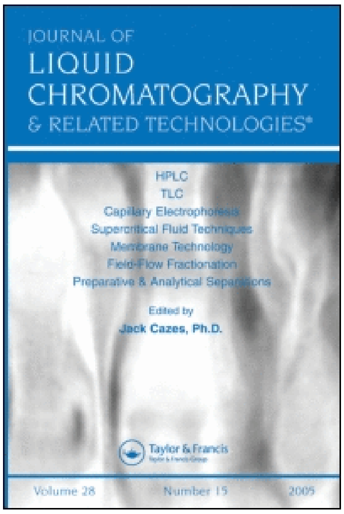

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Chemometric Account for Retention of Lysosomotropic Alkanolamines in Planar Chromatography Systems

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CHEMOMETRIC ACCOUNT FOR RETENTION OF LYSOSOMOTROPIC ALKANOLAMINES IN PLANAR CHROMATOGRAPHY SYSTEMS

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ABSTRACT

Planar chromatography retention data (in form of thermodynamically meaningful R_M coefficients) for twelve model alkanolamines, indicating lysosomotropic activity, were determined in eighty chromatographic systems using eight different silica gel layers with varied chemically bonded stationary phases. In performed thin layer chromatography experiments, aqueous binary mobile phases containing methanol or acetonitrile as organic modifier, in five different proportions, were applied. Additionaly, nine quantum chemical descriptors were calculated for test solutes by a semi-empirical molecular modelling procedure (AM1) to describe their structural properties. Chemometric technique by the multivariate method of principal components analysis (PCA) was appplied to the all completed chromatographic retention and computational chemistry data for identification of the most selective and useful planar chromatography systems as well as molecular interactions of solutes which affect their retention phenomena in the particular separation mode. The statistically significant relationship was revealed between quantitative measure of antimicrobial activity of alkanolamines and values of calculated scores of principal components.

INTRODUCTION

Some alkanolamines, preferably in the uncharged form, indicate lysosomotropic activity since *in vivo* penetrate the native lysosomal and cytoplasmic membranes leading to inhibition of choline transport,[1] norepinephrine uptake,[2] phosphostranferase activity,[3] or modification and enrichmment of membrane phospholipids polar head groups.[4] Such feature allows use of alkanolamines as the protectors of hematopoietic precursor cells against some cytotoxic compounds[5] or, like N-ethyl-3-carbazole carboxaldehyde phenylhydrazones[6] and 4-aza-5α-sitostanes,[7,8] as the specific antimicrobial agents.[9,10] Moreover, the structural factors govering the membrane transport process of alkanolamines are still not rationalized[11] and unable to identify such compounds useful for the property oriented *in vivo* chemotherapy. However, the increasing application of advanced chemometric methods employing a large set of physical and chemical parameters in structure-biological property studies[12,13] gives quite real possibility to establish more reliable models of such defined activity of alkanolamines. For instance, by chemometric evaluation of chromatographic retention data, it was possible to recognize[14-20] the continuity of changes in alkanolamines hydrophobicity and its relation with Wiener topological index, expressing ability of these solutes for the non-specific molecular interactions.

A chemometric technique by the method of principal components analysis (PCA) is the multivariate statistical procedure which uses the well-defined linear transformation

$$y = \boldsymbol{W} * x$$

changing vectorial description of the stationary stochastic process $x = R^K$ into the vector $y = R^K$ by applying the intermediate matrix $\boldsymbol{W} \varepsilon R^{K * N}$, where K

$<< N$, in such manner that the output surface with reduced dimensions still retain all the principal informations referring to the considered process. Accurate statistical theory implies that PCA transformation creates the set of K orthogonal vectors which have great impact into variance of input data. The great amount of information included in the mutually correlated input data is changed during PCA transformation into the set of statistically independent minor components K arranged according their validity.[21] PCA transformation can be referred to as the form of the disadvantage (loss) compression of input data, which is known in communications theory as the Karhunen-Loeve transformation.[22]

PCA as most frequently employed in chemical applications is the special case of factorial methods of data analysis[23] which, in chromatography, have been used in such areas, distinguished and reviewed recently by Kaliszan,[24,25] as elucidation of molecular mechanism of separation in chromatographic systems, prediction of retention based on the structural descriptors of solutes, evaluation of the separation properties of stationary phase materials and mobile phase composition, optimization of separation conditions in multivariable chromatographic systems, prediction of biological activities, and pharmacological classification of xenobiotics based on their retention in diversified chromatographic systems.

Especially, in case of planar chromatography, the different factorial methods of multivariate analysis were introduced to classify the strength of mobile phases in RPTLC systems for separation of triazine derivatives,[26] characterize suitability of the impregnating agents for TLC determination of lipophilicity of phytotoxic 2-nitro-4-cyanophenyl esters,[27] evaluate effects of support matrix[28] or organic modifier[29] on the TLC estimation of hydrophobicity parameters of peptides or commercial anticancer drugs, respectively.

Application of PCA to TLC data made it possible to establish the procedure for precise identification of near 450 drugs and their metabolites[30,31] or optimization of TLC separation of flavonoids.[32] Use of the PCA procedure to data matrix, founded on the TLC retention values and topological molecular descriptors, enabled parametrization of molecular activity of amino acids[33] and alkanolamines.[14,16,17,20]

In the present studies, it was shown that systematic information extracted by performed PCA analysis of retention behaviour of alkanolamines in diverse planar chromatography systems not only lead to the explanation of molecular mechanisms of their selective separation but also has direct relevance to the potential pharmacological properties of such solutes.

EXPERIMENTAL

Materials and Methods

HPTLC measurements were performed on methanolic solutions (*ca.* 10 mg/mL) of the following twelve test alkanolamines with chemical structures shown in Figure 1:

> 2-aminoethanol (coded as MEA), bis(2-hydroxyethyl)amine (DEA), tris(hydroxymethyl)aminomethane (APD) (POCh, Gliwice, Poland), bis(2-hydroxypropyl)amine (DIP), N-(2-hydroxyethyl)ethylenediamine (NAE), (Merck-Schuchardt, Hohenbrunn, Germany), N,N'-bis(2-hydroxyethyl)-methylamine (MeDEA), N,N'-bis(2-hydroxyethyl)-*n*-butylamine (BuDEA), N,N'-bis(2-hydroxyethyl)-*tert*-butylamine (tBuDEA), N,N'-bis(2-hydroxyethyl)-aniline (PhDEA), N,N'-bis(2-hydroxyethyl)-p-toluidine (pTDEA), tris(2-hydroxyethyl)amine (TEA) (Fluka, Buchs, Switzerland) and N,N'-bis(2-hydroxyethyl)-o-toluidine (oTDEA) (Bayer, Leverkusen, Germany).

HPTLC experiments was performed with 10 x 10 cm, glass-backed HPTLC plates, precoated with silanized silica, coded as C2 (Merck, Darmstadt, Germany; #5746); bonded octylsiloxane silica, coded as C8 (Merck; #13725); bonded octadecylsiloxane silica, coded as C18 (Baker, Phillipsburg, NJ, USA, #7012-01); wettable (partially silanized) bonded octadecylsiloxane silica, coded as WC18 (Macherey-Nagel, Düren, Germany; #811 075); bonded diphenyl silica, coded as DPH (Whatman, Maidston, England; #44814-820); bonded cyanopropylsiloxane silica, coded as CN (Merck, #16464); bonded aminopropylsiloxane silica, coded as NH2 (Merck, #12572); and bonded propyldiol silica, coded as DIOL (Merck, # 12668).

The plates were used as received. Mixtures of deionized water containing HPLC grade methanol or acetonitrile (Merck) in the proportions 20:80, 40:60, 60:40, 80:20 and 90:10 (v/v) were applied as mobile phases. Before use, the appropriate mixture of solvents was left to stand 1h at room (21°C) temperature.

Samples of solutes (0.5 μL) were spotted individually with a type 701 Hamilton (Reno, USA) 10 μL microsyringe. The plates were developed horizontally in DS-II sandwich type, all-Teflon tanks (Chromdes, Lublin, Poland)[34] until the solvent front reached a point 0.8 cm from the upper edge of the plate.

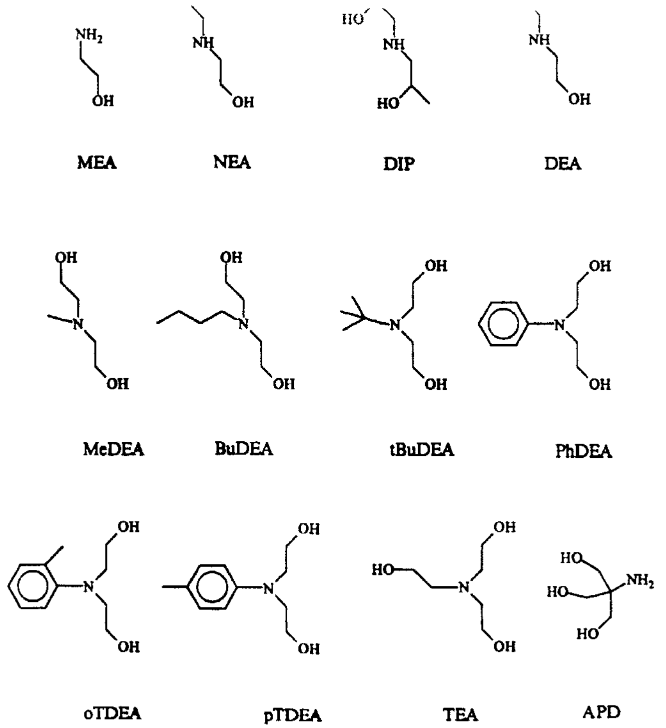

Figure 1. Structure of alkanolamines studied. For abbreviation see Table 1.

Spots of the separated alkanolamines were visualized with ninhydrin (Merck) solution (0.4 g in *n*-butanol-acetic acid (d = 1.04), 100:1 (*v/v*)) and heating the plate at 80°C for 60 min. The chromatograms on the TLC plates were densitometrically evaluated with the 16 (4-bit) grayscale scanner ScanJet 3p (Hewlett-Packard, Warsaw, Poland) connected to IBM PC/486-DX2 microcomputer and working under Quantiscan v.2.0 (Biosoft, Cambridge, U.K.) software.[35]

The retention data in planar chromatography systems (#38, 39, and 40 in Table 7) were obtained in measurements performed on Whatman 1 cellulose layers with n-butanol + acetic acid + water (77:6:17 v/v) in system 38, pyridine + ethyl acetate + water + acetic acid (42:25:25:8) in system 39, or n-butanol saturated with 0.1% ammonia solution in system 40 as mobile phase. Spots visualization was identical as in TLC experiments.

Table 1

Structural Parameters of Model Alkanolamines Derived From Molecular Calculations

				Alkanolamine*					
No.	**Parameter**	**Unit**	**Code**	**MEA**	**NAE**	**DIP**	**DEA**	**MeDEA**	**BuDEA**
1	Molecular Weight	a.m.u.	M.W	61.08	104.15	133.19	105.14	119.16	161.24
2	Total Energy	kcal/mol	TE	-20306.9	-32575.9	-42062.9	-34878.8	-38459.5	-49238.0
3	Binding Energy	kcal/mol	BE	-937.7	-1652.6	-2157.1	-1596.5	-1865.5	-2708.8
4	Electronic Energy	kcal/mol	EE	-62018.9	-132660.7	-196274.7	-134710.4	-166961.5	-262843.8
5	Heat of Formation	kcal/mol	HE	-58.6	-58.3	-118.1	-107.1	-101.1	-119.6
6	Energy of HOMO	eV	HOMO	-9.8453	-9.4624	-9.1483	-9.5388	-9.3220	-9.2232
7	Energy of LUMO	eV	LUMO	3.1720	2.7349	3.0755	2.6272	2.5151	2.4658
8	Maximum Charge	electrons	MAX	0.211	0.1493	0.1994	0.2017	0.2012	0.2008
9	Minumum Charge	electrons	MIN	-0.3490	-0.3465	-0.3344	-0.3293	-0.3305	-0.3353
10	Dipole Moment	debye	D	1.310	2.650	1.129	2.413	2.660	2.420

Table 1 (continued)

Structural Parameters of Model Alkanolamines Derived From Molecular Calculations

				Alkanolamine*					
No.	Parameter	Unit	Code	tBuDEA	PhDEA	pTDEA	oTDEA	TEA	APD
1	Molecular Weight	a.m.u.	M.W.	161.24	181.23	195.26	195.26	149.19	121.14
2	Total Energy	kcal/mol	TE	-49228.4	-53821.1	-57434.2	-57415.5	-49444.6	-42260.7
3	Binding Energy	kcal/mol	BE	-2699.1	-2770.7	-3072.2	-3053.4	-2249.2	-1688.8
4	Electronic Energy	kcal/mol	EE	-275136.9	-293915.1	-338942.9	-328753.3	-235332.3	-184140.0
5	Heat of Formation	kcal/mol	HE	-109.9	-48.2	-74.5	-55.8	-150.7	-140.5
6	Energy of HOMO	eV	HOMO	-9.0876	-8.0646	-9.1318	-7.9409	-9.3556	-10.1574
7	Energy of LUMO	eV	LUMO	2.3916	0.6804	0.4272	0.7089	2.3072	2.9622
8	Maximum Charge	electrons	MAX	0.2005	0.2056	0.2000	0.2057	0.2023	0.1854
9	Minumum Charge	electrons	MIN	-0.3350	-0.3328	-0.3358	-0.3346	-0.3336	-0.3397
10	Dipole Moment	debye	D	1.327	1.082	2.023	1.244	2.258	2.392

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* Abbreviations: MEA = 2-aminoethanol; NEA = N-(2-hydroxyethyl)ethylenediamine; DIP = bis(2-hydroxypropyl)amine; DEA = bis(2-hydroxyethyl)amine; MeDEA = N,N'-bis(2-hydroxyethyl)methylamine; BuDEA = N,N'-bis(2-hydroxyethyl)-n-butylamine; tBuDEA = N,N'-bis(2-hydroxyethyl)-tert-butylamine; PhDEA = N,N'-bis(2-hydroxyethyl)aniline; o-TDEA = N,N'-bis(2-hydroxyethyl)-ortho-toluidine; pTDEA = N,N'-bis(2-hydroxyethyl)-para-toluidine; TEA = tris(2-hydroxyethyl)amine; APD = tris(hydroxymethyl)aminomethane.

Thermodynamically correct values of R_M coefficients for test solutes were calculated from triplicate measurements of R_F parameter using the Bate-Smith and Westall formula:[36]

$$R_M = \log [(1/R_F) - 1] = \log k' \qquad (1)$$

Structural Calculations

For each test solute, the nine structural parameters (*i.e.* total energy, binding energy, electronic energy, heat of formation, energy of HOMO, energy of LUMO, maximum charge, minimum charge and dipole moment) were calculated with HyperChem 4.5 (Hypercube, Inc., Waterloo, Ontario, Canada) molecular modeling software installed on an IBM-compatible 486/DX2-66 MHz personal computer. These calculations were performed on the *in vacuo* optimized geometry of molecular structure of test solutes (as presented in Fig. 1), after employing a semi-empirical technique AM1 with restricted Hartree-Fock (RHF) mode. The Polak-Ribiere conjugate gradient procedure was used for optimization. The values of dipole moments for analytes were calculated using the molecular mechanics (MM+) method.

Chemometric Calculations

Retention data registered in the eighty planar chromatograpy systems (see Tables 2-9) as well as structural data for the test solutes (compare Table 1) were subjected to the multivariate statistical procedure by principal components analysis (PCA).[21,23] The previously reported retention data for test solutes in twenty six HPTLC[14,17] and three paper chromatography[37] systems were included in these calculations.

All calculations were performed with Statistica 4.3 (StatSoft, Inc., Tulsa, OK, USA) software implemented on an IBM-compatible 486/DX2-66 MHz personal computer.

RESULTS AND DISCUSSION

PCA of Structural Data

In Table 1, the values of structural descriptors calculated by the molecular

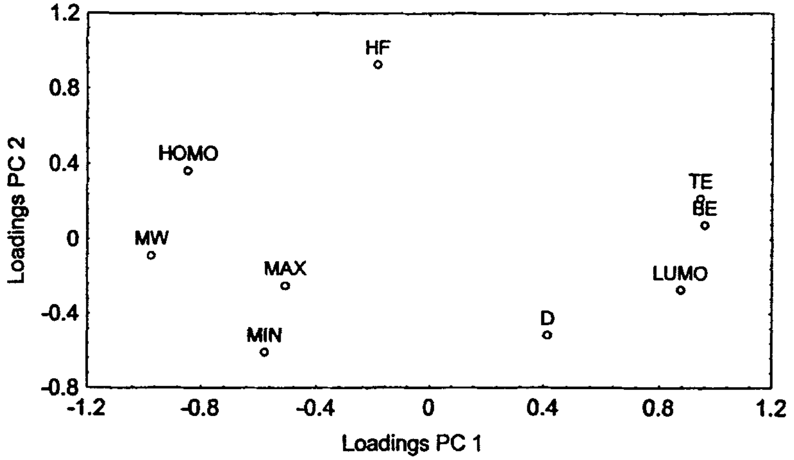

Figure 2. Two-dimensional plot of loadings input to calculate first two principal components (PC1 and PC2) due to the structural parameters from PCA of molecular data in Table 1. For abbreviation on the plane see Table 1.

modelling package, for a set of model alkanolamines, were presented. It can be assumed[24,38] that differences in molecular size or relative ability of individual solutes to undergo nonspecific, dispersive intermolecular interactions have been reflected by the molecular descriptors such as molecular weight MW; total energy TE; binding energy BE; electronic energy EE; and heat of formation HE. The ability of solutes to undergo charge transfer and/or hydrogen bonding interactions has been expressed by the two respective structural parameters: energy of highest occupied molecular orbital (HOMO) and the energy of lowest unoccupied molecular orbital (LUMO). The ability of solutes for electrostatic interactions as the dipole-dipole and dipole-induced dipole type have been quantified, respectively, by maximum/minimum excess charge on individual atoms the solute molecule (MAX and MIN, respectively and dipole moment D.

For assessment of molecular mechanism of observed retention of alkanolamines, the 12 x 10 matrix determined for 12 solutes and 10 structural descriptors was subjected to PCA. In Fig. 2, the loadings of calculated two principal components are presented. In this case, the summarized accounts of both PC1 and PC2 for the total input data variance was near 78 %. The PC1 explained 59.5 % of variance as well as PC2 only 18.5 %.

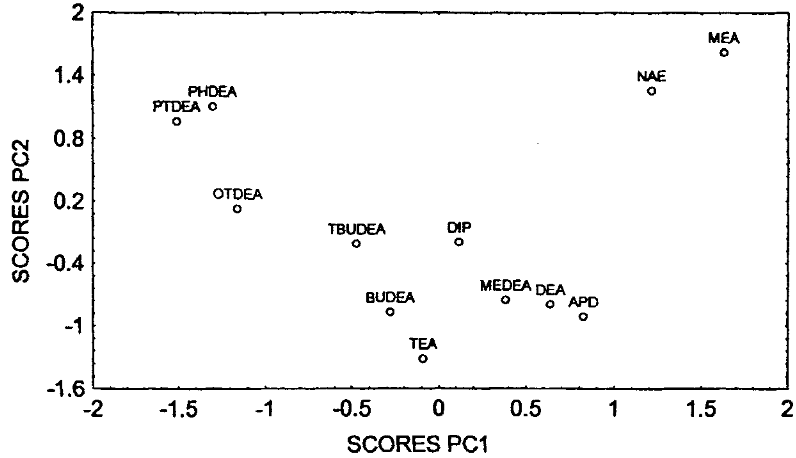

Figure 3. Two-dimensional plot of scores input to calculate first two principal components (PC1 and PC2) due to the individual solutes from PCA of molecular data in Table 1, from PCA of retention data of alkanolamines in Tables 2-9. For abbreviation on the plane see Table 1.

The PC1 is determined mostly by structural parameters associated with descriptors of molecular size of solutes, *i.e.* total energy TE, binding energy BE, electronic energy EE and slightly with such electronic descriptors as energy of LUMO and dipole moment D. It can be assumed that PC1 can be physicochemically interpreted as measure of solutes' ability to undergo the weak non-specific van der Waals interactions (dipole-dipole, dipole-induced dipole and dispersion type) supported by the more specific hydrogen bond formation. The sequence of molecular descriptors on the PC1 axis indicate that dispersion forces prevailing on the sum of both type of dipole-dipole interactions. Contribution of hetero- and homo-intermolecular hydrogen bond formation into potential associative interactions of alkanolamines must lead to a change of their dipole moment as suggest the close proximity of their respective decriptors TE and LUMO on the loading plane in Fig. 2. Similarly, PC2 is loaded mostly by structural parameters related to other descriptors of solute molecular size, such as the heat of formation HF and electronic descriptor as the energy of HOMO, which suggests that PC2 can be related to the quantitative measure of the second characteristic interaction pattern during associations of alkanolamines, *i.e.* specific electron pair donor-electron pair acceptor (charge transfer) interactions supported by the long distance dispersion interactions.

The ability of formation of such charge transfer complexes is dependent on the vector sum of the dipole moments of donor and acceptor molecules[38] as confirmed by proximity of the respective molecular descriptors (HOMO with MIN/MAX) on the loading plane in Fig. 2. The existence of n-n, n-π and π-π type of intermolecular interactions can be predicted by the presence of electron rich substituents in the alkanolamines moiety, in the stationary phase structures and in the molecules of organic modifiers of aqueous binary mobile phase.

Preliminary conclusions drawn from Fig. 2 on the possible interactions pattern of alkanolamines have been confirmed by the scores plot presented in Fig. 3. Alkanolamines containing a phenyl-like substituent connected to the tertiary amine group, *i.e.* PhDEA, oTDEA and pTDEA (see Fig. 1), giving ability for the charge transfer associations, have most extreme values of PC2, and they are alkanolamines which posses the lowest values of PC1. It is well known that the polarizability for π-electron systems in phenyl substituent is high, which favors the molecular orientation and possibility for dispersion interactions, so the PCA classified properly the mentioned solutes in the same cluster.

The PC1 and PC2 are both loaded very high by solutes MEA and NAE (see Fig. 1), containing the lone pairs of the n-electrons, as well as the primary hydroxyl and amine substituents which are both the strongest proton donor group. For seven alkanolamines in the central part of the scores plot (Fig. 3), the relative increase of contribution of all the three van der Waals interactions (orientation, inductive along with dispersive) during the chromatographic process can be predicted.

This conclusion is supported by the highest values of calculated dipole moments (see Table 1) and polarizability[18] for these solutes. For bis(2-hydroxypropyl)amine (DIP), located in the centre of the scores plane, it can be postulated that this molecule is possessed of very limited contribution of charge transfer interactions or hydrogen bond formation during any possible association process. This solute can be probably considered as the standard compound possesing molecular ability to participate exclusively in the non disturbed van der Waals interactions. One can also observe that PC1 is near parabolic function of the molecular mass of examined solutes.

Thus revealed here, PCs divides excellently the two more chemical, in nature, intermolecular interactions patterns which dominate for alkanolamines association and their close relations with the more physical type of van der Waals interactions.

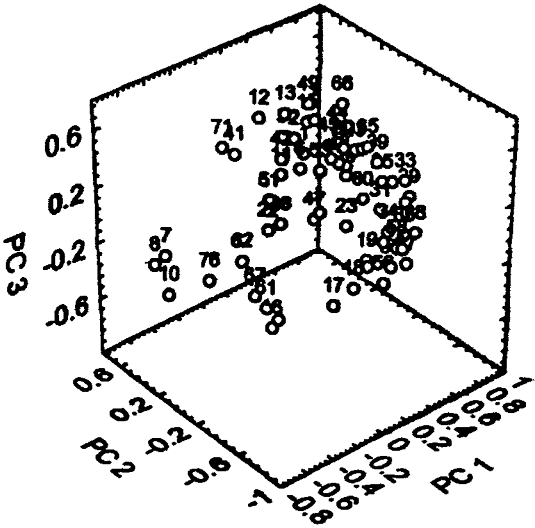

Figure 4. Spatial plot of loadings input to calculate first three principal components (PC1, PC2 and PC3) due to the individual planar chromatography systems from PCA of retention data of alkanolamines in Tables 2-9. The numbers on the plane refer to the code of chromatographic systems in Tables 2-9.

PCA of Retention Data

The values of R_M coefficients for twelve test alkanolamines were measured on the eight diversified hydrocarbonaceous silica gel stationary phases using ten different aqueous binary mobile phases (compare Tables 2-9). The retention data of solutes in the three paper chromatography[37] systems (see Table 7) were also included in these considerations. The purpose of PCA was to find the structure established in such a retention data matrix containing 12 x 80 elements. Assuming that the relative errror of measured R_M coefficients was independent of the employed chromatographic conditions (mainly in reversed-phase mode), a PCA transformation gives the matrix values with a constant variance.

With the use of PCA, a few hidden variables, such as principal components (PCs), can be calculated which explain almost all the observed variance of the retention data set. It appears that the calculated first three principal components, together, explained nearly 59% of the total variance of the initial data matrix, the former accounting for 32.5 %, the second 15.5 %, and third only 11.0 %.

In Fig. 4 the loading inputs to calculated first, second and third principal component, PC1, PC2, PC3 respectively, due to the individual planar chromatography system, are shown. The structure of two-dimensional loading plot in Fig. 5 can be related to the planar chromatographic systems design, *i.e.* composition of binary mobile phase and type of stationary phase. It appears that the main PC1 can be associated with the water fraction in the binary mobile phase as well as changing of the organic modifier from methanol to acetonitrile. This means that PC1 explains the variations in the retention data of alkanolamines caused by mobile phase strenght and solvent strenght selectivity. The second PC2 can be asssociated with type of stationary phase, especially increasing polar character of such phase, and its selectivity. The PC3 is highly loaded (see Fig.4) by retention data registered on the cyano- and aminopropylsiloxane bonded silica layers, and can be related with contribution of the strong, specific interactions of solutes with the stationary phase. Thus, in accordance with the dynamic nature of any chromatographic process, the PCA revealed the three fundamental factors for the systematic variance of registered retention data of alkanolamines as the mobile phase strenght, stationary phase polarity and functionalities in solute molecule.

In Fig. 5, close to the origin of both PC1 and PC2 scales (point with coordinates 0,0), retention data referring to the planar chromatography system number 61 are located. Data for this system (see Table 6) can be considered as the hydrophobicity parameters R_{Mw} of solutes have since been calculated as the water extrapolated (acetonitrile free mobile phase) R_M values on the octadecylsiloxane bonded silica HPTLC plates. Thus the (0,0) point in the loadings plane (Fig. 5) can be regarded as the indicator of diminished contribution of the polar (silanophilic) interactions of stationary phase with solutes during chromatographic retention. The fact that octylsiloxane and diphenylsilica layers, used in systems 16 and 30, respectively, indicate greater bonded phase surface area and bonding density than octadecylsiloxane silica,[39-41] imply further reduction of silanophilic interactions and cause the location of both mentioned TLC systems at the same place in Fig. 5.

The PCA differentiated very well between reduced and enhanced potency to the silanophilic as well as proton donor interactions of the cyano- and aminopropylsiloxane bonded phases[39] by grouping them in the opposite sides of Fig. 5, in the right- and left-upper corner, respectively. Conversely, the other pair of polar stationary phases, *i.e.* propyldiol and cyanopropylsiloxane bonded silica, have been located in the one cluster (at the upper-right corner) indicating

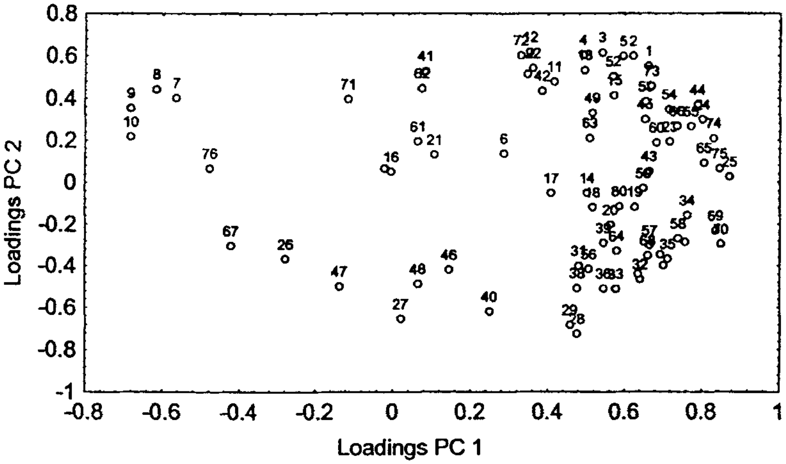

Figure 5. Two-dimensional plot of loadings input to calculate first two principal components (PC1 and PC2) due to the individual planar chromatography systems from PCA of retention data of alkanolamines in Tables 2-9. The numbers on the plane refer to the code of chromatographic systems in Tables 2-9.

their similar strong solvatation by the mobile phase components in a surface phase which diminishes the effect of the presence of polar substituents in these chemically bonded phases on the overall sorbent selectivity. Poole *et al.*[40,41] reported an analogous phenomenon on the results of inspection of retention properties of solid-phase extraction cartridges filled with the mentioned polar silica packings. The enhanced and comparable access of considerable fraction of the original pore volumes of the cyano- and propyldiol- bonded silica by small molecules has been observed[39,40] in contrast to the octadecylsiloxane bonded silica in which the pores are near completly blocked after chemical modification.[23,39] Thus, the chromatographic systems with the octadecylsiloxane phases comprising different ability for solvatation by sorption of organic modifier from mobile phase[42] were grouped in a separate place (the lower-right corner) in Fig. 5.

The position of the paper chromatography systems (numberd as 38, 39, 40, see also Table 7) in Fig. 5, close to the TLC systems (numbered as 31, 66, 28, 58), with alkyl or diphenyl bonded silica and employing rather high concentration of organic modifier in mobile phase, reflects similar predominant significance of the solutes' solvatation in the bulk mobile phase on their

Table 2

Retention Data (log k') for Alkanolamines Determined on Propyldiol Silica Layers (DIOL) with Aqueous-Methanol or Aqueous-Acetonitrile Mobile Phases

		Methanol Concentration (% v/v)					Acetonitrile Concentration (% v/v)				
No.	Solute	20	40	60	80	90	20	40	60	80	90
1	MEA	-1.269	-0.846	-0.804	-0.823	0.901	-1.680	-1.366	-1.024	-0.217	-0.485
2	NAE	0.583	-0.294	-1.000	-1.074	-0.876	-1.122	0.010	0.734	0.590	0.498
3	DIP	-0.967	-0.925	-0.849	-0.861	-0.944	-1.063	0.745	1.957	-0.112	-0.298
4	DEA	-1.372	-0.837	-0.467	0.001	-0.876	1.800	1.500	1.121	-0.578	0.471
5	MeDEA	1.292	1.270	1.118	1.115	0.984	1.295	1.355	1.446	1.374	1.369
6	BuDEA	1.561	1.425	1.272	1.238	1.090	1.555	1.596	1.528	1.325	1.343
7	tBuDEA	1.761	1.635	1.555	1.423	1.264	1.050	0.826	0.960	1.150	1.382
8	PhDEA	-0.858	-0.803	-0.808	-0.838	-0.901	-1.056	-1.066	-1-163	-0.676	-0.587
9	oTDEA	-0.820	-0.807	-0.761	-0.823	-0.990	0.990	-0.251	-1.390	-0.625	-0.556
10	pTDEA	-0.998	-0.762	-0.753	-0.700	-0.886	-0.580	-0.620	-0.650	-0.694	-0.457
11	TEA	0.927	0.846	-0.761	0.603	0.444	0.863	0.873	0.838	1.021	1.121
12	APD	-1.045	-0.903	-0.864	-0.879	-0.955	-0.711	-1.283	-1.000	-0.500	0.498
TLC System No.:		1	2	3	4	5	41	42	43	44	45

Table 3

Retention Data (log k') for Alkanolamines Determined on Aminopropylsiloxane Silica Layer (NH) with Aqueous-Methanol or Aqueous-Acetonitrile Mobile Phases

		Methanol Concentration (% v/v)					Acetonitrile Concentration (% v/v)				
No.	Solute	20	40	60	80	90	20	40	60	80	90
1	MEA	-0.620	-0.590	-0.544	-0.500	-0.471	1.065	0.920	0.010	0.159	0.400
2	NAE	-0.520	-0.752	-0.798	-0.738	-0.513	-0.380	-1.331	-1.100	-0.147	1.043
3	DIP	-1.137	-1.068	-0.873	-0.895	0.093	-1.060	-1.129	-1.550	-1.195	-0.779
4	DEA	-1.430	-1.348	-0.992	-0.779	-0.781	-1.153	-1.409	-0.470	0.944	-0.244
5	MeDEA	-1.065	-0.475	0.031	0.220	0.137	-1.454	-1.206	-0.661	0.864	-0.125
6	BuDEA	-0.824	-0.937	0.178	-0.410	-0.894	-0.820	-2.230	-1.387	1.037	-0.850
7	tBuDEA	-1.032	-0.522	-0.256	-0.679	-0.979	-1.347	-1.973	-1.710	1.737	1,742
8	PhDEA	-1.078	1.071	1.687	1.841	1.874	-1.324	-1.438	-1.765	-0.528	-1.200
9	oTDEA	-0.983	1.043	1.742	1.921	1.916	-1.312	-1.121	-1.796	-1.428	-1.615
10	pTDEA	0.811	1.116	1.773	1.930	1.950	-0.846	-1.048	-1.060	-1.297	-1.070
11	TEA	0.137	0.146	-0.700	-0.794	-1.161	-1.195	-1.928	-1.638	1.391	-0.021
12	APD	-1.200	-0.888	-0.535	-0.656	-0.830	-1.001	-1.196	-0.840	-0.329	-0.400
TLC System No.:		6	7	8	9	10	46	47	48	49	50

Table 4

Retention Data (log k') for Alkanolamines Determined on Cyanopropylsiloxane Silica Layer (CN) with Aqueous-Methanol or Aqueous-Acetonitrile Mobile Phases

		Methanol Concentration (% v/v)					Acetonitrile Concentration (% v/v)				
No.	Solute	20	40	60	80	90	20	40	60	80	90
1	MEA	1.114	1.160	1.188	1.192	1.198	0.640	0.714	0.677	0.578	0.518
2	NAE	1.301	0.925	0.904	1.034	1.089	-1.253	0.205	1.369	1.414	1.366
3	DIP	1.138	-1.019	-0.904	-1.177	-0.687	1.737	1.778	2.053	1.200	0.778
4	DEA	-0.378	-0.535	-0.606	-0.808	-1.031	-0.546	-0.670	-0.807	-0.912	-1.063
5	MeDEA	1.106	1.252	1.232	1.085	-.910	1.275	1.450	1.409	1.167	1.076
6	BuDEA	1.146	1.396	1.449	1.226	1.104	1.588	1.520	1.577	1.211	0.936
7	tBuDEA	1.325	1.682	1.692	1.475	1.292	1.836	1.885	1.926	1.544	1.161
8	PhDEA	0.423	0.202	-0.097	-0.478	-0.811	0.317	-0.041	-0.457	-0.808	-1.088
9	oTDEA	0.921	0.417	-0.386	-0.449	-0.746	0.471	0.041	-0.356	-0.533	-1.101
10	pTDEA	0.662	0.873	0.773	0.693	0.536	0.430	0.072	-0.450	-0.694	-1.143
11	TEA	0.816	0.874	0.773	0.693	0.536	0.840	0.852	0.955	0.790	0.597
12	APD	-1.130	-1.151	-1.075	1.900	-1.604	-0.671	-1.620	-2.230	-1.973	-1.146
TLC System No.:		11	12	13	14	15	51	52	53	54	55

Table 5

Retention Data (log k') for Alkanolamines Determined on Diphenylsilica Layer (DPH) with Aqueous-Methanol or Aqueous-Acetonitrile Mobile Phases

		Methanol Concentration (% v/v)					Acetonitrile Concentration (% v/v)				
No.	Solute	20	40	60	80	90	20	40	60	80	90
1	MEA	-0.459	-0.600	-0.984	-1.075	-1.068	1.115	0.842	0.930	0.491	0.518
2	NAE	0.738	0.895	0.952	0.976	0.697	0.968	0.803	1.072	1.089	1.366
3	DIP	1.266	1.338	0.984	0.593	0.496	1.394	1.704	1.737	1.444	-0.778
4	DEA	-0.260	-0.529	-0.876	-1.090	-1.054	-0.693	-0.994	-1.916	-2.459	-1.063
5	MeDEA	0.738	0.719	0.831	0.720	0.132	0.713	0.779	0.784	0.812	1.076
6	BuDEA	0.940	1.010	0.565	0.078	-0.583	0.968	0.928	0.864	0.882	0.936
7	tBuDEA	1.004	1.035	0.925	1.430	1.595	0.817	1.099	1.195	1.153	1.161
8	PhDEA	0.489	0.363	-0.230	-0.715	-0.893	0.193	-0.125	-0.693	-1.444	-1.088
9	oTDEA	1.844	0.301	-0.117	-0.612	-0.888	0.396	-0.011	-0.629	-1.625	-1.101
10	pTDEA	0.782	0.339	-0.113	-0.596	-0.883	0.358	-0.027	-0.623	-1.374	-1.143
11	TEA	0.107	0.157	-0.010	-0.074	-0.633	0.200	0.573	0.528	-0.609	-1.200
12	APD	1.397	1.418	1.460	1.430	1.348	1.635	1.576	1.510	1.625	-1.146
TLC System No.:		16	17	18	19	20	56	57	58	59	60

Table 6

Retention Data (log k') for Alkanolamines Determined on Wetted Silica Layer (WC18) with Aqueous-Methanol or Aqueous-Acetonitrile Mobile Phases

		Methanol Concentration (% v/v)					Acetonitrile Concentration (% v/v)					
No.	**Solute**	**20**	**40**	**60**	**80**	**90**	**0**	**20**	**40**	**60**	**80**	**90**
1	MEA	-0.509	-0.371	-0.376	-0.230	0.514	-0.652	-0.862	0.157	0.153	0.984	0.838
2	NAE	0.703	0.546	0.437	0.781	1.114	0.099	0.389	0.674	0.771	0.852	1.161
3	DIP	1.305	0.843	0.211	-0.144	1.183	2.049	1.680	1.200	1.525	1.062	-0.635
4	DEA	0.088	-0.253	-0.483	-0.847	-0.744	-0.427	0.441	0.325	-0.061	-0.241	0.057
5	MeDEA	0.317	0.369	0.325	0.736	0.888	0.084	0.497	0.424	0.051	0.978	1.438
6	BuDEA	0.804	0.561	0.398	0.772	0.893	0.509	0.730	0.438	0.061	0.706	0.928
7	tBuDEA	0.898	0.775	0.451	-.785	1.039	0.532	0.914	0.895	0.102	1.121	1.508
8	PhDEA	0.481	0.098	-0.301	-0.603	-0.862	0.356	0.376	-0.010	-0.694	-1.205	-1.048
9	oTDEA	0.733	0.320	-0.146	-0.512	-0.782	0.560	0.648	0.115	-0.578	-0.928	-0.928
10	pTDEA	0.873	0.420	-0.062	-0.431	-0.723	0.594	0.711	0.178	-0.491	-0.785	-0.744
11	TEA	0.565	0.344	0.178	0.431	0.833	-0.054	0.364	0.233	0.020	0.807	1.331
12	APD	0.868	-0.522	0.146	-0.246	-0.231	-0.254	-0.505	-0.349	-0.164	-0.984	-0.785
TLC System No.:		21	22	23	24	25	61	62	63	64	65	66

Table 7

Retention Data (log k') for Alkanolamines Determined on Octadecylsiloxane Silica Layer (C18) with Aqueous-Methanol or Aqueous-Acetonitrile Mobile Phases and in Paper Chromatography Systems

		Methanol Conc'n (% v/v)				Acetonitrile Conc'n (% v/v)				Paper Chromatography		
No.	**Solute**	**40**	**60**	**80**	**90**	**40**	**60**	**80**	**90**	**A***	**B****	**C#**
1	MEA	1.541	1.534	1.453	1.358	0.788	0.547	1.518	1.457	0.630	-0.070	0.327
2	NAE	1.621	1.512	1.450	1.431	0.624	1.056	1.423	1.638	0.825	0.052	0.176
3	DIP	1.504	1.116	0.790	1.171	1.002	1.204	1.416	1.539	0.231	-0.477	-0.410
4	DEA	2.685	1.417	0.029	1.472	0.718	-1.003	-1.924	-0.851	0.575	-0.213	0.052
5	MeDEA	1.721	1.072	0.555	1.504	1.123	1.204	1.367	1.327	0.477	-0.212	-0.288
6	BuDEA	1.748	1.064	0.459	1.490	0.405	0.845	1.306	1.225	-0.035	-0.525	-0.865
7	tBuDEA	-1.740	-0.954	-0.126	1.735	-0.501	0.103	1.618	1.638	0.176	-0.432	-0.825
8	PhDEA	0.673	0.080	-0.525	1.215	0.689	-0.380	-1.437	-1.457	-0.908	-1.995	-0.688
9	oTDEA	1.777	0.780	-0.171	1.967	2.594	-0.335	-1.924	-1.225	0.001	-0.630	-0.908
10	pTDEA	1.173	0.343	-0.323	1.542	0.483	-0.320	-1.119	-1.005	-0.005	-0.650	-0.930
11	TEA	1.453	0.733	0.151	1.741	0.449	1.101	1.253	1.220	0.550	-0.213	0.087
12	APD	1.324	1.435	1.540	1.867	1.287	1.518	1.732	1.766	0.658	-0.454	-0.035
TLC System No.:		26	27	28	29	67	68	69	70	38	39	40

Table 8

Retention Data (log k') for Alkanolamines Determined on Octylsiloxane Silica Layer (C8) with Aqueous-Methanol or Aqueous-Acetonitrile Mobile Phases

		Methanol Concentration (% v/v)				Acetonitrile Concentration (% v/v)				
No.	Solute	40	60	80	90	20	40	60	80	90
1	MEA	1.358	1.442	1.525	1.706	0.146	0.898	1.369	1.690	1.499
2	NAE	1.431	1.751	1.751	1.789	-0.066	-0.370	0.097	1.462	1.256
3	DIP	1.171	1.930	1.930	1.282	-2.000	-1.500	-0.129	1.195	1.905
4	DEA	1.142	1.802	1.802	1.841	0.845	-0.538	-0.611	-0.771	-0.201
5	MeDEA	1.504	1.317	1.317	0.769	0.880	1.269	1.697	1.905	1.578
6	BuDEA	1.490	1.440	1.440	1.100	0.810	1.252	1.662	1.987	1.721
7	tBuDEA	1.735	1.262	1.262	1.162	1.290	1.681	2.083	1.955	1.727
8	PhDEA	1.215	0.405	-0.387	-0.781	0.760	0.236	-0.311	-0.812	-0.784
9	oTDEA	1.967	1.580	-0.205	-0.535	0.582	0.183	-0.238	-0.622	-0.601
10	pTDEA	1.542	0.681	-0.185	-0.628	0.498	0.156	-0.203	-0.532	-0.702
11	TEA	1.741	1.418	1.085	0.739	0.579	1.217	1.469	1.591	1.557
12	APD	1.867	1.805	1.751	1.730	0.794	-0.506	-0.574	-0.664	-0.188
TLC System No.:		30	31	32	33	71	72	73	74	75

Table 9

Retention Data (log k') for Alkanolamines Determined on Silanized Silica Layer (C2) with Aqueous-Methanol or Aqueous-Acetonitrile Mobile Phases

		Methanol Concentration (% v/v)				Acetonitrile Concentration (% v/v)				
No.	Solute	40	60	80	90	20	40	60	80	90
1	MEA	1.271	1.415	1.510	-0.082	0.696	0.769	0.720	1.076	1.602
2	NAE	1.952	0.950	-0.070	1.119	-0.677	1.209	1.562	1.613	-1.603
3	DIP	1.634	1.229	0.792	0.670	0.943	1.442	1.540	1.740	1.729
4	DEA	-0.783	-0.873	-0.886	-1.369	0.502	0.036	-0.923	-1.301	-0.194
5	MeDEA	1.212	0.903	0.586	0.343	1.510	1.450	1.351	1.362	1.580
6	BuDEA	1.291	0.877	0.438	0.135	1.187	0.087	-0.567	-0.978	1.507
7	tBuDEA	1.594	1.061	0.492	0.220	-0.005	1.012	1.462	1.513	1.908
8	PhDEA	-0.053	-0.349	-0.670	-1.015	1.610	-0.040	-0.567	-1.431	-1.187
9	oTDEA	0.653	0.010	-0.602	-0.984	1.801	0.163	-0.549	-1.613	-0.909
10	pTDEA	0.131	-0.185	-0.586	-0.874	1.709	0.041	-0.501	-0.581	-0.615
11	TEA	1.043	0.610	0.176	-0.010	1.196	1.106	1.061	1.301	1.421
12	APD	1.251	1.573	1.915	1.826	1.738	1.623	1.549	1.919	1.987
TLC System No.:		34	35	36	37	76	77	78	79	80

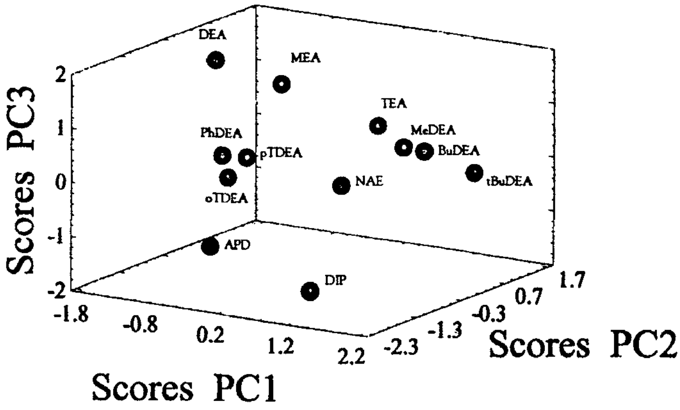

Figure 6. Spatial plot of scores input to calculate first three principal components (PC1, PC2 and PC3) due to the individual solutes from PCA of retention data of alkanolamines in Tables 2-9. For abbreviation on the plane see Table 1.

retention. Moreover, in mentioned TLC systems the transport of solute molecules from the bulk mobile phase to the non-polar chemically bonded stationary phase is less favored by competitive sorption of molecules of organic modifiers from binary mobile phase in the surface phase.[17,20,23,42]

To explain which original variables have high impact on the above-calculated PCs, their respective scores have been investigated. Fig. 6 gives a calculated scores plot, which shows the contribution of the twelve solutes to the original Pcs. Here, it is seen that solutes such as MeDEA, BuDEA, tBuDEA and NAE, DIP, TEA (see Table 1 for codes) are most extreme on the mobile phase strenght axis PC1. The tBuDEA and BuDEA load very high on the both first and second PC as shown in Fig. 7.

The solutes containing phenyl substituent in their structures, *i.e.* PhDEA, oTDEA and pTDEA, load the second PC2, explaining that their retention is driven, in fact, by presence or lack of hydrophobic interactions with stationary phase. The PC3 is loaded by such polar solutes as MEA, DEA and TEA which contain exposed hydroxy group(s), giving possibility for the specific interactions, *i.e*,. hydrogen bond formation, with the polar stationary phase, *e.g.*, cyano- and aminopropylsiloxane or propyldiol bonded silica. On results of PCA, one can assume method development for optimizing chromatographic

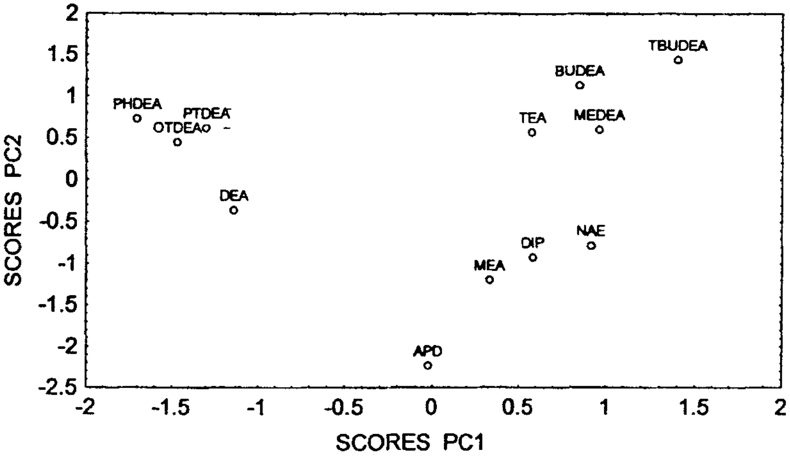

Figure 7. Two-dimensional plot of scores input to calculate first two principal components (PC1 and PC2) due to the individual solutes from PCA of retention data of alkanolamines in Tables 2-9. For abbreviation on the plane see Table 1.

separation of the three groups of alkanolamines in which the retention process can be regulated, primarily, by molecular interactions in the mobile phase (for the N-alkyl substituted solutes loading PC1), or by interactions with the stationary phase (for the N-phenyl substituted solutes loading PC2), as well as by modification (derivatization) of solute structure (for solutes loading PC3). However, the values of explained variance of experimental retention data by each calculated PC, indicates the relatively low and comparable contribution of the stationary phase polarity and its ability to hydrogen bond formation to the overall retention of solutes in the particular planar chromatography system employing aqueous-methanol or aqueous-acetonitrile binary mobile phase.

A similar conclusion was drawn for retention on HPLC columns with diversified bonded functional groups by Snyder *et al*[43] on the basis of gradient elution theory as well as Park *et al.*[44] and Abraham *et al.*[45] using linear solvation energy relationships. Similarly, on the basis of thermodynamically formulated theory of chromatographic processes[46] the dominating role of intermolecular association between solute and more polar components of bulk mobile phase during retention of alkanolamines have been previously indicated[15,19,20] after inspection of the limited retention data registered in adsorption TLC systems.

Prediction of Alkanolamines Bioactivity

Systematic information revealed by chemometric analysis with PCA of retention data, determined in various planar chromatography systems, can be useful to clarify the observed lysosomotropic activity of alkanolamines. The statistically significant relationship has been calculated by a multiregression method between the logarithm of minimal bactericidal concentration (log *MBC*)[9] of alkanolamines and calculated principal components scores presented in Fig. 6 :

$$\log MBC = -0.465\ (+/-\ 0.100) * PC1 + 0.438\ (+/-\ 0.100) * PC2 \\ - 0.209\ (+/-\ 0.100) * PC3 - 1.635\ (+/-\ 0.105) \qquad (2)$$

$$n = 12;\ R = 0.932;\ F_{(3,8)} = 15.021;\ SE = 0.364;\ p < 0.001$$

The moderate level of antimicrobial activity against *Pseudomonas pseudoalcaligenes* observed for BuDEA and DEA,[9] which were significantly related with scores calculated here for PC1 and PC2, respectively, can be explained by their ability to hydrogen bond formation for the former compound and electrostatic interactions for the second compound, both accompanied with dispersive interactions, during penetration of bacterial membrane. Thus, the established above model equation (2) indicates that some modification of structural features of alkanolamines, especially in kind of substituents connected to the tertiary amine group, which causes any disturbances in their subtle balanced ability to specific molecular interactions leads to the sharp changes of lysosomotropic and antimicrobial activity of such compounds. Similar effects have been observed for the highly molecular bactericidal agents 4-aza-5α-sitostanes in which extended lipophilicity leads to increase of their antimicrobial activity.[7,8]

CONCLUSION

Principal Components Analysis provides a useful interpretation of the retention characteristics of the planar chromatography systems used for alkanolamines separation, under reversed-phase conditions, in terms of optimization of their selectivity, and the structural features of solutes that affect retention in particular chromatographic systems. The polar bonded silica layers, containing cyanopropylsiloxane or propyldiol functionalities, give near comparable separation properties of ionizable alkanolamines as the standard, commercially available octadecylsiloxane phase. The performed PCA select the three planar chromatography systems in which the pure hydrophobic interactions dominate the retention process of alkanolamines. In spite of the limited set of solutes used in these studies, the conclusions drawn on the PCA of retention data of alkanolamines were useful for the correct prediction of their antimicrobial activity.

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