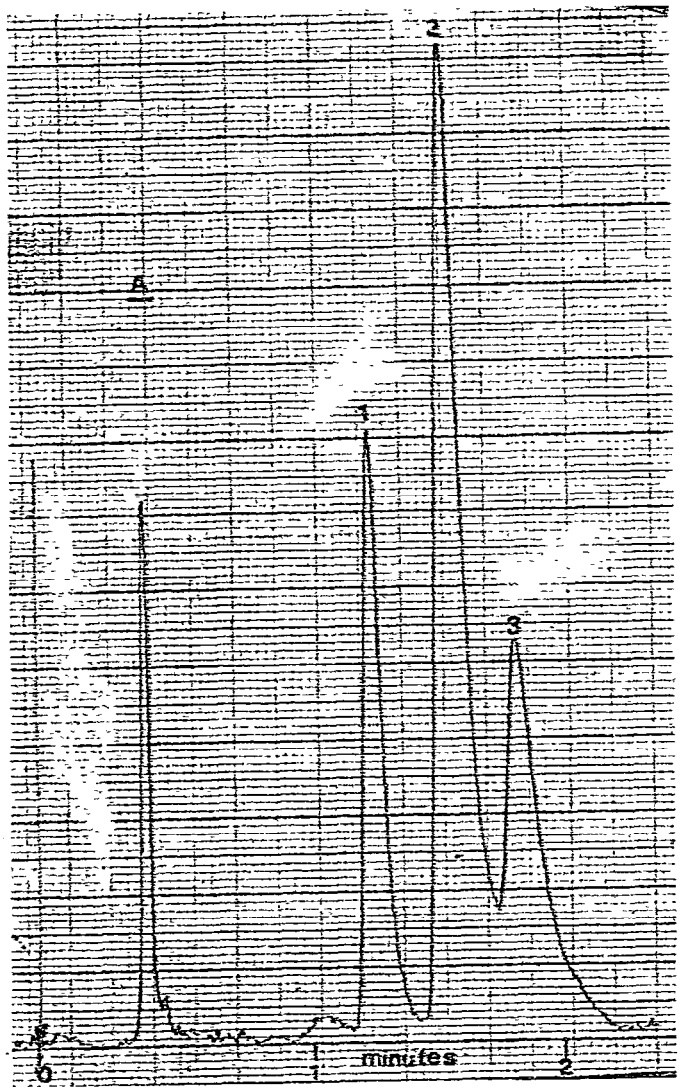


Fig. 18.

(Continued on p. 198)

A clinical control has been developed in our laboratory for the quantitative determination of phenobarbital using treated silica gels. The internal standard is barbital. Fig. 19 shows a chromatogram of a standard sample, a chromatogram of a human blood serum and a chromatogram of red blood on LiChrosorb SI 60 treated at 600°.

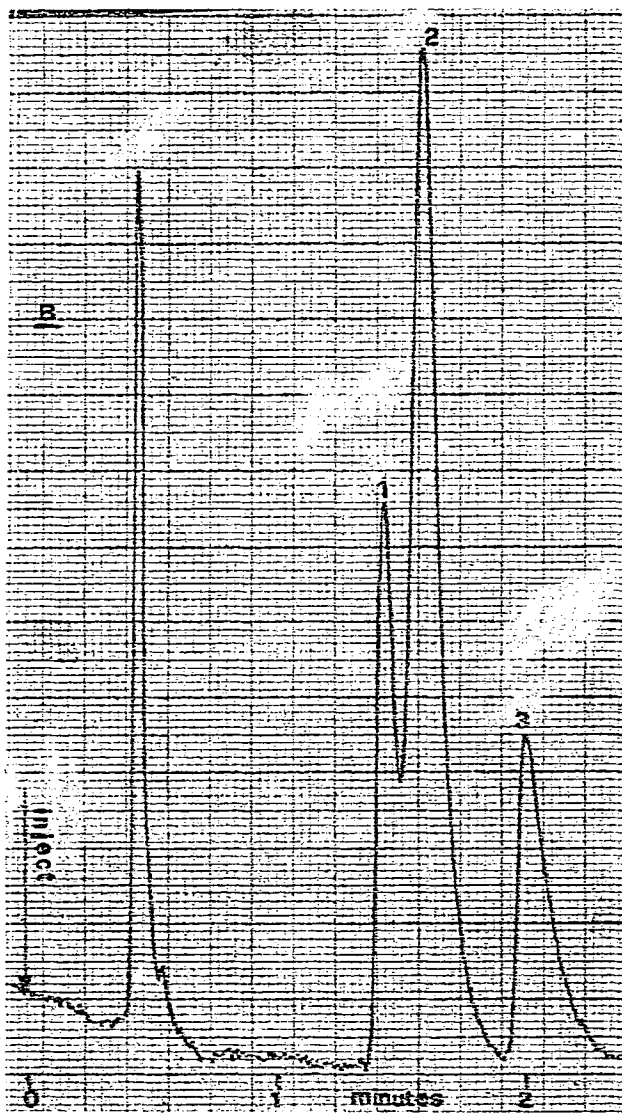


Fig. 18.

CONCLUSION

With the two modes of deactivation, addition of water and thermal treatment, we can reduce the influence of strong site adsorption. Firstly, water provides a greater modification of retention. Secondly, although thermal treatment may lead to unde-

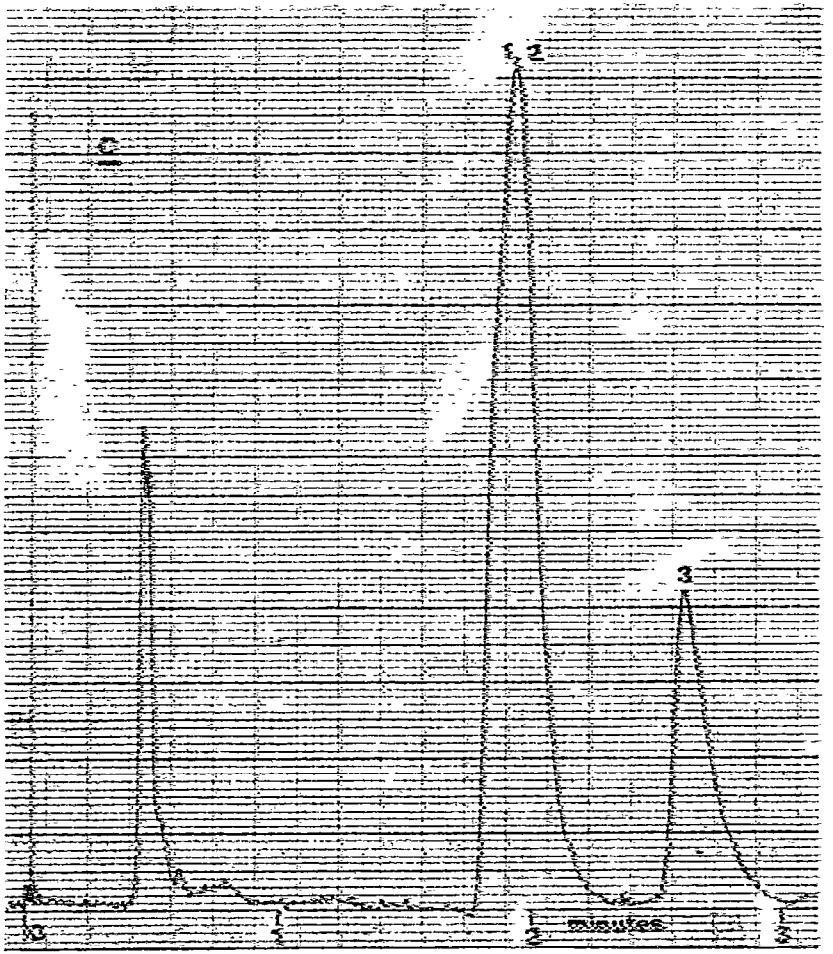


Fig. 18. Separation of barbiturates on thermally modified silica gel. Column, 10 cm × 4.6 mm I.D. LiChrosorb SI 60. Isocratic elution, room temperature. Mobile phase: dichloromethane–isopropanol (98:2). Linear velocity, 0.37 cm/sec. A, untreated silica gel; B, treatment temperature 450°, ΔP = 50 bar; C, treatment temperature 500°, ΔP = 60 bar. Peaks: 1, amobarbital; 2, phenobarbital; 3, barbital.

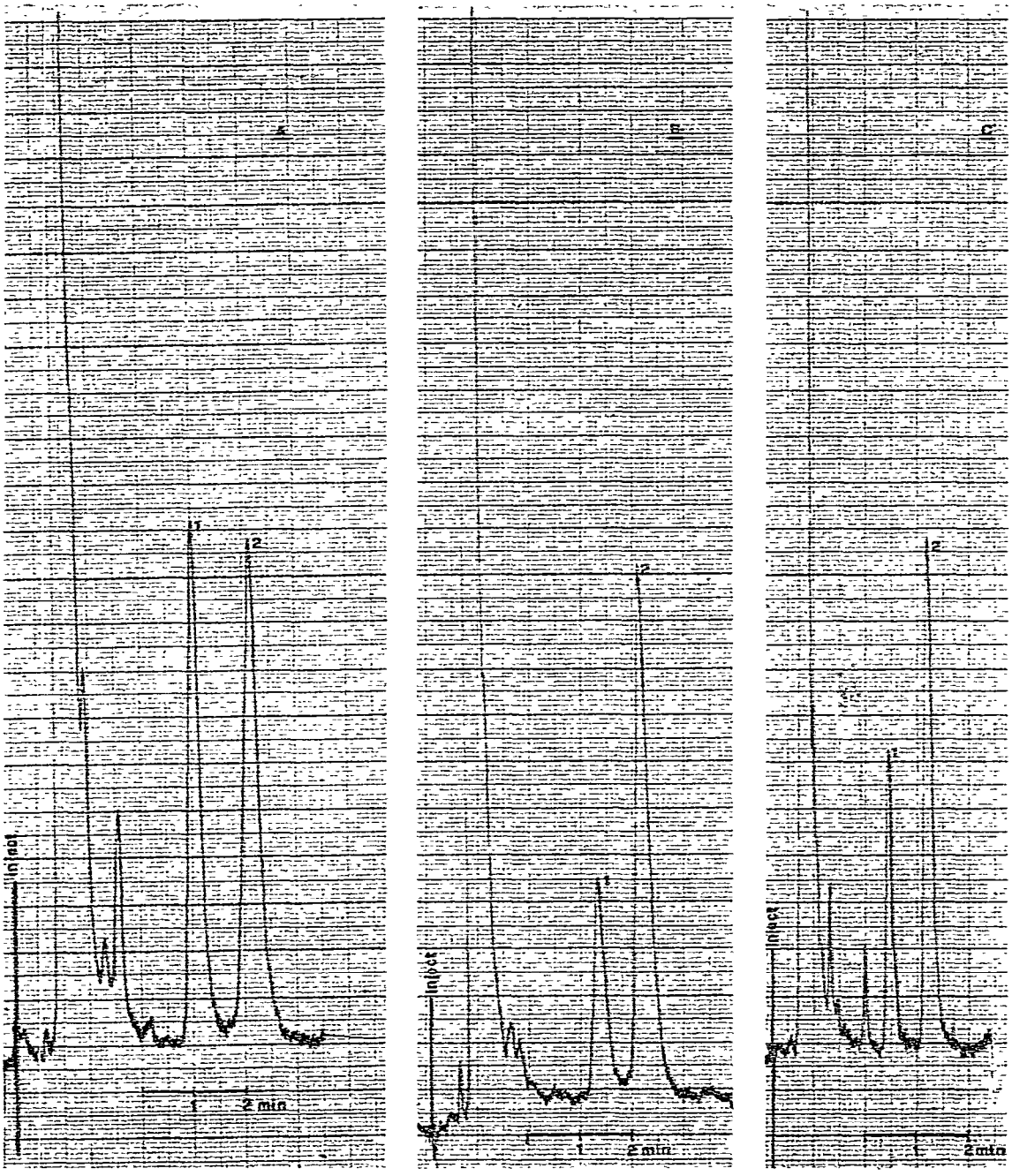


Fig. 19. Quantitative analysis of phenobarbital. A, standard sample; B, serum sample; C, red blood sample. Column, 10 cm × 4.6 mm I.D. LiChrosorb SI 60. Treatment temperature, 600°. Mobile phase, dichloromethane–isopropanol (98:2). UV detector, $\lambda = 254$ nm; $S = 0.01$. Flow-rate, 1.2 ml/min; $\Delta P = 40$ bar. Sample, 5 μl. Peaks: 1, phenobarbital; 2, barbital.

sirable geometric modification of the silica, it provides faster equilibrium kinetics, which can be of particular interest on a preparative scale or in a gradient elution mode.

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