Prediction of Chromatographic Parameters for Some Anilines by Molecular Connectivity


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Key Words
Thin-layer chromatography
RF values
Anilines
QSAR

Summary
The possible relation existing between RF values obtained by thin-layer chromatography for a group of anilines with connectivity indices proposed by Kier and Hall has been studied. Using multivariable regression the corresponding connectivity functions, selected for their respective correlation coefficients, standard deviations, Snedecor's F and Student's t were obtained. Regression analysis of the connectivity functions gives a correct prediction of the experimental elution sequence for this group of substances on silica gel stationary phases and various mobile phases of different polarity. The corresponding random and stability studies of the different prediction models selected were carried out, showing good stability and null randomness in all cases.

Introduction
Quantitative structure-activity relationship (QSAR) studies have been used for the prediction of physical-chemical [1, 2] and pharmacological [3–5] properties of drugs. These methods transcribe the molecular structure to a graph from which the topological indices which characterize a specific substance are obtained [6]. The molecular connectivity indices have been used for the prediction of parameters related to the biological and pharmacological activity of drugs [7].

The degree of retention in a chromatographic experiment depends on the structure and properties of the stationary phase and the molecular characteristics of the solute (molecular volume, temperature, distribution coefficient of each substance, etc.). The experimental retention data of some groups of substances on a specific stationary phase can be correlated with parameters describing the molecular structure [1, 2, 8].

It has been shown in recent articles that molecular connectivity satisfactorily predicts chromatographic parameters like retention times in gas-liquid chromatography [2], and RF in thin layer chromatography (TLC) [1, 2].

In this investigation, the relationship between different RF values in TLC and the connectivity indices of a group of anilines has been studied.

Experimental

Theory
Molecular connectivity indices are calculated from the formula of suppressed hydrogens of the molecule, following the method proposed by Kier and Hall [6] and are defined for a type t subgraph with m ties, represented as \( m^t \). The molecular connectivity indices are obtained as a result of the sum of the terms corresponding to the different subgraphs:

\[
mx_t = \sum_{j=1}^{nm} m^t \delta_j
\]

(1)

where \( nm \) is the number of type t and m order subgraphs and \( m^t \delta_j \) is a quantity calculated for each subgraph from the following expression:

\[
m^t \delta_j = \left[ \prod_{i=1}^{m+1} (\delta_i) \right]^{\frac{1}{2}}
\]

(2)

where \( j \) defines a specific subgraph and \( \delta_i \) is the valence of a vertex, which depends on the type of subgraph, that is, the subgraphs are defined by \( m + 1 \) vertices, \( m \) being the order of the subgraph.

The valence connectivity indices are calculated from the following:

\[
\delta^v = Z^v - N_H
\]

(3)
where \( Z_v \) is the number of valence electrons of the atom and \( N_H \) is the number of hydrogens attached to it [7].

To obtain the relationship between the parameters of thin-layer chromatography and the connectivity indices, a multilineal regression analysis is used, obtaining:

\[
P = A_0 + \sum m \cdot t \cdot A_{m,t}
\]  

(4)

where \( P \) is the property (independent variable), and \( A_0 \) and \( A_{m,t} \) represent the regression coefficients of the obtained equation.

Equation (4) is obtained by a multilineal regression using the 9R program of the BMDP (biomedical computer programs) statistical package [9]. The equations are selected based on statistical parameters like correlation coefficient (\( r \)), standard error of estimate (\( s \)), F-Snedecor and Student's t (statistical significance).

Random and stability tests were carried out for the equations selected.

a) Randomness: the values of the independent variables which intervene in the equation are modified randomly. The value of the property (independent variable) is randomly modified in the same way. After each modification the BMDP 9R is executed. Subsequently, the correlation coefficient obtained after realizing the random modifications is compared with that obtained in the selected equation [10].

b) Stability (cross-validation): is carried out using the jackknife method [11], eliminating \( n \) observations by means of a random process and subsequently executing the regression program, repeating the process as many times as necessary until the observations have been eliminated a minimum of once and a maximum of 4 times (in the cases where the number of eliminations > one). The correlation coefficients, standard deviations and residuals obtained are subsequently compared with those of the selected equation.

**Chemicals and Reagents**

All aniline derivates were from Lancaster Laboratory (Barcelona, Spain), except 4-pentylaniline, 4-octylaniline, 2-naphthylamine from Aldrich Laboratory (Madrid, Spain) and 1-naphthylamine from Riedel-de Haën (Madrid, Spain). Silica gel chromatoplates type RP-8 F-254s 10 x 20 cm from Merck (Darmstadt, Germany). Ethanol, p-dimethylaminobenzaldehyde, glacial acetic acid, acetonitrile, potassium dihydrogen phosphate and disodium hydrogen phosphate from Panreac (Barcelona, Spain).

**Chromatographic Measurements**

The different experimental \( R_F \) values were obtained using inverse-phase, fluorescent silica gel Merck chromatoplates type RP-8 F-254s of 10 x 20 cm, as support. The spotting was 1.5 cm from the beginning of the plate and development was carried out in an area of 21 x 21 x 9 cm, previously saturated with 100 mL mobile phase for 12 h. The development was 8 cm in all cases. All experiments were at 20 °C, using different stationary and mobile phases in each case (Table I). Visualisation was by drying the chromatogram with warm air and subsequently spraying it with a 1 % p-dimethylaminobenzaldehyde in ethanol, with 3 mL glacial acetic acid added for each 100 mL liquid. The amines tested were prepared in 0.1 % (P/V) solutions in 70 % ethanol.

Six chromatograms for each substance and for each of the systems employed were carried out, calculating the mean and standard error of each of the measures obtained (Table II).

**Results and Discussion**

The experimental \( R_F \) values and molecular connectivity indices of the 13 anilines used in this study appear in Table II.

The equations selected for \( R_{FA} \), \( R_{FB} \) and \( R_{FC} \) of the compounds studied are:

\[
R_{FA} = 0.756 - 0.115 \cdot \chi \\
n = 13 \quad r = 0.981 \quad s = 0.027 \quad F = 274.23
\]  

(5)

\[
R_{FB} = 0.660 - 0.102 \cdot \chi \\
n = 13 \quad r = 0.967 \quad s = 0.031 \quad F = 160.69
\]  

(6)

\[
R_{FC} = 0.672 - 0.100 \cdot \chi \\
n = 13 \quad r = 0.942 \quad s = 0.041 \quad F = 85.92
\]  

(7)

### Table I. Stationary and mobile phases in study of experimental \( R_F \) values.

<table>
<thead>
<tr>
<th>TLC</th>
<th>SP</th>
<th>MP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Fluorescent Silica Gel Merk Chromatoplates type RP-8, F-254s</td>
<td>Acetonitrile-Sørensen Solution 1/15 M 50 : 50 (V/V) pH = 6.2</td>
</tr>
<tr>
<td>B</td>
<td>Fluorescent Silica Gel Merk Chromatoplates type RP-8, F-254s</td>
<td>Acetonitrile-Sørensen Solution 1/150 M 50 : 50 (V/V) pH = 6.2</td>
</tr>
<tr>
<td>C</td>
<td>Fluorescent Silica Gel Merk Chromatoplates type RP-8, F-254s</td>
<td>Acetonitrile-Sørensen Solution 1/150 M 50 : 50 (V/V) pH = 7.5</td>
</tr>
</tbody>
</table>

SP: Stationary Phase.  
MP: Mobile Phase.