Structural Properties Governing Retention Mechanisms on Immobilized Artificial Membrane (IAM) HPLC Columns

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The aims of this study were to investigate whether three commercially available immobilized artificial membrane (IAM) HPLC columns yield collinear data for neutral compounds, and whether IAM scales are distinct from the log P_{oct} (partition coefficient in the octanol/H_2O system) scale. With these objectives, the retention mechanisms on the IAM HPLC columns were analysed by linear solvation free-energy relationships (LSERs). A set of 68 neutral model compounds with known solvatochromic parameters and log P_{oct} values was investigated, allowing a regular and broad exploration of property space. The resulting solvatochromic equations clearly indicate that the three IAM stationary phases retain small neutral solutes by a balance of intermolecular forces closely resembling those underlying partitioning in octanol/H_2O and retention on a reversed-phase LC-ABZ HPLC column. For all systems, the solute's size and hydrogen-bond-acceptor basicity are the two predominant factors, whereas dipolarity/polarisability and hydrogen-bond-donor acidity play only minor roles.

1. Introduction[1]). – To exert its pharmacological and therapeutic effects, a drug must reach its sites of action in sufficient concentration. Thus, depending on the route of administration and on its target sites, a drug may have to cross several biological membranes, *e.g.*, in the intestine, skin, and blood-brain barrier (BBB) [1–3]. Lipophilicity, as expressed by the octanol/H_2O partition coefficient (log P_{oct}) has often been considered a key parameter to model passive diffusion, as unambiguously demonstrated in numerous quantitative structure-permeability relationship (QSPR) studies [4]. For complex compounds, such as most drugs, however, the log P_{oct} is not always a good indicator of biodistribution [5]. Thus, the development of membrane-like HPLC stationary phases has been of particular interest, as it combines fast analytical HPLC methodology with partitioning in artificial lipid membranes [6]. Immobilized artificial membranes (IAMs) are prepared by linking synthetic phospholipid analogues at monolayer densities covalently to silica particles, to mimic the lipid environment of a fluid cell membrane on a solid matrix. Solute capacity factors (log

[1]) *Abbreviations:* IAM: Immobilized artificial membrane; IAM.PC.MG: IAM prepared with a diacylated phosphatidylcholine analogue and end-capped with methyl-glycolate (MG); IAM.PC.DD: IAM prepared with a single-chain phosphatidylcholine analogue lacking the glycerol backbone and end-capped with C_3- and C_{10}-anhydrides (DD = Drug Discovery); IAM.PC.DD2: IAM prepared with a diacylated phosphatidylcholine analogue and end-capped with C_3- and C_{10}-anhydrides; log *P:* log of partition coefficient; Λ: global parameter of polarity; α, β, and π^*: solvatochromic parameters; log *k:* log of capacity factor.

k_{IAMw}) can thus be effective data for prediction of drug partitioning into biological membranes.

Three IAM HPLC columns are currently commercially available, namely the IAM.PC.MG, the IAM.PC.DD2, and the IAM.PC.DD stationary phases (*Fig. 1*). The IAM.PC.MG and the IAM.PC.DD2 chromatographic surfaces contain double-chain phosphatidylcholine (PC) ligands that are covalently bound to propylamine-silica and differ only in the end-capping of the residual amino groups. The IAM.PC.DD surface, on the other hand, is made by linking single-chain ligands to the silica particles, and it also lacks the glycerol moiety of natural phospholipids.

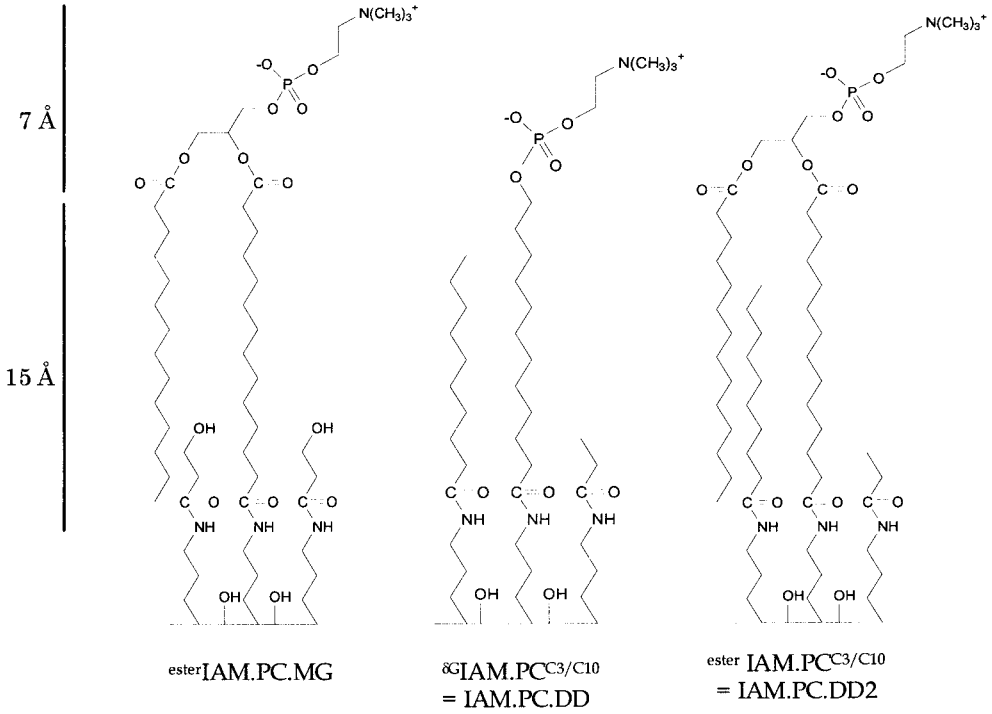

Fig. 1. *Chemical structures of commercially available immobilized artificial membranes* (IAMs)

At present, it is not clear whether the different IAM stationary phases yield collinear data, and whether IAM capacity factors for neutral compounds are distinct from partition coefficients measured in the octanol/H_2O system. In fact, only a few investigations on small series of homologous compounds have been performed to date to identify the mechanisms governing the retention on IAMs.

As in reversed-phase (RP) HPLC [7], both partitioning and adsorption mechanisms may be implied in the retention on IAMs. According to *Ong* and *Pidgeon* [8], partitioning seems to be the principal retention mechanism, and, thus, also polar interactions with the solvated layer(s) of the stationary phases [9][10] and the headgroups of the immobilized phospholipids [8] are reflected in IAM retention besides hydrophobic/solvophobic interactions.

The linear solvation-free-energy relationships (LSERs) based on the solvatochromic parameters are a powerful tool to unravel structural determinants governing chromatographic retention or partitioning of solutes. Indeed, LSERs are extensively used [11–13] to factorize some given molecular properties (S_p) of neutral organic solutes in terms of structural parameters such as the calculated molecular volume (V_W) and the so-called solvatochromic parameters (dipolarity/polarizability π^*, H-bond-donor acidity α, and H-bond acceptor basicity β).

$$S_p = v \cdot V_w + p \cdot \pi^* + a \cdot \alpha + b \cdot \beta + c \quad (1)$$

In fact, the linear *Eqn. 1* reflects a solvation model constructed with two factors, namely the endoergic creation of a cavity in the solvent (as reflected by the V_w term accounting for dispersive and solvophobic/hydrophobic forces) and the introduction of the solute in the cavity, which leads to exoergic polar interactions (as reflected by the π^*, α, and β terms). In the above equation, v, p, a, and b are the regression coefficients, which reflect the relative contribution of each parameter to S_p.

The LSER approach has been used to evaluate and identify the intermolecular interaction forces underlying the partitioning of solutes in various solvent/H_2O biphasic systems [14–16], as well as retention on RP-HPLC stationary phases [17–20]. Recently, LSERs were also applied to identify the recognition forces responsible for the retention on IAM stationary phases [21][22]. Capacity factors measured in RP-HPLC often correlate with log P_{oct} values, and, indeed, the derived LSER equations often reflect a comparable balance (comparable values of the coefficients v, p, a, and b in *Eqn. 1*) between structural properties [17–19]. On the contrary, for the retention on IAM stationary phases, both published LSER equations show a small but positive value for the coefficient a [21][22]. Thus, it seems that, in contrast to partitioning in octanol and retention on RP stationary phases, the solute's H-bond-donor acidity α is favorable for their retention on IAMs.

A quantitative comparison of the above differences is difficult, since the compounds used to generate the respective LSER models are limited in number and only partly comparable. Thus, the resulting statistical artefacts may mask or exaggerate some structural information. For this reason, *Pagliara et al.* [18] used cluster analysis to define a set of structurally diverse, well-distributed model compounds, the solvatochromic parameters of which are known, and which allows a regular and broad exploration of property space.

In this work, LSER equations were derived from the log k_{IAMw} values determined for the optimal set of compounds [18], in order to assess the molecular determinants governing the retention mechanisms on IAMs and to compare the relative retention of neutral compounds on the three IAM phases to octanol/H_2O partitioning and to capacity factors measured on an LC-ABZ HPLC column [18].

Previous studies have shown that lipophilicity can be factorised into a hydrophobic term characterized by the molecular volume and a polarity term called Λ [23–25]. This factorization was also applied to the IAM.PC.DD2 log k_{IAMw} values to investigate more closely the polar structural features responsible for retention on the IAM HPLC columns under study.

2. Results and Discussion. – 2.1. *Structural Diversity of the Investigated Solutes.* As mentioned above, the optimal set of 80 compounds derived by cluster analysis from 253 candidates [18] had to be restricted to 68 compounds (*Table 1*). Before deriving the solvatochromic equations for the retention behaviour of solutes on the three IAM stationary phases investigated, it was thus necessary to check whether the smaller set still allowed a well-balanced exploration of the 5D space defined by log P_{oct}, V_w, α, β, and π^*.

The LSER of log P_{oct} was used to test the relevance of the reduced set. The optimal set of 80 compounds was characterized by the LSER model of *Eqn. 3* with a relative contribution of 53% for V_w, 35% for β, 10% for π^*, and 2% for α [26]:

$$\begin{aligned}\log P_{oct} = {} & 3.22 \cdot 10^{-2}(\pm 0.13 \cdot 10^{-2}) \cdot V_w - 0.67(\pm 0.12) \cdot \pi^* \\ & + 0.21(\pm 0.16) \cdot \alpha - 4.10(\pm 0.23) \cdot \beta + 0.17(\pm 0.19) \qquad (3) \\ n = 80;\ r^2 = 0.99;\ q^2 = 0.98;\ s = 0.18;\ F = 1246 & \end{aligned}$$

In this and the following equations, 95% confidence limits are given in parentheses, n is the number of compounds, r^2 the squared correlation coefficient, q^2 the cross-validated correlation coefficient [27], s the standard deviation, and F the *Fischer*'s test.

The final set of 68 compounds yielded the following LSER model for their log P_{oct} values (*Eqn. 4*)

$$\begin{aligned}\log P_{oct} = {} & 3.29 \cdot 10^{-2}(\pm 0.15 \cdot 10^{-2}) \cdot V_w - 0.64(\pm 0.14) \cdot \pi^* \\ & + 0.22(\pm 0.15) \cdot \alpha - 4.12(\pm 0.24) \cdot \beta + 0.09(\pm 0.18) \qquad (4) \\ n = 68;\ r^2 = 0.99;\ q^2 = 0.99;\ \mathrm{s} = 0.16;\ F = 1275 & \end{aligned}$$

with the relative contributions of 52% for V_w, 36% for β, 9% for π^*, and 3% for α.

Eqns. 3 and *4* have similar regression coefficients and relative contributions of the variables. Indeed, even the smaller set of 68 solutes yields a solvatochromic equation with the correct balance of intermolecular forces controlling octanol/H_2O partitioning, the slightly broadening of 95% confidence limits being the most visible consequence of the reduced number of data. In summary, the reduction of the set to 68 compounds yielded no fundamental difference from the optimal set of 80 solutes. Indeed, the above LSER models (*Eqns. 3* and *4*) show similar intermolecular contributions to partition coefficients.

2.2. *Chromatographic Behaviour of the Compounds on IAMs.* To obtain experimental conditions as close as possible to the physiological pH and compatible with the stability of the IAM stationary phases, the log k_{IAMw} values were determined at pH 7.0 (except for the acidic compounds at pH 3.0). All the capacity factors measured for the 68 compounds on the three investigated IAM HPLC-columns are reported in *Table 1*.

The chromatograms of relatively nonpolar solutes (*e.g.*, 1,1′-biphenyl or acridine) and strong H-bond donors such as phenol show very symmetrical peaks. It is, thus, suggested that only partitioning is involved in the retention of neutral compounds, as already reported [8].

Table 1. *Investigated Compounds in the Optimal Set*

No.[a])	Solute			log k_{IAMw}				
		V_w	log P_{oct}[b])	MG	DD	DD2	A_{DD2}[c])	A_{oct}[d])
1	C_5H_{12}	96.8	3.39	1.88	0.47	2.28	0.00	0.00
2	CH_2Cl_2	58.7	1.15	0.31	−0.47	0.11	1.28	1.01
3	$CHCl_3$	73.7	1.94	0.63	−0.11	0.62	1.14	0.68
4	CCl_4	88.2	2.63	1.06	0.27	1.21	0.91	0.44
5	CH_2ClCH_2Cl	75.8	1.48	0.44	−0.32	0.34	1.48	1.21
6	$CHCl_2CHCl_2$	105.5	2.39	1.14	0.31	1.28	1.27	1.21
7	$Me(CH_2)_3-Cl$	94.7	2.64	0.92	0.07	1.05	1.23	0.63
8	Et_2O	87.8	0.89	0.25	−0.77	−0.06	2.17	2.17
9	Pr_2O	120.3	2.03	0.36	−0.19	0.78	2.13	2.03
10	MeCOOMe	71.4	0.18	−0.62	−0.94	−0.66	2.36	2.37
11	MeCOOEt	88.6	0.73	−0.35	−0.77	−0.25	2.38	2.35
12	MeCOOBu	121.3	1.82	0.41	−0.13	0.62	2.31	2.27
13	MeCN	47.6	−0.34	0.05	−1.50	−0.70	1.81	2.16
14	$MeCH_2CN$	64.3	0.10	0.10	−0.87	−0.35	1.88	2.23
15	$MeCONMe_2$	92.6	−0.77	−0.66	−1.02	−0.47	2.70	3.98
16	$MeCONEt_2$	125.9	0.34	−0.15	−0.65	0.12	2.93	3.89
17	EtOH	52.6	−0.25	0.04	−1.25	−0.62	1.86	2.22
18	PrOH	69.9	0.28	0.09	−0.93	−0.42	2.09	2.22
19	*t*-BuOH	86.0	0.36	0.10	−0.99	0.37	1.69	2.64
20	$C_5H_{11}OH$	103.9	1.40	0.40	−0.30	0.33	2.18	2.15
21	$MeCH_2C(Me_2)OH$	103.1	0.93	0.19	−0.70	−0.08	2.57	2.60
22	1-$C_6H_{13}OH$	121.0	2.03	0.73	0.05	0.83	2.10	2.05
23	HCOOH	36.6	−0.54	−0.97	−1.25	−0.65	1.50	2.02
24	MeCOOH	53.2	−0.24	−0.91	−1.15	−0.68	1.93	2.23
25	PrCOOH	88.0	0.79	−0.33	−0.53	−0.08	2.20	2.27
26	BuCOOH	104.7	1.39	0.06	−0.19	0.38	2.15	2.19
27	$BuNO_2$	98.8	1.47	0.50	−0.25	0.48	1.90	1.93
28	Tetrahydrofuran	77.3	0.46	0.15	−0.82	−0.18	2.03	2.27
29	PhMe	103.9	2.69	1.04	0.51	1.17	1.34	0.86
30	PhCOMe	122.1	1.58	0.76	0.33	0.88	2.08	2.54
31	$PhNO_2$	107.2	1.85	0.89	0.45	0.97	1.62	1.81
32	PhOMe	111.4	2.11	0.90	0.39	0.95	1.74	1.68
33	PhCOOEt	147.7	2.64	0.83	0.85	1.48	2.11	2.27
34	PhCOEE	139.4	2.20	1.09	0.60	1.23	2.15	2.45
35	$PhCOOCH_2Ph$	206.2	3.97	2.69	2.05	3.04	2.00	2.74
36	2-Cl−$C_6H_4NO_2$	120.6	2.24	1.28	0.74	1.44	1.48	1.83
37	$PhCH_2CN$	122.4	1.56	0.53	0.16	0.82	2.14	2.56
38	$PhCH_2COMe$	139.2	1.44	0.32	−0.02	0.38	3.00	3.20
39	$Ph(CH_2)_2OCOMe$	164.5	2.30	1.06	0.53	1.21	2.80	3.12
40	Pyridine	82.4	0.65	0.29	−0.26	0.32	1.65	2.24
41	Acridine	174.9	3.40	2.03	1.90	2.54	1.72	2.35
42	1-Naphthoic acid	158.7	3.10	1.73	1.61	2.13	1.73	2.15
43	Naphthalen-2-amine	145.3	2.28	1.50	1.01	1.77	1.76	2.55
44	$PhNH_2$	98.4	0.90	0.27	−0.07	0.30	2.07	2.48
45	PhNHEt	132.6	2.16	0.96	0.36	1.06	2.16	2.28
46	2-Cl−$C_6H_4NH_2$	113.2	1.91	0.96	0.51	1.03	1.71	1.93
47	2-NH_2−C_6H_4Ph	173.3	2.84	1.72	1.46	2.13	2.09	2.86
48	4,4′-$(NH_2)_2$-1,1′-Biphenyl	186.0	1.53[e])	1.28	1.11	1.46	3.07	4.56
49	4-NO_2−$C_6H_4NH_2$	118.3	1.39	0.98	0.89	1.03	1.83	2.61
50	PhOH	93.7	1.49	0.59	0.46	0.65	1.60	1.75
51	3-Cl−C_6H_4OH	108.5	2.49	1.38	1.04	1.70	0.92	1.21

Table 1 (cont.)

No.[a])	Solute			log k_{IAMw}				
		V_w	log P_{oct}[b])	MG	DD	DD2	Λ_{DD2}[c])	Λ_{oct}[d])
52	Me–C_6H_4COOH	129.0	2.37	1.01	0.79	1.43	1.70	1.96
53	$PhCH_2COOH$	128.6	1.46	0.41	0.17	0.76	2.35	2.86
54	3-Cl–$C_6H_4CH_2COOH$	143.2	2.09	1.06	0.70	1.47	2.01	2.68
55	$Ph(CH_2)_3COOH$	162.2	2.42	1.07	0.72	1.52	2.43	2.93
56	$PhCH_2OH$	111.2	1.08	0.29	– 0.01	0.34	2.35	2.70
57	4-Cl–$C_6H_4CH_2OH$	126.7	1.96	0.93	0.48	1.06	2.01	2.30
58	4-NO_2–C_6H_4OH	113.8	1.92	0.94	0.75	1.28	1.47	1.94
59	1,3-$C_6H_4Cl_2$	116.4	3.48	2.08	1.25	2.48	0.34	0.46
60	1,1′-Biphenyl	163.3	3.90	2.72	1.90	3.14	0.84	1.49
61	MeSOMe	70.5	– 1.35	– 1.01	– 1.18	– 1.09	2.77	3.87
62	Me_3N	75.1	0.22	–	–	–	–	2.44
63	Et_3N	126.3	1.36	–	–	–	–	2.89
64	Pr_3N	175.9	2.79	–	–	–	–	2.99
65	$PhCH_2NMe_2$	151.1	1.91	–	–	–	–	3.10
66	3-Cl–C_6H_4OCOMe	144.3	2.32	–	–	–	–	2.48
67	$PhOCH_2COOEt$	171.8	1.41	–	–	–	–	4.24
68	$PhOCH_2CONMe_2$	175.4	0.77	–	–	–	–	4.99
69	$Ph(CH_2)_3OMe$	162.4	2.70	–	–	–	–	2.66
70	PhOPr	145.5	3.18	–	–	–	–	1.66
71	Naphthalene	133.9	3.35	2.12	1.66	2.47	0.78	1.13
72	1,3,5-$C_6H_3Me_3$	138.0	3.84	2.17	1.33	2.61	0.74	0.77
73	$C_9H_{19}COOH$	190.4	4.09	2.81	2.30	2.86	1.78	2.13
74	2-$C_{12}H_9Cl$	178.7	4.30	–	–	–	–	1.56
75	1,2,4,5-$C_6H_2Cl_4$	146.4	4.51	3.03	2.12	3.50	0.06	0.36
76	C_7H_{16}	131.1	4.66	2.88	1.87	3.20	0.00	0.00
77	$Ph(CH_2)_2Ph$	196.6	4.80	3.24	2.48	3.77	1.03	1.62
78	C_6HMe_3	170.9	4.56	2.77	1.87	3.32	0.84	1.06
79	2,5-$C_2H_8Cl_2$	193.2	5.10	–	–	–	–	1.21
80	1-$C_{12}H_{25}OH$	223.7	5.13	–	–	–	–	2.12

[a]) The same numbering as in [18] was used. [b]) The log P_{oct} values were taken from [12]. [c]) Parameter accounting for the global polarity of the solutes, calculated for the retention on the IAM.PC.DD2 HPLC column according to *Eqn. 11*. [d]) Parameter accounting for the global polarity of the solutes, calculated for the partitioning in octanol with the equation published in [24]. [e]) The log P_{oct} determined in this work by potentiometric titration.

With all three IAM stationary phases, most of the compounds eluted in a reasonable time with a completely aqueous mobile phase. For the other solutes, extrapolation of the log k_{IAMw} values by linear regression was necessary. At least four (only three for 1,2,4,5-tetrachlorobenzene) different percentages of MeCN were used for each compound; over the eluent range studied, good linear relationships ($r^2 > 0.990$) between log k_{IAM} and the percentage of organic modifier (v/v) were observed.

The log k_{IAMw} values of the neutral acidic solutes (measured at pH 3.0) are different from the values calculated by correcting for ionization the capacity factors of the anions measured at pH 7.0. It appears that, for anionic compounds, the partitioning mechanism in IAMs is perturbed by specific interactions between the stationary phase and charged solutes. It is, thus, obvious that, for immobilized artificial membranes, the neutral form of acidic compounds has to be measured directly. This was also true for the LC-ABZ column investigated by *Pagliara et al.* [18].

The IAM stationary phases, like most silica based HPLC columns, are not suitable for strongly basic compounds. Indeed, correcting the capacity factors of protonated species for ionization leads to erratic results. The direct determination of capacity factors of neutral forms is impossible, as these stationary phases are not stable under basic pH conditions.

2.3. *Retention Mechanisms on the IAM Stationary Phases.* The application of linear solvation free-energy relationships (LSERs) to the 68 compounds of the optimal set for which capacity factors were measured during this study gave statistically significant equations which describe the structural properties governing the retention mechanisms on the three investigated IAM stationary phases. *Eqn. 5* was derived for the IAM.PC.MG column, *Eqn. 6* for the IAM.PC.DD2 stationary phase and *Eqn. 7* for the IAM.PC.DD chromatographic surface:

$$\log k_{\text{IAM.MGw}} = 2.29 \cdot 10^{-2}(\pm 0.25 \cdot 10^{-2}) \cdot V_w - 0.23(\pm 0.29) \cdot \pi^* + 0.09(\pm 0.30) \cdot \alpha - 2.71(\pm 0.47) \cdot \beta - 0.58(\pm 0.35) \quad (5)$$

$$n = 68;\ r^2 = 0.91;\ q^2 = 0.89;\ s = 0.31,\ F = 151$$

$$\log k_{\text{IAM.DD2w}} = 2.79 \cdot 10^{-2}(\pm 0.21 \cdot 10^{-2}) \cdot V_w - 0.24(\pm 0.28) \cdot \pi^* + 0.28(\pm 0.29) \cdot \alpha - 3.07(\pm 0.50) \cdot \beta - 0.90(\pm 0.29) \quad (6)$$

$$n = 68;\ r^2 = 0.94;\ q^2 = 0.93;\ s = 0.29;\ F = 253$$

$$\log k_{\text{IAM.DDw}} = 2.38 \cdot 10^{-2}(\pm 0.21 \cdot 10^{-2}) \cdot V_w + 0.12(\pm 0.26) \cdot \pi^* + 0.51(\pm 0.30) \cdot \alpha - 2.38(\pm 0.40) \cdot \beta - 1.72(\pm 0.23) \quad (7)$$

$$n = 68;\ r^2 = 0.93;\ q^2 = 0.92;\ s = 0.27;\ F = 215$$

These equations clearly show that V_w and β are the principal structural descriptors contributing to log k_{IAMw} values on all three columns, while the contributions of π^* and α are not significant. Thus, it can be concluded, in contrast to the results of other studies [21][22], that the H-bond-donor acidity of solutes does not influence their partitioning in IAMs.

The comparison of the above derived LSER models to the solvatochromic equation obtained for partitioning in octanol/H_2O (*Eqn. 4*) for the same series of solutes shows a close analogy between log P_{oct} and capacity factors measured on the three IAM stationary phases. These results are confirmed by the similarity of the relative contributions of each variable to the LSER models (*Table 2*).

A closer look at *Eqns. 4–7* shows, however, that the positive v coefficients (indicating the preference of solutes to transfer from the more-cohesive aqueous to the less-cohesive organic phase [28]) are significantly smaller in the LSER models derived for IAM retention compared to the equation obtained for octanol/H_2O partitioning. This indicates that immobilized artificial membranes are more cohesive than bulk octanol and can be explained by IAMs being ordered systems, containing amphiphilic

Table 2. *Relative Contributions of Each Variable to the LSER Models Established for Octanol/H_2O Partitioning, Retention on IAM HPLC Columns, and on a* Supelcosil LC-ABZ *Stationary Phase*

S_p[a])	V_w[b])	π^*[b])	α[b])	β[b])	n[c])
log P_{oct}	52.2	9.4	2.6	35.9	68
log $k_{IAM.MGw}$	57.2	5.1	1.6	36.2	68
log $k_{IAM.DD2w}$	57.8	4.4	3.9	34.0	68
log $k_{IAM.DDw}$	58.1	2.7	8.2	31.0	68
log k_w ABZ[d])	53.2	7.7	5.2	33.9	60

[a]) Organic solvent: oct = octanol; stationary phases: IAM = immobilized artificial membrane; MG = endcapped with methyl glycolate; DD2 = drug discovery 2; DD = drug discovery; ABZ = *Supelcosil LC-ABZ*. [b]) Contribution of each variable in %. [c]) Number of compounds included in the LSER model. [d]) Taken from [18].

molecules with polar head-groups. Indeed, the cohesive energy increases with polarity [28].

Further analysis of the above solvatochromic equations shows that the second most important parameter in the LSER models, the *b* coefficient (which is proportional to the difference in H-bond-donor acidity between the organic and aqueous phases) is smaller for retention on IAMs than for partitioning in octanol. This indicates that the IAM columns may provide a more favorable environment for the partitioning of strong H-bond-acceptor solutes than octanol, was they are more H-bond-acidic than the bulk organic solvent.

A closer look at the constant term *c* (*Eqn. 1*) in *Eqns. 4–7* shows that it is significantly different from zero in the solvatochromic equations obtained for IAM retentions, and that this difference is largest for the single-chain IAM.PC.DD stationary phase. In fact, the constant term includes information on the chromatographic-phase ratio (V_m/V_s) for the respective HPLC column. This ratio relates the capacity factor to the solute's partition coefficient between the mobile and the stationary phase. The more negative the constant term in *Eqn. 1*, the higher the phase ratio. When the retention measurements with different columns are conducted in the same chromatographic system, the constant terms are directly related to the respective volumes of the stationary phases as V_m ($t_0 \times$ flow) will be the same for all columns. In the present study, two different chromatographic systems were used, and, thus, the constant terms obtained in *Eqns. 5–7* for the three IAM HPLC columns could not be used to deduce information about the volumes of the respective stationary phases.

The relationships obtained for the three columns between log P_{oct} and log k_{IAMw} values are shown in *Fig. 2*. It can be seen that, for all columns, a curvature appears around log P_{oct} of *ca.* 0.0, leading to a slope of almost zero for polar compounds. This implies that no correlation between log k_{IAMw} and log P_{oct} exists for hydrophilic solutes having a log P_{oct} value smaller than zero, since their retention becomes insignificant and is not longer discriminative. Furthermore, for all columns, the relationships generated from experimental values lie below the ideal correlation indicated by the broken lines. Thus, for a compound with a given log P_{oct} value, a smaller log k_{IAMw} value will be measured. However, it should be borne in mind that the capacity factors determined on IAMs are not partition coefficients, and, thus, a proper comparison of the two lipophilicity indices in terms of absolute values is not possible.

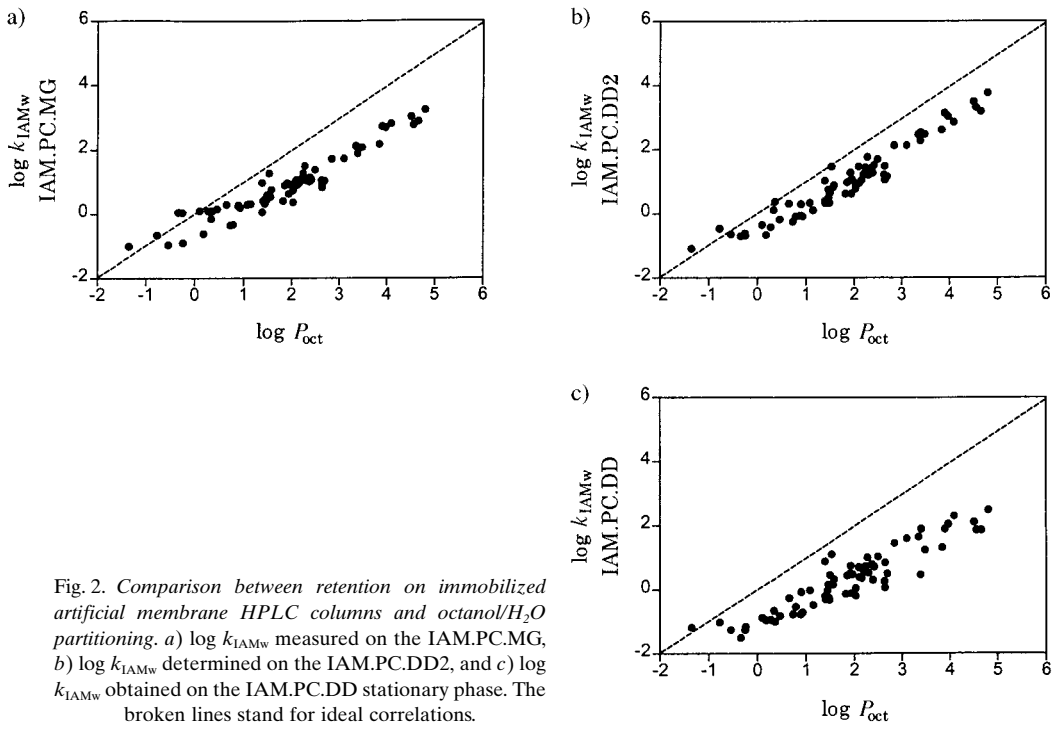

Fig. 2. *Comparison between retention on immobilized artificial membrane HPLC columns and octanol/H_2O partitioning. a*) log k_{IAMw} measured on the IAM.PC.MG, *b*) log k_{IAMw} determined on the IAM.PC.DD2, and *c*) log k_{IAMw} obtained on the IAM.PC.DD stationary phase. The broken lines stand for ideal correlations.

The relative contributions of each variable to the LSER models obtained for IAM retention (*Table 2*) indicate that the three IAM columns yield collinear data, and that the differences existing in their chemical structure (*Fig. 1*) do not influence the retention of small neutral compounds. This agrees with the results of *Ong et al.* [29] who also found a good correlation between capacity factors measured on different IAMs.

The lipophilicity indices measured on the IAM stationary phases were also compared to the capacity factors determined on a LC-ABZ HPLC column by *Pagliara et al.* [18]. The relationship between the two descriptors, considering the 60 common solutes, is illustrated by *Eqn. 8*, which relates the capacity factors measured on the IAM.PC.DD2 stationary phase to the log k_w values determined on the LC-ABZ HPLC column.

$$\log k_{IAM.DD2w} = 0.87(\pm 0.08) \cdot \log k_{ABZw} - 0.49(\pm 0.14) \quad (8)$$
$$n = 60;\ r^2 = 0.90;\ s = 0.29;\ F = 522$$

It can be seen from *Eqn. 8* that the two columns are quite similar. This is not surprising, since *Pagliara et al.* [18] have shown that the LC-ABZ stationary phase encodes the same structural information and could be a promising surrogate for

log P_{oct}. However, in terms of hydrophobicity, the IAM surface resembles a *RP-C_3* HPLC column [30][31], whereas the LC-ABZ is a *RP-C_{18}* stationary phase. It can, thus, be supposed that the latter provides a more hydrophobic environment for solute partitioning.

2.4. *Derivation of the Λ Parameter on the IAM.PC.DD2 Stationary Phase.* Previous studies have shown that lipophilicity (*e.g.*, log P) can be factorized into a hydrophobic term characterized mainly by molecular volume and a polarity term called Λ [23–25]. The hydrophobic term encodes all intermolecular forces proportional to the compound's size (*e.g.*, mainly hydrophobic forces [25] between the solute and the aqueous phase), while the polar term expresses *Van der Waals* forces and particularly H-bonds between the solute and both phases.

To gain further insight into the polar features responsible for IAM retention, this factorization was applied to the log k_{IAMw} values determined on the IAM.PC.DD2 stationary phase, according to *Eqn. 9:*

$$\log k_{IAM.DD2w} = \text{hydrophobicity} - \Lambda = (a \cdot V_w + c) - \Lambda \quad (9)$$

For nonpolar compounds such as the alkanes (pentane to nonane) included in this study ($\Lambda = 0$), the coefficient a in *Eqn. 9* is the slope of the regression line relating log k_{IAMw} and V_w, and c is the intercept. Thus, the hydrophobic term can easily be determined for these compounds and was calculated in *Eqn. 10* with the experimentally determined log k_{IAMw} values measured on the IAM.PC.DD2 stationary phase (*Table 3*) and the molecular *Van der Waals* volumes V_w.

$$\log k_{IAM.DD2w}\,(\text{alkane}) = 2.47 \cdot 10^{-2}(\pm 0.30 \cdot 10^{-2}) \cdot V_w - 0.06(\pm 0.41) \quad (10)$$
$$n = 5;\ r^2 = 0.996;\ s = 0.05;\ F = 665$$

Table 3. *Capacity Factors (*log k_{IAMw}*) Determined on the IAM.PC.DD2 HPLC Column and Molecular Volumes of Linear Alkanes*

	V_w [Å^3][a])	log k_{IAMw}
Pentane	96.8	2.28
Hexane	113.9	2.82
Heptane	131.1	3.20
Octane	148.3	3.61
Nonane	165.4	4.00

[a]) Calculated according to *Sect. 2.3.*

The combination of *Eqns. 9* and *10* leads to *Eqn. 11*, which allows the calculation of the polarity terms for the compounds included in the solvatochromic analysis:

$$\Lambda_{IAM.DD2} = 2.47 \cdot 10^{-2} \cdot V_w - 0.06 - \log k_{IAM.DD2w} \quad (11)$$

Eqn. 11 means that the polarity parameter of any solute is the difference between the log k_{IAMw} of a virtual alkane of identical volume (calculated according to *Eqn. 10*) and the log k_{IAMw} of this solute. With *Eqn. 11*, the Λ values of the 66 polar model compounds

(all solutes given in *Table 1* with the exception of pentane (**1**) and heptane (**76**)) were determined from their molecular volumes and the log k_{IAMw} values measured on the IAM.PC.DD2 column (*Table 1*).

Eqns. 6 and *9* suggest that the $\Lambda_{IAM.DD2}$ values must correlate with β (H-bond-acceptor basicity of solutes). Indeed, a rather good relationship between the two parameters was found (*Eqn. 12*) and is shown in *Fig. 3*.

$$\Lambda_{IAM.DD2} = 2.84(\pm 0.38) \cdot \beta + 0.71(\pm 0.17) \quad (12)$$
$$n = 66;\ r^2 = 0.78;\ s = 0.29;\ F = 225$$

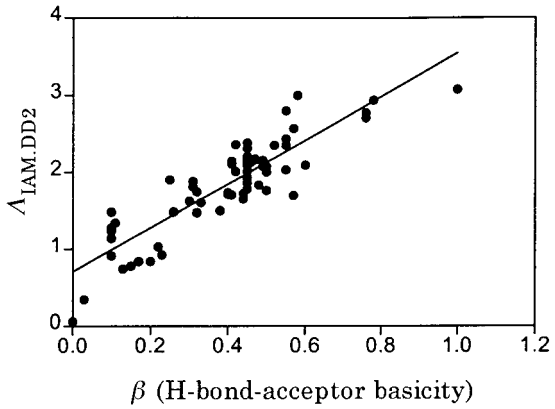

Fig. 3. *Relationship between the Λ parameter calculated for the 66 polar model compounds under study* (all compounds listed in *Table 1* with the exception of pentane (**1**) and heptane (**76**)) *and their H-bond-acceptor basicity* (β). The regression line was calculated according to *Eqn. 12*.

However, a closer look at *Fig. 3* shows that several values of Λ are observed for a given value of β. Thus, it seems that the IAM polarity term contains additional information beside the solute's H-bond-acceptor basicity. This cannot be the H-bond acidity (α) or dipolarity/polarisability (π^*), as they do not influence the retention on the IAM.PC.DD2 stationary phase (*Eqn. 6*) as also shown by *Eqn. 13:*

$$\Lambda_{IAM.DD2} = 2.95(\pm 0.51) \cdot \beta - 0.27(\pm 0.25) \cdot \alpha - 0.05(\pm 0.26) \cdot \pi^* + 0.76(\pm 0.27) \quad (13)$$
$$n = 66;\ r^2 = 0.79;\ q^2 = 0.76;\ s = 0.29;\ F = 78$$

For further analysis the Λ_{oct} parameters were calculated as described above by means of the equation given in [24]. The resulting values are listed in *Table 1*, and, based on the similarity observed between *Eqns. 4* and *6*, the Λ_{oct} terms, too, should correlate with the solutes' H-bond-acceptor basicity. This is confirmed by *Eqn. 14:*

$$\Lambda_{oct} = 4.23(\pm 0.29) \cdot \beta + 0.45(\pm 0.13) \quad (14)$$
$$n = 66;\ r^2 = 0.93;\ s = 0.22;\ F = 855$$

Comparing *Eqn. 12* and *Eqn. 14*, it can be suggested that some other polar interactions, maybe due to the zwitterionic properties of the polar head-groups of the immobilized phospholipids and not well-expressed by the solvatochromic parameters, are encoded in the Λ term calculated for the retention on the IAM.PC.DD2 stationary phase. These results may invite further investigations.

3. Conclusions. – We have demonstrated that three commercially available IAM stationary phases yield collinear data and retain small neutral solutes by a balance of intermolecular forces closely resembling those underlying partitioning in octanol/H_2O and retention on a reversed-phase LC-ABZ HPLC column. The comparison of the resulting LSER models shows that for all systems the size (favourable for partitioning and retention) and the H-bond-acceptor basicity of the solutes (unfavourable for partitioning and retention) are the two predominant factors, whereas dipolarity/polarisability and H-bond-donor acidity play only minor roles. In fact, this seems to be a general trend among systems where hydrophobic interactions control solute partitioning from an aqueous phase into an apolar organic phase. The close resemblance of the balances of intermolecular forces revealed by solvatochromic analysis may also be explained by interactions of the solutes with the solvated layer(s) of the stationary phases involved in HPLC retention and by octanol being H_2O-saturated.

A closer look at our results also shows that the IAM stationary phases are more cohesive and slightly more H-bond-acidic than bulk octanol. Furthermore, some additional polar interactions not well-expressed by the solvatochromic parameters seem to be involved in IAM retention.

Experimental Part

1. *Chemicals.* All compounds were obtained from commercial sources (*Merck*, D-Darmstadt; *Fluka Chemie*, CH-Buchs; *Janssen*, B-Beerse; Aldrich, D-Steinheim) and were of the highest available purity. MeCN of superpure quality for HPLC was bought from *Romil Chemicals* (Cambridge, UK). All other chemicals were of anal. grade. Deionized water was used throughout.

2. *Multivariate Statistical Analysis.* The LSER models were generated by multivariate regression with both the TSAR program [32] and the QSAR module in the SYBYL software [33], running on *Silicon Graphics Indy R4400* 175 MHz, *O_2 R5000* 180 MHz, and *Origin 2000 R10000* 195 MHz workstations. The relative contributions of each variable to the LSER models were obtained by *Mager*'s standardization [26].

3. *Selection of an Optimal Set of Solutes by Cluster Analysis.* The optimal set previously described by *Pagliara et al.* [18] is composed of 80 compounds and was derived by cluster analysis from 253 neutral organic compounds whose solvatochromic parameters are known [12]. The original solvatochromic parameters (α, β, π^*) were used for this study, while the *Van der Waals* volumes (V_w) were calculated with the standard software MOLSV (QCPE No 509) and the atomic radii of *Gavezzotti* [34]. The geometries used to generate them were optimized with the MMFF 94s force field [35][36].

Due to the unavailability of some compounds (**66**–**70**), the poor solubility in mobile phases of others (**74**, **79**–**80**), and experimental problems associated with basic solutes (**62**–**65**), the optimal set had to be reduced to 68 compounds (*Table 1*).

The *Van der Waals* volumes (V_w) of a series of linear alkanes (pentane – nonane), needed for the calculation of the Λ parameters (*Sect. 2.4*), were obtained as described above.

4. *Measurements of Capacity Factors.* 4.1. *Chromatographic Systems.* Retention measurements for the 68 compounds of the optimal set (and for hexane, octane, and nonane on the IAM.PC.DD2 chromatographic surface) were performed on three IAM HPLC columns (*Regis Technology*, Morton Grove, IL, USA), namely an IAM.PC.MG (150 × 4.6 mm, 12 μm, 300 Å), an IAM.PC.DD (100 × 4.6 mm, 5 μm, 300 Å), and an IAM.PC.DD2 (100 × 4.6 mm, 12 μm, 300 Å) stationary phase.

The capacity factors on the IAM.PC.MG and IAM.PC.DD stationary phases were measured with a *600E* liquid chromatograph (*Waters-Millipore*, Milford, MA, USA) equipped with a *7125 Rheodyne* injection valve. The detection was performed either by a *486 UV* detector (*Waters*) set at the λ of maximal absorbance for each compound or by a *RID-10A* refractive-index detector cell thermostated at 40° (*Shimadzu*, Kyoto, Japan), and the chromatograms were recorded by a *746 Data Module* (*Millipore*). Both RI- and UV-detection modes were employed for a number of UV-active solutes to verify the results calculated by means of the RI-detection method.

The IAM.PC.DD2 capacity factors were obtained with a liquid chromatograph consisting of a pump type *LC 414-T Kontron Analytica* (*Kontron Instruments*, CH-Zürich) equipped with an *Uvikon 730 S LC Kontron* UV spectrophotometer (*Kontron*) set at 254 or 220 nm. For the UV-inactive compounds, a refractive index (RI) detector with the detector cell thermostated at 40° (*Erma* refractometer, Tokyo, Japan) was used, and the RI detection method was validated as above. The chromatograms were recorded by an integrator type *3390 A* from *Hewlett Packard* (Avondale, PA, USA).

4.2. *Chromatographic Conditions.* The eluents were either 0.1M phosphate buffer, pH 7.0, except for the acidic compounds (pH 3.0), or mixtures of MeCN and buffer in different flow rates ranging from 0.5 ml/min to 2.4 ml/min according to the compounds' lipophilicity. For a number of nonionizable compounds, capacity factors were determined at pH 7.0, 4.5, and 3.0 in order to verify that the retention of neutral solutes at pH 3.0 was not affected by the changing ionization state of the phosphate group of the immobilized phospholipids. The aq. portion of the mobile phases was filtered through 0.45-μm *HA Millipore* filters (*Millipore*, Milford, MA, USA), and the eluent mixtures was prepared manually, with degassing prior to use. MeCN was chosen as the org. modifier because the use of MeOH is not recommended for the IAM.PC.MG stationary phase. The chromatographic experiments were carried out in a laboratory with temp. thermostated at $20 \pm 2°$ or performed with a water-bath circulator (*Haake*, *Digitana*, CH-Lausanne) and a column jacket in order to maintain the column temperature at $25 \pm 2°$.

Stock solns. (10^{-2} M) of the compounds were prepared in MeOH, MeCN, or directly with the mobile phase. When necessary, the solutes were diluted with the respective eluent before analysis and end-concentrations ranging from 10^{-5} M to 10^{-2} M were used. The injection volume was 20 μl, and all samples were injected at least three times for each mobile phase. Citric acid (10^{-3} M in buffer, pH 7.0) was utilized for the determination of the column void volume.

The chromatographic retention data are the mean of at least three determinations and are expressed by the logarithm of the capacity factor, $\log k_{IAM}$, defined as:

$$\log k_{IAM} = \log[(t_r - t_0)/t_0] \qquad \text{[Eq. 2]}$$

where t_r and t_0 are the retention times of the solute and of the unretained compound (citric acid), resp.

For elution of the more lipophilic compounds, mixtures of MeCN/buffer were used containing up to 40% (*v*/*v*) org. modifier (or up to 50% (*v*/*v*) cosolvent for the apolar alkanes). The $\log k_{IAMw}$ values at 100% aq. phase were obtained by linear extrapolation, plotting the isocratic $\log k_{IAM}$ values *vs.* the percentage (*v*/*v*) of org. modifier in the eluent mixtures.

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