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Characterization of dynamically prepared phospholipid-modified reversed-phase columns

Irena Tsirkin, Eli Grushka*

Department of Inorganic and Analytical Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel

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Abstract

We have modified a reversed-phase (RP8) column by passing through it an aqueous solution of phosphatidylcholine-based liposomes. The phospholipids from the liposomes adsorb onto the octyl chain of the stationary phase, thus altering the nature of the stationary phase and of the chromatographic interactions. The properties of the phospholipid-modified column were investigated using solutes belonging to several chemical classes. We found that the retention factors of negatively and positively charged solutes decreased as the amount of phospholipid in the column was increased. For the solutes studied here the extent of the decrease was smaller for the positive solutes. With neutral solutes, the retention factors of some (benzenediols) increased markedly while with others (ketones) the retention factors decreased. The selectivities between the various solutes on the phospholipid-modified column were different than on the original reversed-phase column. The retention behavior of the solutes can be explained in terms of (1) effective shielding of the hydrophobic part of the stationary phase by the polar head groups of the phospholipids and (2) hydrogen bond formation between the solutes and the carbonyl oxygens as well as the non-ester phosphate oxygens in the polar head groups of the phospholipids.

Keywords: Phospholipids; Phosphatidylcholine; Stationary phases, LC; Liposomes

1. Introduction

Selectivity in HPLC is controlled by manipulating the partition coefficients of the solutes to be separated. Such control of the selectivity is achieved by changing either the temperature of the chromatographic system or the difference in the solute's standard chemical potential in the stationary and the mobile phases; i.e. by changing the nature of the mobile and/or the stationary phase(s). The latter approach is most often favored in HPLC over changes in the temperature.

One approach to change the nature of the mobile phase is to add a component that can interact selectively with one of the solutes and alter its partition coefficient relative to other solutes. The presence of the added component affords an additional chemical equilibrium, the so-called secondary chemical equilibrium, over and above the partitioning equilibrium of the solute between the two phases. In the present paper, we report on the use of a phospholipid as an additive to the stationary phase in RPLC.

In an aqueous solution, phospholipids aggregate to

*Corresponding author. Tel.: +972-2-658-5311; fax: +972-2-658-6201.

E-mail address: eliga@chem.ch.huji.ac.il (E. Grushka).

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form liposomes. Liposomes are spherical vesicles of bilayered lipid membrane. The bilayered form of liposomes gives them substantial internal stability, thus extending their useful life span from weeks to months. The additional stability makes it easier to manipulate the liposomes experimentally.

Some of their properties render the liposomes as ideal tools in analytical chemistry. Due to the similarity of the liposomes structure to the natural membranes, they have been used as membrane models for more than 30 years and much information is known about their properties (viz. [1–6]). A relatively large number of solutes such as phenols [7], imidazoline derivatives [8] and alkylaminobenzoates [9] undergo partition between liposomes and the aqueous medium that contains the liposomes. Traditionally, these partitioning effects were exploited for drug delivery systems. The use of liposomes in analytical chemistry is much scarcer. Some of the analytical applications that were reported in the literature are spectroluminescence analysis [4], flow injection immunoassay [10,11], immunomigration analysis [12], and fluorescence based sensors [13,14].

In separation science the use of liposomes is attractive due to the partitioning of solutes in and out of the liposomes as mentioned above. The selectivity of the separations can be manipulated, at least in theory, by adding liposomes to the separation system. For example, several research groups have attempted to characterize liposomes as additives in capillary electrophoresis (e.g. Refs. [15–19]). Malek and Khaledi [20] have used liposomes as a carrier of the detection reagent in capillary electrophoresis.

Liquid chromatography (LC) should also benefit from the use of liposomes. LC has been used to characterize the liposomes themselves as well as their components. For example, TLC has been used to characterize the chromatographic behavior of liposomes containing various compounds (e.g. Refs. [21,22]). Size exclusion chromatography has been used to study the stability of liposomes (e.g. Refs. [23–25]). HPLC has been employed to quantitate the lipid components of vesicles (e.g. Ref. [26]) as well as to examine the contents of liposomes (e.g. Refs. [27,28]). However, of much greater interest is the use of liposomes to affect the quality of the separation.

Two general approaches can be found in the literature for the utilization of liposomes and liposome-like materials in liquid chromatography. One approach immobilizes whole liposomes on the chromatographic support. Lundahl and co-workers first described this approach [29] and have used it successfully in many applications (e.g. Refs. [30–37]). They have immobilized liposomes on gel beads (mostly agarose) either by coating them on the beads or by entrapping them inside the beads (see Refs. [38,39] and references therein). In addition, several other groups have described the coating of liquid chromatographic support with liposomes or liposome-related material (e.g. Refs. [40–42]).

The second approach bonds covalently phospholipids, or related compounds, to the chromatographic support, most often silica gel. Pidgeon and his co-workers have championed this approach, which they have termed immobilized artificial membrane (IAM)–HPLC (e.g. Refs. [43–51]). Pidgeon and co-workers maintain that IAM is easier to use than unbound liposomes and that IAM systems mimic better solid-phase membranes. They have used IAM to study the interactions between a large number of compounds of biological interest and phospholipids. Several other research groups have used and studied IAM systems (e.g. Refs. [52–55]).

All the above-mentioned papers indicate that liquid chromatographic stationary phases that contain liposomes or liposomal components, are useful both for a wide range of separations and for studying solute–membrane interactions. Great efforts were made to prove the validity of using phospholipid-based stationary phases as a model to study interactions between various solutes and cell membrane. Little attention was given to the chromatographic characterization of these stationary phases as media for selective separations. In the present work we extend the technique by coating phosphatidylcholine-based liposomes on conventional reversed-phase columns. In our experiments we observed that liposomes are adsorbed strongly to alkane chains of the reversed-phase stationary phase in the column. The adsorbed species change drastically the nature of the chromatographic interactions, leading to a different retention behavior and to different selectivities. The purpose of this paper is to characterize chromatographically the phospholipid-modified stationary

phase using some small solutes with varied functional groups. At this stage of the research we are interested in the general description of the interactions between a wide variety of analytes and the phospholipid layer.

2. Experimental section

2.1. Chemicals

The water used throughout was purified and deionized with Seradest SD 2000 system (Germany). All the solutions were filtered through 0.20 μm RC-58 filters (Schliecher & Schuell) before being introduced to the HPLC system.

$Na_2HPO_4 \cdot 7H_2O$ was purchased from Merck (Darmstadt, Germany). Egg phosphatidylcholine used for liposome preparations was obtained from Lipoid (Ludwigshafen, Germany). The solutes studied were structural isomers of benzenediol (Sigma, USA), isomers of toluic acid (Aldrich, USA), D-tryptophan (Sigma), benzylamine (Merck), *p*-anisidine (Sigma), acetone, 2-butanone, 2-pentanone, *p*-phenylenediamine (Aldrich), and sodium nitrate (J.T. Baker, USA). HPLC grade methanol, used as the organic modifier, was purchased from J.T. Baker (The Netherlands). In some of the studies sodium dodecylsulfate (Sigma) was used as a mobile phase additive.

The buffer for the mobile phase was prepared by titrating 0.05 *M* disodium hydrogen phosphate salt solution with phosphoric acid (J.T. Baker, The Netherlands) to pH 7.0 or 6.2 depending on the experiment done. The mobile phase consisted of the above phosphate buffer and methanol in the ratio of 95:5 v/v.

Solute solutions for injection were prepared by dissolving the compounds in the mobile phase. The concentration of these solutions varied between 2×10^{-4} *M* and 4×10^{-4}, except in the case of D-tryptophan whose concentration was 3×10^{-5} *M*.

2.2. Instrumentation

The HPLC system was a Perkin-Elmer Series 4 HPLC equipped with a variable wavelength UV detector model LC-85B operated at 252 nm. The mobile phase flow-rate was 1 ml/min. Data collection was carried out using a Hitachi D-2000 integrator.

The reversed-phase columns used were LiChrosphere 100 RP-8, 125×4 mm, packed with 5 μm particles (Merck). The column was immersed in a water bath whose temperature was kept constant at 50°C, using a model TEP-1 temperature controller (Freed Electric, Israel).

2.3. Methods

2.3.1. Void volume measurement

$NaNO_3$, dissolved in the mobile phase at a concentration of 1×10^{-3} *M*, was injected in order to measure the void volume of the column.

2.3.2. Liposomes preparation

Egg phosphatidylcholine was dissolved in the mobile phase in the concentration of 10% of lipid by weight. Complete dissolution of the lipid was ensured using a vortex mixer. After the dissolution of the lipid, the mixture was passed through a mini-extruder (Avanti Polar Lipids Inc. USA) equipped with a 50 nm pore size filter. This process resulted in a solution containing small unilamellar vesicle (SUV) type liposomes, having a diameter of 50 nm.

2.3.3. Column preparation

Mobile phase containing liposomes in the concentration of 0.03% was passed through the reversed-phase column for given time intervals. At the end of each time interval the properties of the column were studied.

2.3.4. Ion-pair studies

The phospholipid-modified column was compared with an ion-pair system where the surface-active component was sodium dodecylsulfate (SDS). The mobile phase in this case was a mixture of 95% phosphate buffer (0.05 *M*, pH 6.2) containing SDS and 5% methanol. All the chromatographic measurements were made at 30°C. The column was first saturated with SDS by passing through it the mobile phase containing 7 m*M* SDS. Then, for the actual runs the mobile phase contained 1 m*M* SDS.

2.3.5. Verification of phospholipid adsorption on the RP-8 column

To verify that liposomes were adsorbed onto the stationary phase, we used electron probe microanalysis (EPMA, Joel JXA-8600). For that purpose, we used the packing from a used column that was modified with liposomes. For comparison, we also examined the packing from a column that was not treated with a liposome solution.

3. Results and discussion

In the experiments described here, a mobile phase solution containing liposomes was passed through a reversed-phase column. The results, to be discussed shortly, show that the liposomes are adsorbed onto the reversed-phase material in the column. However, the exact fate of the adsorbed liposomes is not entirely obvious. Lundahl and co-workers maintain in some of their papers (viz. [35]) that the liposomes remained intact on the stationary phase gels. At this stage of our research we cannot state with certainty whether the liposome structure remains intact or collapses in the column. Furthermore, if we assume that the liposome structure is disrupted, we do not know if the adsorbed layer is ordered or randomly distributed on the reversed-phase foundation. However, the long-term stability of the adsorbed layer (k' values varied about 2–3% over a 2 to 3-week period of heavy use) suggests that the liposomes break down and that the phospholipid molecules adsorb onto the reversed-phase packing (RP8) in the column. In addition, the retention behavior of the solutes studied here indicates that the liposomes are adsorbed with the hydrophobic chains towards the bonded C8 and with the polar heads being solvated by the mostly aqueous mobile phase.

3.1. Verification of phospholipid adsorption on the RP-8 column

To verify that phospholipids were adsorbed on the stationary phase we removed the packing from one of the columns that were loaded with the liposome solution and subjected it to electron probe microanalysis. Fig. 1 compares the X-ray emission from packing taken from a column that was not treated with liposomes and packing from a column through which a liposome solution was passed. The mobile phase used with both columns was a phosphate buffer. While both packings show a peak of silicon, a positive phosphorus signal was found only in the case of the column treated with the liposome solution. This phosphorus signal indicates that the phospholipid moieties were adsorbed onto the reversed-phase packing.

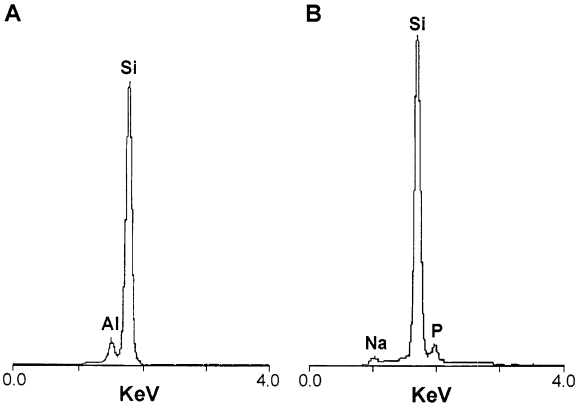

Fig. 1. The results of examination of column packing by electron probe microanalysis. The left panel is of a column packing that was not exposed to liposomes. The right panel is of a packing treated with liposomes. While both packing materials were exposed to phosphate buffer, only the packing exposed to phospholipids shows a peak of phosphorus.

To ascertain typical amounts of phospholipid in the modified columns, we washed out one of the columns with methanol, which we then evaporated. The residue was subjected to phosphorus analysis using ICP. We found that 0.175 g of phosphatidylcholine were retained on the column as a result of the loading process.

3.2. Changes in the chromatographic behavior of test solutes with the adsorption of phospholipids on the column

In the following experiments the columns were loaded with the phospholipid by passing through them, at a constant flow-rate, a mobile phase containing liposomes. The amount of phospholipid adsorbed onto the stationary phase is proportional to the length of the time of loading. Therefore, we expect that changes in the chromatographic prop-

erties, such as retention times and retention factors, will be a function of the loading time as well. Miyake et al. [41], who studied silica gel columns loaded with phospholipids, observed this dependency between the amount loaded and the retention factor.

The phospholipid adsorption process required that a substantial volume of the liposomes containing buffer be pumped through the column. For example, we see from Fig. 2 that it took over 1000 min to reach saturation. Since the flow-rate of the loading buffer is 1 ml/min, that means that about a litre of solution had to be passed through the column to saturate the stationary phase with the phospholipids.

The chromatographic behavior that we have observed in the present study will be presented according to the charge of the solutes examined. Firstly, we will describe the experimental results and then we will discuss the chromatographic reasons for the observations.

3.2.1. Void volume

The adsorption of phospholipids on the column decreases significantly the void volume of the column. Fig. 2 plots the retention time t_m of the void volume marker versus the time of column loading. The decrease in t_m with increasing amounts of phospholipids can be explained in terms of a decreased access to support pores due to the shielding effect of the adsorbed layer(s) of phospholipids.

The sigmoid shape of the curve in Fig. 2 is indicative of an adsorption isotherm. The plateau at long coating times is due to the saturation of the stationary phase with the phospholipids. A similar sigmoidal behavior was found with the dependence of the retention times and retention factors of the various solutes studied here on the amount of adsorbed phospholipid.

3.2.2. Negatively charged solutes

Of the solutes studied here the two isomers of toluic acid and D-tryptophan were negatively charged at the condition of the experiments (phosphate buffer at pH 7.0). The precision of the retention data was between 1 and 1.5% RSD. Fig. 3 shows that the retention factors, k', of these solutes decrease with an increase in the adsorption time, that is, with an increase in the amount of adsorbed phospholipids. As with t_m, the retention factors approach an asymptote at high levels of adsorbed phospholipid. The rate of decrease in k' is solute dependent, which means that the selectivity of the chromatographic system is also a function of the amount of phospholipid in the column. A case in point is the selectivity between tryptophan and *p*-toluic acid. At zero or low amounts of phospholipid the retention order of these two solutes is tryptophan before *p*-toluic acid. However, at high levels of phospholipid in the column the elution order is reversed. Fig. 4 plots the selectivity between tryptophan and *p*-toluic

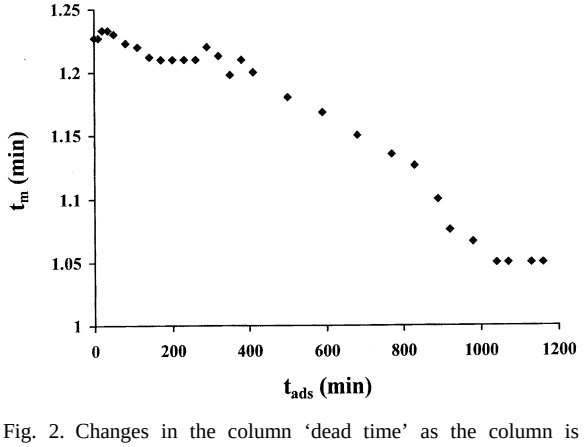

Fig. 2. Changes in the column 'dead time' as the column is loaded with phospholipids. t_{ads} represents the time that it took to pass the liposome solution through the reversed-phase column at a flow-rate of 1 ml/min.

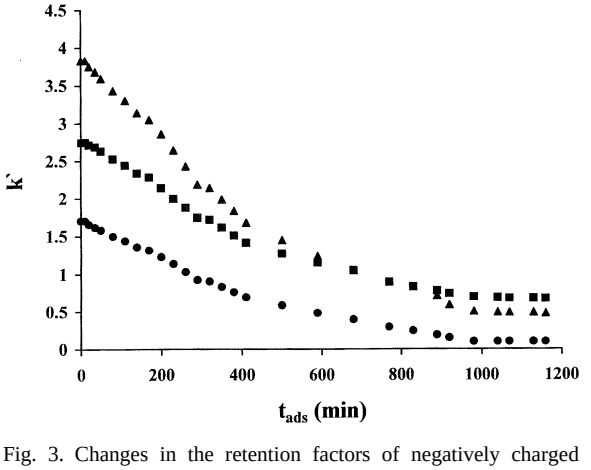

Fig. 3. Changes in the retention factors of negatively charged solutes as the amount of phospholipid in the column is increased. ▲, *p*-toluic acid; ●, *o*-toluic acid; ■, D-tryptophan. For the meaning of t_{ads} see the text as well as the caption of Fig. 2.

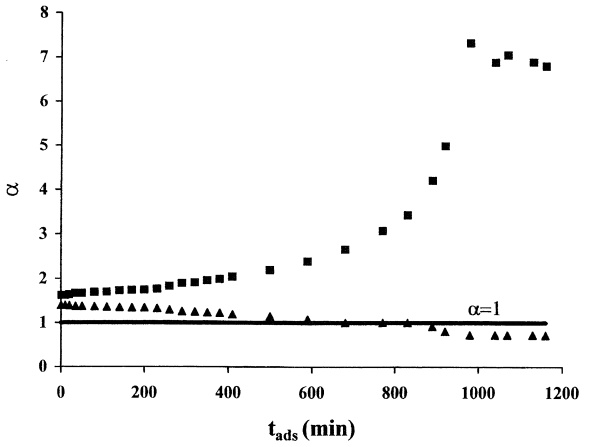

Fig. 4. Changes in the selectivity between tryptophan and *p*-toluic acid (▲) and between tryptophan and *o*-toluic acid (■) as a function of the phospholipid loading in the column. Values of the selectivity below the line $\alpha=1$ indicate retention reversal of the solutes in the pair. For the meaning of t_{ads} see the text as well as the caption of Fig. 2.

acid and between tryptophan and *o*-toluic acid. The change in the α value for the tryptophan–*p*-toluic acid pair from $\alpha>1$ to $\alpha<1$ is due to the retention order reversal indicated above. The seemingly large increase in the selectivity between the solutes in the second pair should be examined more carefully. The retention factor of *o*-toluic acid decreases to values very close to zero. Therefore, although the precision of the data is good, a small error in the determination of either the retention time or t_m will give an error in k', which will cause a large error in α. Still, the selectivity values seem self-consistent, including the plateau in α at high amounts of adsorbed phospholipid.

Fig. 5 shows five chromatograms that include the above three solutes at different phospholipid loadings. We see clearly the decrease in the retention times of all three solutes as the amount of adsorbed phospholipid is increased. The retention order change between tryptophan and *p*-toluic acid is also evident in the figure.

3.2.3. Positively charged solutes

The solutes that we used for this section of the study were *p*-anisidine, *p*-phenylenediamine and benzylamine. To ensure the positive charge of the solute, the experimental conditions were modified slightly. The mobile phase was still a 95:5 mixture of phosphate buffer and methanol but here the pH of the aqueous component was 6.2 rather than 7 as in the previously described experiments. At this pH the benzylamine is 100% ionized, *p*-phenylenediamine is 47% ionized and *p*-anisidine is 11% ionized. In this set of experiments we did not follow the

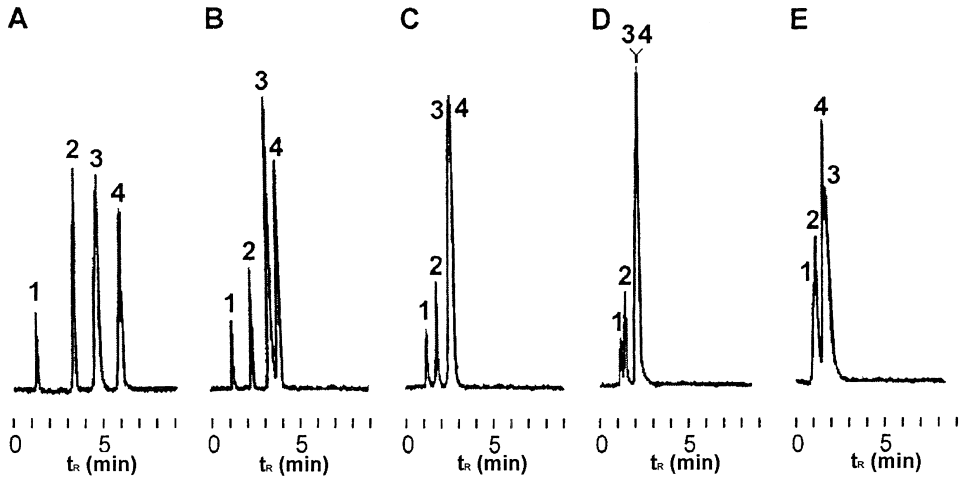

Fig. 5. The effect of the amount of phospholipid in the column on the chromatographic behaviour of *o*-toluic acid, tryptophan and *p*-toluic acid. Each panel represents a different phospholipid loading volume: (A) 0 ml (the original RP8 separation), (B) 290 ml, (C) 590 ml, (D) 830 ml, (E) 1160 ml. Solute identification is as follows (1) $NaNO_3$ as void volume marker, (2) *o*-toluic acid, (3) tryptophan and (4) *p*-toluic acid.

complete phospholipid adsorption curve but rather we first fully loaded the column with the phospholipid and then injected the solutes. In essence, we collected the chromatographic data at the beginning and at the plateau region of the phospholipid adsorption curve.

In Table 1 we compare the k' values of the three positively charged solutes as obtained on a reversed-phase column and on the phospholipid-modified column. As can be seen, the retention factors decrease although at a slower rate than that observed with the negatively charged solutes. The selectivities between the solutes change as well. For the pair *p*-phenylenediamine/benzylamine α increases from about 3.6 to 6 while for the solute pair benzylamine/*p*-anisidine α decreases from about 3.9 to 2.8.

3.2.4. Neutral solutes

The chromatographic behavior of the neutral solutes in the phospholipid-modified column is more complicated than that observed with the negatively and positively charged solutes. The neutral solutes used in the study belong to two categories: alcohols and ketones.

Under the conditions of the experiment (phosphate buffer mobile phase, pH 7), benzenediols are uncharged. The behavior of these solutes in the phospholipid-modified column is drastically different than the behavior of both the negatively and positively charged solutes. As seen in Fig. 6, the retention factors of the solutes increase substantially with increasing amounts of the adsorbed phospholipid. As in the previous cases, at high phospholipid loadings the retention factors of the benzenediols approach limiting values. The selectivity between the two benzenediols (Fig. 7) decreases slightly as the amount of phospholipid in the column is increased. The maximum in the selectivity at adsorption time of about 300 min is most likely due to experimental fluctuations in the behavior of the individual retention factors of the two solutes. We see from Fig. 6 that at about 300 min into the adsorption process the retention factor of the *o*-benzenediol increases slightly faster than the retention factor of the *m*-ben-

Table 1
Retention factors of (a) positively charged solutes and (b) some ketones obtained on a native RP8 column and on the same column loaded with phospholipid. The mobile phase: phosphate buffer, 0.05 *M*, pH 6.2 with 5% of methanol

Solute	RP8 column	Modified column
(a) Positively charged solutes		
p-Phenylenediamine	0.92	0.38
Benzylamine	3.35	2.26
p-Anisidine	13.21	6.22
(b) Ketones		
Acetone	1.66	0.53
2-Butanone	3.69	1.49
2-Pentanone	15.42	4.15

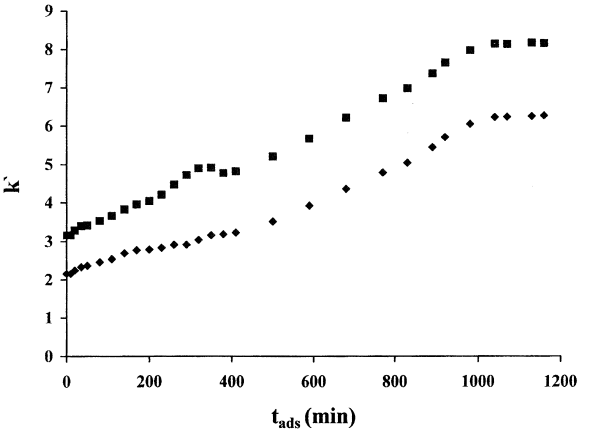

Fig. 6. The behavior of the retention factors of benzenediols as a function of the amount of phospholipid in the column. ♦, *m*-benzenediol; ■, *o*-benzenediol. For the meaning of t_{ads} see the text as well as the caption of Fig. 2.

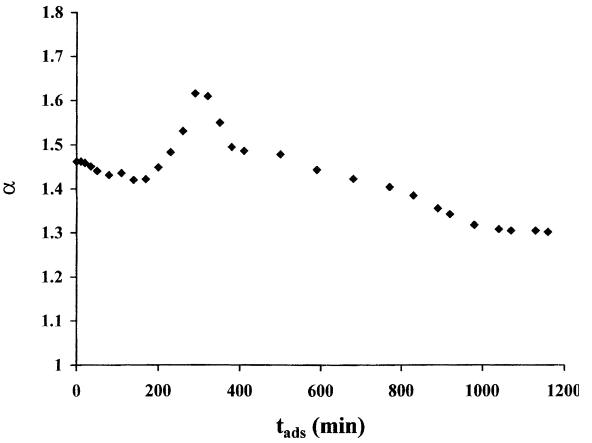

Fig. 7. The dependence of the selectivity between *m*-benzenediol and *o*-benzenediol as a function of the amount of phospholipid in the column. For the meaning of t_{ads} see the text as well as the caption of Fig. 2.

zenediol. This difference in the rate of k' increase is manifested in the maximum in the selectivity plot in Fig. 7.

The three ketones studied here behave differently than the diols discussed above. Their retention factors decrease rather than increase. In Table 1 we show the retention factors of the ketones on the RP8 column and on the phospholipid-modified column. The selectivity between the three solutes is different in the two columns. For the butanone/acetone pair the α value increases from about 2.2 on the RP8 column to 2.8 on the phospholipid column. For the butanone/pentanone pair the selectivity decreases from 4.2 to 2.8.

3.3. Retention mechanism

It is clear that the presence of the phospholipid affects greatly the retention of the solutes studied here. Importantly, the magnitude of the effect is proportional to the amount of the phospholipid loaded on the column. Thus, whatever the change in the column properties is, that change occurs gradually as the amount of the phospholipid in the column is increased. The actual nature of the change in the chromatographic behavior between the native reversed-phase column and the phospholipid-loaded column is a function of the chemical nature of the solutes. The partition coefficients of the charged solutes, both positive and negative, decreased upon the introduction of the phospholipid. Neutral solutes showed both an increase and a decrease in their partition coefficients.

Phospholipids, having a polar head group and a hydrophobic tail, are reminiscent of the conventional ion-pair chromatographic reagents such as sodium dodecylsulfate (SDS). However, the retention characteristic of the phospholipid-modified column is not at all similar to the retention behavior in ion-pair systems. Table 2 shows a comparison between the retention behavior of some of the solutes studied here in a phospholipid-modified column and in an SDS ion-pair column. At the conditions of the experiments the SDS is negatively charged.

The behavior of the ion-pair column is well understood in terms of electrostatic interactions. The positively charged solutes are greatly retained on the negatively charged SDS modified column while all other solutes are minimally affected. With the phospholipid column *negative and positive solutes* elute faster than on the reversed-phase column. An increase in the retention is observed with some *neutral solutes*. Clearly, the ion-pair column and the phospholipid column behave differently. Should we have used a positively charged ion-pair reagent we would have observed an increase in the retention of the anionic solutes while the rest of the solutes would have been minimally affected.

The retention behavior of the solutes on the phospholipid-modified column can be explained as follows. The mobile phase used contained a very small amount of methanol (5%). With this mobile phase, before the loading with the liposomes, the C8 chains of the reversed-phase material are most likely collapsed on the silica gel surface. The solutes in this case encounter a hydrophobic stationary phase. Upon loading the column with the liposomes, the long alkyl side chains of the phospholipids adsorb strongly to the collapsed C8 phase by hydrophobic interactions. The polar head groups of the phospholipid,

Table 2
A comparison in the retention factors of several solutes in a reversed-phase column, a phospholipid-modified column and an ion-pair column (SDS). The mobile phase used with all three columns was a mixture of 95% phosphate buffer (pH 6.2) and 5% methanol

Solute	k' on native RP8 column	k' on phospholipid-modified column	k' on SDS modified column
o-Toluic acid	2.15	0.41	0.31
p-Toluic acid	5.79	1.71	1.32
Tryptophan	3.81	1.44	5.61
o-Benzenediol	3.92	14.4	5.83
m-Benzenediol	3.22	12.3	2.04
p-Phenylenediamine	0.92	0.38	13.6
Benzylamine	3.35	2.26	30.7

which point away from the hydrophobic sections, are solvated by the aqueous mobile phase in the column. Theoretically, the solutes can interact with the polar heads and/or the hydrophobic part of the adsorbed phospholipid layer (see for example Ref. [49] and references therein). Solutes that interact strongly with the polar heads of the phospholipids cannot penetrate to the looser, more fluid-like, hydrophobic chains and therefore cannot partition into the liposome vesicles or pass through cellular membranes. Pidgeon and co-workers (viz. [48,49]) as well as Aguilar and co-workers [55,56] have used immobilized phospholipids to study chromatographically these two types of solute–phospholipid interactions and to differentiate between them.

In the present work the phospholipids were not bonded to the silica gel surface. Rather, the phospholipid molecules were dynamically adsorbed onto the reversed-phase material in the column. The decrease in the retention factors of most of the solutes studied here, in the presence of the phospholipids, is in agreement with the notion that the polar head groups, which point towards the mobile phase, shield very effectively the hydrophobic section of the stationary phase. As a result, the interaction between the solutes and the stationary phase weakens, resulting in lower partition coefficients and shorter retention times. The exceptions are those solutes that are capable of interacting strongly with the polar head groups, for example via hydrogen bonding with either the phospholipid carbonyl oxygens or the non-ester phosphate oxygens (e.g. Refs. [57,58]). Indeed, the benzenediols, which are hydrogen bond donors, are retained to a much greater extent on the phospholipid-modified column. In fact, we found the same phenomenon with several other phenols that we injected to the column (not detailed in the present paper); namely, the retention factors of the phenols on the phospholipid-modified column were higher than on the original RP8 column. Other workers have observed hydrogen bond effects on the chromatographic behavior of appropriate solutes in phospholipid-mediated chromatography; see for example the work of Miyake et al. who studied the properties of phospholipids adsorbed on silica gel [41].

Partial hydrogen bond interactions between the positive (amines) solutes and the phospholipid in the column may explain the smaller decrease in their retention factors as the amount of the phospholipids in the column is increased. The solutes possessing a ketone functionality can also form hydrogen bonds. However, since ketone is a hydrogen bond acceptor it cannot form hydrogen bonds with the phospholipids. As a result, these solutes interact minimally with the phospholipid stationary phase and their retention factors decrease in comparison to RP8.

4. Conclusions

It is clear from the results reported here that the passage of an aqueous phase containing phosphatidylcholine-based liposomes through a reversed-phase column alters the stationary phase, yielding in the process a new chromatographic column. While it is not clear from our results whether the liposomes are retained as a whole on the reversed-phase material in the column or whether they collapse as a result of hydrophobic interactions, the fact remains that liposome-related material is adsorbed onto the stationary phase. The loading of the column with the phospholipid required that a substantial volume of the liposome solution be pumped through the column before reaching saturation, typically 1 liter of solution containing 0.03% liposomes.

The adsorbed phospholipids change the chromatographic behavior of the column. We found that solutes that rely mainly on hydrophobic interactions for their retention elute faster on the phospholipid-modified column. Solutes that can interact with the polar head group of the phospholipid, for example, by forming hydrogen bonds, will be retained longer on the modified column. The results indicate that the phospholipids adsorb onto the reversed-phase material predominantly by hydrophobic interactions. The adsorbed phospholipid is arranged on the stationary phase in such a way that the polar heads are (a) pointing towards, and being solvated by, the aqueous mobile phase and (b) shielding effectively the underlying hydrophobic surface. The extent of the changes in the retention factors is a function of the functional groups of the solutes. As a result of changes in the retention factors of the solutes, the selectivities between them change as the column is loaded with the phospholipids.

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