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# Potential of immobilized artificial membrane chromatography for lipophilicity determination of arylpropionic acid non-steroidal anti-inflammatory drugs

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**Abstract**

Molecular lipophilicity can be expressed by $\log P$ or more conveniently by $\log k$, i.e. determined by the traditional shake-flask technique or by liquid chromatography. The $\log k$ of 11 arylpropionic non-steroidal anti-inflammatory drugs (NSAIDs) was determined at pH 7.4 of the eluent using two stationary phases i.e. octadecylsilane phase and an immobilized artificial membrane (IAM.PC.MG) packing. The chromatographic retention factors extrapolated to 100% aqueous phase ($\log k_{wODS}$ and $\log k_{wIAM}$) were correlated with *n*-octanol/water lipophilicity parameters ($\log P$) and with *n*-octanol/water partition coefficients corrected for ionization at pH 7.4 ($\log D_{7.4}$). In this series of compounds, significant linear correlations ($r > 0.94$) between the chromatographic parameters ($\log k_{wIAM}$) and the reference lipophilicity data ($\log P$ and $\log D_{7.4}$) were described. Moreover, regression analysis between the lipophilicity parameters and some pharmacokinetic data for the drugs under study were performed. The $\log k_{wIAM}$ parameter over *n*-octanol/water partition data seems to provide a good model to obtain lipophilicity parameters of arylpropionic acid NSAIDs for quantitative structure-activity relationships studies.


*Keywords:* Lipophilicity; Immobilized artificial membrane chromatography; Arylpropionic acid; Partition coefficient

## 1. Introduction

The partition coefficient for the *n*-octanol/water system ($\log P$) was used first as the molecular lipophilicity parameter in quantitative structure-activity relationship (QSAR) studies of bioactive compounds [1,2]. Then, reversed-phase high performance liquid chromatography (RP-HPLC) has been widely used as an alternative to the direct measurement of $\log P$ through a linear relation-

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ship of the retention factor ($\log k$) and $\log P$ [3–5]. Chromatographic experiments present several practical advantages over the direct determination of *n*-octanol/water partition coefficients, i.e. small amounts of material are required, impurities can be separated during the measurements and the process can be easily automated. However, to measure a wide range of lipophilicity by RP-HPLC, it is necessary to determine $\log k$ values at several mobile phase compositions and extrapolate back to pure aqueous mobile phase ($\log k_w$) [6]. Moreover, the nature and specific properties of the stationary phase used for chromatographic determination of lipophilicity have a prevailing effect on the data obtained. Any given $\log k$ value is specific to one HPLC system [7]. The stationary phase generally used in such lipophilicity determinations is a standard octadecylsilane packing (ODS).

For few years, the immobilized artificial membranes (IAMs) have been introduced as chromatographic packing materials. IAMs consist of phosphatidyl choline residues covalently bound to silica propylamine and consequently, mimic fluid phospholipid bilayers [8,9]. Firstly, these physical similarities between IAMs and fluid membranes led to use these IAM surfaces to predict drug transport across membranes [10]. Then, chromatographic retention factors of drugs on IAM columns were shown to correlate with their biological activities [11] or their pharmacokinetic parameters [12]. The penetration of the cell membrane by arylpropionic non-steroidal anti-inflammatory drugs (NSAIDs) has been reported to be an important aspect of their activity [13].

Consequently, in this work, the IAM retention data ($\log k_{wIAM}$) of arylpropionic NSAIDs have been compared with $\log D_{7.4}$ and $\log k_{wODS}$ values to various pharmacokinetic parameters in a QSAR study.

## 2. Experimental

### 2.1. Chemicals

The 11 studied arylpropionic acids are depicted in Fig. 1. Carprofen, fenoprofen, indoprofen, ketoprofen, naproxen, pirprofen and suprofen were purchased from Sigma Aldrich (St. Quentin Fallavier, France). Alminoprofen (E. Bouchara, Levallois, France), flurbiprofen and ibuprofen (Boots, Nottingham, UK) and tiaprofenic acid (Roussel Uclaf, Romainville, France) were generously supplied. The samples were prepared as solutions of 0.5 mg/ml in methanol and appropriate dilutions were made in water. All chemicals and solvents were of analytical grade or HPLC grade. Water was obtained from a Milli-Q purification system.

### 2.2. Apparatus

The chromatographic apparatus (ThermoFinnigan™, San Jose, CA) was equipped with a constant flow pump M 100, a Model 150 ultraviolet detector operating between 225 and 290 nm and a Datajet® integrator. The detection was performed at the maximum wavelength of each compound.

### 2.3. Columns

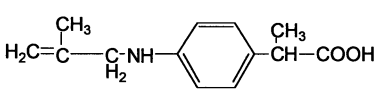
The ODS column (30 cm × 3.9 mm i.d.) was prepacked with μBondapak® C18, particle size 10 μm (Waters, Milford, MA). The IAM.PC.MG column (15 cm × 4.6 mm) was filled with phosphatidylcholine (PC) residues convently bonded to silica (Regis Technologies, Inc., Morton Grove, IL). The MG indicates that the silica surface was end-capped with methylglycolate.

### 2.4. Mobile phase preparation

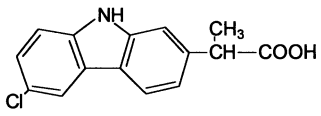
The mobile phases were prepared volumetrically from combinations of methanol (ODS column) or acetonitrile (IAM.PC.MG column) and phosphate buffer (0.016 M, pH 7.4) in the range 30–60% for methanol and 4–27% for acetonitrile. The flow rate was 1.5 ml/min for the ODS column and 1.0 ml/mn for the IAM.PC.MG column.

### 2.5. Determination of retention factor (*k*)

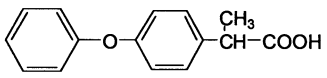
According to their chromatographic behaviour, the retention time ($t_r$) of each compound was



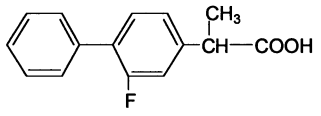
1- Alminoprofen



2- Carprofen

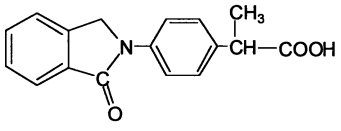


3- Fenoprofen

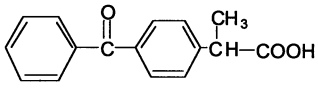


4- Flurbiprofen

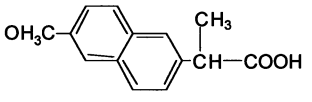
5- Ibuprofen



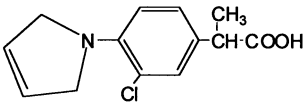
6- Indoprofen



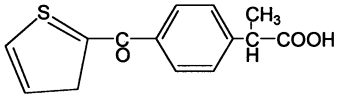
7- Ketoprofen



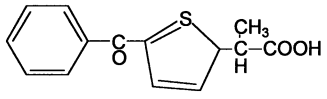
8- Naproxen



9- Pirprofen



10- Suprofen



11- Tiaprofenic acid

Fig. 1. Chemical structures of the arylpropionic acids NSAIDs.

determined in triplicate at six different organic modifier-buffer mobile phase mixtures. At each mobile phase composition, the retention factor was calculated through the formula: $k = (t_r - t_0)/t_0$, where $t_0$ is the column dead-time of the system and was measured as the time from the injection to the first distortion of the baseline after drug injection.

The $\log k$ values at 100% aqueous mobile phase ($\log k_w$) were obtained from the $y$-intercept of

plots $\log k$ versus percent of organic modifier in the eluent.

### 2.6. Calculation of log D

The apparent distribution coefficients ($\log D_{7.4}$) of the 11 arylpropionic acids were calculated at pH 7.4 according to the formula:

$$\log D_{7.4} = \log P + \log(1 + 10^{7.4 - \mathrm{p}K_a}),$$

with $\log P$ the *n*-octanol/water partition coefficient and $\mathrm{p}K_a$ the dissociation constant.

In a previous study [14], the *n*-octanol/water partition coefficients ($\log P$) of these 11 arylpropionic acids were determined by the traditional shake-flask technique, and their dissociation constants ($\mathrm{p}K_a$) were performed using a classical potentiometric method.

Correlation studies were performed using a statistical program (GraphPad PRISM®).

## 3. Results and discussion

### 3.1. Lipophilicity parameters

In agreement with previous results [15,16], the increase in $\log k$ values with decreasing methanol or acetonitrile percentage in the eluent was linear with the ODS packing and the IAM packing, respectively. In our study, the straight lines of $\log k$ versus organic modifier concentration ($\Phi$) display *r* values ranging from 0.991 to 0.999. Consequently, the $\log k$ values could be extrapolated linearly to 100% water content (Fig. 2), yielding the $\log k_w$ values reported in Tables 1 and 2, respectively.

The *n*-octanol/water partition coefficients ($\log P$) and their corresponding calculated $\log D$ (at pH 7.4) are reported in Table 3.

#### 3.1.1. ODS stationary phase

The linear relationship between *n*-octanol/water partition coefficients ($\log P$ values) and the extrapolated retention factors ($\log k_{wODS}$) values, as dependant variable is reported in the following equation.

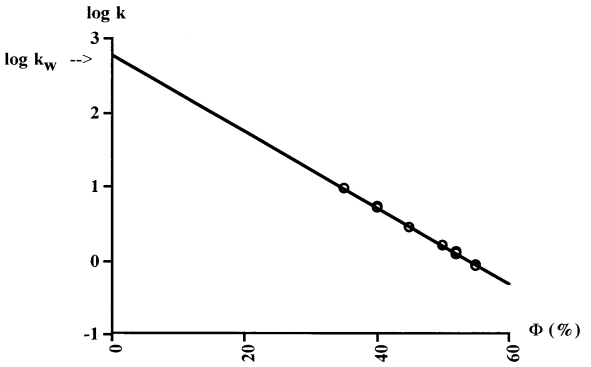



Fig. 2. Plot of logarithm of capacity factors determined on μBondapak® C18 column ($\log k$) at different percentages of methanol ($\Phi$) (compound: indoprofen).

Table 1
Linear correlations $\log k = S\Phi + \log k_{wODS}$, obtained by RP-HPLC for the arylpropionic acid derivatives on an octadecylsilane (ODS) column

| Compounds | $\log k_{wODS}$ | *S* | *r* |
|---|---|---|---|
| Alminoprofen | 1.85 | −0.037 | 0.998 |
| Carprofen | 3.85 | −0.055 | 0.991 |
| Fenoprofen | 3.54 | −0.055 | 0.996 |
| Flurbiprofen | 2.91 | −0.042 | 0.995 |
| Ibuprofen | 3.75 | −0.054 | 0.994 |
| Indoprofen | 2.77 | −0.051 | 0.999 |
| Ketoprofen | 2.83 | −0.050 | 0.994 |
| Naproxen | 2.99 | −0.067 | 0.992 |
| Pirprofen | 2.72 | −0.046 | 0.994 |
| Suprofen | 2.46 | −0.048 | 0.998 |
| Tiaprofenic acid | 2.66 | −0.050 | 0.998 |

*S*, slope of the linear relationship; *r*, correlation coefficient.

$$\log k_{wODS} = 0.504(\pm 0.099)\log P + 1.517(\pm 0.296) \quad (1)$$

$n = 11$; $r = 0.860$; $s = 0.31$.

In this and the following equations, *r* is the correlation coefficient, *s* is the standard error of the estimate. Numbers in parentheses account for the standard error of the regression coefficients.

Considering Eq. (1), a weak correlation between $\log P$ and $\log k_{wODS}$ was found.

As noted previously [17], it is essential to correlate the distribution coefficient $\log D$ and $\log k$ determined at the same pH. Most of the

Table 2
Linear correlations $\log k = S\Phi + \log k_{wIAM}$, obtained for the arylpropionic acid derivatives on an IAM.PC.MG packing

| Compounds | $\log k_{wIAM}$ | $S$ | $r$ |
|---|---|---|---|
| Alminoprofen | 0.33 | −0.045 | 0.998 |
| Carprofen | 1.81 | −0.047 | 0.993 |
| Fenoprofen | 1.21 | −0.052 | 0.998 |
| Flurbiprofen | 1.58 | −0.060 | 0.995 |
| Ibuprofen | 1.57 | −0.066 | 0.997 |
| Indoprofen | 1.10 | −0.070 | 0.998 |
| Ketoprofen | 1.02 | −0.058 | 0.992 |
| Naproxen | 1.07 | −0.055 | 0.997 |
| Pirprofen | 0.95 | −0.048 | 0.999 |
| Suprofen | 1.05 | −0.065 | 0.997 |
| Tiaprofenic acid | 1.05 | −0.060 | 0.995 |

$S$, slope of the linear relationship; $r$, correlation coefficient.

Table 3
Physicochemical parameters of the 11 arylpropionic acids studied [14]

| Compounds | $pK_a$ | $\log P$ | $\log D_{7.4}$ |
|---|---|---|---|
| Alminoprofen | 5.02 | 0.618 | −1.762 |
| Carprofen | 4.36 | 4.128 | 1.088 |
| Fenoprofen | 5.70 | 3.449 | 0.549 |
| Flurbiprofen | 4.20 | 3.769 | 0.569 |
| Ibuprofen | 4.55 | 3.686 | 0.836 |
| Indoprofen | 4.25 | 2.391 | −0.759 |
| Ketoprofen | 4.18 | 2.683 | −0.5 |
| Naproxen | 4.20 | 2.998 | −0.202 |
| Pirprofen | 4.64 | 1.765 | −0.995 |
| Suprofen | 4.11 | 2.659 | −0.631 |
| Tiaprofenic acid | 3.80 | 2.858 | −0.742 |

time, the pH of interest for pharmacological testing is the physiological pH 7.4, which is within the pH limitations for most reversed-phase packing.

A better correlation is obtained between $\log D_{7.4}$ and $\log k_{wODS}$:

$$\log k_{wODS} = 0.612(\pm 0.083)\log D_{7.4} + 3.083(\pm 0.073) \quad (2)$$

$n = 11$; $r = 0.925$; $s = 0.23$.

When the $\log k_{wODS}$ values were correlated with the corresponding $\log P$ values for the non-ionic form of these NSAIDs a poor correlation coefficient was obtained (Eq. (1)). This behaviour may suggest that the NSAIDs retention on this ODS packing depends not only on the hydrophobic interactions by also on the compound's ionization degree [18]. This observation was supported by the fact that this correlation was better ($r > 0.92$) between $\log k_{wODS}$ values and the apparent distribution coefficient ($\log D_{7.4}$) values. At pH 7.4, these 11 acidic compounds exist in their fully ionized form (Table 3, [14]).

### 3.1.2. IAM stationary phase

The significant relationship between $\log P$ (independent variable) and $\log k_{wIAM}$ for these NSAIDs is given by the following equation:

$$\log k_{wIAM} = 0.373(\pm 0.042)\log P + 0.114(\pm 0.124) \quad (3)$$

$n = 11$; $r = 0.948$; $s = 0.13$.

A good linear correlation was also observed between the apparent distribution coefficient ($\log D_{7.4}$) and the chromatographic parameter $\log k_{wIAM}$:

$$\log k_{wIAM} = 0.414(\pm 0.053)\log D_{7.4} + 1.262(\pm 0.046) \quad (4)$$

$n = 11$; $r = 0.934$; $s = 0.15$.

In this HPLC approach on IAM.PC.MG column (Eqs. (3) and (4)), the compound's $\log P$ or $\log D_{7.4}$ were linearly related to their retention factor obtained with a 100% aqueous phase, $\log k_{wIAM}$. Moreover, the slope values of these two linear relationships were very close. The ranking order on IAM for these arylpropionic acids is governed by their intrinsic lipophilicity and is not affected by the presence of an electric charge on the molecule.

Our results are agree with those reported by several authors [11,19–22]. They underlined that the correlations between logarithms of retention factor determined on the IAM columns, $\log k_{wIAM}$, and the reference parameter of lipophilicity, $\log P$, generally exited for structurally related compounds.

Finally, in this homologous series of compounds supporting a carboxylic function, different methods of lipophilicity determination were described ($\log D_{7.4}$, $\log k_{wODS}$ and $\log k_{wIAM}$). We chose extrapolations to 100% aqueous phase ($\log k_w$) to compare retention factors independently of the amount and type of organic modifier. Classically, for estimating lipophilicity by RP-HPLC, methanol is considered as the most suitable organic solvent [3]. With an IAM.PC.MG packing material, the manufacturer recommended the use of acetonitrile. According to a previous study [23], no significant difference was observed between the $\log k_{wIAM}$ values (at pH 7) extrapolated from either methanol or acetonitrile. The acetonitrile percentage should not be more than 30% since this would disrupt the water structure.

As illustrated in Fig. 3, the lipophilicity values ranked as follow $\log k_{wODS} > \log k_{wIAM} > \log D_{7.4}$. The retention properties of the IAM.PC.MG sorbent was shown to be different to octadecylsiloxane-bonded silica sorbent. The IAM columns offer polar heads as the first site of contact between the solute and consequently, they are more realistic models for biomembranes [24]. Consequently, the chromatographic indices of drugs on immobilized stationary phases could be correlated with their pharmacological activity [25].

### 3.2. Log $k_w$ and log D correlations to various pharmacokinetic parameters

We investigated potential relationships between various pharmacokinetic data of arylpropionic NSAIDs and the lipophilicity parameters, described above ($\log k_{wODS}$, $\log k_{wIAM}$, $\log D_{7.4}$).

After administration, the arylpropionic acid NSAIDs are rapidly distributed into most body tissues and fluids and their volume of distribution is generally 0.10–0.25 l/kg at the usual therapeutic concentrations. All exhibit an extensive binding to plasma protein: over 95%. In patients with normal renal function, these NSAIDs and their metabolites are rapidly and almost completely excreted in urine. With the exception of carprofen and naproxen, their elimination half-lifes are less than 7 h (Table 4). The possibility of establishing relationships between the lipophilicity values and three pharmacokinetic parameters (volume of distribution ($V$), percent of binding to plasma protein (B%) and half-life time ($t_{1/2}$)) was evaluated in a second-order polynomial model. Table 5 shows the statistical analysis of this QSAR study.

In this series of ionizable compounds, the lipophilicity measured on an IAM column provided better correlations with the pharmacokinetic data than the other lipophilicity parameters. These

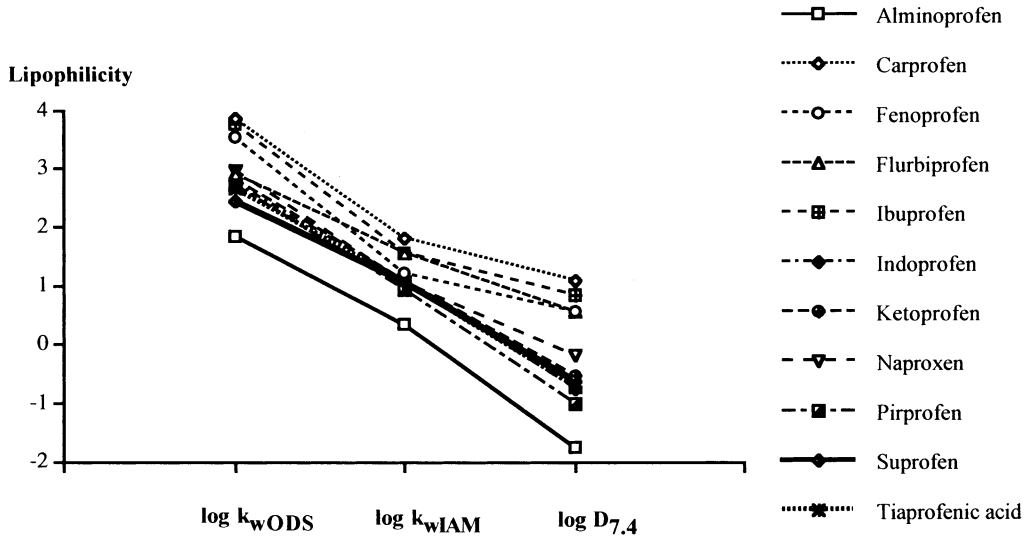



Fig. 3. Comparison of $\log k_{wODS}$, $\log k_{wIAM}$ and $\log D_{7.4}$ for the series of 11 arylpropionic NSAIDs.

Table 4
Pharmacokinetic parameters used for the QSAR study ([13,26–29])

| Compound | $V$ (l/kg) | $t_{1/2}$ (h) | $B$ (%) |
|---|---|---|---|
| Carprofen | 0.24 | 19.5 | 91.4 |
| Fenoprofen | 0.10 | 2.5 | 99.8 |
| Flurbiprofen | 0.10 | 4.0 | 99.7 |
| Ibuprofen | 0.10 | 2.2 | 98.8 |
| Indoprofen | 0.16 | 3.2 | 95.6 |
| Ketoprofen | 0.11 | 2.5 | 98.5 |
| Naproxen | 0.10 | 10 | 97.2 |
| Pirprofen | 0.18 | 6.5 | – |
| Suprofen | – | 5.8 | 99.7 |
| Tiaprofenic acid | 0.17 | 4.2 | 98 |

Table 5
Comparisons of correlations ($Y = a + bX + cX^2$) between pharmacokinetic parameters ($Y$) and lipophilicity values ($X$) of arylpropionic acid NSAIDs; with $n$ number of compounds, $r$ correlation coefficient, $s$ standard error of estimate

| $Y$ | $X$ | $n$ | $r$ | $s$ |
|---|---|---|---|---|
| log $V$ | log $k_{wIAM}$ | 9 | 0.862 | 0.085 |
| | log $D_{7.4}$ | 9 | 0.878 | 0.080 |
| | log $k_{wODS}$ | 9 | 0.846 | 0.090 |
| log $t_{1/2}$ | log $k_{wIAM}$ | 10 | 0.839 | 0.185 |
| | log $D_{7.4}$ | 10 | 0.404 | 0.311 |
| | log $k_{wODS}$ | 10 | 0.332 | 0.321 |
| log $B$% | log $k_{wIAM}$ | 9 | 0.911 | 0.006 |
| | log $D_{7.4}$ | 9 | 0.886 | 0.006 |
| | log $k_{wODS}$ | 9 | 0.694 | 0.010 |

results are agree with those of Kaliszan et al. [30] and Caldwell et al. [12], who already reported significant regression analysis between log $k$ determined on a IAM.PC.MG column and pharmacokinetic parameters in a series of β-adrenolytics.

## 4. Conclusion

In this study, we have compared different methods of lipophilicity determination in a homologous series of compounds supporting a carboxylic function. The lipophilicity values ranked as follow $\log k_{wODS} > \log k_{wIAM} > \log D_{7.4}$. The $\log k_{wIAM}$ parameter determined with a buffer of physiological pH correlated well with the ionization-corrected reference lipophilicity parameter from the $n$-octanol–water system. Moreover, correlations between pharmacokinetic data reported for these NSAIDs demonstrated the performance of $\log k_{wIAM}$ to be as good as that of the reference partition coefficient in predicting bioactivity. There is an important advantage of the $\log k_{wIAM}$ parameter over the log $P$ data which are tedious to measure: $\log k_{wIAM}$ is derived in a simple, fast and reproducible manner.

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