Interrelation of Retention Factor of Amino-Acids by QSPR and Linear Regression

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The interrelation between retention factors of several L-amino acids and their physico-chemical and structural properties can be determined in chromatographic research. In this paper we describe a predictor for retention factors with various properties of the L-amino acids. Eighteen L-amino acids are included in this study, and retention factors are measured experimentally by RP-HPLC. Binding energy (E_b), hydrophobicity ($log\ P$), molecular refractivity (MR), polarizability (α), total energy (E_t), water solubility ($log\ S$), connectivity index (χ) of different orders and Wiener index (w) are theoretically calculated. Relationships between these properties and retention factors are established, and their predictive and interpretive ability are evaluated. The equation of the relationship between retention factors and various descriptors of L-amino acids is suggested as linear and multiple linear form, and the correlation coefficients estimated are relatively higher than 0.90.

Key Words : Amino-acids, QSPR, Linear regression

Introduction

With the advent of inexpensive and rapid computational power, there has been a remarkable growth in interest in the area of quantitative structure-property/activity relationships (QSP/AR, this abbreviation is used frequently for a designation of such equations),[1-6] which use multivariate methods to model relevant properties as a function of molecular structure parameters (called descriptors). A large number of descriptors (structural, topological, constitutional, electronic and geometric) and physico-chemical properties have been proposed in the literature.[7,8] Therefore, the knowledge of the relation between retention and these descriptors makes possible the design of substances with predetermined properties, which is an important task in chemistry and biology.

Meanwhile, retention in chromatography is the result of a competitive distribution process of the solute between the mobile and stationary phase, in which the partitioning of the solute between these phases is largely determined by the molecular structure. Based on this approach, much of the literature describes linear or multiple linear regression models for predicting RP-HPLC retention, using different kinds of molecular descriptors and physico-chemical properties.[9-13] Theoretical calculations of various molecular descriptors and physico-chemical properties can be obtained with special computer programs.[14-16] In the present study, the retention of L-amino acids is assumed to be governed by their physico-chemical properties [binding energy (E_b), hydrophobicity ($log\ P$), molecular refractivity (MR), polarizability (α), total energy (E_t), water solubility ($log\ S$)] and the theoretical topological indexes [connectivity index (χ) of different orders and Wiener index (w)] are calculated. Especially, the topological indexes (connectivity or Wiener) have been used very widely for the description and representation of the molecular structures.[17] So, molecular connectivity can be used to easily and quickly characterize the molecular structures. Also, they can be correlated with many physical, chemical and biological properties of molecules by obtaining the QSPR relationships. This method allows the description of a wide spectrum of molecular properties, establishing the correlation dependences between the properties and values of topological indices. Modern science uses topological indices for the description of far more complicated systems and their properties, including reactivity.

In the present paper, quantitative relationships between the retention factors of several L-amino acids and their various physico-chemical properties and topological indices are studied. Furthermore, the evaluation of the predictive retention factor of L-amino acids was done by linear and multiple linear empirical equations.

Experimental Section

Reagents. Eighteen standard L-amino-acids were purchased from Sigma (St. Louis, MO, USA). HPLC grade solvent, acetonitrile was obtained from Ducksan Pure Chemical (Kyungki province, Korea). Trifluoroacetic Acid (TFA) was purchased from Sigma (St. Louis, MO, USA). Water was filtered by a Milipore ultra pure water system (Milipore, Bedford, MA, USA).

Sample preparation. Eighteen standard L-amino-acids (5 mg) were each dissolved in 1 mL of water, then the solutions were mixed, respectively. A constant injection volume (20 μL) for the mixture solutions, was used throughout.

Apparatus and method. HPLC was performed using Waters 600S solvent delivery system (Waters, Milford, MA, USA) and the 2487 UV dual channel detector was used (Waters, Milford, MA, U.S.A.). The data acquisition system was Millenium^{32} (Waters) installed in an HP Vectra 500 PC. The mobile phases were degassed with helium. The flow rate of the mobile phases was 1 mL/min and monitored at the fixed wavelength of 215 nm. The column was purchased

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from Alltech Co. The column size was 0.46 × 15 cm and packed by C18, 100 Å, 5 μm. All the experimental runs were carried out in ambient temperature. The dead time (t_m) was determined as the retention volume of 20 mL of acetonitrile.

Mobile phase composition. In isocratic mode, the binary system of 0.5% TFA in water and 0.1% TFA in acetonitrile was used. In gradient mode, the first mobile phase composition was water with 0.5% TFA/acetonitrile with 0.5% TFA, 90/10 vol.%, after 10 min. The second composition of the mobile phase was changed to 80/20 vol.% after 20 min.

Theoretical Background

A. Retention Factor. The retention factor (k) of L-amino acids was defined as

$$k = (t_R - t_m)/t_m \quad (1)$$

where t_R and t_m are the retention times of the solute and an unretained compound, respectively.

B. Physico-chemical Properties. The calculations of physico-chemical properties were performed using the ChemSW[14] and HyperChem program.[15,16] The molecular refractivity (MR) and polarizability (α) of all L-amino acids have been obtained by a semi-empirical method, AM1, in the package HyperChem. The values of binding energy (E_b), hydrophobicity ($log\ P$), total energy (E_t) and water solubility (S) were calculated by the ChemSW.

***Binding energy*:** The binding energy (E_b) is the energy of all the chemical binding of molecules. This parameter characterizes energy that is necessary for the destruction of all chemical bonds between atoms in a molecule.[18]

***Molecular refractivity*:** The molecular refractivity (MR) is the molar volume corrected by the refractive index. It was represented by size and polarizability of a fragment or molecule. The molecular refractivity can be rewritten as follows:

$$MR = ((n^2 - 1)M)/(n^2 + 2)d \quad (2)$$

Where n is the refractive index, M the molecular weight, d the density.[19]

***Polarizability*:** Polarizability (α) is the ease of distortion of the electron cloud of a molecular entity by an electric field (such as that due to the proximity of a charged reagent). It is experimentally measured as the ratio of induced dipole moment (μ_{ind}) to the field (E), which induces it

$$\alpha = \mu_{ind}/E \quad (3)$$

In ordinary usage the term refers to the "mean polarizability", *i.e.*, the average over three rectilinear axes of the molecule. Polarizability is seen in certain modern theoretical approaches as a factor influencing chemical reactivity, etc.[19]

***Total energy*:** Total energy (E_t) is the general complete energy of a molecule. Thus, total energy takes all types of the intramolecular energetic interactions.[20] Total energy in a vacuum approximation can be written as follows

$$E_t = E_b - E_e - E_n \quad (4)$$

where E_b is the binding energy, E_e the electronic energy, E_n the nuclear energy.

Water solubility. Water solubility ($log\ S$), also known as aqueous solubility, is the maximum amount of a substance dissolved in water at equilibrium at a given temperature and pressure. Water solubility values are usually expressed as moles of solute per liter. Water solubility has been correlated to various chemical parameters used to determine the fate of chemicals in the environment.[21]

C. Structure Properties. Connectivity indexes (χ) of the different orders and Wiener index (w) were calculated by the ChemSW.

***Connectivity indexes*:** In the present work we have used connectivity indices.[22,23] All these indices may be derived from the adjacency matrix and they are defined as

$$^n\chi = \sum_{g=1}^{n+1} (\delta_i)_j^{-1/2} \quad (5)$$

where n is the subgraph order, *i.e.* the number of edges in the subgraph, j denotes the particular set of edges that constitutes the subgraph and δ_i vertices valence.[24]

***Wiener index*:** The Wiener index (w) is one of the oldest molecular-graph-based structure descriptors and its chemical applications are well documented.[25] If G is a molecular graph, u and v are two vertices of G, and $d(uv|G)$ is their distance (= number of edges in a shortest path connecting u and v), then the Wiener index is defined as

$$w = w(G) = \sum_{u<v} d(uv|G) \quad (6)$$

with the summation over all pairs of vertices of G.[26] The fact that there are good correlations between a variety of physico-chemical properties of organic compounds (boiling point, heat of evaporation, heat of formation, chromatographic retention times, surface tension, vapor pressure, partition coefficients, etc.) could be rationalized by the assumption that w is roughly proportional to the Van-der-Waals surface area of the respective molecule.

D. Regression Equations. In many cases the characteristic can be well written as follows

$$R = a - b(P) \quad (7)$$

where R is a parameter describing chromatographic retention, P is a structural or physico-chemical parameter (descriptors), a and b are coefficients. As a rule, theoretical recommendations for the selection of descriptors are absent, and these terms are taken as parameters whose values are determined from the data of a limited set of studied molecules. The regression equations were obtained by correlating the experimental data of retention factors with the calculated physico-chemical and structural descriptors through linear regression analysis, which led to eq. (7).

Results and Discussion

In this paper, the quantitative relationships between the

Table 1. Name and chemical structure of L-amino acids

Compound	Structure
Alanine	CH_3-CH(NH_2)-COOH
Arginine	HN=C(NH_2)-NH-(CH_2)$_3$-CH(NH_2)-COOH
Aspartic acid	HOOC-CH_2-CH(NH_2)-COOH
Cysteine	HS-CH_2-CH(NH_2)-COOH
Glutamic acid	HOOC-(CH_2)$_2$-CH(NH_2)-COOH
Glycine	NH_2-CH_2-COOH
Histidine	NH-CH=N-CH=C-CH_2-CH(NH_2)-COOH
Isoleucine	CH_3-CH_2-CH(CH_3)-CH(NH_2)-COOH
Leucine	(CH_3)$_2$-CH-CH_2-CH(NH_2)-COOH
Lysine	H_2N-(CH_2)$_3$-CH(NH_2)-COOH
Methionine	CH_3-S-(CH_2)$_2$-CH(NH_2)-COOH
Phenylalanine	Ph-CH_2-CH(NH_2)-COOH
Proline	NH-(CH_2)$_3$-CH-COOH
Serine	HO-CH_2-CH(NH_2)-COOH
Threonine	CH_3-CH(OH)-CH(NH_2)-COOH
Tryptophan	Ph-NH-CH=C-CH_2-CH(NH_2)-COOH
Tyrosine	HO-p-Ph-CH_2-CH(NH_2)-COOH
Valine	(CH_3)$_2$-CH-CH(NH_2)-COOH

chromatographic retention data of L-amino acids and several descriptors are studied. Table 1 shows the name and structure of the amino acids. The retention factor (k) was obtained by RP-HPLC. To investigate the relationship of the retention factor in terms of descriptors, several experimental runs were performed on isocratic and gradient modes. The retention factors of L-amino acids are summarized in Table 2, and the chromatogram of the L-amino acids are shown in Figure 1.

In this work, one of the eluent components of the mobile phase was water, which does not interact with the hydrophobic adsorbent surface. And it does not compete with the L-amino acids for adsorption. Acetonitrile can interact with the adsorbent surface and compete with L-amino acid molecules for adsorption sites. The optimum mobile phase of L-amino acids was selected in this research. The selected gradient of a mobile phase allows conducting the analysis of L-amino acids for a reasonable time and gives satisfactory selectivity of separation.

The absence of a strict homology in a series of investigated molecules is a serious obstacle in the creation of an QSPR model in RP-HPLC of L-amino acids. In this connection, the simple linear correlation equations are not always applicable to the description of retention within the limits of a nonhomologous series. The best outcomes can be obtained by formal division of all investigated substances into groups which have some common features.

All investigated L-amino acids were divided into three groups. The ten L-amino acids containing nonpolar, various alkyl- or phenyl-radicals were incorporated in the first group. Compounds of the nonpolar category were characterized by low dielectric permeability, weak dipole moment, small

Table 2. Retention factors with isocratic and gradient modes eluting of L-amino acids

Compound	Mobile phase composition			
	*Gradient mode	Isocratic mode		
	k	k**(100/0)	k (95/5)	k (90/10)
Glycine	0.105	0.104	0.074	0.081
Serine	0.162	0.117	0.070	0.086
Aspartic acid	0.208	0.161	0.104	0.117
Lysine	0.231	0.200	0.108	0.106
Alanine	0.250	0.187	0.095	0.110
Glutamic acid	0.255	0.209	0.102	0.109
Cysteine	0.269	0.209	0.095	0.128
Threonine	0.276	0.207	0.095	0.095
Histidine	0.285	0.233	0.132	0.113
Arginine	0.542	0.383	0.171	0.112
Proline	0.596	0.497	0.169	0.142
Valine	1.046	1.392	0.405	0.256
Tyrosine	1.284	5.179	0.909	0.325
Methionine	1.404	1.682	0.521	0.319
Isoleucine	2.309	4.237	0.980	0.490
Leucine	2.467	5.065	1.213	0.592
Phenylalanine	4.544	10.31	2.075	0.904
Tryptophan	7.113	29.53	4.723	1.712

*The first mobile phase composition was water with 0.5% TFA/acetonitrile with 0.5% TFA, 90/10 vol.%, then after 10 min, the second composition of mobile phase was changed to 80/20 vol.%, after 20 min. **(A/B) = (A: 0.5% TFA with water, B: 0.5% TFA with acetonitrile)

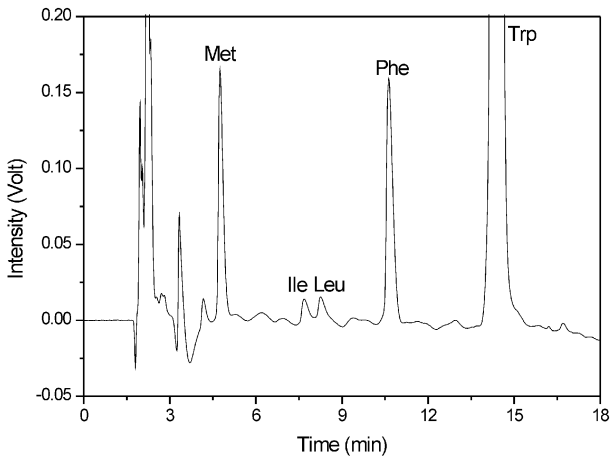

Figure 1. Chromatogram of the L-amino acids with gradient mode.

values of empirical parameters describing specific intermolecular interactions and not capable of playing the role of the donor of hydrogen bond. Such L-amino acids very poorly interact with the mobile phase, as in them only non-specific interactions can take place. The second group is submitted by three amino acids, which have hydroxyl groups. The L-amino acids with polar amino-groups were incorporated in the third group. Polar L-amino acids are characterized by high dielectric capacitivity, marked dipole moment and great values of empirical parameters describing the specific intermolecular interactions. They are good donors of electronic pairs and, hence, can be interactive in the mobile phase.

Table 3. Several physico-chemical properties of L-amino acids

Group	No.	Property / Compound	E_b *[Atomic Units]	*log P* [–]	*MR* [Å^3]	α [Å^3]	E_t [Atomic Units]	*log S* [mol/M^3 water]
1	1	Glycine	-3.694	-1.070	16.00	6.520	-66.46	4.117
	2	Alanine	-4.938	-0.530	20.50	8.350	-75.15	3.914
	3	Cysteine	-5.086	-0.730	28.17	11.35	-86.06	3.195
	4	Proline	-7.280	-0.140	28.06	11.25	-91.09	2.950
	5	Valine	-7.427	0.340	29.49	12.02	-92.52	3.192
	6	Methionine	-7.575	-0.550	37.83	15.02	-103.4	2.258
	7	Isoleucine	-8.672	0.730	34.09	13.86	-101.2	2.672
	8	Leucine	-8.671	0.660	34.17	13.86	-101.2	2.672
	9	Phenylalanine	-11.07	1.150	45.12	18.01	-120.8	1.381
	10	Tryptophan	-13.43	0.930	56.20	23.28	-147.2	1.162
2	11	Serine	-5.300	-1.320	22.04	8.990	-93.59	4.902
	12	Threonine	-6.555	-0.910	26.46	10.83	-102.3	4.699
	13	Tyrosine	-11.45	0.870	46.81	18.65	-139.3	2.348
3	14	Aspartic acid	-6.864	3.070	23.20	10.46	-113.0	4.322
	15	Lysine	-9.266	-1.210	35.99	14.63	-113.5	2.870
	16	Glutamic acid	-7.751	3.320	33.11	12.29	-127.7	3.584
	17	Histidine	-9.097	-1.150	38.38	15.75	-121.2	2.964
	18	Arginine	-10.46	-1.030	47.13	18.07	-136.9	2.859

*(1 Atomic Units = 9.3145×10^7 Electron volts/m)

Table 3 summarizes the several physico-chemical properties that were calculated in this work. Table 4 illustrates the topological indexes (connectivity of the different orders and wiener) used to obtain Eq. (5) and Eq. (6), respectively. It is to be noted the presence of the terms $^0\chi$ and $^1\chi$, which are null for L-leucin and L-isoleucine. As generally known, these indices take into account first-order interactions only ($^1\chi$) of the neighboring atoms, otherwise ignoring these interactions ($^0\chi$) totally. From this point of view, the connectivity indexes of the zero and first orders are not distinguishable for any of the structural isomers. The connectivity indices of the higher ($^2\chi$, $^3\chi$...) orders take account of distant intramolecular interactions. Therefore, these indices guarantee the full description of the structural characteristic property of the

Table 4. Topological indexes of L-amino acids

Group	No.	Index / Compound	$^0\chi$	$^1\chi$	$^2\chi$	$^3\chi$	$^4\chi$	*w
1	1	Glycine	9.284	2.270	1.802	0.817	0	101.2
	2	Alanine	12.15	2.643	2.488	1.333	0	193.3
	3	Cysteine	12.86	3.181	2.630	1.782	0.471	242.6
	4	Proline	14.98	3.805	3.289	2.343	1.624	353.6
	5	Valine	17.73	3.553	3.347	2.103	0.770	476.1
	6	Methionine	18.28	4.181	3.364	2.151	0.859	675.3
	7	Isoleucine	20.44	4.091	3.489	2.593	1.029	722.7
	8	Leucine	20.44	4.037	3.851	1.981	1.016	735.2
	9	Phenylalanine	19.97	5.698	4.961	3.568	2.221	915.5
	10	Tryptophan	22.84	7.182	6.503	5.294	3.900	1469
2	11	Serine	12.86	3.181	2.630	1.782	0.471	235.8
	12	Threonine	15.73	3.553	3.347	2.103	0.770	378.3
	13	Tyrosine	20.84	6.092	5.583	3.979	2.363	1045
3	14	Aspartic acid	14.44	4.037	3.851	1.981	1.016	396.6
	15	Lysine	21.98	4.681	3.717	2.401	1.050	992.6
	16	Glutamic acid	17.15	4.537	4.192	2.337	0.930	544.0
	17	Histidine	17.27	5.198	4.607	3.318	2.044	629.4
	18	Arginine	23.56	5.537	4.900	2.829	1.352	1375

*(0.5 × sum of interatomic distances)

Table 5. Linear equations of various physico-chemical properties and topological indexes with groups

Descriptor	Group	P_i	a_i	b_i	r^2
Physico-chemical in table 3	1	E_h	-3.717	-0.736	0.907
		log P	1.829	2.300	0.634
		MR	-3.957	0.181	0.870
		α	-3.930	0.445	0.888
		E_t	-7.246	-0.094	0.911
		log S	7.796	-2.103	0.802
	2	E_h	-0.895	-0.189	0.990
		log P	0.814	0.528	0.993
		MR	-0.906	0.047	0.994
		α	-0.963	0.120	0.992
		E_t	-2.257	-0.025	0.992
		log S	2.305	-0.435	0.999
	3	E_h	-0.352	-0.076	0.606
		log P	0.320	-0.026	0.211
		MR	-0.166	0.013	0.711
		α	-0.227	0.037	0.663
		E_t	-1.132	-0.012	0.754
		log S	0.672	-0.111	0.269
Structure in table 4	1	$^0\chi$	-5.000	0.415	0.650
		$^1\chi$	-4.121	1.509	0.932
		$^2\chi$	-3.858	1.643	0.941
		$^3\chi$	-2.073	1.704	0.896
		$^4\chi$	-0.111	1.784	0.854
		w	-1.123	0.005	0.935
	2	$^0\chi$	-1.852	0.147	0.929
		$^1\chi$	-1.091	0.389	0.999
		$^2\chi$	-0.955	0.397	0.980
		$^3\chi$	-0.789	0.520	0.998
		$^4\chi$	-0.154	0.606	0.997
		w	-0.214	0.001	0.995
	3	$^0\chi$	-0.171	0.025	0.486
		$^1\chi$	-0.618	0.192	0.683
		$^2\chi$	-0.669	0.229	0.706
		$^3\chi$	-0.007	0.121	0.209
		$^4\chi$	0.209	0.074	0.062
		w	0.080	0.0003	0.681

molecules. The maximum discriminating capacity in a series of investigated L-amino acids (except for L-leucin and L-isoleucine) has a connectivity index of zero order.

Retention factors were estimated by the mathematical equations expressed as a linear relationship in terms of physico-chemical and/or structural properties of the target compounds. For a property (P_i) with a retention factor k in the form of a linear equation, the equation can be written as

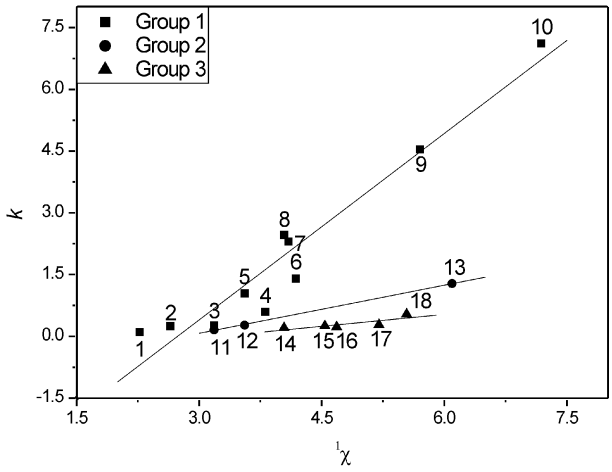

Figure 2. Correlation of k in descriptor of $^1\chi$ with groups (■ : Group 1. ● : Group 2. ▲ : Group 3).

$$k = a_i + b_i P_i \tag{8}$$

where k is the retention factor, P_i is the structural or physico-chemical parameter (descriptor), and a_i and b_i are coefficients. The resulting correlations for three groups of L-amino acids are suggested in Table 5. A rating of the quality of the correlation equations was conducted with the help of a correlation coefficient r^2. As can be observed in many cases the linear models were adequate ($0.70 \le r^2 \le 1.00$). Figure 2 shows the relationships between the connectivity indices of the first order of the L-amino acids and their retention data. The trend demonstrates the linear relationship. However, in the third group of L-amino acids, the simple linear equation gave a low regression coefficient, less than 0.75. Therefore, the multiple linear statistical analysis was used to find other reliable expressions. The retention factors with various physico-chemical and/or structural properties of L-amino acids from first and third groups are represented in the form,

$$k = a_0 - \sum a_i(P_i) \tag{9}$$

Eq. (9) was tested and the numeric coefficients were determined by linear regression. After investigating the effect of descriptors on retention factors, the five major ones were found to be E_h, *MR*, E_t, $^1\chi$ and w. The expression and regression coefficient for the combined properties with retention factor k is shown in Table 6. The good quantitative agreement of the experimental data and the calculated values by Eq. (9) is seen in Figures 3 and 4. The correlation coefficient was more than 0.95; therefore, the empirical models of the chromatographic retention (Eqs. (8) and (9)) might be used to predict the retention factors as well as the

Table 6. Combined retention factor in multiple linear form in the first and third groups

$k = a_0 + a_1E_h + a_2MR + a_3E_t + a_4{}^1\chi + a_5w$	Group	a_0	a_1	a_2	a_3	a_4	a_5	r^2
	1	0.055	0.066	-0.110	0.065	2.683	0.003	0.959
	3	-0.152	0.350	-0.009	0.002	0.705	0.0008	0.975

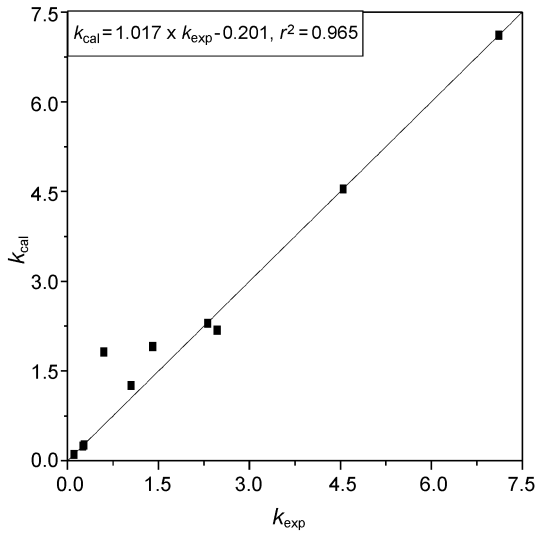

Figure 3. Comparisons of the experimental data and the calculated retention factors in the fisrt group.

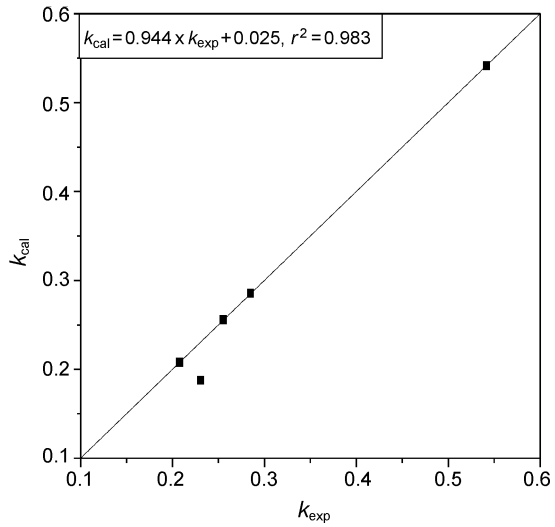

Figure 4. Comparisons of the experimental data and the calculated retention factors in the third group.

eluting order of compounds. The correlation equations can describe and predict the chromatographic retention of L-amino acids. This demonstrates the statistical availability of the equations. If this parameter set is correctly selected, it should be applied to other relationship processes, such as the k value determined with various eluent systems containing other organic modifiers and the k value obtained in different partitioning systems.

In light of our results, we conclude that the adequate selection of descriptors may give rise to a mathematical model, Eqs. (8) and (9), which is useful not only for the prediction of retention factors but also for the prediction of other characteristics of L-amino acids. Although we have considered only one class of molecules, namely the L-amino acids, the method proposed can also be adapted to other molecules. Finally, it can be concluded that QSPR equations are a very interesting formalism for the prediction of chromatographic retention and even in the search for new properties of L-amino acids within line of the structural homologies.

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