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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF CARDIAC GLYCOSIDES

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SUMMARY

Highly efficient, short columns have been prepared for the separation of mixtures of cardiac glycosides at relatively small pressure drops. The selectivities of silica adsorbents with different chemically modified surface layers for cardiac glycosides were determined. The influence of the structure of cardiac glycoside molecules on their retention characteristics in liquid chromatography was investigated. The relative number of hydrophobic and hydrophilic groups in the glycoside molecules as well as the configurations are very important for the separation.

INTRODUCTION

The chromatographic investigation of cardiac glycosides is of great interest, first because these natural biologically active compounds are used increasingly in medicine and secondly because the large number of compounds in this class with different structures enables the investigation of the relation between their structures and retention volumes. Moreover, in the course of optimizing the chromatographic separation of glycosides it is also possible to investigate some physico-chemical problems of adsorption from dilute solution and of the surface chemistry of the adsorbents.

Although a number of studies[1–9] have been devoted to the chromatographic analysis of cardiac glycosides, many problems remain to be solved, one of which is the correlation between their structures and retention volumes. The determination of such correlations in liquid chromatography requires the use of adsorbents with homogeneous surfaces, as in gas chromatography[10].

Hydroxylated silica and silicas with chemically modified surfaces are generally used as adsorbents in liquid chromatography[11–13]. Most often, long hydrocarbon chains are attached to the silica surface and it is assumed that these form a "brush" on the surface. The use of diphenyldichlorosilane for the chemical modification of silica surfaces makes it possible to obtain a hydrophobic surface with a large carbon content that is very important for increasing the capacity factor. At the same time the phenyl groups are rigid and they can only vibrate around the carbon–silicon bond.

The chemical modification of silica surfaces by diphenyldichlorosilane is carried out in toluene at 80–100°C. The surface is first covered by the electron donor

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triethylamine which accelerates the reaction. A convenient method of qualitative and quantitative control of the reaction is infrared (IR) spectroscopy which enables the evaluation of the surface concentration of phenylsilyl groups, α, and observation of the attached phenyl groups. Since α for LiChrosorb Si 100 (particle size 30 μm) with attached phenylsilyl groups was determined earlier independently of the carbon content on the surface, and since the extinction coefficient, K, is the same for all samples, it is possible to evaluate the concentration of attached diphenylsilyl groups by the spectroscopic method.

Fig. 1 shows the IR spectra of silica with different specific surface areas after chemical modification by diphenyldichlorosilane. The band at $\nu = 3083\ \mathrm{cm}^{-1}$ may be used for the evaluation of the surface concentration of phenylsilyl groups. The optical density, D, is related to the surface concentration, α, by

$$D = \ln I_0/I = K\alpha\varrho s \qquad (1)$$

where K is the extinction coefficient, α is the surface concentration (μmol/m^2), ϱ is related to the thickness of the tablet (g/cm^2) and s is the specific surface area (m^2/g) of the adsorbent under investigation. If D, ϱ and s are known it is possible to determine the products $K\alpha$ and to compare them for different chemically modified adsorbents. Such a comparison makes it possible to evaluate the relative degree of chemical modification of surfaces.

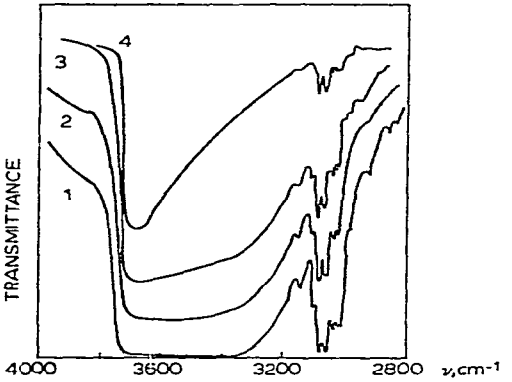


Fig. 1. Infrared spectra of silica gels modified by diphenyldichlorosilane evacuated at 200°C and in carbon tetrachloride. Adsorbents: 1 = LiChrosorb Si 60; 2 = LiChrosorb Si 100; 3 = LiChrospher Si 500; 4 = LiChrospher Si 1000.

For the spectroscopic investigation a Zeiss UR-20 infrared spectrometer was used. The spectra were taken in the region 4000–2700 cm^{-1} with a LiF prism. The tablet of adsorbent under investigation was evacuated at 200°C to 10^{-5} Torr. It was then placed in carbon tetrachloride to reduce the scattering and the spectrum of the sample was taken.

In Table I the values of $K\alpha$ and α are presented for different modified silicas. It is seen that the surface coverage of attached groups is practically the same for the different silicas. Thus for the preparation of modified adsorbents with reproducible

TABLE I

SURFACE CONCENTRATIONS OF DIPHENYLSILYL GROUPS ATTACHED TO SILICA
Spectral data from Fig. 1.

Adsorbent	d_p (μm)	s (m^2/g)	D	ϱ (g/cm^2)	$10^4 K\alpha$	α ($\mu mol/m^2$)
LiChrosorb Si 60	5	500	3.16	0.0418	0.15	1.56
LiChrosorb Si 100	30	285	1.21	0.0215	0.20	2.04
LiChrosorb Si 100*	7	285	0.74	0.0136	0.19	1.98
LiChrospher Si 500	10	50	0.68	0.0651	0.21	2.16
LiChrospher Si 1000	10	20	0.14	0.0185	0.38	3.91

* Spectral data from Fig. 2.

surface properties it is possible to use the reaction of silica with diphenyldichlorosilane in the presence of triethylamine as electron donor in a liquid phase.

It is interesting to compare the separation of cardiac glycosides on adsorbents with attached phenyl and octadecyl groups. The adsorbent was LiChrosorb Si 100 (particle size 7 μm) modified by diphenyldichlorosilane and octadecyltrichlorosilane in the presence of triethylamine under the same conditions. Fig. 2 shows the IR spectra of the initial adsorbent and after the modification with phenyl and octadecyl groups. The spectra exhibit the bands of phenyl and octadecyl groups and the bands of hydrogen-bonded hydroxyl groups which do not take part in the reaction of chemical modification.

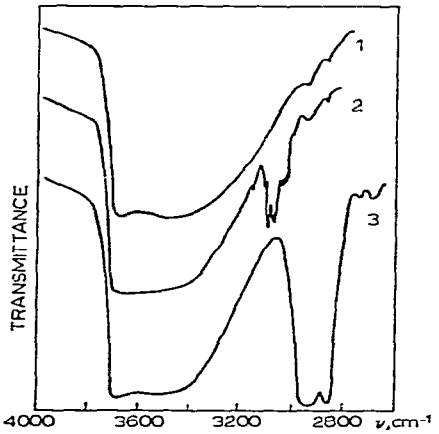
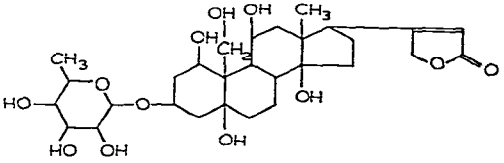
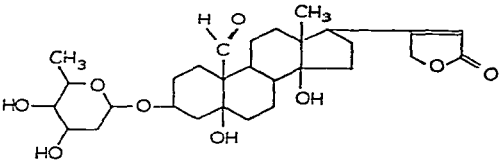
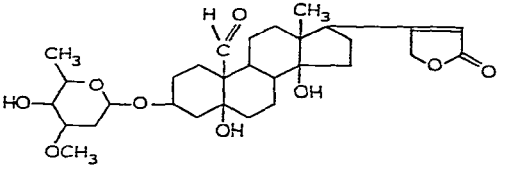
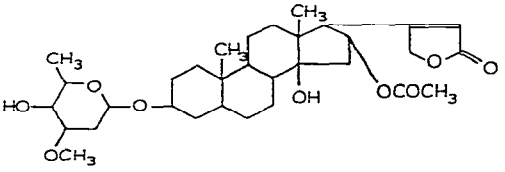
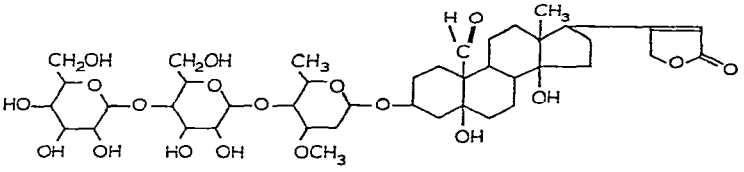
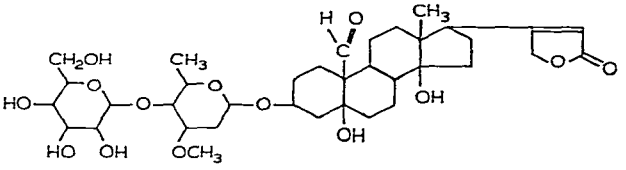
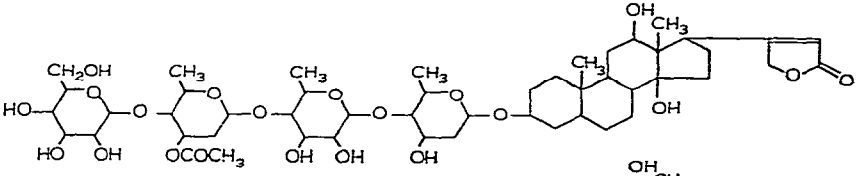
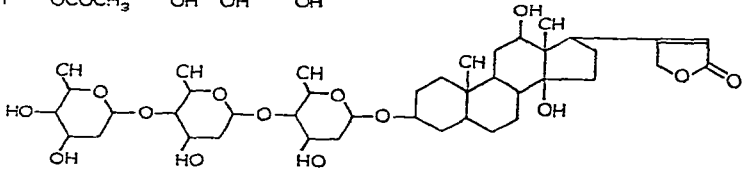


Fig. 2. Infrared spectra of LiChrosorb Si 100 (1) and of the same silica modified by diphenyldichlorosilane (2) and octadecyltrichlorosilane (3) evacuated at 200°C and placed in carbon tetrachloride.

To determine the differences in the adsorption properties of silica surfaces having different functional groups we investigated the effect of temperature and eluent composition on the retention volumes of some cardiac glycosides in columns of these adsorbents. A mixture of glycosides having a monosaccharide in the glycone part of the molecules was chosen. To determine the main correlation between the retention volumes and the structure of the glycoside molecules the eight-component

TABLE II

INVESTIGATED CARDIAC GLYCOSIDES

Name	*Structural formula*
G-Strophanthin	
Erysimin	
Cymarin	
Neriolin	
K-Strophanthoside	
K-Strophanthin	
Isolanid	
Digoxin	

mixture of cardiac glycosides was separated on modified silica adsorbents. In Table II the structural formulae of the cardiac glycosides are presented.

A Spectra-Physics liquid chromatograph was used. The separation was effected with water–ethanol eluents at temperatures from 20 to 60°C. The temperature of the columns was maintained within ± 0.1°C by a thermostated water-jacket. Fig. 3 shows the separation of G-strophanthin, erysimin, cymarin and neriolin on LiChrosorb Si 100 (7 μm) with columns of 125 mm length and 4.8 mm I.D. The first glycoside to be eluted from the columns is G-strophanthin, *i.e.*, the most hydrophilic molecule[6], then erysimin and cymarin which have the same steroid part but different glycone part of the molecules. Cymarose in cymarin is more hydrophobic than digitoxose in erysimin. Thus cymarin is eluted later than erysimin. Neriolin, which contains a more hydrophobic aglycon oleandrigenin than aglycon strophanthidin in cymarin and erysimin, is eluted last.

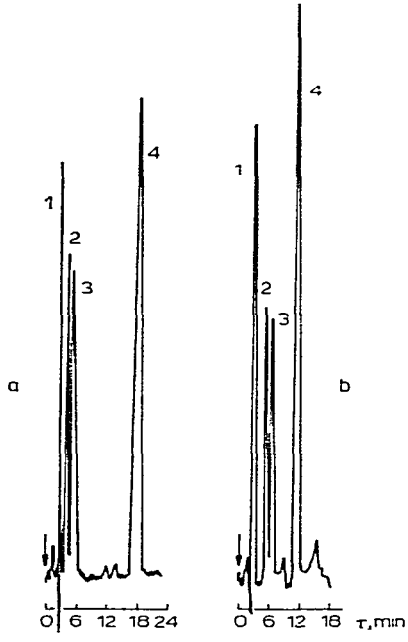


Fig. 3. Separation of cardiac glycoside mixture on LiChrosorb Si 100 (7 μm) modified by octadecyltrichlorosilane (a) and diphenyldichlorosilane (b). Column: 125 × 4.8 mm; temperature 40°C. Eluent: ethanol–water (40:60); flow-rate 0.7 cm^3/min. Peaks: 1 = G-strophanthin; 2 = erysimin; 3 = cymarin; 4 neriolin.

Figs. 4 and 5 show the dependences of the logarithm of retention volume of these cardiac glycosides on inverse temperature and on the composition of the eluent for columns of silica modified by octadecyltrichlorosilane (Fig. 4) and diphenyldichlorosilane (Fig. 5). It is seen that increasing the ethanol content in the eluent decreases the retention volume of glycosides on the adsorbent with octadecyl groups faster than on the adsorbent containing phenyl groups. But increasing the temperature decreases the retention volumes on the adsorbent with phenyl groups faster than on

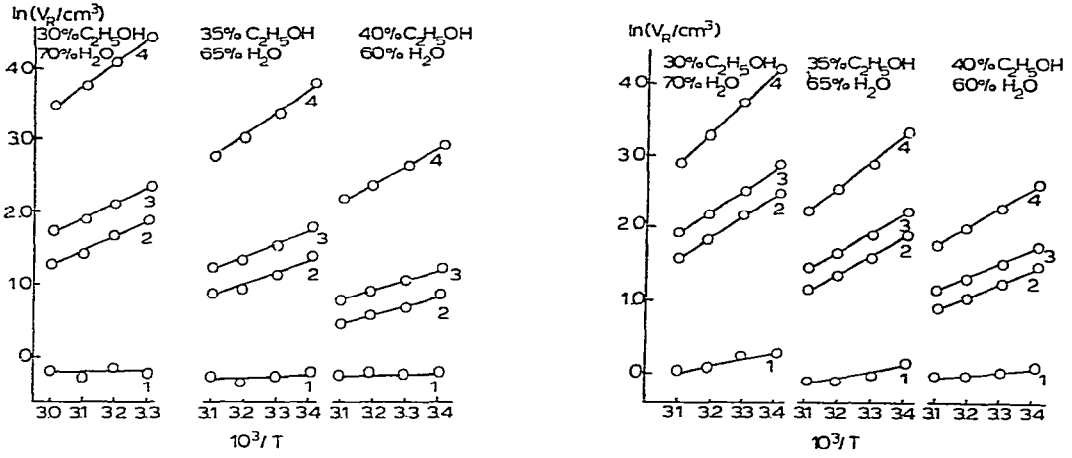


Fig. 4. Dependences of retention volume on temperature and eluent composition for cardiac glycosides on a column of LiChrosorb Si 100 (7 μm) modified by octadecyltrichlorosilane. Glycosides as in Fig. 3.

Fig. 5. Dependences of retention volume on temperature and eluent composition for cardiac glycosides on a column of LiChrosorb Si 100 (7 μm) modified by diphenyldichlorosilane. Glycosides as in Fig. 3.

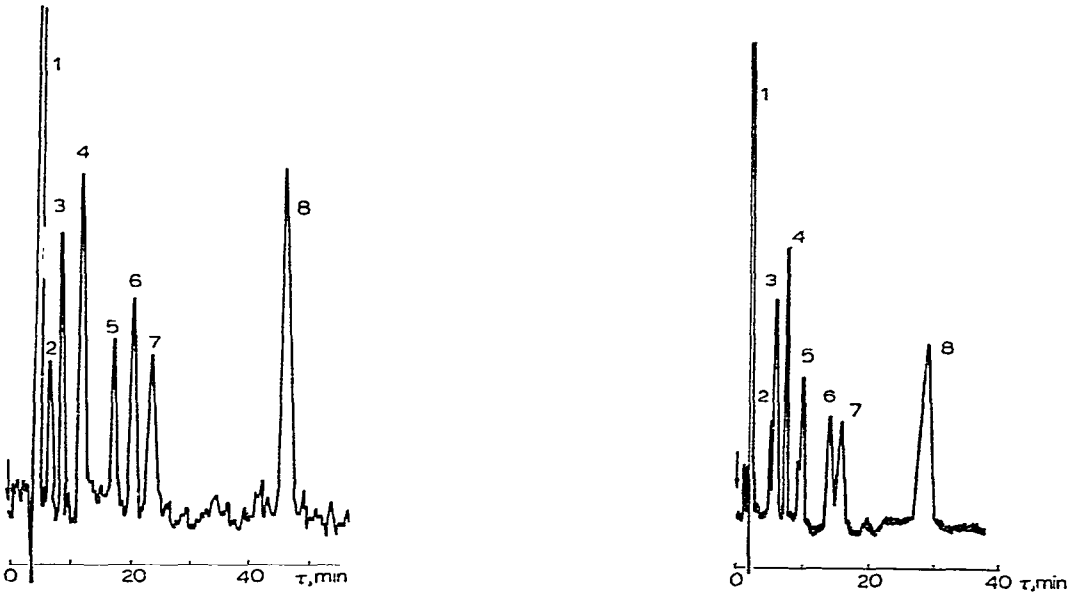


Fig. 6. Separation of cardiac glycoside mixture on LiChrosorb Si 60 (5 μm) modified by diphenyldichlorosilane. Column: 125 × 4.8 mm; temperature 50°C. Eluent: ethanol–water (40:60); flow-rate 0.34 cm³/min. Pressure drop 38 atm. Peaks: 1 = G-strophanthin; 2 = K-strophanthoside; 3 = K-strophanthin; 4 = erysimin; 5 = cymarin; 6 = isoland; 7 = digoxin; 8 = neriolin.

Fig. 7. Separation as in Fig. 6 but on LiChrosorb Si 100 (7 μm) modified by diphenyldichlorosilane. Column: 125 × 4.8 mm; temperature 40°C. Eluent: ethanol–water (30:70); flow-rate 0.7 cm³/min. Pressure drop 35 atm.

the adsorbent with octadecyl groups. Thus for the separation of cardiac glycosides on silica with octadecyl groups it is better to change the composition of eluent, but on silica with phenyl groups it is better to change the temperature.

Figs. 6 and 7 show the separations of eight components of cardiac glycosides on LiChrosorb Si 60 ($d_p = 5$ μm, $s = 500$ m^2/g) and on LiChrosorb Si 100 ($d_p = 7$ μm, $s = 285$ m^2/g) with attached phenyl groups. To reduce the analysis time on the LiChrosorb Si 60 column the eluent with a higher ethanol content was used in comparison with LiChrosorb Si 100, the capacity factor of which is smaller. For comparison, the same mixture was separated (Fig. 8) on the HiBar pre-packed column of LiChrosorb RP-8 (Merck).

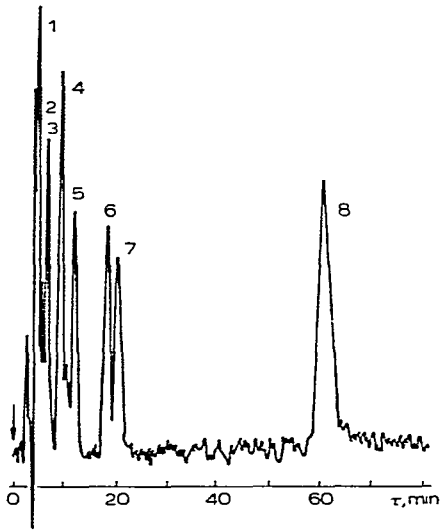


Fig. 8. Separation as in Fig. 6 but on HiBar pre-packed column of LiChrosorb RP-8 (7 μm). Column: 250 × 3 mm; temperature 50°C. Eluent: ethanol–water (30:70); flow-rate 0.34 cm^3/min. Pressure drop 135 atm.

It has been shown previously[14–17] that a particle size of 7 μm and a column of 100–150 mm length and ≈ 5 mm I.D. are optimal as regards the pressure drop in the column, the efficiency and the time of analysis. In the case of the cardiac glycosides, the column packed with LiChrosorb Si 100 ($d_p = 7$ μm) with attached phenyl groups gave better separations.

The elution order of glycosides from the columns under investigation is determined by the relative content of hydroxyl and hydrophobic groups in the molecules, but not by molecular weight.

Fig. 9 shows the selectivity, α, of the columns packed with silica modified by octadecyltrichlorosilane and diphenyldichlorosilane. It is seen that α for erysimin, cymarin and neriolin relative to G-strophanthin decreases with increasing ethanol concentration and with increasing temperature on both adsorbents. The dependence on ethanol concentration was stronger for the silica modified by octadecyltrichlorosilane.

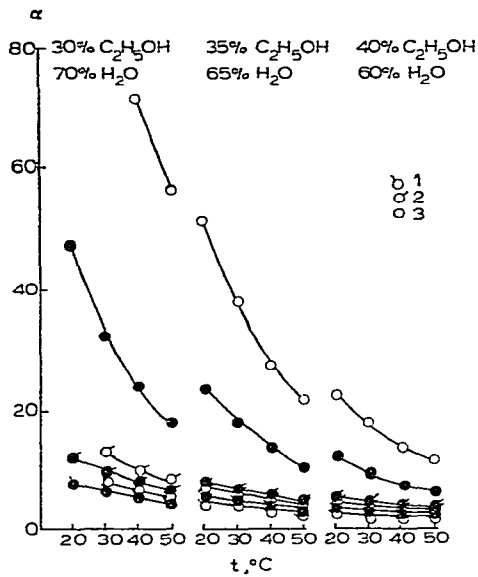


Fig. 9. Dependence of selectivity, α, on temperature and eluent composition for columns packed by LiChrosorb Si 100 (7 μm) modified by octadecyltrichlorosilane (open symbols) and diphenyldichlorosilane (closed symbols). Curves: 1 = erysimin; 2 = cymarin; 3 = neriolin, all relative to G-strophantin.

Such a large difference in the dependence of α on the ethanol content in the eluent is probably connected with the nature of the modifying layer. The layer formed by long hydrocarbon chains changes thickness with the ethanol concentration in water, while phenyl groups attached to the surface form a stable layer the structure of which is not dependent on the eluent composition. In the latter case the adsorption mechanism of separation operates, but in the former case the separation mechanism is quasi-partition and will depend on the eluent composition, although one probably cannot regard the long hydrocarbon chains attached to the surface as stationary liquid phase on the surface because the density of the hydrocarbon "brush" is less than that of the hydrocarbon liquid and because the chains are attached at one end. In a non-polar solvent, for example some hydrocarbons, these attached chains may

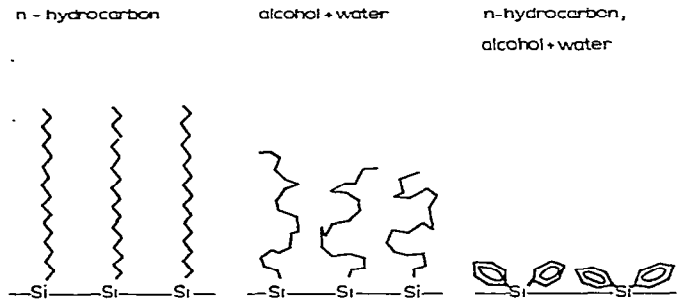


Fig. 10. Schematic picture of the dependence of the possible conformations of the functional groups attached to the silica surface on the eluent composition.

indeed form a "brush", but in the presence of water the chains may roll up and interact with each other and the thickness of the modifying layer will change.

Fig. 10 shows possible structures of modifying layers consisting of octadecyl and phenyl groups. Owing to conformational mobility, the hydrocarbon chains may be arranged in different ways near the surface, but in the case of attached phenyl groups the modifying layer will be independent of eluent composition.

The high carbon content of the phenyl rings attached to the surface results in a high capacity factor and a stable modifying layer in any eluent.

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