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Aminosilica chemically modified with dodecamolybdophosphoric acid as stationary phase in high-performance liquid chromatography

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Abstract

A commercially available aminosilica stationary phase, after being chemically modified with dodecamolybdophosphoric acid, can be used for the preparation of new columns for high-performance liquid chromatography. Aminosilica dodecamolybdophosphate mixed phase was obtained by reaction of LiChrosorb NH_2 (Merck) with dodecamolybdophosphoric acid (DMPA). The structure of this stationary phase was established by chemical and elemental analysis. The capacity factors for various organic compounds were determined using mixtures of tetrahydrofuran–*n*-hexane as mobile phase. The results were compared with the data obtained on a LiChrosorb NH_2 column. This new mixed chemically bonded stationary phase which contains electron-donor and electron-acceptor groups afford high selectivity for organic compounds with polar functional groups. © 1998 Elsevier Science B.V.

Keywords: Stationary phases, LC; Dodecamolybdophosphoric acid; Aminosilica

1. Introduction

Workers using high-performance liquid chromatography (HPLC) with nonaqueous mobile phases (‘normal-phase separation’) can choose among silica, alumina, or various polar bonded-phase columns [1]. Usually polar bonded phases carry functional groups with terminal polar non-ionizable substituents such as alcoholic hydroxyl, nitro, cyano and amino groups. The polar substituents are linked through a short hydrocarbon spacer (usually a propyl group) to the surface of the support. Bonded phases applied in normal-phase liquid chromatography are most useful for the analysis of organic soluble solutes and in particular for isomers. The polar character of the bonded phases dominates when mobile phases of low-to-moderate polarity are used in accordance with the eluotropic series of solvents and solvent mixtures introduced by Snyder [2].

Using derivatization techniques, aminosilica, as with amines, can be chemically modified in order to

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make it more suitable for a particular analytical procedure. It is known that the alkylammonium ions (mono, di, tri) react with dodecamolybdophosphoric acid (DMPA) $H_3PMo_{12}O_{40}\cdot 24H_2O$. The alkylammonium 12-molybdophosphates were quantitatively precipitated at room temperature [3]. We also observed that DMPA reacts with aminopropylsilica, producing a blue compound.

In this paper we present some experimental data obtained by using aminopropylsilica chemically modified with dodecamolybdophosphoric acid as stationary phase in high-performance liquid chromatography.

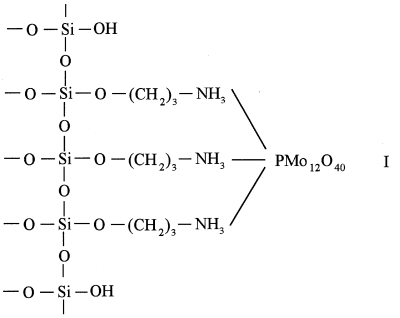
2. Experimental

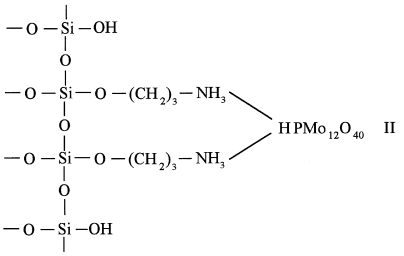
The synthesis of aminopropylsilica dodecamolybdophosphate constitutes a simple reaction between LiChrosorb NH_2 (Merck) and DMPA. The reaction takes place at room temperature by adding an excess of 0.001 *M* DMPA solution to LiChrosorb NH_2 followed by filtration and washing the obtained product with distilled water until the reaction for DMPA is negative [3]. The reaction product is air-dried and is insoluble in organic solvents commonly used in liquid chromatography.

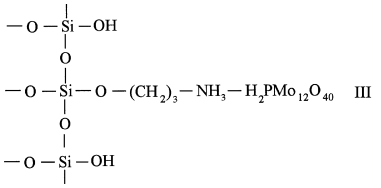
Similar to LiChrosorb NH_2, aminosilica dodecamolybdophosphate is stable in 0.1 *M* phosphate buffer between pH 2.5 and 8.5. The chemical and elemental analyses of the compound show the following quantitative results: DMPA 21.10%; C 3.30%; N 1.25%; P 0.27%; Mo 10.70%.

Taking into account the fact that the amount of S_{BET}LiChrosorb NH_2 used is 300 m^2/g and that the surface NH_2 concentration used is 4.2 $\mu M/m^2$, this results in a molar ratio between the $-NH_2$ groups and DMPA of 2.2:1. The fact that this ratio is not equal to 3:1, as in the case of ammonium ion or amines, can be explained by the different distribution of $-NH_2$ groups on the LiChrosorb NH_2 surface [3–5].

According to the spatial distribution of the NH_2 groups, we can predict three possible different chemical bonds between LiChrosorb NH_2 and dodecamolybdophosphoric acid $H_3[PMo_{12}O_{40}]$ as follows:







These three possible types of chemical bonds cannot be quantitatively estimated, but the fact that the molar ratio of the amino groups bonded to the acid is 2.2:1 is an indication that bonding types I and II are predominant.

3. Chromatographic experiments

The chromatographic experiments were performed with a Hewlett-Packard 1084 B liquid chromatograph equipped with variable wavelength detector (190–600 nm) with scanning capabilities, a variable volume injection system, a built-in printer/plotter and a digital integrator.

The columns (250×4.6 mm I.D.) were packed with aminosilica dodecamolybdophosphate obtained from LiChrosorb NH_2 10 μM (Merck) and DMPA (ANALAR) using a slurry packing procedure.

A modular packing system consisting of an air-driven pump (Haskel DST 122 C), a solvent reservoir, a commercially available, roughly 20-ml capacity filling tube and a working pressure range of 0–600 bar was used.

The slurry medium, isopropanol, and solvent packing, *n*-heptane, were LiChrosolv type (Merck). The column blank was filled with slurry medium (the slurry concentration was 12.5% w/v).

Before packing the suspension and solvent were treated for at least 3 min in a normal ultrasonic bath (Bransonic 2000, Danbury, CT, USA).

Good columns were obtained by pumping about 100 ml *n*-heptane at 500–600 bar. Tetrahydrofuran (THF) and *n*-hexane used as mobile phase were of LiChrosolv purity (Merck). All organic compounds and drugs used were analytically pure (0.1% w/v in THF and 10 μl injection volume).

Determinations were performed with a variable wavelength detector at 254 nm.

4. Results and discussions

Various organic compounds (aromatic hydrocarbons and derivatives of aromatics with different functional groups) were chromatographically separated on columns with LiChrosorb NH_2 and LiChrosorb NH_2 chemically modified with dodecamolybdophosphoric acid.

Experimental capacity factor (k') values for various solutes on these columns, using tetrahydrofuran–*n*-hexane as mobile phase, are reported in Tables 1 and 2.

The number of theoretical plates per meter calculated according to Snyder et al. [6], for some representative solutes on LiChrosorb NH_2 and LiChrosorb NH_2–DMPA columns used, are shown in Table 3, using as a mobile phase a mixture of tetrahydrofuran–*n*-hexane (50:50, v/v).

The results from Table 3 show that the values of the number of theoretical plates per meter (N) for LiChrosorb NH_2 and LiChrosorb NH_2–DMPA columns are comparable and indicate good efficiency and packing procedure for these columns.

The following observations can be made: the dependence of the experimental capacity factor (k') values on tetrahydrofuran (THF)–*n*-hexane ratio (v/v) is linear (higher concentrations of THF gave lower k' values). Such a dependence for various solutes on a LiChrosorb NH_2–DMPA column is illustrated in Figs. 1 and 2.

The capacity factor values of aromatic hydrocarbons were higher on a LiChrosorb NH_2–DMPA column at all ratios of THF–*n*-hexane used.

The strong retention of the aromatic hydrocarbons on the LiChrosorb NH_2–DMPA column as compared to LiChrosorb NH_2 can be explained by the fact that aminopropylsilica–DMPA contains electron-acceptor groups ($^{+}NH_3$) while, on the other hand, there are possible interactions between π orbitals of aromatic hydrocarbons and d orbitals of molybdenum from molybdophosphate.

The fact that the aminopropylsilica–DMPA col-

Table 1
Experimental capacity factors (k') values for various solutes on a LiChrosorb NH_2 column

Solute	Tetrahydrofuran–*n*-hexane (v/v)				
	10:90	20:80	30:70	40:60	50:50
Benzene	0.27	0.20	—	—	—
Naphthalene	0.30	0.22	—	—	—
Anthracene	0.54	0.41	0.25	—	—
Pyrene	0.89	0.72	0.56	0.38	0.23
Nitrobenzene	1.29	0.83	0.63	0.45	0.28
Phenol	—	—	10.97	8.23	6.13
Nitrophenol	—	—	12.80	10.34	8.44
Hydroquinone	—	—	13.24	10.85	8.91
Aniline	—	—	3.90	2.52	1.30
Benzoic acid	—	—	9.44	7.72	6.00
Methylanthranilic acid	—	—	8.00	6.52	5.10

Mobile phase: tetrahydrofuran–*n*-hexane; flow-rate, 1 ml/min.

Table 2
Experimental capacity factors (k') values for various solutes on a LiChrosorb NH_2–DMPA column

Solute	Tetrahydrofuran–*n*-hexane (v/v)				
	20:80	40:60	50:50	60:40	70:30
Benzene	1.36 (6.8)[a]	1.26	1.15	1.00	—
Naphthalene	1.57 (7.1)[a]	1.47	1.40	1.36	—
Anthracene	1.87 (4.6)[a]	1.66	1.53	1.42	—
Pyrene	2.12 (2.9)[a]	1.82 (4.8)[a]	1.70 (7.4)[a]	1.62	—
Nitrobenzene	2.65 (3.2)[a]	2.24 (5.0)[a]	2.10 (7.5)[a]	1.90	—
Phenol	2.97	2.31 (0.28)[a]	1.95 (0.32)[a]	1.70	—
Nitrophenol	—	2.98 (0.29)[a]	2.48 (0.29)[a]	2.00	—
Hydroquinone	—	4.00 (0.37)[a]	3.40 (0.38)[a]	2.65	2.05
Aniline	—	7.40 (2.94)[a]	5.80 (4.6)[a]	4.00	2.32
Benzoic acid	—	6.00 (0.78)[a]	4.92 (0.82)[a]	3.61	2.46
Methylanthranilic acid	—	5.10 (0.78)[a]	4.25 (0.83)[a]	3.48	2.50

Mobile phase: tetrahydrofuran–*n*-hexane; flow-rate, 1 ml/min.
[a]Ratio of *k* values for DMPA packing vs. aminosilica packing.

Table 3
Number of theoretical plates per meter (N) for some solutes on LiChrosorb NH_2 and Lichrosorb NH_2–DMPA volumes

Solute	$N \times 10^3$ LiChrosorb NH_2	$N \times 10^3$ LiChrosorb NH_2–DMPA
Pyrene	12.8	13.0
Nitrobenzene	12.8	13.1
Phenol	13.2	13.0
Nitrophenol	13.4	13.1
Hydroquinone	13.5	13.3
Aniline	13.0	13.5
Benzoic acid	13.2	13.1
Methylanthranilic acid	13.2	13.1

Mobile phase THF–*n*-hexane (50:50, v/v); flow-rate, 1 ml/min.

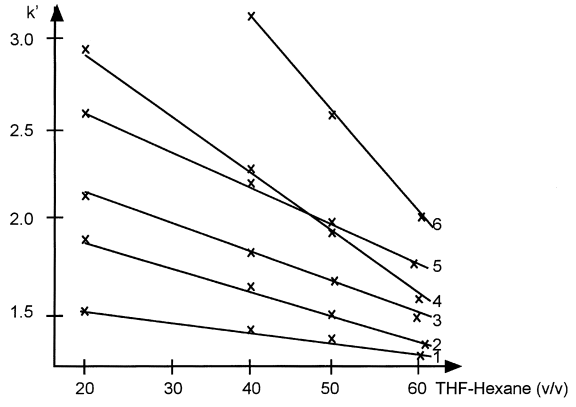


Fig. 1. Dependence of capacity factors (k') on THF–*n*-hexane ratio (v/v); flow-rate, 1 ml/min; column, LiChrosorb NH_2–DMPA. 1, naphthalene; 2, anthracene; 3, pyrene; 4, phenol; 5, nitrobenzene; 6, nitrophenol.

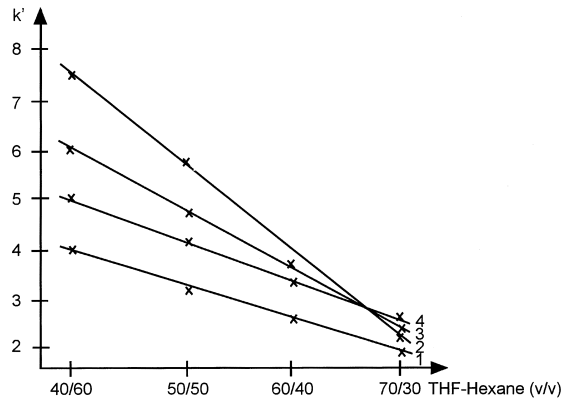


Fig. 2. Dependence of capacity factors (k') on THF–*n*-hexane ratio (v/v); flow-rate, 1 ml/min; column, LiChrosorb NH_2–DMPA. 1, hydroquinone; 2, aniline; 3, benzoic acid; 4, methyl anthranilic acid.

umn contains electron-acceptor and electron-donor groups shows the possibility of selective interactions with acidic as well as basic solutes. Accordingly, the stronger retention of the aniline on aminopropylsilica–DMPA and the reverse elution order of methylanthranilic acid and of aniline on the LiChrosorb NH_2 and LiChrosorb NH_2–DMPA columns can be seen.

However, the organic compounds with acidic groups (phenol, nitrophenol, hydroquinone, benzoic acid, methylanthranilic acid) are better retained on the column with LiChrosorb NH_2 compared to the column with LiChrosorb NH_2–DMPA.

The strong interaction of these solutes with the LiChrosorb NH_2 column may be evidently attributed to their ability to form hydrogen bonds, while in the LiChrosorb NH_2–DMPA column these types of interactions are diminished by steric hindrance effects due to molybdophosphate groups.

The fact that organic compounds with basic functional groups (amines, ketones, ethers, esters) are weakly retained on the LiChrosorb NH_2 column [7], while they are more strongly retained on aminosilica dodecamolybdophosphate due to strong interactions, persuaded us to use the LiChrosorb NH_2–DMPA column for purity determination of some methylated xanthines (caffeine, theophylline), estrogen and corticosteroid hormones, in order to check the potential of the new stationary phase in the pharmaceutical analysis and separation of drugs.

These separations were performed rapidly by normal-phase liquid chromatography using an aminosilica dodecamolybdophosphate column and a tetrahydrofuran–*n*-hexane mixture as mobile phase (Figs. 3–5).

It is of interest to mention that, under the chromatographic conditions applied in Figs. 3–5, these solutes are not separated by the LiChrosorb NH_2 column.

Fig. 3 shows the chromatogram of a mixture of estriol, estradiol and estrone on a LiChrosorb NH_2–DMPA column, while Fig. 4 shows the separation of the caffeine and theophylline, and Fig. 5 illustrates the chromatogram of a hydrocortisone sample.

It should be taken into account that these separations should be regarded only as an attempt to demonstrate the utility of aminosilica dodecamolybdophosphate as stationary phase in normal-phase liquid chromatography as a potential stationary phase which could be useful in pharmaceutical analysis.

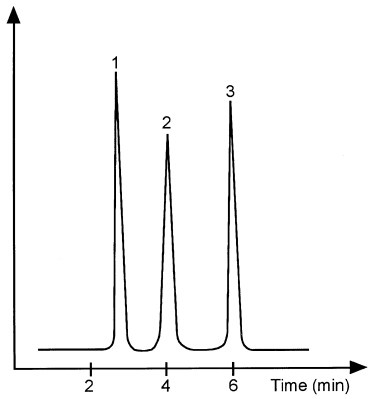


Fig. 3. Separation of a mixture of hormones on a stainless-steel column (25 cm×4.6 mm I.D.) packed aminopropylsilica dodecamolybdophosphate (10 μm); mobile phase, THF–*n*-hexane (60:40, v/v); flow-rate, 1 ml/min; detector, UV 254 nm. 1, estriol; 2, estradiol; 3, estrone.

5. Conclusions

The reaction product between aminopropylsilica and dodecamolybdophosphoric acid (DMPA) produces an interesting stationary phase which can be used in normal-phase liquid chromatography. This new stationary phase was found to have comparable

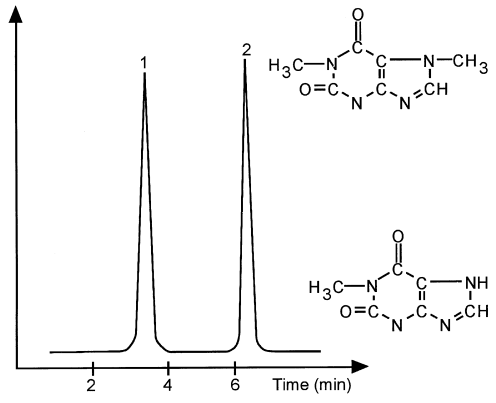


Fig. 4. Separation of a mixture of caffeine and theophylline (conditions as in Fig. 3); mobile phase, THF–*n*-hexane (70:30, v/v); flow-rate, 1 ml/min. 1, caffeine; 2, theophylline.

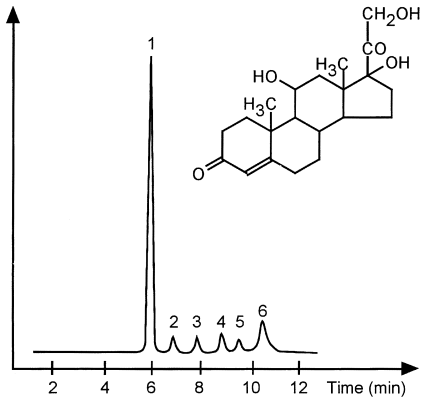


Fig. 5. Purity determination of a hydrocortisone sample (conditions as in Fig. 3). 1, hydrocortisone; 2,3,4,5,6, unidentified components.

efficiency when compared with the aminopropylsilica phase.

The fact that the aminopropylsilica–DMPA contains electron-acceptor and electron-donor groups gives the possibility for selective interactions with the acid as well as the basic solutes used in this study; this was shown by improved selectivity.

Further investigation is currently in progress to exploit the potential of this mixed stationary phase for enantioselective resolution utilised in chiral chromatography.

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