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New stationary phases for the high-performance liquid chromatographic separation of nucleosides and cyclic nucleotides Synthesis and chemometric analysis of retention data

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Abstract

Eleven new chemically bonded silica stationary phase materials were synthesized and characterized physicochemically in search for the separation systems suitable for analysis of natural nucleosides and nucleotides. A set of 11 fundamental nucleosides and cyclic nucleotides was analyzed in 15 reversed-phase high-performance liquid chromatographic (RP-HPLC) systems. The HPLC systems comprised additionally 4 reference columns of different chemical structure. For the test solutes 10 structural descriptors were derived by molecular modelling. Sets of retention parameters and of structural descriptors were subjected to chemometric analysis by the method of principal component analysis (PCA). It was demonstrated that of the total number of 11 newly synthesized stationary phase materials 8 hydrocarbon-bonded silica phases had separation properties regarding nucleosides and nucleotides similar to a standard octylsilica phase. The phases which comprised aromatic fragments in their ligands did not differ significantly from the aliphatic hydrocarbon-silica materials. The new phases comprising methylimidazole and to a lesser extent pyridine and cyano moiety, had specific properties regarding the separation of nucleosides and cyclic nucleotides. It has been demonstrated that PCA of structural descriptors of solutes obtained by molecular modelling facilitates identification of structural features which determine retention in individual HPLC systems.

Keywords: Stationary phases, LC; Chemometrics; Principal component analysis; Structural analysis; Molecular modelling; Factor analysis; Nucleosides; Nucleotides, cyclic

1. Introduction

Optimization of separation of natural nucleosides and cyclic nucleotides is important in analytical biochemistry. To achieve this aim new separation systems are designed and tested. In high-performance liquid chromatography (HPLC) the most effective separation factor is the chemical structure of the stationary phases. Numerous stationary phase materials are available. However, for a defined set of solutes like natural nucleosides and nucleotides, the commonly used phases need not be optimal. If new stationary phase materials are obtained the question

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arises how to objectively evaluate their advantages and shortages.

Normally scientists try to isolate the systems studied from mutual influences of many variables and select two (three at the very most) individual variables for observation keeping at the same time the remaining factors constant. In chemistry this is seldom possible. In effect, a lot of potential information hidden in numerous data collected laboriously all over the world is left unexploited. Chemometric analysis provides a means to extract systematic information dispersed over numerous and diversified data.

Factorial methods of data analysis are applied in chemistry to determine the 'intrinsic dimensionality' of certain experimentally determined chemical properties, that is, the number of 'fundamental factors' required to account for the variance [1]. This number should be small enough to be manageable to the human mind. Once the number of factors has been determined, the next step is to try to identify these abstract factors with physically significant parameters. The advantage that factor analysis has over, e.g., regression analysis is that individual physical factors can be tested for possible identification with the abstract factors without simultaneously identifying all the other fundamental factors.

Factorial methods of data analysis have been applied in chromatography since the early 1970s [2,3]. The following areas of application of factorial analysis of chromatographic data can now be distinguished:

- Optimization of separation conditions (mostly the composition of mobile phase) in multivariable chromatographic systems ([4–6] and references cited therein)
- Prediction of toxicity [7–11], other biological activities [12,13] and pharmacological classification of xenobiotics based on their chromatographic behaviour in diversified separation systems [14–16]
- Elucidation of molecular mechanism of separation in given chromatographic systems and prediction of retention based on structural parameters of the solutes [17–27]
- Evaluation of the separation properties of various stationary phase materials ([28–30] and the references regarding other works by the group cited therein, [31,32]).

Authors of this paper have since long been engaged in a search for HPLC systems best suited for the evaluation of differences in bioactivity of various classes of solutes [33–35] and in the analysis of stationary phases [36–40]. In due course several novel silica-based, chemically bonded stationary phase materials have been synthesized and their performance was tested, along with some original commercially available columns, regarding the separation of nucleosides and cyclic nucleotides. Here we report the synthesis and results of chemometric analysis of HPLC data for a test series of nucleosides and cyclic nucleotides aimed at an objective classification of the stationary phase materials studied and at the identification of structural features of the solutes which determine the differences in their chromatographic behaviour.

2. Experimental

2.1. Materials

Eleven test solutes were used in this study. cAMP, cGMP and cIMP were purchased from the BioLog Life Science Institute (Bremen, Germany). cTMP and cCMP were from Sigma (Deisenhofen, Germany). Adenosine (ADO), guanosine (GUO), cytidine (CYT), inosine (INO), uridine (URD) and thymidine (THD) were obtained from Pharma Waldhof (Düsseldorf, Germany).

Chemical structures of the stationary phase materials used in this work are given in Fig. 1. The following commercially available columns (125 mm×4 mm I.D.) were purchased from their respective producers: RP-8, LiChrospher RP-Select B (Merck, Darmstadt, Germany); ALU, Aluspher RP-Select B (Merck, Darmstadt, Germany); CARB, Hypercarb (Shandon, Astmoor, Runcorn, UK); and IAM, IAM.PC.MG (Regis, Morton Grove, IL, USA).

2.2. Synthesis of new stationary phases

The following new stationary phases were obtained in this work (Fig. 1): OD2, 1NAPH, BN, CN,

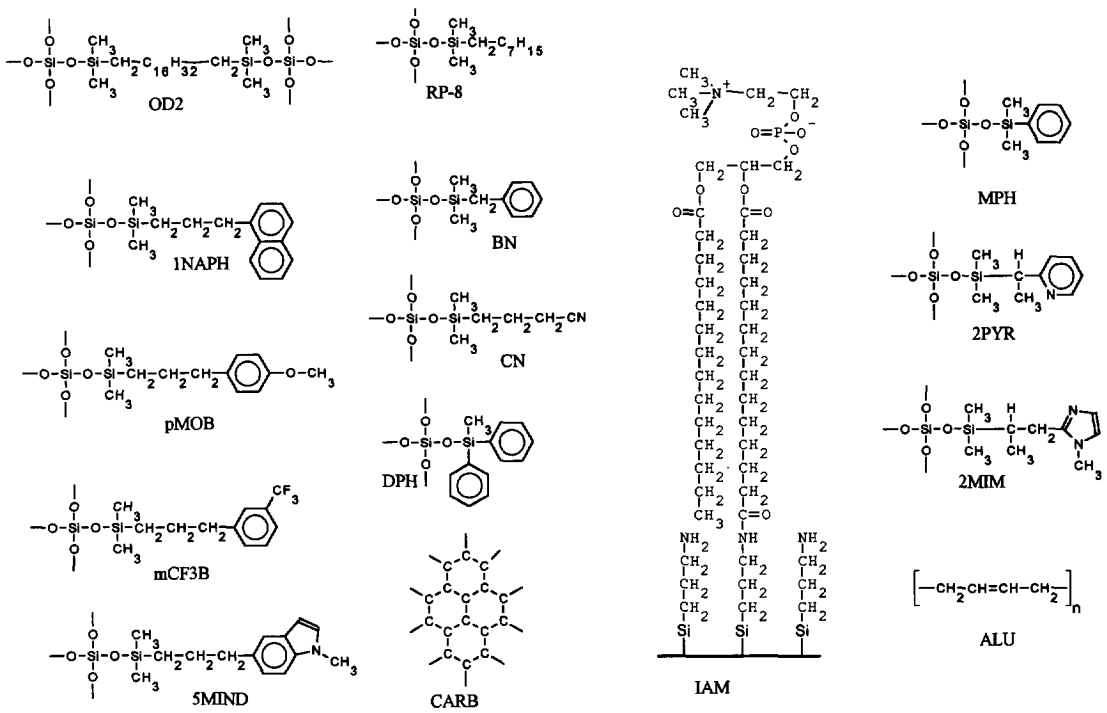

Fig. 1. Chemical structures of the stationary phases studied.

pMOB, DPH, mCF3B, 5MIND, MPH, 2PYR and 2MIM. For 7 of them new silanes were synthesized. To prepare the remaining 4 new silica-based stationary phase materials the respective commercially available silanes were used.

Chemicals

Hexane, pyridine, methanol, LiChrosorb silica gel 60, LiChrosorb NH_2, diethyl ether, 2-bromonaphthalene, toluene, acetone, tetrahydrofuran, methyl iodide, magnesium and sodium chloride, ethyl bromide and isopropanol were analytical reagent quality products from Merck (Darmstadt, Germany). Dimethylethoxysilane and dimethylchlorosilane were kindly provided by Wacker-Chemie (Burghausen, Germany). 5-Bromoindole, 2-vinylpyridine, N-methylimidazole, allylbromide, 3-bromotrifluoromethylbenzene, 1,12-dibromododecane and 4-allylanisole were from Janssen Chimica (Beerse, Belgium). Chlorodimethylphenylsilane was from Aldrich (Milwaukee, WI, USA). Chloromethyldiphenylsilane, benzylchlorodimethylsilane and chloro-3-cyanopropyldimethylsilane were obtained from Petrach Systems (Bristol, PA, USA).

Synthesis of silanes

The newly synthesized stationary phases were all prepared by using monofunctional silanes without end-capping. All the synthesized chemical entities were identified based on the ^{1}H-NMR, IR and MS analysis. The stationary phases obtained in this work were characterized by elemental analysis and by alkali fusion and a subsequent GC–MS analysis [36–40]. A standard BET procedure was applied to determine the specific surface area of newly prepared phases. Results of elemental analysis and the BET analysis are given in Table 1.

Table 1
Density of organic coverage of new stationary phase materials determined by elemental analysis and specific surface area determined by the BET method

Stationary phase	Elemental analysis			Specific surface area (m^2/g)
	Element	Content (%)	Coverage density (μmol/mg)	
OD2	C	14.53	0.542	152
	H	2.81	0.585	
	Cl	<<0.20	-	
pMOB	C	13.49	0.936	173
	H	1.97	1.036	
2PYR	C	4.20	0.388	184
	H	1.18	0.800	1
	N	0.20	0.150	
1NAPH	C	15.98	0.887	180
	H	2.01	1.058	
BN	C	10.90	1.009	189
	H	1.52	1.169	
CN	C	8.04	1.116	185
	H	1.59	1.325	
	N	1.55	1.107	
MPH	C	9.19	0.957	178
	H	1.30	1.181	
DPH	C	10.64	0.682	225
	H	1.21	0.931	
mCF3B	C	12.78	0.887	178
	H	1.81	1.131	
	F	5.30	0.929	
2MIM	C	6.45	0.597	189
	H	1.86	1.000	
	N	1.19	0.430	
5MIND	C	3.96	0.235	238
	H	0.88	0.440	
	N	0.30	0.214	

Phase OD2

Octadecadiene In a 500 ml three-necked flask filled with nitrogen, 250 ml diethyl ether and 50 ml tetrahydrofuran (both dried and distilled over Na) were placed. Then, 15.3 g (0.64 mol) magnesium was added and the solution was cooled down to 0°C. Next, 27.3 ml (0.32 mol) allylbromide was added drop by drop with stirring. The resulting white solution was heated at room temperature with stirring for 1 h. After that 34.5 g (0.105 mol) 1,12-dibromododecane was dissolved in 50 ml of dry tetrahydrofuran and added slowly to the Grignard solution. The solution was stirred for 12 h at 50°C and the solvent was evaporated. In effect a white solid precipitated. The substance was dissolved in a mixture of water and heptane (1:1:1) in a separating funnel. Organic phase was detached and compressed. A yellowish, viscous liquid remained in the flask. After a GC–MS analysis the liquid was subjected to distillation at reduced pressure (ca. 0.1 Torr). 1,17-octadecadiene was distilled off at 110°C. The yield was 42.6%.

Chlorodimethyl-18-(chlorodimethylsilyl)-octadecylsilane An amount of 14.73 g (0.0588 mol) 1,17-octadecadiene was mixed with 200 μl solution of 0.01 *M* $H_2PtCl_6 \cdot 6H_2O$ in isopropanol in a three-necked flask under dry nitrogen. The lightly yellow solution was heated on an oil-bath for 1 h at 100°C. Then, a triple excess of chlorodimethylsilane (19.59 ml, 16.62 g, 0.1764 mol) was added slowly drop by

drop at a constant temperature. Next, the solution was stirred for 5 h at 100°C. After characterizing the compound by GC–MS, a high vacuum distillation (ca. 0.1 Torr) followed. The chlorodimethyl-18-(chlorodimethylsilyl)-octadecyl silane solidified as white crystals. The yield was ca. 40%.

Phase 1NAPH

The starting substrate which reacted with allylbromide was 1-bromonaphthalene. The resulting product was 1-allylnaphthalene. The next steps of synthesis were as described for chlorodimethyl-18-(chlorodimethylsilyl)-octadecylsilane. The yield was 80%.

Phase mCF3B

The starting substrate which reacted with allylbromide was bromotrifluoromethylbenzene. The resulting product was allyl-3-trifluoromethylbenzene. The next steps of the synthesis were as described for chlorodimethyl-18-(chlorodimethylsilyl)-octadecylsilane. The yield was 80%.

Phase pMOB

Commercially available 4-allylanisole was subjected to the subsequent synthesis steps as described for chlorodimethyl-18-(chlorodimethylsilyl)-octadecylsilane. The yield was 70%.

Phase 2MIM

The starting substrate which reacted with allylbromide was N-methylimidazole. The next synthesis steps were as described for chlorodimethyl-18-(chlorodimethylsilyl)-octadecylsilane, except that dimethylethoxysilane was used instead of chlorodimethylsilane. The yield was not measured.

Phase 2PYR

Commercially available 2-vinylpyridine was subjected to the subsequent synthesis steps as described for chlorodimethyl-18-(chlorodimethylsilyl)-octadecylsilane, except that dimethylethoxysilane was used instead of chlorodimethylsilane. The yield was 80%.

Phase 5MIND

The bromoindole was methylated to obtain 5-bromo-N-methylindole, which was next used in the reaction with allylbromide. The next synthesis steps were as described for chlorodimethyl-18-(chlorodimethylsilyl)-octadecylsilane, except that dimethylethoxysilane was used instead chlorodimethylsilane. The yield was 50%.

Phases DPH, CN, BN and MPH

The silanes used to prepare the phases DPH, CN, BN and MPH were obtained from commercial sources indicated in the Chemicals section.

Coupling of silanes to silica gel

All the newly synthesized stationary phases were obtained by coupling the silanes to the silica gel after the same last step of the synthesis. The example described below regards the phase OD2.

A volume of 150 ml dry toluene was placed in a 250 ml two-necked flask flushed with nitrogen. 6 g silica gel, dried over P_2O_5 at 180°C under high vacuum, was added. At first, 970 μl (12 mmol) pyridine (dried over CaH_2) and then 2.64 g chlorodimethyl-18-(chlorodimethylsilyl)-octadecylsilane (6 mmol) were added. The mixture was stirred for 7 days under back flow with agitation. Then, the silica gel was separated by filtration, rinsed with dimethoxyethanol, mixed with water and stirred for 24 h. Next, the silica gel was repeatedly rinsed with the following solvents: water, methanol, acetone, toluene and hexane. Finally, the silica gel was dried in a dessicator under high vacuum (0.1 Torr).

2.3. Chromatographic measurements

The chromatographic apparatus consisted of a Model L-6200A pump, a Model L-4250 UV–VIS detector and a Model D-2500 chromato-integrator (all from Merck-Hitachi, Vienna, Austria). The new stationary phase materials synthesized for this project were slurry packed in typical HPLC columns 125 mm×4 mm I.D. (OD2, pMOB, MPH, BN, 2PYR) or 250 mm×4 mm I.D. (5MIND, DPH, 1NAPH, 2MIM, CN, mCF3B). Mobile phase flow-rate was 1 ml/min.

Test solutes were chromatographed in 15 HPLC systems. Mobile phase compositions were adjusted experimentally to obtain measurable retention data for each member of the series in each system. Excepting CARB the proportion of buffer (50 m*M* KH_2PO_4/KOH) to acetonitrile was 98:2 (v/v). With the CARB column the volume ratio of the buffer to acetonitrile was 70:30 (v/v). Normally the pH of the

Table 2
HPLC capacity factors for a set of nucleosides and cyclic nucleotides from 15 chromatographic systems

Solute	Capacity factors from HPLC systems														
	RP-8	ALU	CARB	OD2	pMOB	5MIND	MPH	BN	DPH	1NAPH	2MIM	CN	2PYR	mCF3B	IAM
INO	1.865	0.190	0.153	4.760	2.158	0.848	0.955	2.321	1.401	6.502	0.315	0.442	0.138	3.705	0.409
THD	3.565	0.133	0.267	10.632	4.491	0.828	1.866	4.195	2.552	11.290	0.309	0.829	0.240	8.236	0.430
URD	0.870	0.084	−0.223	1.974	1.063	0.335	0.630	0.911	0.727	2.105	0.075	0.345	0.079	1.607	0.077
CYT	0.774	0.208	−0.035	1.110	0.802	0.391	0.236	0.756	0.526	1.297	0.303	0.287	0.110	0.918	0.077
ADO	7.913	0.403	0.970	20.496	9.437	1.805	4.695	10.785	7.753	17.395	0.521	1.932	0.850	12.209	0.587
GUO	2.104	0.358	0.313	5.662	2.685	0.758	1.016	2.447	1.665	6.111	0.391	0.725	0.579	4.272	0.483
cIMP	1.909	0.018	0.396	5.570	2.072	0.504	1.057	2.264	1.335	6.952	1.659	0.362	0.110	4.048	0.052
cTMP	2.513	−0.035	0.861	7.311	3.595	0.543	1.618	3.439	1.783	10.865	1.603	0.452	0.157	6.605	0.035
cCMP	1.000	0.027	−0.166	2.575	0.928	0.380	0.707	0.923	0.788	2.096	1.809	0.277	0.114	1.680	0.017
cGMP	1.978	0.080	0.488	5.351	2.613	0.721	1.094	2.504	1.618	5.989	2.869	0.479	0.173	4.073	0.098
cAMP	7.035	0.124	2.555	19.268	6.649	1.710	4.130	8.785	6.058	15.830	3.376	1.250	0.752	12.013	0.427

buffer used to prepare mobile phases was 6.0. Working with the immobilized artificial membrane column (IAM.PC.MG) we used pure buffer of pH 6.8 as the mobile phase.

Capacity factors, k', for 11 test solutes determined in 15 HPLC systems are presented in Table 2. To calculate k' the dead time measures were used as obtained by injection of sodium nitrate.

2.4. Structural analysis

The test solutes were subjected to molecular modelling by HyperChem (Hypercube, Waterloo, Canada). For nucleosides calculations were performed for the non-ionized forms of the compounds. For cyclic nucleotides respective calculations were done for the ionized (−1) forms of the solutes.

For the geometry optimized structures numerous structural parameters were obtained. Selected data for the test solutes are collected in Table 3. The first five structural parameters considered (molecular mass, total energy, binding energy, electronic energy and heat of formation) reflect basically the differences in molecular size ('bulkiness') among the solutes. In other words, they are expected to quantify the differences in ability of individual solutes to take part in non-specific, dispersive intermolecular interactions with stationary phase ligands. The next two structural parameters, energy of the highest occupied molecular orbital (HOMO) and energy of the lowest unoccupied molecular orbital (LUMO), are consid-

Table 3
Structural parameters of a set of nucleosides and cyclic nucleotides obtained from molecular modelling

Solute	Molecular mass	Total energy (kcal/mol)	Binding energy (kcal/mol)	Electronic energy (kcal/mol)	Heat of formation (kcal/mol)	Energy of HOMO (eV)	Energy of LUMO (eV)	Maximum charge (electrons)	Minimum charge (electrons)	Dipole moment (D)
INO	268.2	−89351.6	−3217.7	−547431	−133.8	−8.9996	−0.5158	0.381	−0.335	6.61
THD	242.2	−80479.2	−3155.2	−478466	−193.1	−9.6600	−0.3404	0.408	−0.390	4.06
URD	244.2	−84280.3	−2978.4	−492188	−231.8	−10.0402	−0.4232	0.410	−0.405	3.22
CYT	243.2	−81968.7	−3025.9	−485049	−173.8	−9.3789	−0.1656	0.359	−0.366	4.21
ADO	267.2	−87056.3	−3281.5	−547501	−92.0	−8.8900	−0.2815	0.251	−0.356	1.48
GUO	283.2	−94442.4	−3378	−603868	−129.0	−8.7641	−0.4762	0.399	−0.348	5.69
cIMP(−1)	329.2	−106551	−3493.7	−673476	−319.3	−6.7569	1.5764	2.537	−1.123	13.26
cTMP(−1)	303.2	−97666.5	−3419.3	−609258	−366.7	−6.4108	2.0273	2.533	−1.138	14.91
cCMP(−1)	304.2	−99163.2	−3297.3	−607488	−354.6	−6.4881	1.8086	2.539	−1.103	18.90
cGMP(−1)	344.2	−111630	−3642.7	−726843	−303.2	−6.7370	1.5998	2.530	−1.140	13.55
cAMP(−1)	328.2	−104241	−3543.3	−670199	263.4	−6.6709	1.7611	2.532	−1.141	16.14

ered as reflecting the abilities of solutes to participate in the so-called electron-pair-donor–electron-pair-acceptor interactions ('charge transfer' interactions) and/or hydrogen bonding interactions. Maximum excess charge, minimum excess charge on given atoms in the solute molecule and dipole moment are presumed to quantify differences among the solutes regarding the electrostatic interactions of the type dipole–dipole and dipole–induced dipole.

2.5. Chemometric analysis

Retention data (Table 2) as well as structural data for solutes (Table 3) were subjected to statistical analysis by the principal component analysis (PCA) method [42]. PCA was applied as the method of choice [41,42]. The reason was that dealing with a matrix of the HPLC capacity factors we were concerned with quantitative (not qualitative) aspects of the data. PCA was also chosen because all the retention measurements were positively correlated and expressed in the same units (undimensional). Besides, other factorial methods require non-negative data and we were interested in both positive and negative values of capacity factors (chromatographic exclusion).

Calculations employing Statgraphics package (Manugistics, Rockville, MA, USA) were run on a personal computer.

First principal component (PC1) accounted for 79.6% and the second principal component (PC2) for 13.7% variance within the 11×15 matrix of retention data (capacity factors from Table 2). The inputs to PC1 and PC2 due to the individual HPLC system and due to the solutes are displayed in Fig. 2 (loadings) and Fig. 3 (scores).

In case of 11×10 matrix of structural parameters of nucleosides and cyclic nucleotides (Table 3) PC1 accounted for 86.6% and PC2 for 10.4% of data variance. The inputs to PC1 and PC2 by the molecular modelling derived structural descriptors and by individual solutes are presented in Fig. 4 (loadings) and Fig. 5 (scores).

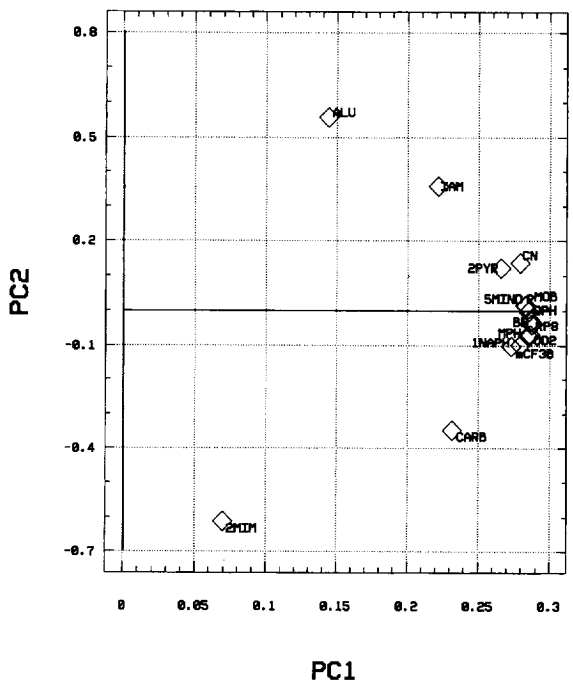

Fig. 2. Plot of inputs (loadings) of the first two principal components (PC1 and PC2) obtained from the analysis of retention data from Table 2 for the variables of the chromatographic systems.

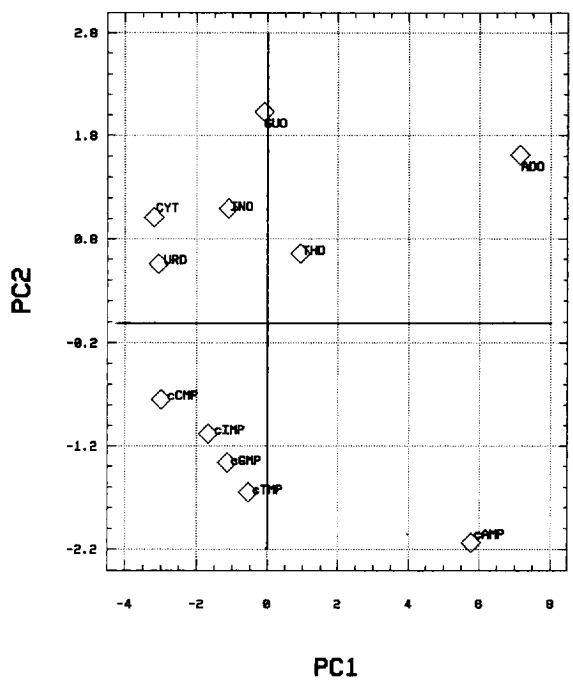

Fig. 3. Plot of inputs (scores) of the first two principal components (PC1 and PC2) obtained from the analysis of retention data from Table 2 for individual nucleosides and cyclic nucleotides.

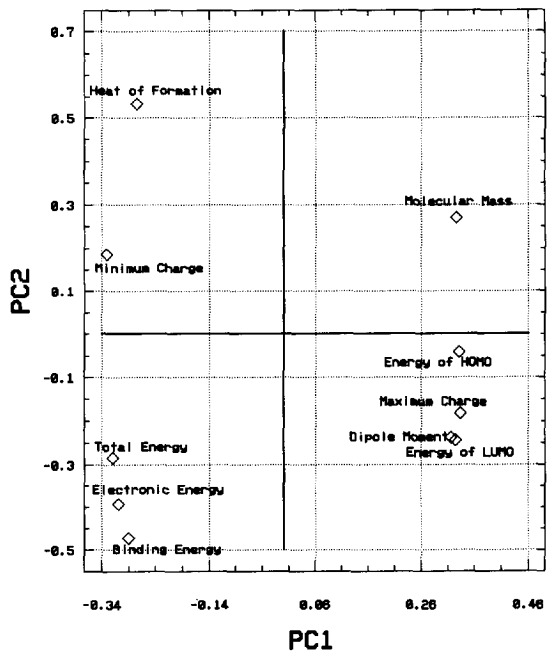

Fig. 4. Plot of inputs (loadings) of the first two principal components (PC1 and PC2) obtained from the analysis of structural data of solutes from Table 3 considering individual structural descriptors.

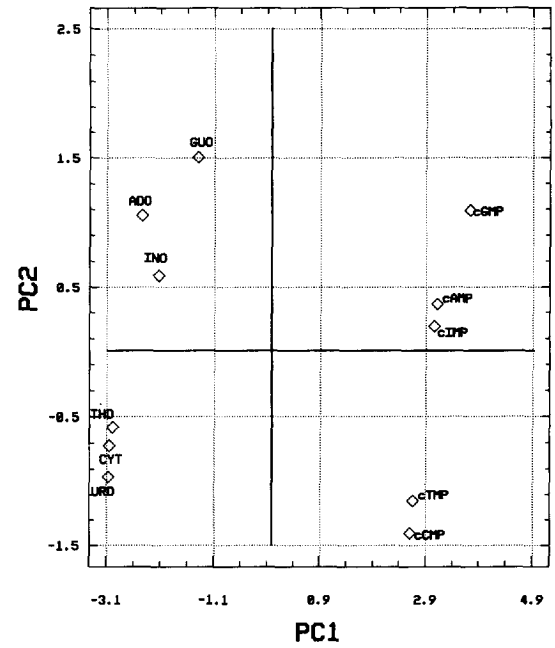

Fig. 5. Plot of inputs (scores) of the first two principal components (PC1 and PC2) obtained from the analysis of structural data of solutes from Table 3 for individual nucleosides and cyclic nucleotides.

3. Results and discussion

For the sake of comparison four recently introduced commercially available stationary phases were selected: RP-8, CARB, ALU, and IAM (Fig. 1). These phases represent distinctive types of reversed-phase materials. RP-8 is a standard hydrocarbonaceous (C_8) silica-based phase. CARB is a graphitized carbon material of unique separation properties [23,43]. The IAM column was designed to model biological membranes [44]. The ALU column is packed with a polybutadiene-encapsulated alumina and, similarly to CARB, can be operated at acidic, neutral and alkaline conditions.

Distribution of 15 HPLC systems on a plane determined by the first two principal components (Fig. 2) reveals a compact cluster of hydrocarbonaceous silica-based phases. Close distances between points corresponding to 1NAPH, mCF3B, OD2, MPH, BN, DPH and RP-8 indicate similar discriminative properties of the phases toward the test nucleosides and cyclic nucleotides. There seems to be not much difference in the separating properties between the aromatic and aliphatic hydrocarbon phases. To the same cluster belong also the phases pMOB and 5MIND. Evidently, single heteroatoms (ether oxygen in pMOB and tertiary nitrogen in 5MIND) do not change the overwhelmingly hydrocarbonaceous character of these phases. On the other hand, cyano functionality in CN and pyridine moiety in 2PYR provide specific interactions which are significant enough to separate these phases from the cluster of purely hydrocarbonaceous phases.

The most striking specifity can be assigned to the imidazoline derivative phase 2MIM. It appears that 2MIM phase has an enhanced affinity to the ionized forms of cyclic nucleotides. Opposite properties have the phases ALU and IAM. They seem to prefer nucleosides.

Data on density of organic coverage and on specific surface area (Table 1) do not distinguish phases of unique selectivity. The hydroorganic mobile phase was the same (with one exception) for all the HPLC systems studied. Thus, differences in

retention of test solutes can be attributed to the differences in selective interactions between solutes and stationary phase ligands.

Conclusions drawn from distribution of stationary phases (variables) are supported by the distribution of test solutes (objects) on the plane determined by the two first principal components (Fig. 3). It must be noted here that the assigning of a physical meaning to complex abstract PCA factors is in most instances not straightforward. Analyzing Fig. 3 one can not speculate on some features of structure of solutes extracted by PC1 and PC2. It appears that a positive value of PC2 is typical for the basic nucleosides whereas negative values are observed for acidic cyclic nucleotides. Thus, PC2 can be assumed to extract information on acid–base properties of solutes. On the other hand, the highest inputs to PC1 are provided by ADO and cAMP and the lowest by CYT, URD and cCMP. Referring to a standard measure of hydrophobicity, logarithm of *n*-octanol–water partition coefficient (log *P*), available for two nucleosides [45], one can assume that PC1 extracts information on hydrophobicity of solutes: log P for ADO is −1.10 and for URD log P is −1.98.

Certainly, hydrophobicity is not a simple one-dimensional property and the *n*-octanol–water partition system does not provide a unique, universal hydrophobicity scale. However, if PC1 averages information on hydrophobic properties of solutes from 15 HPLC systems then one can conclude that hydrophobicities of nucleosides are closely similar to the hydrophobicities of the corresponding cyclic nucleotides. This means that the expected increase of hydrophobicity of cyclic nucleotides with respect to the corresponding nucleosides due to increased molecular size is fully compensated for by the increased polarity (hydrophilicity) due to the phosphate moiety. Results of principal component analysis of retention data illustrated in Fig. 2 and Fig. 3 provide an objective statistical proof for the intuitive assumption that separation in reversed-phase HPLC systems is a net effect of non-specific (hydrophobic) and specific (polar) intermolecular interactions between the solutes and both the stationary and the mobile phase. Similar conclusions were drawn from PCA of the reversed-phase HPLC data by other authors [24–29].

As far as the newly synthesized stationary phases are concerned the 2MIM material appears especially interesting from the point of view of separation of nucleosides and cyclic nucleotides. Also, the 2PYR and CN may provide distinguishable separation patterns. Other new phases seem to have no clear-cut advantages over the classical hydrocarbonaceous stationary phase materials.

To further explain the mechanism of HPLC separations of nucleosides and cyclic nucleotides a set of molecular descriptors of the compounds was subjected to PCA. In Fig. 4 the 'loadings' of PC1 and PC2 by individual structural variables of the test solutes are displayed. PC1 is determined mostly by electronic parameters: maximum and minimum atomic excess charge, energies of HOMO and LUMO and dipole moment. PC2 is determined by structural descriptors related to molecular size ('bulkiness'): heat of formation, molecular mass, and the total, electronic and binding energies.

Conclusions drawn from Fig. 4 are fully confirmed by a PCA plot for the solutes (Fig. 5). Polar cyclic nucleotides have high values of PC1 and nucleosides posses low values of PC1. Distribution of nucleosides and of cyclic nucleotides along the PC2 axis is according to their molecular mass (provided that the two subgroups are considered separately). However, PC2 is not a simple function of molecular mass because heavier cyclic nucleotides have lower values of PC2 than their corresponding nucleosides.

A comparison of the distribution of nucleosides and cyclic nucleotides in Fig. 3 and Fig. 5 appears interesting, which resulted from PCA of the experimental retention parameters and of the theoretically calculated structural parameters, respectively. One can notice that distribution of solutes along PC1 in Fig. 3 resembles to some extent their distribution along PC2 in Fig. 5. Analogously, localization of solutes along PC2 in Fig. 3 is similar to that along PC1 in Fig. 5. Of course, the sequence of individual solutes differs in some instances. However, PCA demonstrates a general relationship between chromatographic behaviour of solutes in various HPLC systems and their characteristics provided by the computational chemistry methods.

Results of this study can be summarized as follows:

- Of 11 newly synthesized stationary phase materials 8 hydrocarbon-bonded silica phases have separating properties closely similar to a standard,

commercially available octylsilica phase. The phases comprising aromatic fragments in their ligands do not differ significantly from the aliphatic hydrocarbon materials

- New stationary phases possessing the methylimidazole, pyridine and cyano moiety in their structure have specific, distinguishable properties regarding the separation of nucleosides and cyclic nucleotides
- PCA of retention parameters provides an objective quantitative means to mutually compare separation properties of a large number of diversified HPLC systems
- PCA of structural descriptors of solutes obtained by molecular modelling facilitates the identification of structural features that most strongly affect retention in individual HPLC systems.

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