Immobilized Artificial Membrane Chromatography: Quantitative Structure-Retention Relationships of Structurally Diverse Drugs

Juan M. Luco,*,† Adriana P. Salinas,‡ Angel A. J. Torriero,§ Rodolfo Nieto Vázquez,§ Julio Raba,§ and Eduardo Marchevsky§

Laboratorio de Alimentos, Area de Farmacotécnia, and Departamento de Química, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco y Pedernera 5700 San Luis, Argentina

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The chromatographic capacity factors (log k') for 32 structurally diverse drugs were determined by high performance liquid chromatography (HPLC) on a stationary phase composed of phospholipids, the so-called immobilized artificial membrane (IAM). In addition, quantitative structure-retention relationships (QSRR) were developed in order to explain the dependence of retention on the chemical structure of the neutral, acidic, and basic drugs considered in this study. The obtained retention data were modeled by means of multiple regression analysis (MLR) and partial least squares (PLS) techniques. The structures of the compounds under study were characterized by means of calculated physicochemical properties and several nonempirical descriptors. For the carboxylic compounds included in the analysis, the obtained results suggest that the IAM-retention is governed by hydrophobicity factors followed by electronic effects due to polarizability in second place. Further, from the analysis of the results obtained of two developed quantitative structure-permeability studies for 20 miscellaneous carboxylic compounds, it may be concluded that the balance between polarizability and hydrophobic effects is not the same toward IAM phases and biological membranes. These results suggest that the IAM phases could not be a suitable model in assessing the acid-membrane interactions. However, it is not possible to generalize this observation, and further work in this area needs to be done to obtain a full understanding of the partitioning of carboxylic compounds in biological membranes. For the non-carboxylic compounds included in the analysis, this work shows that the hydrophobic factors are of prime importance for the IAM-retention of these compounds, while the specific polar interactions, such as electron pair donor−acceptor interactions and electrostatic interactions, are also involved, but they are not dominant.

INTRODUCTION

The ability of a compound to traverse biological membranes, usually by passive diffusion, has long been recognized as a principal factor contributing to the biological activity of bioactive compounds.[1,2] Among the several physicochemical parameters proposed to mimick the in vivo situation of the transport processes, the hydrophobic parameter, log *Poct*, the logarithm of octanol−water partition coefficient, is the most popular and commonly reported descriptor that has been used to explain the drug-membrane interactions.[3] A number of experimental models, such as the shake-flask technique, have been proposed for their experimental determination. However, log *Poct* measurements are laborious, time-consuming, and are prone to errors and experimental problems. Furthermore, to obtain reliable experimental data, their values are limited to a certain range,[4] that is, $-3 < \log Poct < 3$. Alternative methods, such as chromatographic ones, have been proposed for estimating this important molecular parameter. These methods have the advantages of high speed of determination, reproducibility, ease of use, and instrumentation available for automation. Reversed-phase liquid chromatography (RPLC) has been widely recognized as a valuable method to obtain lipophilicity parameters which are extensively used in studies of quantitative structure−activity relationships (QSAR).[5−8] However, if the reason is to use the chromatographic systems to model processes in the biophase, the components of both chromatographic and biological systems must be comparable. As a consequence, membrane-like systems, such as the so-called immobilized artificial membranes (IAMs), have been developed and introduced in the market as RPLC column packing materials.[9−11] These stationary phases, which contain phosphatidylcholine or similar lipid-like ligands covalently bonded to silica propylamine particles, appear to be reliable and convenient models of drug-membrane interactions.

The IAM chromatography has been successfully applied for predictions of drug permeability across several biological barriers such as human skin, intestinal epithelium, epithelial Caco-2 cell line, and the vascular endothelium of the brain microvessels.[12−14]

In the present work, the retention factors for a set of 32 structurally diverse drugs were determined using IAM chromatography. In addition, quantitative structure-retention relationships (QSRR) were developed in order to explain the dependence of retention on the chemical structure of the neutral, acidic, and basic compounds considered in this study. The obtained retention data were modeled by mean of

* Corresponding author fax: +54-2652-430224; e-mail: jmluco@unsl.edu.ar.
† Laboratorio de Alimentos.
‡ Area de Farmacotécnia.
§ Departamento de Química.

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multiple regression analysis (MLR) and partial least squares (PLS) techniques. The PLS approach is one of the most useful techniques for molecular modeling in drug design,[15] and it has been successfully applied to several quantitative structure−activity and structure−property studies (QSAR and QSPR).[16−21]

The structures of the compounds under study were characterized by means of calculated physicochemical properties and several nonempirical descriptors, such as topological and quantum chemical indexes. The main purpose of this work was to construct several QSRR models in order to obtain some insight into the mechanism of retention on IAM chromatography.

EXPERIMENTAL SECTION

Materials. The biogenic amines and derivatives (epinephrine, norepinephrine, dopamine, serotonin, tryptamine, indole-3-acetic acid, indole-3-carboxylic acid, indole-3-pyruvic acid, 3-methoxy-4-hydroxyphenylglycol (MHPG), isoproterenol, 6-hydroxydopamine, 3,4-dihydroxybenzylamine (DHBA), and α-methyl-m-tyrosine) were purchased from Sigma Chemical Company and used without further purification. The followings drugs were gifts: atenolol (from Gador S.A., Argentine); metoprolol (from Novartis, Argentine); terbutaline (from Astra AB, Sweden); and olsalazine (from Pharmacia & Upjohn AB Sweden). The other group of drugs were of the best available quality from the Department of Pharmacy of University of San Luis: morphine, ethylmorphine, atropine, nicotine, lidocaine, aspirin, salicylic acid, antipyrine, caffeine, theophylline, theobromine, warfarin, vanillin, phenol, and *o*-nitrophenol. All other chemicals, i.e., phosphoric acid, sodium hydroxide, sodium nitrate, and phosphate saline were from Merck (grade proanalysis). Milli-Q water (Milli-Q UF Plus system, Millipore Corporation) was used to prepare the mobile phases, which were filtered through a 0.45 μm membrane and degassed by ultrasonication immediately before use.

Instrumental. The experiments were performed with a Beckman HPLC system (model 332) coupled with a Varian 4290 integrator and equipped with a variable wavelength detector (model Beckman 164) operated at range 220−300 nm depending of the analyzed compound. The injecting valve was fitted with a 50 μL sample loop. A commercially available column was used in all experiments: IAM.PC.MG 12 μm (150 × 4.6 mm), which was purchased from Regis Chemical Company (Morton Grove, IL).

HPLC Procedure. Drugs were dissolved in the mobile phase at concentrations ranged from 0.1 at 1 mg mL^{-1}, depending on the solubility of the compound. The chromatography was carried out at room temperature (26 ± 1 °C), and the injection volume was 50 μL for all experiments. The flow-rate was 1 mL min^{-1}, and the mobile phase consisted of 0.035 M phosphate buffer at pH 7.1. The column dead time (To) was estimated from the retention time of sodium nitrate measured at 210 nm. The obtained retention data were used to derive the values of capacity factor (k'), which was calculated in the usual manner; that is, $k' = (\mathrm{Tr} - \mathrm{To})/\mathrm{To}$. All capacity factors given represent the mean of 2−4 determinations of each sample solution. The reproducibility of retention times varied from 0.5%−5% (RSD) within a period of 4 weeks of experiments.

Molecular Descriptors. The following indexes based on molecular topology[22,23] were considered: the Wiener index (W), the valence and connectivity molecular indexes ($^{m}\chi^{n}$), the kappa shape indexes (κ), and the geometrical indexes recently introduced by Galvez et al. (*E*, *S*, and *L*). The other group of variables included the following: the count of electron pairs on oxygen and nitrogen atoms (*HBA*), the count of O−H and N−H bonds (*HBD*), molar volume (V_m), molecular weight (*Mw*), calculated octanol−water partition coefficient (log P_{cal}), calculated octanol−water apparent distribution constant (log D_{cal}, pH 7.0), and the calculated refraction index expressed as $f_{cal} = (\eta^2 - 1)/(\eta^2 + 2)$. The last group of structural descriptors considered in this study were several quantum chemical indexes. The compound starting geometries were built in their neutral form within HyperChem package (release 5.11 for Windows). The 3D molecular structures were obtained by energy minimization using the MM+ molecular mechanics potential-energy function. In a follow-up procedure, a complete optimization of the geometrical parameters was carried out by using the AM1 method implemented in the standard version of MOPAC 6.0. The following quantum chemical indexes were considered: total energy (E_{total}), heat of formation (ΔH_f), energy of highest occupied molecular orbital (E_{HOMO}), energy of lowest unoccupied molecular orbital (E_{LUMO}), dipole moment (μ), the most positive partial charge on a hydrogen atom (qH^+), and the most negative partial charge in the molecule (q^-). At this point, is important to highlight that the molecular descriptors such as E_{total} and ΔH_f reflect basically the differences in bulkiness among the solutes. That is, these descriptors encode the differences in ability of individual solutes to participate in nonspecific, dispersive intermolecular interactions with stationary phase ligands. On the other hand, the E_{HOMO}, E_{LUMO}, μ, qH^+, and q^- descriptors were used as measures of the ability of compounds to participate in specific polar interactions.

Statistical Methods. The multiple regression analysis (MLR) together with partial least squares projections in latent variables (PLS) were the methods here used to construct the QSRR models. In relation to the MLR analysis, and taking into account the large number of descriptors considered, a stepwise multiple regression procedure based on the forward-selection and backward-elimination methods was used for inclusion or rejection of descriptors in the screened models. To avoid overestimations or difficulties in interpretation of the resulting models, pairs of variables with an $r \geq 0.70$ were classified as intercorrelating ones, and only one of these was included in the screened model. The other statistical method utilized was PLS,[15] which is a projection technique that relates the information in the response matrix Y to the systematic variation in the descriptor matrix X. In the present work, the response matrix Y consisted of logarithms of capacity factors (log k'_{IAM}), while the matrix X consisted of several structural descriptors. Determinations of the significant number of model dimensions was made by cross-validation. For the QSRR analysis developed in the present study, the novel filtering technique called orthogonal signal correction[24] (OSC) was used. The OSC technique removes the variability of matrix X which is orthogonal to the matrix Y. This reduction of irrelevant systematic information from the data set leads to more powerful PLS models. This is possible because OSC uses the matrix Y to construct a filter

Table 1. Retention Data and Physicochemical Parameters for the Set of Compounds Analyzed

no.	drug	log $k'_{(IAM)}$	log *Poct*[a]	log *Doct*[a]	pK_a value(s)[b]	nature of drug
1	adrenaline	−0.272	−0.63	−3.00	8.57/10.01	amphoteric
2	dopamine	−0.077	0.12	−2.30	8.85/10.31	amphoteric
3	tryptamine	0.843	1.38	−1.60	10.20	basic
4	serotonin	0.501	0.21	−2.50	9.80/11.13	amphoteric
5	noradrenaline	−0.389	−0.88	−3.90	8.53/9.78	amphoteric
6	indole-3-acetic acid	0.160	1.43	−0.80	4.54	acidic
7	indole-3-piruvic acid	0.518	2.06	−1.20	3.10[c]	acidic
8	indole-3-carboxylic acid	0.132	1.82	−1.10	5.25	acidic
9	MHPG	−0.219	−1.00	−1.00	9.95[c]	acidic
10	isoproterenol	0.099	0.25	−2.10	8.55/10.04	amphoteric
11	6-hydroxydopamine	−0.141	−0.70	−2.50	8.78/10.30	amphoteric
12	vanillin	0.532	1.19	1.10	7.40	acidic
13	α-methyl-m-tyrosine	−0.503	0.73	−1.80	2.2/9.1/10.1[c]	amphoteric
14	phenol	0.558	1.48	1.50	9.90	acidic
15	*o*-nitrophenol	0.654	1.71	1.50	7.23	acidic
16	DHBA	−0.205	−0.25	−2.20	8.65/10.01[c]	amphoteric
17	caffeine	0.134	−0.07	−0.10	0.60	neutral
18	theophylline	−0.009	0.06	0.00	8.54	acidic
19	theobromine	−0.240	−0.78	−1.50	10.05	acidic
20	aspirin	−0.586	1.19	−2.20	3.48	acidic
21	salicylic acid	0.091	2.06	−1.70	2.97	acidic
22	antipyrine	0.462	0.27	0.30	1.44	neutral
23	nicotine	0.346	0.72	−1.40	8.01	basic
24	morphine	0.563	1.06	−0.30	8.18/9.26	amphoteric
25	ethylmorphine	0.974	2.36	0.80	7.90	basic
26	lidocaine	0.970	2.36	0.80	7.84	basic
27	terbutaline	0.424	0.48	−2.00	8.7/10.0/11.1	amphoteric
28	atenolol	0.207	0.10	−2.40	9.60	basic
29	metoprolol	0.947	1.76	−0.70	9.75	basic
30	warfarin	1.388	3.47	1.00	5.10	acidic
31	atropine	0.815	1.53	−1.20	9.70	basic
32	olsalazine	1.285	3.94	−1.10	2.73/3.43[c]	acidic

[a] Calculated values. [b] Experimental values from the ACD/I-Lab Web service (ACD/pK_a DB 6.00). [c] Calculated values by using the ACD/I-Lab Web service (ACD/pK_a 6.00).

of matrix X. In recent years, different OSC algorithms have been developed, and, in this study, the OSC algorithm as implemented in SIMCA P 7.01 (Umetri, Sweden) was used.

The computer software (Molconn-X) used to calculate the molecular connectivity-type topological indexes was obtained from L. H. Hall, Eastern, Nazarene College, Quincy, MA. The molecular descriptors (molar volume, molecular weight, and refraction index) and log P_{cal} were computed with ACD/ChemSketch-4.0 and ACD/LogP-3.5 softwares, respectively (Advanced Chemistry Development Inc., Toronto, Canada). The values of log D_{cal} (computed at pH 7.0) were obtained using the ACD/I-Lab Web service. The PLS analysis was carried out using the SIMCA P 7.01 software package obtained from Umetri AB, Box 7960, 907 19 Umea, Sweden. Regression analysis was performed by using the 7.0 version of Statgraphic Plus. All statistical and other calculations were carried out on a PC-IBM Computer.

RESULTS AND DISCUSSION

To obtain experimental conditions as close as possible to the physiologic pH and compatible with the stability of the stationary phase, the determination of the capacity factors on the IAM.PC.MG HPLC-column were determined at pH 7.1 using a 0.035 M phosphate buffer as mobile phase.

To evaluate the ability of the IAM phase in assessing lipophilicity of the compounds under study, it was of interest to study to what extent the log k_{IAM} values are related to the calculated log P_{cal} and log D_{cal} values. Table 1 shows the chromatographic data and the corresponding physicochemical parameters of the compounds under study. The regression analysis for the whole set of compounds yielded the following equations:

$$\log k'_{(IAM)} = \underset{(0.828)}{0.016}\ (0.074) + \underset{(0.000)}{0.321}\ (0.048) \log Poct$$

$$R^2 = 0.592,\ r = 0.769,\ r_{cv} = 0.744,\ s = 0.332,\ n = 32,\ F = 43.54,\ W(\log Poct) = 1.586 \quad (1)$$

$$\log k'_{(IAM)} = \underset{(0.000)}{0.544}\ (0.093) + \underset{(0.000)}{0.222}\ (0.054) \log Doct$$

$$R^2 = 0.358,\ r = 0.598,\ r_{cv} = 0.535,\ s = 0.417,\ n = 32,\ F = 16.72,\ W(\log Doct) = 1.198 \quad (2)$$

In these and the following equations, n is the number of compounds, s is the standard deviation, R^2 is the squared correlation coefficient, r is the correlation coefficient, r_{cv} is the cross-validation correlation coefficient and F is the Fisher F-statistic. The figures in parentheses are the standard deviations and P-values of coefficients, and W is the standardized regression coefficient obtained when the variables are scaled to the same numerical range (0,...1).

The poor statistics of eq 1 is due to the worse fitting of the seven compounds containing a carboxyl group in their molecule. In contrast, the residuals are well distributed in eq 2 although its statistical quality is quite inferior to eq 1. To illustrate this fact, the relationship between the log k_{IAM} and log P_{cal} values is depicted in Figure 1.

This shows that the carboxylic compounds, which are almost completely in the anionic form at pH 7.11, clearly

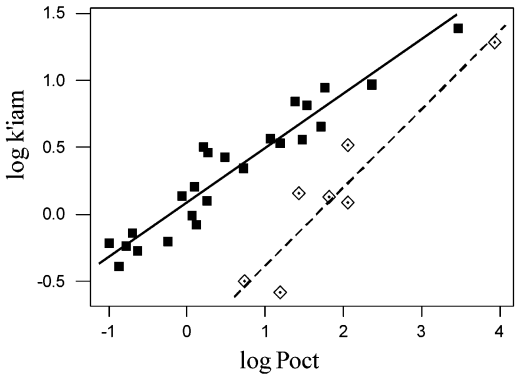

Figure 1. Relationship between log $k'_{(IAM)}$ and log *Poct* for the 32 compounds shown in Table 1. Key: (■) non-carboxylic compounds; (diamond shaped symbol containing a dot) carboxylic compounds.

form a subgroup that behaves differently in IAM chromatography. It is important to note that these results are in agreement with the findings by other authors[25,26] who have noted that when carboxylic compounds are treated separately to basic and neutral compounds, improved relationships between log k_{IAM} and log *P* (octanol/water) are observed. In view of this, it seems reasonable to consider a common interaction mechanism between the carboxylic compounds and the phospholipids of the IAM stationary phases. Thus, to explore fundamental intermolecular interactions that govern the retention of carboxylic compounds on the IAM phases, several QSRR equations were derived. It should be highlighted that these studies are of relevance for the pharmaceutical industry since many pharmaceutical drugs contain one or more carboxyl groups in its molecule.

Quantitative Structure-Retention Relationships for Carboxylic Compounds. To elucidate the mechanism driving the interaction between the carboxylic compounds and the IAM phases, we related the log $k'_{(IAM)}$ values of compounds **6**, **7**, **8**, **13**, **20**, **21**, and **32** to the respective calculated *n*-octanol/water partition coefficients and some others molecular descriptors mentioned in the Experimental Section. After some consideration, the following equations were obtained:

$$\log k'_{(IAM)} = \underset{(0.006)}{-0.923\ (0.198)} + \underset{(0.002)}{0.570\ (0.093)} \log Poct$$

$$R^2 = 0.882,\ r = 0.939,\ r_{cv} = 0.899,\ s = 0.238,\ n = 7,\ F = 37.22,\ W(\log Poct) = 1.828 \quad (3)$$

$$\log k'_{(IAM)} = \underset{(0.008)}{-3.145\ (0.491)} + \underset{(0.001)}{0.490\ (0.056)} \log Poct + \underset{(0.025)}{6.456\ (1.836)} f_{cal}$$

$$R^2 = 0.971,\ r = 0.985,\ r_{cv} = 0.938,\ s = 0.132,\ n = 7,\ F = 67.07,\ W(\log Poct) = 1.574,\ W(f_{cal}) = 0.568 \quad (4)$$

The statistical quality of eqs 3 and 4 is good and accounts for 88−97 of the variance in log $k'_{(IAM)}$. The log *Poct* and f_{cal} parameters are weakly correlated ($r = 0.40$) which is important to reach a correct physicochemical interpretation of the equation.

The analysis of eq 3 suggests that the IAM retention of carboxylic compounds is governed by their intrinsic lipophilicity, although the acids are almost completely ionized under the experimental conditions used. However, the correlation found between log *Poct* and log $k'_{(IAM)}$ does not allow to infer that the acids/phospholipids interactions are uniquely lipophilicity based. Thus, although the log *Poct* parameter is evidently necessary to obtain a reasonable correlation, it is not enough to explain completely the observed retention variation. Equation 4 shows that the f_{cal} parameter is an important contributor for a suitable explanation of retention behavior of these compounds. This parameter is a refractive index function, and, therefore, it is related to the polarizability of the solute with regards to the interactions due to the presence of polarizable electrons. It should be noted that the relatively large coefficient associated with the f_{cal} term is due to the small range of the f_{cal} values as shown in Table 2. The standardized regression coefficients (*W*) obtained for the log *Poct* and f_{cal} variables illustrate this situation (1.574 and 0.568, respectively).

Table 2. Observed and Calculated log $k'_{(IAM)}$ Values and Molecular Parameters Used in Eq 7

drug	log $k'_{(IAM)}$ exp	log $k'_{(IAM)}$ calc	log *Poct* [b]	f_{cal}	*L*
valproic acid	−0.745[a]	−0.571	2.72	0.261	6
ibuprofen	0.409[a]	0.439	3.68	0.303	9
ketoprofen	0.496[a]	0.487	2.79	0.338	10
indomethacin	1.452[a]	1.339	4.23	0.351	12
tolfenamic acid	1.790[a]	1.729	5.70	0.368	8
indole-3-acetic acid (**6**)	0.160	0.101	1.43	0.383	7
indole-3-piruvic acid (**7**)	0.518	0.632	2.06	0.406	8
indole-3-carboxylic acid (**8**)	0.132	0.288	1.82	0.397	6
α-methyl-m-tyrosine (**13**)	−0.503	−0.548	0.73	0.342	7
aspirin (**20**)	−0.586	−0.694	1.19	0.318	6
salicylic acid (**21**)	0.091	−0.080	2.06	0.349	5
olsalazine (**32**)	1.285	1.373	3.94	0.377	11

[a] Experimental values taken from ref 25. [b] Calculated log *Poct* values.

One weakness of eqs 3 and 4 is related to the small number of compounds used to obtain these equations. Therefore, one way of evaluating their quality is to derive new regression equations based on the same variables as used in eq 4 but incorporating other carboxylic compounds into the analysis. To do this, the IAM.PC retention data published on five carboxylic compounds were utilized[25] (aspirin and salicylic acid are repeated and were not included). It should be noted that the similarity of the stationary phase and other experimental conditions permits to generate a common data set. The new regression equations, relative to 12 data points, were as follows:

$$\log k'_{(IAM)} = \underset{(0.012)}{-0.880\ (0.289)} + \underset{(0.000)}{0.465\ (0.095)} \log Poct$$

$$R^2 = 0.704,\ r = 0.839,\ r_{cv} = 0.793,\ s = 0.460,\ n = 12,\ F = 23.81,\ W(\log Poct) = 1.155 \quad (5)$$

$$\log k'_{(IAM)} = \underset{(0.000)}{-4.243\ (0.544)} + \underset{(0.000)}{0.469\ (0.043)} \log Poct + \underset{(0.000)}{9.589\ (1.508)} f_{cal}$$

$$R^2 = 0.946,\ r = 0.973,\ r_{cv} = 0.938,\ s = 0.207,\ n = 12,\ F = 79.06,\ W(\log Poct) = 1.167,\ W(f_{cal}) = 0.695 \quad (6)$$

It is encouraging to observe that the physicochemical information contained in eqs 5 and 6 is quite similar to that

obtained from eqs 3 and 4, both regarding statistical quality and signs of the coefficients. Comparison between eqs 3−4 and 5−6 shows a difference in magnitude of the f_{cal} and log *Poct* coefficients, which probably is due to the structural diversity of the compounds incorporated into the analysis. Finally, it is possible to introduce a new variable, the *L* index (the chemical graph length), which significantly improved eq 6 and generates a more general model for carboxylic compounds. The new regression equation was as follows:

$$\log k'_{(IAM)} = \underset{(0.000)}{-4.48}\ (0.35) + \underset{(0.000)}{0.393}\ (0.034) \log Poct + \underset{(0.000)}{8.910}\ (0.978)\, f_{cal} + \underset{(0.006)}{0.09}\ L\ (0.02)$$

$$R^2 = 0.980,\ r = 0.990,\ r_{cv} = 0.971,\ s = 0.133,\ n = 12,$$
$$F = 132.37W\ (\log Poct) = 0.978,\ W\ (f_{cal}) = 0.646,$$
$$W(L) = 0.299 \quad (7)$$

The derived equation shows a very good correlation as well as low standard deviation at a high level of significance. Intercorrelations for the pairs of variables log *Poct* vs f_{cal}, log *Poct* vs *L*, and f_{cal} vs *L* are $r = 0.02$, 0.59, and 0.14, respectively. It should be noted that a similar fit is obtained when the reported log $k'_{(IAM)}$ values for aspirin and salicylic acid (−1.022 and −0.101)[25] are used to derive eqs 5−7 instead of our data, that is −0.586 and 0.091, respectively. Table 2 shows the observed and calculated log $k'_{(IAM)}$ values and the molecular parameters used in eq 7. The presence of *L* in eq 7 suggests that the more elongated molecules will have a higher *L* value and will consequently interact more strongly with the phospholipid chains of the IAM phase.

Validation of the Obtained Equations for Carboxylic Compounds. The above-discussed on eqs 3−7 is valid only within the limitations of the present data set. Therefore, to validate and provide greater robustness to the obtained results, a new data set of carboxylic compounds was analyzed. The validity of the derived equations was demonstrated based on the IAM.PC.MG retention data reported by Barbato et al.[27] for 17 nonsteroidal antiinflammatory drugs. It should be noted that, due to the hydrophobicity of some analyzed compounds, the logarithms of the capacity factors were determined using a phosphate-buffered saline at pH 7.0 but extrapolating to zero the different percentages of acetonitrile used in the mobile phases (log $k'w_{(IAM)}$). Using the same molecular parameters as used in eq 7, the following equation was obtained:

$$\log k'w_{(IAM)} = \underset{(0.000)}{-4.61}\ (0.67) + \underset{(0.000)}{0.739}\ (0.037) \log Poct + \underset{(0.007)}{6.537}\ (2.026)\, f_{cal} + \underset{(0.000)}{0.13}\ L\ (0.02)$$

$$R^2 = 0.976,\ r = 0.988,\ r_{cv} = 0.978,\ s = 0.169,\ n = 17,$$
$$F = 177.25,\ W\ (\log Poct) = 3.377,\ W\ (f_{cal}) = 0.582,$$
$$W(L) = 0.930 \quad (8)$$

The statistical quality of this equation is very good and accounts for 97.6% of the variance in log $k'w_{(IAM)}$. Intercorrelations between the pairs of descriptors used are negligible: log *Poct* vs f_{cal} ($r = 0.22$); log *Poct* vs *L* ($r = 0.13$); and f_{cal} vs *L* ($r = 0.23$). Table 3 shows the observed and calculated log $k'_{(IAM)}$ values and the molecular parameters employed in this equation (the log *Poct* experimental values were used in this case). The evaluation of the parameter weights shows that the physicochemical information contained in this equation is qualitatively similar to that obtained from previous equations. However, a comment is needed with respect to the physical meaning of f_{cal} parameter. Their presence in the QSRRs derived indicates that polarizability effects play an important role in the IAM retention process of carboxylic compounds. Moreover, such additional forces may be associated to electronic interactions between the ionized carboxyl group and the polar headgroups of phospholipids of the IAM phases. This analysis is in agreement with the hypothesized by Kubinyi[28] and Hansch et al.[29] which stated that in a QSAR equation, a positive sign for a parameter encoding information about the molecular polarizability can be explained as a drug-polar surface interaction while a negative sign indicates a steric hindrance in the interaction site. Another important point to highlight is that f_{cal} is weakly correlated with the so-called bulkiness descriptors. For example, for the data set used in eq 8, the intercorrelation between the characteristic volume (V_x) calculated by McGowan's method[30] and f_{cal} is poor ($r = 0.47$). In contrast, there is a statistically significant correlation between f_{cal} and the polarizability parameter, R_2, developed by Abraham et al.[31] ($r = 0.84$), which implies that both variables encode similar information. This is to be expected since R_2 is an excess molar refraction that represents the tendency of a compound to interact with a phase through π or lone electron pairs.

From the point of view of mechanism of retention, one can conclude that the highest contribution to the IAM retention of carboxylic compounds comes invariably from the hydrophobicity factors followed by electronic effects due to polarizability in second place. With respect to the *L* index, their presence in the above equations probably suggests a contribution of the molecular shape in the retention process. However, this observation must be viewed with caution since *L* and V_x are intercorrelated in this data set ($r = 0.87$). Finally, although additional studies are required to generalize the conclusions obtained about the acids/phospholipids interac-

Table 3. Observed and Calculated log $k'_{(IAM)}$ Values and Molecular Parameters Used in Eq 8

drug	log $k'_{(IAM)}$ exp[a]	log $k'_{(IAM)}$ calc	log *Poct*[a]	f_{cal}	*L*
salicylic acid	0.05	0.006	2.27	0.349	5
aspirin	−0.95	−0.903	1.13	0.319	6
indoprofen	1.17	1.250	2.77	0.361	11
tolmetin	1.13	1.083	2.79	0.333	11
ketoprofen	1.12	1.225	3.12	0.338	10
naproxen	1.26	1.439	3.34	0.346	10
fenbufen	1.66	1.671	3.39	0.335	12
sulindac	1.80	1.949	3.42	0.374	12
ibuprofen	1.12	1.146	3.50	0.303	9
flurbiprofen	2.02	1.920	4.16	0.327	10
indomethacin	2.39	2.424	4.27	0.351	12
diclofenac	2.43	2.245	4.40	0.370	9
diflunisal	2.33	2.099	4.44	0.343	9
mefenamic acid	2.46	2.582	5.12	0.360	8
flufenamic acid	2.86	2.649	5.25	0.335	9
tofenamic acid	2.75	3.043	5.70	0.365	8
piroxicam	1.85	1.624	3.00	0.392	11

[a] Experimental log $k'_{(IAM)}$ and log *Poct* values taken from ref 27.

Table 4. Apparent Permeability Coefficients and Molecular Parameters Used in Eqs 9 and 10

drug	log P_{app}[a]	log *Mw*	log *Poct*[b]	f_{cal}	*L*
salicylic acid	−3.668	2.140	2.06	0.348	5
phenylacetic acid	−3.585	2.134	1.50	0.319	6
benzoic acid	−3.587	2.087	1.89	0.325	5
decanoic acid	−3.506	2.236	3.97	0.265	10
octanoic acid	−3.530	2.159	2.90	0.261	8
hexanoic acid	−3.578	2.065	1.84	0.256	6
butyric acid	−3.726	1.945	0.78	0.248	4
prostaglandin E_1	−4.081	2.550	2.18	0.316	17
prostaglandin E_2	−4.167	2.547	1.81	0.323	17
prostaglandin F_2	−4.377	2.550	2.14	0.327	17

[a] Experimental log P_{app} (cm/s) values taken from ref 32. [b] Calculated log *Poct* values.

Table 5. Apparent Absorption Clearances and Molecular Parameters Used in Eq 11

drug	log P_a[a]	*Mw*	log *Poct*[b]	f_{cal}	*L*
indoleacetic acid	0.739	175	1.43	0.384	7
indomethacin	1.171	358	4.27	0.351	12
ketoprofen	0.854	254	3.12	0.338	10
salicylic acid	0.804	137	2.27	0.349	5
tolmetin	0.852	256	2.79	0.333	11
naproxen	0.924	230	3.34	0.346	10
phenylacetic acid	0.910	136	1.50	0.319	6
phenyllactic acid	1.117	164	1.05	0.331	7
fenbufen	0.957	254	3.39	0.335	12
5-BOIAA[c]	0.786	281	3.00	0.377	12

[a] Experimental log P_a (cm^2/s) values taken from ref 33. [b] Calculated log *Poct* values. [c] 5-(Benzyloxy)indoleacetic acid.

tions, the fact that structurally different compound sets yield equations with similar structural information provides further confidence that the results obtained are not artifactual.

Modeling Biopartitioning Processes of Carboxylic Compounds. The partitioning of drugs into lipid bilayers and the biological membranes is the basis for drug and metabolite uptake, passive transport across membranes, and bioaccumulation. It therefore seemed interesting to establish whether and to what extent the IAM chromatographic measures would be of value to represent the partitioning of carboxylic compounds in biological membranes. Thus, two quantitative structure-permeability relationship (QSPR) studies were performed. The QSPR models were based on the same variables as used in QSRRs 7 and 8, to compare the significance and signs of the parameter weights obtained in the derivation of both kinds of models, this is, the QSRR and QSPR ones, respectively.

The first data set included the rat jejunum permeabilities for 10 miscellaneous carboxylic compounds studied by Lien and co-workers.[32] Table 4 shows the apparent permeability coefficients and the molecular descriptors used in the derivation of the QSPR models. The two equations with the best statistics are shown below:

$$\log P_{app} = \underset{(0.017)}{-1.179\ (0.381)} + \underset{(0.011)}{0.160\ (0.046)} \log Poct - \underset{(0.000)}{1.311\ (0.174)} \log Mw$$

$$R^2 = 0.895,\ r = 0.946,\ r_{cv} = 0.871,\ s = 0.114,\ n = 10,\ F = 29.69,\ W(\log Poct) = 0.255,\ W(\log Mw) = -0.396 \quad (9)$$

$$\log P_{app} = \underset{(0.000)}{-3.599\ (0.106)} + \underset{(0.009)}{0.159\ (0.044)} \log Poct - \underset{(0.000)}{0.054\ (0.007)} L$$

$$R^2 = 0.903,\ r = 0.950,\ r_{cv} = 0.901,\ s = 0.109,\ n = 10,\ F = 32.74,\ W(\log Poct) = 0.254,\ W(L) = -0.354 \quad (10)$$

Intercorrelations between the pairs of descriptors used are as follows: log *Poct* vs log *Mw* ($r = 0.24$); log *Poct* vs *L* ($r = 0.24$); and log *Mw* vs *L* ($r = 0.98$). The above regressions clearly show that the hydrophobic and size factors are of prime and approximately equal importance in the intestinal absorption for this set of carboxylic compounds. The negative dependence of log P_{app} on the molecular size, as reflected by the log *Mw* and *L* descriptors (note that both are highly collinear), can be rationalized based on the inverse relation that exists between the diffusion coefficient of a solute and its molecular weight. It should be noted that for this data set, the f_{cal} parameter does not exhibit a significant correlation with log P_{app}.

The second data set included the intestinal absorption of 10 miscellaneous carboxylic compounds reported by Sugawara and co-workers.[33] The permeability data were measured on rat jejunum using the in situ single-pass perfusion technique. Table 5 shows the apparent absorption clearances (P_a) and the molecular descriptors used in the derivation of the QSPR models. The equation with the best statistic is shown below:

$$\log P_a = \underset{(0.000)}{2.492\ (0.29)} - \underset{(0.001)}{0.547\ (0.09)} \log Poct + \underset{(0.000)}{0.115\ (0.02)} (\log Poct)^2 - \underset{(0.009)}{3.014\ (0.80)} f_{cal}$$

$$R^2 = 0.920,\ r = 0.959,\ r_{cv} = 0.701,\ s = 0.048,\ n = 10,\ F = 22.74,\ W(\log Poct) = -1.761,\ W((\log Poct)^2) = 1.965,\ W(f_{cal}) = -0.196 \quad (11)$$

For this group of carboxylic compounds, a parabolic relation exists between hydrophobicity and intestinal absorption. In this case, the f_{cal} parameter is significant, although it also has a negative coefficient. The analysis of this equation again suggests that hydrophobicity plays a main role in the passive diffusion processes.

On the basis of the obtained results, and comparing the significance and signs of the parameter weights in the obtained QSRR-QSPR models, it may be concluded that the balance between polarizability and hydrophobic effects is not the same toward IAM.PC.MG phases and biological membranes, at least, for the series of carboxylic compounds under study. Thus, although eqs 9−11 have been derived from only 20 data points, this comparison provides evidence that the IAM phases could not be a suitable model to represent the partitioning of carboxylic compounds in biological membranes. However, it is not possible to generalize this observation due to the limited amount of studied compounds. Thus, further work in this area needs to be done to obtain a full understanding of the partitioning of carboxylic compounds in biological membranes.

Quantitative Structure-Retention Relationships for Non-Carboxylic Compounds. According to Figure 1, when log $k'_{(IAM)}$ is related to log *Poct*, a clear class separation is observed between the carboxylic compounds and the remaining ones. To evaluate the factors responsible for the retention of non-carboxylic compounds on the IAM.PC.MG phases, a QSRR study using the partial least squares technique (PLS) was performed. Prior to the PLS study, it was of interest to analyze to what extent the log k_{IAM} values are related to the calculated log P_{cal} values. The corresponding equation for the whole set of non-carboxylic compounds was as follows:

$$\log k'_{(IAM)} = \underset{(0.008)}{0.097}\ (0.033) + \underset{(0.000)}{0.397}\ (0.025) \log Poct$$

$$R^2 = 0.913,\ r = 0.955,\ r_{cv} = 0.949,\ s = 0.144,\ n = 25,$$
$$F = 240.95,\ W(\log Poct) = 1.774 \quad (12)$$

This equation shows that a mainly lipophilic mechanism governs the IAM retention of all the structurally unrelated compounds included in the analysis. However, because the log $k'_{(IAM)}$ values were determined at pH 7.1, all basic compounds were almost fully ionized, whereas the remaining ones were either neutral, positively, or partly negatively charged (see pK_a values in Table 1). Thus, although the log *Poct* parameter is evidently necessary to obtain a reasonable correlation, it is not enough to explain completely the interactions occurring between the compounds at different ionization degree and the phospholipid-IAM/water system.

PLS Regression of IAM-Retention for Non-Carboxylic Compounds. All variables used in the PLS calculations were initially autoscaled to zero mean and unit variance to give each descriptor equal importance in the PLS analysis. The statistical significance of the screened models was judged by the parameters already mentioned. The predictive ability was evaluated by the cross-validation coefficient (Q) which is based on the prediction error sum of squares (PRESS). The PRESS statistic is computed as the squared differences between observed and predicted values when the observations are kept out of the derived model. This procedure is repeated several times until every observation has been kept out once and only once. Although the PLS method offers the advantage of handling data set in which the number of independent variables is greater than the number of observations, considerably worse predictions are obtained if many irrelevant descriptors are included in the PLS model. Because of the large number of structural descriptors considered in this study, the VIP (variable importance in the projection) parameter was used to unravel which descriptor variables were the most relevant to explain the IAM-retention data of the non-carboxylic compounds considered in this study (refer to Simca Manual for details about VIP calculations).

As previously mentioned, the OSC−PLS methodology was applied in this study. Thus, the OSC analysis was carried out using the centered and scaled X matrix. After extracting four OSC-components, 40.74% of the original sum of squares remained in the corrected X matrix. The PLS analysis of the corrected data set resulted in a significant one-component model with the following statistics: $R^2 = 0.959$, $Q = 0.974$, $r = 0.979$, $s = 0.09$, and $F = 537.97$. Comparison of the quality between the obtained model and eq 13 shows that the OSC−PLS model has a better fitting capacity and a lower

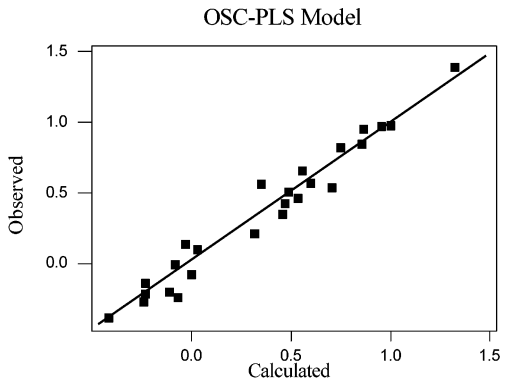

Figure 2. Relationship between the observed and calculated log $k'_{(IAM)}$ values for the 25 non-carboxylic compounds shown in Table 1.

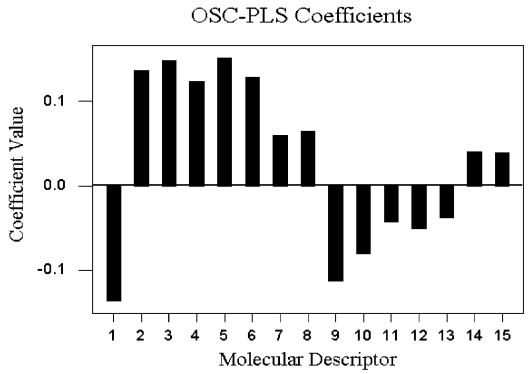

Figure 3. The OSC−PLS pseudoregression coefficients. Descriptors key: **1**: E_{total}, **2**: *Mw*, **3**: V_m, **4**: $\kappa 0$, **5**: $\kappa 1$, **6**: $\kappa 2$, **7**: $\kappa 3$, **8**: ΔH_f, **9**: *HBD*, **10**: *HBA*, **11**: E_{HOMO}, **12**: E_{LUMO}, **13**: qH^+, **14**: q^-, **15**: μ.

standard deviation at a high level of significance. As can be seen in Figure 2, the agreement between measured and calculated data is very satisfactory.

Figure 3 shows the 15 selected descriptors and the corresponding OSC−PLS pseudoregression coefficients. From these values, it can be seen how much a single variable contributes to the modeling of the retention data. According to these values, it can be inferred that, as expected, both the molecular size and the solute's capacity to form H-bonds play a predominant role in the retention behavior of these compounds, since bulkiness descriptors (E_{total}, ΔH_f, V_m, *Mw*, κ_0, κ_1, and κ_2) and hydrogen-bonding terms (*HBA* and *HBD*) are among the most influential descriptors. On the other hand, it appears that the electronic descriptors derived from semiempirical calculations concentrate basically on structural information related to specific polar interactions of the compounds studied. Moreover, on analyzing the OSC−PLS coefficients, it may be concluded that these variables reflect a compromise between the ability of the compounds to participate in electron pair donor−acceptor interactions (E_{HOMO}, E_{LUMO}), on one hand, and the electrostatic interactions (μ, qH^+, q^-) on the other. However, as shown in Figure 3, the low relative values of the coefficients of these descriptors indicate that they serve as a fine-tuning in the developed OSC−PLS model.

CONCLUSION

Two important consequences emerge from the present study. First, the quite well developed QSRR equations show

which are the principal factors the governing retention of the carboxylic compounds in IAM-HPLC. The obtained results indicate that both hydrophobic factors and electronic effects due to polarizability are implicated in the IAM-retention of the compounds under study. Further, from the analysis of the results obtained for the developed QSPR studies for 20 miscellaneous carboxylic compounds, it may be concluded that the balance between polarizability and hydrophobic effects is not the same toward IAM phases and biological membranes. Thus, these results suggest that IAM.PC.MG phases could not be a suitable model in assessing the acid-membrane interactions. However, it is not possible to generalize this observation, and further work in this area needs to be done to obtain a full understanding of the partitioning of carboxylic compounds in biological membranes. On the other hand, this work shows that the hydrophobic factors are of prime importance for the IAM-retention of the non-carboxylic compounds under study, while the specific polar interactions are also involved but they are not dominant.

Second, this study provides evidence for the great potential of PLS regression modeling based on the OSC methodology for the development of QSRR models.

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