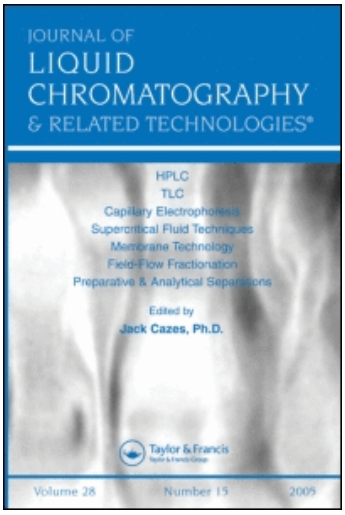

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S. Demare[a]; D. Roy[a]; J. Y. Legendre[a]
[a] UPSA Laboratoires, Rueil-Malmaison, France

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FACTORS GOVERNING THE RETENTION OF SOLUTES ON CHROMATOGRAPHIC IMMOBILIZED ARTIFICIAL MEMBRANES: APPLICATION TO ANTI-INFLAMMATORY AND ANALGESIC DRUGS

S. Demare, D. Roy, J. Y. Legendre*

UPSA Laboratoires
128, rue Danton
92506 Rueil-Malmaison, France

ABSTRACT

The performance of two different types of immobilized artificial membrane (IAM) stationary phases, IAM PC DD 1 and IAM PC DD 2 was studied with a series of model compounds as well as anti-inflammatory and analgesic drugs. The influence of the composition of the mobile phase and sample preparation on the chromatographic retention of selected compounds was investigated. Premature column failure was evidenced for both types of columns by a continuous decrease of k'_{IAM} values over time. pH of the mobile phase strongly influenced k'_{IAM} values of ionizable compounds. The k'_{IAM} value of the neutral form was always 2 to 7 fold higher than the value of the corresponding ionized form, depending on the compound and the type of IAM. A linear relationship was found between log k'_{IAM} and the percentage of acetonitrile in the mobile phase. However, extrapolation of the log k'_{IAM} value at 0% acetonitrile was improved when the pH and the ionic strength of acetonitrile-containing mobile phases were adjusted to the values of the purely aqueous mobile phase.

Finally, k'_{IAM} was determined for a set of 13 anti-inflammatory and analgesic drugs on the IAM PC DD 2 column. Results correlated best (r = 0.834) with the log D values at pH 7, indicating that IAM chromatography can be used to measure hydrophobicity of ionizable substances.

INTRODUCTION

Reliable prediction of drug absorption is becoming a valuable tool to select optimized lead compounds during drug development. As combinatorial chemistry and pharmacological high throughput screening are increasingly adopted by pharmaceutical companies, there is need for rapid screening techniques to evaluate the permeation of drugs. For that purpose, biological assays such as permeation through cell layers[1-3] or across intestinal membranes[3] have been developed. However, these methods are usually time-consuming and not suitable for high throughput screening. It is now well established that the passive diffusion of drugs across intestinal membrane is governed by several physico-chemical parameters including the partition coefficient of the drug between the aqueous phase and the membrane, the pKa, the molecular weight and volume of the drug, as well as, its ability to form hydrogen bonds.[3-6] Development of techniques to evaluate these various parameters may therefore have some value to predict drug absorption. In this scope, immobilized-artificial-membrane (IAM) chromatography was recently introduced to study drug-membrane interactions.[7,8]

IAMs are chromatographic surfaces prepared by covalently immobilizing monolayers of phospholipid analogs on silica particles.[9,10] Chromatographic retention factors of drugs on IAM columns were shown to correlate to some extent with octanol/water partition coefficient,[11-13] membrane permeability,[11,14] and even pharmacological activity.[15,16] Although there has been much effort to assess IAM chromatography as a predictive tool for drug absorption or potency, much less attention has gone into the optimization of the chromatographic conditions using IAM.[17] In this work, the influence of the type of IAM, chromatographic parameters and sample preparation on the retention time of model compounds as well as anti-inflammatory and analgesic drugs was studied. An optimized protocol to measure drug-membrane interactions using IAM chromatography is then discussed.

EXPERIMENTAL

Two types of IAM stationary phases were studied: IAM PC DD 1 and IAM PC DD 2 (Regis Technology, Morton Grove, IL, USA). Structures of both chromatographic supports are fully described elsewhere.[7,10]

Briefly, IAM PC DD 1 consists of a single-chain phosphatidylcholine ligand that lacks a glycerol backbone, whereas IAM PC DD 2 is a diacylated phosphatidylcholine ligand. IAM PC DD stationary phase is C_3 and C_{10} end-capped. Both columns were 100 x 4.6 mm and were used with a pre-column and a filter cartridge.

The chromatographic system consisted of a model 1050/1100 liquid chromatograph equipped with a model 1100 diode array UV detector (Hewlett-Packard Company, MD, USA). For all studies, the injection volume was 10 µL of a 50 µg/mL analyte solution prepared in 0.138 M DPBS (Dulbecco's phosphate buffer saline containing 2.7 mM KCl, 1.5 mM KH_2PO_4, 137 mM NaCl, and 8.1 mM $Na_2HPO_4.7\ H_2O$). In some cases, acetonitrile was added to the analyte solution to help dissolve poorly-soluble compounds. The mobile phase was DPBS adjusted with 10% phosphoric acid to vary the pH. Acetonitrile (10 to 40% v/v) was also sometimes added to the mobile phase. Isocratic conditions were always used at a flow rate of 1 mL/min. Detection was performed at 205 nm. The retention factor k'_{IAM} was calculated according to:

$$k'_{IAM} = (t_r - t_0)/t_0$$

where t_r is the retention time of the analyte and t_0 the hold-up time.

All chemicals were of analytical grade. Model compounds and drugs were all obtained from commercial sources and used as received.

RESULTS

IAM Chromatography Reproducibility

First, a reliable marker to measure the hold-up time was chosen. Indeed, determination of the hold-up time by injecting citric acid as recommended by the column manufacturer led to irreproducible results due to a poor resolution of the peak (data not shown). When using a purely aqueous mobile phase, acetonitrile was used as a suitable marker to determine the hold-up time. Conversely, when the mobile phase contained acetonitrile, water was injected in the column as the void marker. In all cases the hold-up time was between 1.35 and 1.37 min and between 1.31 and 1.32 min for IAM PC DD1 and IAM PC DD 2, respectively.

Injection-to-injection reproducibility was also studied by repeated injections of salicylic acid with up to 40% of acetonitrile in the mobile phase. For both columns, standard deviation of the retention time of salicylic acid was below 1%, regardless of the pH of the mobile phase and the presence of acetonitrile.

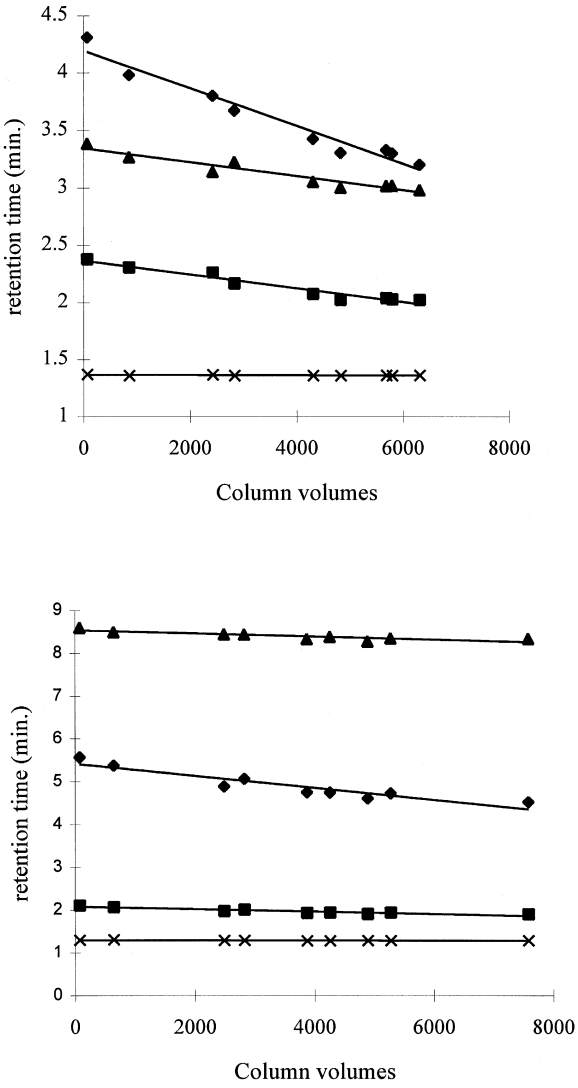

Figure 1. Retention time of salicylic acid (◆), benzoic acid (■), p-toluidine (▲) and hold-up time (✕) as a function of column volumes on the IAM PC DD 1 column (upper panel) and the IAM PC DD 2 column (lower panel).

Table 1

Evolution (%) of k'_{IAM} of Salicylic Acid, Aspirin, Benzoic Acid, and p-Toluidine Between 80 and 5000 Column Volumes on Both IAM and PC DC1 and IAM PC DD2 Columns

	IAM PC DD 1	IAM PC DD 2
Salicylic Acid	-33.2 %	-21.8 %
Aspirin	-33.4 %	-16.0 %
Benzoic Acid	-17.5 %	-22.8 %
p-Toluidine	-19.1 %	-3.4 %

Next, we looked at the reproducibility over time (i.e. experiment-to-experiment reproducibility) of the retention factor k'_{IAM} of model molecules on both IAM PC DD 1 and IAM PC DD 2. Benzoic acid, salicylic acid, and p-toluidine were injected in the IAM PC DD 1 and IAM PC DD 2 columns using DPBS as the mobile phase. k'_{IAM} of benzoic acid, salicylic acid, and p-toluidine as a function of column volumes are plotted in Figure 1.

Values of k'_{IAM} decreased upon column use for all molecules tested and for both types of IAM, indicating a deterioration of the column.

Peak broadening was also observed. The hold-up time was not affected, as its retention time over experiments remained constant (Figure 1). As indicated in Table 1, the percentage of decrease of k'_{IAM} at 5000 column volumes as compared to 80 column volumes ranged between 3% and 33%, depending on the compound and the type of IAM.

Indeed, the extent of the decrease of k'_{IAM} upon column failure was not identical for all the molecules under investigation, which precluded comparisons of k'_{IAM} over time.

Influence of Chromatographic Conditions on the Determination of k'_{IAM}

We next studied the influence of the pH of the mobile phase on the chromatographic retention of a series of structurally different model compounds. These compounds were selected to cover a large range of pKa, i.e. from 1.0 (for p-nitroaniline) to 8.8 (for theophylline) and included both basic and acidic compounds (Table 2).

Three mobile phase pHs were studied: 3, 5.4, and 7. Mobile phases with pH values less than 3 or greater than 7 were not used as recommended by the manufacturer.

Table 2

k'_{IAM} of Model Compounds as a Function of the pH of the Mobile Phase and the Type of IAM

		IAM PC DD1			IAM PC DD2		
	pKa	pH 3.0	pH 5.4	pH 7.0	pH 3.0	pH 5.4	pH 7.0
Benzoic Acid	4.0	5.92	0.71	0.28	11.23	1.96	0.47
Salicylic Acid	2.9	8.51	2.51	1.39	8.80	6.96	2.50
Aspirin	3.5	3.40	0.34	0.24	6.55	1.01	0.29
p-Toluidine	5.3	0.66	1.46	1.74	0.93	3.83	5.39
m-Nitroaniline	2.5	2.79	3.82	3.83	7.69	9.70	9.67
p-Nitroaniline	1.0	8.18	8.27	8.21	14.03	14.25	14.14
Antipyrine	1.5	0.88	0.85	0.82	2.69	2.72	2.59
Theophylline	8.8	0.66	0.66	0.66	1.23	1.26	1.25

The capacity factor k'_{IAM} of the selected molecules are given Table 2. It can be seen that for p-nitroaniline, antipyrine, and theophylline, k'_{IAM} is constant regardless of the pH of the mobile phase. This is explained by the fact that over the pH range studied these compounds are almost exclusively (>99% of the molecules) present as the same form, i.e., neutral for p-nitroaniline and antipyrine and ionized for theophylline.

For the other compounds, the higher the percentage of neutral form present in the mobile phase the higher the retention factor. For example, on IAM PC DD 2, k'_{IAM} of p-toluidine increased 5-6 fold when the compound shifted from the ionized form (pH 3) to the neutral one (pH 7). This observation was made for both IAM PC DD1 and IAM PC DD2.

Poorly-soluble compounds may need to be dissolved with some organic solvent (e.g. acetonitrile) prior to injection in the IAM column. In order to see if such a procedure would impact the k'_{IAM} value of the compound, benzoic acid, p-toluidine and salicylic acid were dissolved in the 0.138M DPBS containing 0 to 40% acetonitrile and 10 µL of each solution were injected in the IAM PC DD 1 column. In that case, the mobile phase was 0.138 M DPBS, pH 3, without acetonitrile. A slight decrease of k'_{IAM} was evidenced for all compounds at 40% acetonitrile in the samples (data not shown). Consequently, samples, when needed, were dissolved prior to injection in the chromatograph with no more than 30% acetonitrile.

IAM chromatography of lipophilic, highly retained compounds require the use of an organic modifier, such as acetonitrile, in the mobile phase. In that case, the $\log k'_{IAM}$ value in 100% aqueous phase can be extrapolated through a linear relationship with the fraction of the organic modifier in the mobile phase.[11,17]

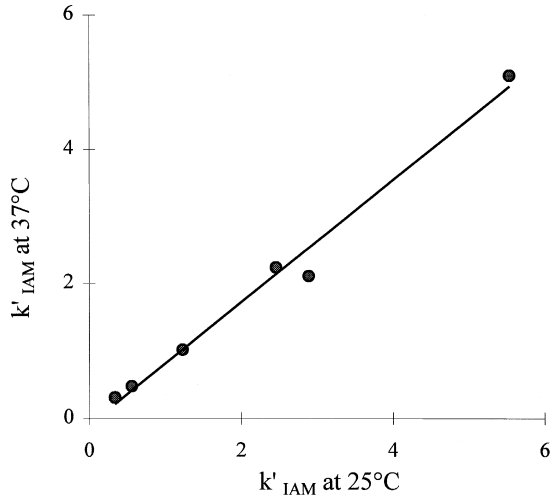

Figure 2. Correlation between k'_{IAM} values determined at 25°C and at 37°C on the IAM PC DD 2 column for a set of model compounds.

The influence of the percentage of acetonitrile in the aqueous mobile phase (ϕ = 0 to 40% v/v) on the retention of model compounds on the IAM PC DD 2 column was studied. The pH and the ionic strength of the acetonitrile-containing mobile phase were or were not readjusted to the values of the purely aqueous phase, i.e., pH 7.0, 0.138M.

Table 3 shows the values of extrapolated $\log k'_{IAM}$ of salicylic acid, benzoic acid, p-nitroaniline, m-nitroaniline, and p-toluidine using 10, 20, 30, and 40% acetonitrile as compared to the $\log k'_{IAM}$ value determined with a purely aqueous mobile phase. Addition of 40% acetonitrile in the aqueous mobile phase induced a shift of pH from 7.0 to 7.6 and of ionic strength from 0.138M to 0.083M.

When the mobile phase was prepared by a simple addition of acetonitrile in the buffer, i.e. when pH and ionic strength were not controlled, a deviation ranging from 2% (for p-nitroaniline) to 68% (for benzoic acid) was seen between the extrapolated $\log k'_{IAM}$ value and the measured value at 0% acetonitrile. The relationship between these two values was:

$$\text{extrapolated } \log k'_{IAM} = 1.238\ (\text{measured } \log k'_{IAM}) - 0.250$$
$$n = 5;\ r = 0.988$$

where n denotes the number of molecules used in the derivation of the regression equation and r is the correlation coefficient.

Table 3

Effect of pH and Ionic Strength of the Mobile Phase on Extrapolated Log k'_{IAM} Values as Compared to Measured Log k'_{IAM} Values in 100% DPBS

Mobile Phase:	100% DPBS	10 to 40% Acetonitrile in DPBS	10 to 40% Acetonitrile in DPBS
pH:	**7.0**	**7.0**	**7.15 to 7.60**
Ionic Strength:	**0.138M**	**0.138M**	**0.24 to 0.083 M**
Benzoic Acid	-0.327	-0.384	-0.549
Salicylic Acid	0.384	0.245	0.226
p-Nitroaniline	1.146	1.138	1.127
m-Nitroaniline	0.980	1.026	1.055
p-Toluidine	0.736	0.761	0.775

Table 4

k'_{IAM} of Model Compounds as a Function of the Temperature of the IAM PC DD2 Column

	Temperature of the Column	
	25°C	**37°C**
Benzoic Acid	0.56	0.48
Salicylic Acid	2.89	2.11
Aspirin	0.34	0.31
p-Toluidine	5.54	5.10
Antipyrine	2.46	2.24
Theophylline	1.23	1.02

However, when pH was maintained at 7 and ionic strength at 0.138M in all mobile phases, the deviation between extrapolated and measured k'_{IAM} values ranged between 1% (for p-nitroaniline) and 36% (for salicylic acid). The relationship is:

extrapolated $\log k'_{IAM} = 1.11 (\text{measured } \log k'_{IAM}) - 0.117$
$n = 5$; $r = 0.998$

The influence of the temperature on the IAM chromatographic retention was also studied. IAM PC DD 2 column was maintained at either 25°C or 37°C. DPBS pH 7.0 was used as the mobile phase. Table 4 indicates that k'_{IAM} of all compounds experienced a decrease when IAM chromatography was performed

Table 5

pKa Values, n-Octanol/Water Partition Coefficients, and k'_{IAM} of Selected Anti-Inflammatory and Analgesic Drugs

	pKa[a]	Log P[a]	Log D_7[b]	Log k'_{IAM}
Salicylic Acid	3.0	2.25	-1.78	0.44
Aspirin	3.5	1.15	-2.35	-0.50
Naproxen	4.1	3.40	0.55	1.53
Diclofenac	4.5	4.40	1.90	2.23
Piroxicam	1.9/5.5	2.65	0.25	1.46
Niflumic Acid	2.3/4.4	4.81	1.33	1.37
Ibuprofen	5.2	3.50	1.69	1.63
Acetaminophen	9.6	0.40	0.40	0.64
Antipyrine	1.5	-0.05	-0.05	0.39
Lidocaine	8.0	2.48	1.44	1.00
Procaine	8.8	1.95	-0.05	0.68
Codeine	8.2	1.19	-0.04	0.19
Caffeine	0.6/14	-0.07	-0.07	0.19

[a] pKa and log P literature values were drawn from a variety of sources and are judged to be of high quality.

[b] log D_7 was calculated according to:

$\log P = \log D_7 - \log [1/(1 + 10^{7-pKa})]$ for acids

$\log P = \log D_7 - \log [1/(1 + 10^{pKa-7})]$ for bases

except for Piroxicam and Niflumic acid for which log D_7 values were obtained from references 22 and 23, respectively.

at 37°C. However, the relationship between k'_{IAM} at 25°C and k'_{IAM} at 37°C was linear (r = 0.9963), indicating that either temperature may be used (Figure 2). For practical reasons, 25°C was selected as the temperature of the IAM column for further studies.

Correlation Between k'_{IAM} and Partition Coefficient

k'_{IAM} of a set of thirteen anti-inflammatory and analgesic drugs were determined on the IAM PC DD 2 column and compared to their partition coefficient values determined by the shake-flask reference technique. The set of selected compounds included 6 acidic molecules (salicylic acid, naproxen, diclofenac, ibuprofen, aspirin, acetaminophen), four basic molecules (codeine, procaine, lidocaine, antipyrine) and three amphoteric molecules (niflumic acid, piroxicam, caffeine). All molecules, except diclofenac, piroxicam, and niflumic acid were eluted from the IAM PC DD 2 column with pure DPBS. Up to 40% acetonitrile in the mobile phase was used to chromatograph diclofenac,

piroxicam, and niflumic acid. log k'_{IAM} as well as pKa, log P and log D_7 values for all drugs tested are given in Table 5. The relationship between log P and log k'_{IAM} is:

$$\log k'_{IAM} = 0.3564 \log P + 0.1461$$
$$n = 13;\ r = 0.788$$

where n denotes the number of molecules used in the derivation of the regression equation and r is the correlation coefficient. However, log k'_{IAM} values were slightly better correlated with the partition coefficient corrected for ionization at pH 7, log D_7:

$$\log k'_{IAM} = 0.4835 \log D_7 + 0.7956$$
$$n = 13;\ r = 0.834$$

DISCUSSION

Premature column failure was evidenced by a continuous decrease of k'_{IAM} of investigated compounds for both IAM PC DD 1 and, to a lower extent, IAM PC DD 2. Such a deterioration of IAM column performance over time was previously reported.[13,17] The reason for such column failure is unclear but a decrease of the k'_{IAM} values over time was seen for both single-and double-chain IAM, with or without a glycerol backbone. Since the extent of the k'_{IAM} decrease varies from compound to compound, the use of an internal standard from experiment to experiment was not possible. Therefore, comparisons between different experiments over time could not be made and it was decided that each further evaluation of a chromatographic factor on the retention of molecules on IAM would be conducted within a single experiment on the same day. Accordingly, when using existing IAM columns, it is advisable to carefully check for premature column failure and make comparisons of k'_{IAM} of different compounds at the same time.

The influence of the pH of the mobile phase on k'_{IAM} was studied by chromatographing structurally diverse compounds including bases and acids with pKa's ranging from 1.1 to 8.9 at three different pHs; 3, 5.4, and 7. k'_{IAM} was always 2 to 7 fold higher for the neutral form than the ionized form, depending on the compound and the IAM stationary phase (Table 2). Obviously, since the ionized form is also partitioning into the stationary phase, $\log k'_{IAM}$ of the neutral form cannot be simply corrected for ionization as done for logP according to the pH partition hypothesis.

Accordingly, $\log k'_{IAM}$ values at a particular pH must be experimentally determined with a mobile phase adjusted to the pH of interest and cannot be extrapolated from values obtained at another pH. The higher retention of the neutral form of ionizable compounds suggests that the retention mechanism on IAM is mainly governed by partition of the drug into the lipid stationary phase

and/or hydrophobic interactions, as previously reported.[17,18] Although solute adsorption to the polar headgroups of IAM by electrostatic interactions may occur, it should not contribute much to the retention mechanism.[10] This distinguishes IAM chromatography from liposome partitioning chromatography.

In the latter technique, electrostatic interactions between the drug and the phospholipid bilayer may play an important role in the partitioning process.[19-21] In addition, for all compounds and pH tested, k'_{IAM} values on IAM PC DD 2 values were higher than the values determined on the IAM PC DD 1 column. As a matter of fact, the double-chain IAM PC DD 2 stationary phase is more lipophilic than the single-chain IAM PC DD 1.[10] Consequently, the hydrophobicity scale of the IAM PC DD 2 column may be more favorable when dealing with unknown new chemical entities. Overall, to perform IAM chromatography in conditions mimicking a physiological environment while preserving the stability of the column, it seems appropriate to use the IAM PC DD 2 column and to maintain the pH of the mobile phase around 7.

Determination of the k'_{IAM} of highly retained compounds in pure aqueous phase is feasible since a linear relationship between k'_{IAM} and the percentage of acetonitrile in the mobile phase ϕ, exists. Values of k'_{IAM} at 0% acetonitrile can therefore be easily extrapolated. We found that 40% acetonitrile was sufficient to chromatograph the most lipophilic compounds among those selected. Such a linear relationship between k'_{IAM} and ϕ was previously reported for IAM chromatography of beta-blockers[17] and anti-inflammatory drugs.[11] However, in previous reports, mobile phases were prepared by simply adding acetonitrile into the aqueous buffer. Particulary, the pH and the ionic strength of the acetonitrile-containing mobile phases were not readjusted to initial values. Indeed, in agreement with our data, Caldwell et al. found up to 40% deviation of the $\log k'_{IAM}$ value determined in 0% acetonitrile as compared to the extrapolated value.[17]

In this work we show that keeping the pH and the ionic strength constant from 0 through 40% acetonitrile in the mobile phase improves the extrapolation of k'_{IAM}. In that case, the correlation coefficient of the linear regression between the extrapolated k'_{IAM} value and the measured k'_{IAM} value in 100% DPBS was higher (0.998 vs 0.988) than when the pH and the ionic strength were not readjusted. More importantly, the slope of the linear regression was closer to 1 (1.113 vs 1.238) when pH and ionic strength were maintained constant, indicating that the extrapolated values were closer to the measured values at 100% DPBS.

Finally, the drug/membrane interactions of a series of thirteen anti-inflammatory and analgesic drugs were measured on the IAM PC DD 2 column. The most hydrophobic drugs were eluted with mobile phases containing various fractions of acetonitrile and again, a linear relationship was found between $\log k'_{IAM}$ and ϕ. k'_{IAM} values obtained for aspirin, salicylic acid, naproxen,

diclofenac, piroxicam, and ibuprofen were comparable to previously reported values by Barbato et al. on an IAM PC MG column.[11] The IAM PC MG column is also characterized by a double acyl chain stationary phase but, in contrast to the IAM PC DD 2 column, it displays a methylglycolate end-capping.[7] Therefore, it seems that the type of end-capping used to mask free propyl-amide groups on the stationary phase has only a very limited influence on the chromatographic indexes of weak acid anti-inflammatory drugs. k'_{IAM} values were then compared to log P and log D_7 values.

Linear regression analysis indicated that the overall correlation between log k'_{IAM} determined on the IAM PC DD 2 column and the partition coefficient measured by the shake-flask method was rather poor. However, the best correlation ($r = 0.834$) was found between log k'_{IAM} and log D_7. This result agrees well with data from Kaliszan et al. who also found a better correlation between log k'_{IAM} and log D_7 rather than between log k'_{IAM} and log P for a series of beta-blockers. In contrast, Barbato et al. showed that correlation of log k'_{IAM} with log P was better than with the ionization-corrected log D for a set of nonsteroidal anti-inflammatory drugs.

Unlike these two previous reports, the set of molecules selected in our study spans a large range of pKa's and chemical structures, thus allowing a better assessment of the correlation between k'_{IAM} and the partition coefficient of structurally diverse compounds, both as neutral and ionized forms. In that case, it was expected that chromatographic indexes on IAM reflect partition coefficient values corrected for ionization. In our hands, it was therefore possible to adequately measure the relative hydrophobicity of a series of anti-inflammatory and analgesic drugs using IAM chromatography. In addition, this method is less cumbersome than the classical shake-flask method and its unique hydrophobicity scale may be more favorable than that of reverse-phase HPLC. However, the predictive value of IAM chromatography for absorption still remains to be demonstrated for the compounds selected in this work and experiments are underway in our laboratory to address this issue.

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REFERENCES

1. E. Walter, S. Janich, B. J. Roessler, J. M. Hilfinger, G. L Amidon, J. Pharm. Sci., **85**, 1070-1076 (1996).

2. P. Artursson, J. Pharm. Sci., **79**, 476-482 (1990).

3. A. L. Ungell, Drug Dev. Ind. Pharm., **23**, 879-892 (1997).

4. E. Lien, Drug Intell. Clin. Pharm., **4**, 7-9 (1970).

5. T. Nook, E. Doelker, P. Buri, Int. J. Pharm., **43**, 119-129 (1988).

6. E. Escribano, A. C. Calpena, T. M. Garrigues, J. Freixas, J. Domenech, J. Moreno, Antimicrob. Agents Chemother., **41**, 1996-2000 (1997).

7. C. Y. Yang, S. J. Cai, H. Liu, C. Pidgeon, Adv. Drug Deliv. Rev., **23**, 229-256 (1996).

8. S. Ong, H. Liu, C. Pidgeon, J. Chromatogr., **728**, 113-128 (1996).

9. H. Liu, S. Ong, L. Glunz, C. Pidgeon, Anal. Chem., **67**, 3550-3557 (1995).

10. C. Pidgeon, C. Marcus, F. Alvarez, "Immobilized Artificial Membrane Chromatography: Surface Chemistry and Applications," in **Applications of Enzyme Biotechnology**, J. W. Kelly, T. O. Baldwin, eds., Plenum Press, New-York, 1991,pp. 201-220.

11. F. Barbato, M. I. La Rotonda, F. Quaglia, J. Pharm. Sci., **86**, 225-229 (1997).

12. M. H. Abraham, H. S. Chadha, R. A. E. Leitao, R. C. Mitchell, W. J. Lambert, R. Kaliszan, A. Nasal, P. Haber, J. Chromatogr., **766**, 35-47 (1997).

13. R. Kaliszan, A. Kaliszan, I. W. Wainer, J. Pharm. Biomed. Anal., **11**, 505-511 (1993).

14. C. Pidgeon, S. Ong, H. Liu, X. Qiu, M. Pidgeon, A. H. Dantzig, J. Munroe, W. J. Hornback, J. S. Kasher, L. Glunz, T. Szczerba, J. Med. Chem., **38**, 590-594 (1995).

15. F. Barbato, M. I. La Rotonda, F. Quaglia, Pharm. Res., **14**, 1699-1705 (1997).

16. R. Kaliszan, A. Nasal, A. Bucinski, Eur. J. Med. Chem., **298**, 163-170 (1994).

17. G. W. Caldwell, J. A. Masucci, M. Evangelisto, R. White, J. Chromatogr. A, **800**, 161-169 (1998).

18. S. Ong, C. Pidgeon, Anal. Chem., **67**, 2119-2128 (1995).

19. A. Avdeef, K. J. Box, J. E. A. Comer, C. Hibbert, K. Y. Tam, Pharm. Res., **15**, 209-215 (1998).

20. S. D. Krämer, C. Jakits-Deiser, H. Wunderli-Allenspach, Pharm. Res., **14**, 827-832 (1997).

21. S. D.Kramer, H. Wunderli-Allenspach, Pharm. Res., **13**, 1851-1855 (1996).

22. K. Takacs-Novak, J. Kökösi, B. Podanyi, B. Noszal, R. S. Tsai, G. Lisa, P. A. Carrupt, B. Testa, Helvet. Chim. Acta, **78**, 553-562 (1995).

23. K. Takacs-Novak, A. Avdeef, K. J. Box, B. Podanyi, G. Szasz, J. Pharm. Biomed. Anal., **12**, 1369-1377 (1994).

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