Modeling of Ibuprofen II: Effect of pH on the Adsorption Behavior on Reversed Phase Liquid Chromatography

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Abstract

The present work involves the study of the influence of pH on the adsorption behavior of ibuprofen on reversed phase liquid chromatography (RPLC). The following four different mobile phases were used for this study at three different pH values: 40% acetonitrile at pH 2.5, 4.38 and 6.72 and 30% acetonitrile at pH 6.5. The adsorption isotherms of ibuprofen are determined by the frontal analysis method and by the inverse method of determining the isotherm. Our results indicate that the adsorption of ibuprofen follows a bi-Langmuir model for the mobile phase containing 40%ACN and at pH 2.5 which is below the pKa value of ibuprofen, while the adsorption pattern follows a bi-Moreau model when mobile phases containing 40% ACN at pH 4.38 (≈ pKa of ibuprofen) and 40% ACN at pH 6.72 and 30%ACN at pH 6.5 (above the pK_a of ibuprofen) were used.

Keywords: adsorption isotherm, ibuprofen, effect of pH, bi-Moreau model, bi-Langmuir model

1. Introduction

Many analytical or preparative separations are now performed by reversed phase liquid chromatography (RPLC). A better understanding of the retention mechanism of the RPLC is required in order to optimize the speed and cost of the separations or the production of the components of interest. To predict the elution times for single-component and multi-component systems, it is required to know the thermodynamics and the kinetics of the phase equilibrium involved in the separation studied. When the solute mass transfer between the stationary and the mobile phases across the column is reasonably fast, elution band profiles are largely controlled by the thermodynamics of phase equilibrium or equilibrium isotherm. For single-component bands, this isotherm is merely the amount of adsorbed solute on the stationary phase or its concentration in the mobile phase. Numerous models of adsorption isotherms are available to fit the adsorption data. The difficulty in using the isotherms is to find a model in which its parameters have a physical meaning. There are many experimental parameters that control the retention and the adsorption isotherms of the compounds on RPLC such as temperature [Kim, 2004, Ahmad, 2007], pressure [Liu, 2003], mobile phase composition [Ahmad, 2006], the concentration and type of the salts added to the mobile phase, the nature of the buffer [Gritti, 2004] and the pH of the mobile phase [Gritti, 2004, Gritti, 2008, Gritti 2009].

The retention behavior of low molecular weight compounds on C_{18} stationary phases using mobile phases containing methanol as modifier has been extensively studied by Guiochon and his co-workers [Gritti 2005, Gritti 2008, Gritti 2009, Gritti 2009]. These studies include acidic compounds like 2-phenylbutyric acid, basic compounds like benzylamine, aniline, amphetamine and neutral compounds like caffeine under different mobile phase compositions and at different pH values. These investigations have shed some light on the adsorption mechanism of analytes. While the previous studies on acidic and basic compounds using methanol as an organic modifier at different pH values have led to very useful conclusions, the literature search reveals that there are no specific studies reported on the effect of pH and mobile phase composition on the behavior of acids and bases on C_{18} stationary phases using acetonitrile as an organic modifier. Therefore, more studies are needed on the retention behavior of ionizable compounds using acetonitrile as an organic modifier.
Ibuprofen [2-(3-isobutylphenyl)propanoic acid] is a non-steroidal anti-inflammatory drug widely used in the treatment of pain and inflammation in rheumatic disease and other musculoskeletal disorders. Millions of pounds of ibuprofen are produced and consumed annually by humans [EPA, 2011]. High performance liquid chromatography (HPLC) is the major technique used for the determination of ibuprofen in pharmaceutical preparations as well as in biological samples [Ghulam 2008]. RP-HPLC is by far the most widely used technique for the determination of ibuprofen and its impurities [USP convention, 2003]. Although most of these RPLC separations are on silica based columns. Zirconia based columns are also used for the determination of ibuprofen, its related compounds and degradation products [Kalafut, 2005; Kalafut, 2009]. Ibuprofen is selected for this study as its pKa of 4.5-4.6 [Higgins] permits the mobile phase pH variation within the working range of the silica based reversed phase C18 column.

This work reported here is part of a series of studies aimed at understanding the retention mechanism of ibuprofen on reversed phase liquid chromatography RPLC using different experimental conditions. The main focus of our previous study (Ahmad 2011) was to find a suitable model for the adsorption behavior of ibuprofen on RPLC using different mobile phase compositions using acetonitrile as an organic modifier at a fixed pH. Two methods are used to determine the isotherm parameters of ibuprofen in this study the frontal analysis method (FA) [Guiochon, Feinger & Shirazi, 2006; Ruthven, 1984, Zong & Guiochon, 1997] and the inverse method of determining the isotherm [Gritti & Guiochon, 2005; Ahmad & Guiochon 2007; Ahmad, 2011].

2.0 Experimental

2.1. Chemicals

Water, acetonitrile, and phosphoric acid (85%) purchased from Fisher Scientific Co. (USA) were all of HPLC grade. The ACS grade acetonitrile after membrane filtration was also used in a limited number of experiments. Ibuprofen and thiourea were from Aldrich/Sigma Chemical Co (USA).

2.2. Chromatographic column

Alltech Altima C18 column 250 mm x 4.6 mm with 10 μm particle sizes was used for this study.

2.3. Software

The software, Origin 7.5 SR6 from Origin Lab Corporation (Northampton, MA, USA) was employed.

2.4. Apparatus

Shimadzu liquid chromatograph, model 20A, equipped with auto sampler (SIL 20A / 20 AC), UV-VIS detector (SPD-20A / SPD-20AV), online degasser (DGU-20 A3 / DGU 20 A5) and system Controller (BM-20 A / 20 A Lite) was used. The extra-column volumes are 0.08 and 0.09 mL as measured from the auto-sampler and from the pump system, respectively, to the column inlet. All the retention data were corrected for these contributions. All measurements were carried out at a constant temperature of 22 °C, fixed by the laboratory air conditioner. The daily variation of the ambient temperature was within +/- 1°C.

2.5.0. Mobile phase preparation

All solutions were membrane filtered before using for the HPLC.

Mobile phases containing aqueous solutions of acetonitrile 30% V/V and 40% V/V were prepared by mixing 300 and 400 mL of acetonitrile respectively with an appropriate amount of hplc water. The pH was adjusted to 2.2 by adding phosphoric acid to the solution.

2.5.2. Phosphate Buffer (pH = 6.00)

Aqueous solutions of 0.05 M each of KH2PO4 and K2HPO4 were prepared. A buffer solution ( pH = 6.00; 0.05 M) was prepared by mixing appropriate amounts of the two phosphate solutions.

2.5.3. Mobile phases containing acetonitrile/ phosphate buffer 30/70 V/V (pH = 6.54) and 40/60 V/V (pH = 6.72 and 4.38)

These mobile phases containing acetonitrile/ phosphate buffer 30/70 V/V (pH = 6.54) and 40/60 V/V (pH = 4.38 and 6.72) were prepared by mixing 300 and 400 mL acetonitrile with 700 and 600 mL of the phosphate buffer (0.05 M), respectively.

Another mobile phase containing 40/60 V/V acetonitrile/ phosphate buffer (pH = 4.38, 0.05 M) was prepared by mixing appropriate amounts of acetonitrile, 0.05M KH2PO4 and 1% H3PO4.
2.5.5 Ibuprofen solutions

Ibuprofen solutions (0.1, 1, 3, 8 or 10 g/L) were prepared daily by dissolving appropriate amounts of ibuprofen respectively in the same mobile phase used for each experiment.

2.6.0 FA for determination of single-component adsorption isotherms:

2.6.1 Choice of the mobile phase composition

Accurate measurements of adsorption isotherm data can be made within a moderate range of the retention factor, $k'$. At low values of $k'$, the difference between retention and hold-up time is small so the isotherm data measured will be inaccurate. At high values of $k'$, the number of data points that can be acquired within a reasonable period of time will be low which can limit the accuracy of the isotherm. Values of $k'$ between 2 and 6 are a good compromise, allowing the measurement of precise and accurate isotherm data. This consideration justifies the choice of the mobile phase composition used in this study (30-40% acetonitrile).

2.6.2 Solubility measurements

Prior to any isotherm measurement, the solubility of ibuprofen in each mobile phase used was measured by the stepwise addition of 0.5 mL of pure mobile phase into 50 mL of a saturated solution containing a small amount of undissolved compound until complete dissolution. Accordingly, the maximum concentrations of ibuprofen used in the FA measurements were 3 g/L and 8 g/L for 30% and 40% ACN /buffer solutions respectively, and 10 g/L for the solutions containing 40% ACN and phosphate buffer solution.

2.6.3 Column conditioning and column hold-up time measurements

Before collecting the adsorption isotherm data, the column was washed with the mobile phase for 45 min to one hour, or until the baseline drift becomes less than 0.5 mau/hr. An injection of thiourea was made just before any frontal analysis run and between successive frontal analysis experiments. The hold-up volume of each daily set of experiments was averaged and used for the calculations made in both the FA and IM methods.

2.6.4 Experimental measurements of breakthrough curves

One of the HPLC pumps was used to deliver a stream of the pure mobile phase while the other pump was used to deliver the ibuprofen sample solution which was prepared in the same mobile phase present in another pump. The total flow rate was kept constant at 1.0 mL/min. Thirty data points were recorded successively for each mobile phase used, with a sufficiently long time (15-30 minutes) gap in between each successive breakthrough curve to allow for the re-equilibration of the column with the pure mobile phase. The sample injection time was 4 min for all the FA measurements. This ensured that, at the end of each FA run, the composition of the eluate was the same as that of the plateau pumped into the column. The detector signals were recorded at 280 nm at all concentrations of ibuprofen. The wavelength was chosen so that the UV signal did not exceed 1200 mau.

2.7 Measurement of the overloaded band profiles

Overloaded band profiles of ibuprofen on Altima-C$_{18}$ were recorded for each mobile phase used, independently from the FA measurements. The injections of ibuprofen solutions were performed with the pump, at three different concentrations, 0.1, 1, 8 and 10 g/L. The band profiles were recorded at 280 nm. The detector response was calibrated from the UV-absorbance value measured on the plateau following each breakthrough curve and the absorbance units (mau) were converted to concentration units (g/L). A third order polynomial was used as a calibration function for all experimental data.

2.8 IM steps

The inverse method was used to derive the best values of the isotherm parameters from overloaded band profiles. To use this method, an isotherm model and initial adsorption parameters are needed. Two to three band profiles were recorded at each mobile phase composition, with injections of ibuprofen at the three different concentrations (see above), for 30 s, shortly after acquisition of the frontal analysis data of ibuprofen. The initial estimates for the adsorption parameters were the values obtained by fitting the frontal analysis data using these isotherm models. These parameters were further optimized to minimize the difference between the experimental and the calculated band profiles. These optimized isotherm parameters were used as initial estimates to calculate the isotherm parameters at different pH and mobile phase composition by the inverse method.
3.0 Results and Discussion

3.1. The effect of pH on the adsorption isotherms of ibuprofen

Figure 1A-H shows the fitting of the adsorption data points of ibuprofen derived from the treatment of each of three series of 30 breakthrough curves recorded during the FA runs on the C18 stationary phase using mobile phases that have different pH values. Two Data sets presented as plots of the isotherm curves (q* versus C, left) and plots of the isotherm cord (q/C versus C, right). The adsorption data of ibuprofen on the C18 stationary phase using a mobile phase of 40% ACN at pH 2.2 were fitted successfully to the bi-Langmuir model (Figure 1A). This behavior is consistent with the results reported earlier [Ahmad et al., 2011] for the same column with a particle size of 5 µm with the same mobile phase mentioned above. Due to the damage of the column that was used in the previous study, a new column with a larger particle size (10 µm) was used in this study. The bi-Moreau model was also used to fit the adsorption data of ibuprofen on C18 using the above mobile phase (40%ACN and pH 2.2) because the same model is used later for the data obtained using other mobile phases at different pHs. The Fisher parameter is approximately the same for both the bi-Langmuir and the bi-Moreau 8467 and 8407 respectively, which made it difficult to decide which model was the best. However, the scatchard plot in Figure 1E has a convex downward shape which confirms that the experimental adsorption data are better modeled by the bi-Langmuir model [Graham, 1953].

\[ q^* = q_{s,1} \cdot \frac{b_1 C}{1 + b_1 C} + q_{s,2} \cdot \frac{b_2 C}{1 + b_2 C} \]  

(1)

This model assumes that the adsorbent surface is heterogeneous and is covered with a quilt of two different surfaces with two saturation capacities, \( q_{s,1} \) and \( q_{s,2} \), corresponding to each one of the two adsorption of sites with two equilibrium constants \( b_1 \) and \( b_2 \).

Additionally, all the shapes of the overloaded band profiles (low and high concentrations) have a Langmuirian shape in which there is a sharp front and a diffused tail without the indication of any anti-Langmuirian behavior. Usually, the compounds with bi-Moreau adsorption isotherms [Moreau, 1991] have band profiles that are Langmuirian at low concentration and anti-Langmuirian at high concentration because of the solute-solute interactions (I >0)

\[ q^* = q_{s,1} \cdot \frac{b_1 C + I_1 b_1^2 C^2}{1 + 2b_1 C + I_1 b_1^2 C^2} + q_{s,2} \cdot \frac{b_2 C + I_2 b_2^2 C^2}{1 + 2b_2 C + I_2 b_2^2 C^2} \]  

(2)

where \( q^* \) and C are the equilibrium concentrations of the compound considered in the adsorbed and the liquid phase respectively, and \( q_{s,1} \), \( q_{s,2} \), \( b_1 \), \( b_2 \), \( I_1 \), and \( I_2 \) are the monolayer saturation capacities, the equilibrium constants, and the adsorbate-adsorbate interaction parameters on the sites of types 1 and 2, respectively.

I, can be written as

\[ I = e^{e_{AA}/RT} \]  

(3)

where \( e_{AA} \) is the interaction energy between two molecules of A adsorbed on close adsorption sites. Note that the bi-Moreau model morphs into the bi-Langmuir model when \( I_1 = I_2 = 0 \), since the equation above is reduced to that of the bi-Langmuir model [Moreau, 1991]. The equilibrium constants \( b_1 \) and \( b_2 \) are associated with the adsorbion energies \( e_{a,1} \) and \( e_{a,2} \), respectively.

Since all the band profiles of ibuprofen using the mobile phase with 40% ACN and at pH 2.2 have Langmuirian shapes, the bi-Langmuir model is the best model to fit the adsorption data of ibuprofen at this pH. The ibuprofen at pH 2.2 is not ionized and therefore it is expected that the solute-solute interactions are negligible. At pH 4.38, the pH is close to pKa, it is expected that the ibuprofen is about 50% ionized and it is expected that there will be some solute-solute interactions. Therefore, we tried to fit the adsorption data using this mobile phase to the bi-Moreau and the s-shaped models. The Fisher parameters are 4842, 6829 and 2814 for the three models bi-Langmuir, bi-Moreau and the S-shaped respectively. This indicates that the bi-Moreau is the best model to fit the adsorption data using this mobile phase. For the other mobile phases, 40% ACN at pH = 6.72 and 30% ACN at pH = 6.5, each of the isotherms has a clear anti-Langmuirian shape and the band profiles have diffused front and sharp rear. The scatchard plots have more than one inflection point for the first mobile phase and it is not very clear for the second mobile phase.
Because of the presence of inflection points in the scatchard plots, the adsorption data fit the bi-Moreau and the S..-shaped models. The Fisher parameter was the highest again for the bi-Moreau model (45000 versus 32000). The values of the solute-solute interactions are large as I, I, are 49.63 and 2.288 for the mobile phase with 40% ACN and pH 6.72, while they are 26.65 and 0.162 for the mobile phase 30% ACN and pH 6.50. For the mobile phase 40%ACN and pH 4.38, the values for I and I, are 2.088 and 0.0267 which are less than when the pH of the mobile phase is higher than the pKa of ibuprofen. This indicates that the solute-solute interactions increase by increasing the pH and the amount of acetonitrile in the mobile phase.

The validities of the bi-Langmuir model for the adsorption data of ibuprofen using the 40% acetonitrile and pH=2.2, and the bi-Moreau model for the mobile phases having 40% acetonitrile at pH = 4.38 or 6.7 and 30% acetonitrile at pH 6.5 are confirmed by the inverse method (IM) results. Figure 2 A-H show an excellent agreement between the experimental and calculated band profiles for ibuprofen using these two models. The parameters of the isotherm calculated by the inverse method are presented in Table 1. It is important to notice from the adsorption isotherms in Figure 1A-D that the amount of ibuprofen adsorbed on the stationary phase is maximum at pH 4.38 (pH ≈ pKa) and minimum at pH 6.72 (pH > pKa). This result can be explained based upon the amount of ibuprofen ionized. At pH 4.38, which is very close to pKa of ibuprofen, the drug is approximately 50% in its ionized form. At pH 6.72 (pH > pKa), most of the drug exists in ionized form so the drug is less retained on the stationary phase at pH 6.72. At pH 2.2 most of the drug is in its neutral form therefore the amount of ibuprofen adsorbed onto the stationary phase at pH 2.2 would be larger than at pH 4.38 and 6.72.

4.0 Conclusions

The adsorption of ibuprofen on Alltech-C18 stationary phase follows the bi-Langmuir adsorption isotherm model at pH 2.2 (below pKa of ibuprofen) and the bi-Moreau adsorption isotherm model for pH 4.38 (close to pKa of ibuprofen), at pH 6.54 and 6.72 (above pKa of ibuprofen). The Fisher parameter, the shapes of the overloaded band profiles and the match between the calculated and experimental band profiles are all evidences that support a bi-Langmuir model for the mobile phase of 40%ACN and pH 2.2 and a bi-Moreau model for mobile phases for the 40%ACN at pH 4.38 and 6.54 or 30%ACN at pH 6.72. The amount of ibuprofen adsorbed onto the stationary phase is maximum at pH 4.38 (near pKa) and minimum at pH 6.72 (above pKa). At pH 4.38, the drug is approximately 50% in its ionized form. At pH 6.72, most of the drug exists in ionized form so that it is less retained on the less polar stationary phase at pH 6.72 (above pKa), due to ionic interactions, as compared to pH 2.2 (below pKa) where most of the drug is in its neutral form. At the pH 6.54 and 6.72 (well above the pka), as most of the drug is in its ionized form, ionic interactions are present among the ions adsorbed onto the stationary phase. At these two pH values 6.54 and 6.72, the active adsorption sites have a large equilibrium constant value as they are saturated quickly and their saturation capacities are less. The less active adsorption sites have more saturation capacities with lower equilibrium constant values.

5.0 Acknowledgments

The authors would like to acknowledge the assistance of Gilles Kouassi in reviewing this manuscript. We also thank Professor Rose McConnell for providing acetonitrile.

6.0 References


Table 1: The best fit of the isotherm parameters to different models using the FA and the IM for ibuprofen using mobile phases at different pH.

<table>
<thead>
<tr>
<th>Mobile Phase</th>
<th>Model</th>
<th>$q_{s1}$</th>
<th>$b_1$</th>
<th>$q_{s2}$</th>
<th>$b_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>40% ACN pH 2.2</td>
<td>bi-Langmuir FA</td>
<td>551.6</td>
<td>0.0223</td>
<td>6.19</td>
<td>1.2808</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>484.52</td>
<td>0.0287</td>
<td>5.01</td>
<td>1.3117</td>
</tr>
<tr>
<td>40% ACN pH 4.38</td>
<td>bi-Moreau FA</td>
<td>461.2</td>
<td>0.0205</td>
<td>2.0875</td>
<td>28.84</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>486.8</td>
<td>0.0197</td>
<td>3.1832</td>
<td>25.13</td>
</tr>
<tr>
<td>40% ACN pH 6.72</td>
<td>bi-Moreau FA</td>
<td>168.9</td>
<td>0.01681</td>
<td>49.6345</td>
<td>20.33</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>207.3</td>
<td>0.01342</td>
<td>44.8752</td>
<td>18.61</td>
</tr>
<tr>
<td>30% ACN pH 6.50</td>
<td>bi-Moreau FA</td>
<td>152.8</td>
<td>0.042</td>
<td>26.65</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>163.4</td>
<td>0.039</td>
<td>22.93</td>
<td>1.54</td>
</tr>
</tbody>
</table>
Fig. 1. Right (A-D) the adsorption data of ibuprofen (circles) derived from single component frontal analysis from the mobile phases containing aqueous solutions that has (A) 40% ACN, pH 2.2, fitted to a bi-Langmuir model (lines) (B) 40% ACN, pH 4.38 (C) 40% ACN, pH 6.72 and (D) 30% ACN, pH 6.5 fitted to the bi-Moreau isotherm model (lines). Left (E-H), the corresponding scatchard plots. Column: C₁₈ Alltech Altima 250 mm, 4.6 mm and 5 µm. Flow rate 1.0 mL/min, wavelength 280 nm, and Temperature = 296 K.
Fig. 2. Experimental (dotted) and calculated (solid line) overloaded band profiles of ibuprofen on C_{18} Alltech Column (250mm, 4.6mm, Particle size-10µ); flow rate 1.0 mL/min; UV-VIS detector, wavelength 280 nm and T=298K.; (A) 0.1 g/L and 1(B)1.0 g/L, mobile phase 40:60 (v/v) ACN/ aqueous H_{3}PO_{4}buffer with final mobile phase pH= 2.2. (C) 1.0 g/L and (D) 8.0 g/L, mobile phase in both A and B is 40:60 (v/v) ACN/ KH_{2}PO_{4}/H_{3}PO_{4}buffer with final mobile phase pH= 4.38;; (E) 1.0 g/L and (F) 8.0 g/L, 40:60 (v/v) ACN/KH_{2}PO_{4}/KH_{2}HPO_{4}buffer with final mobile phase pH= 6.72; and (G) 1.0 g/L and (H) 2.0 g/L, 30:70 (v/v) ACN/KH_{2}PO_{4}/KH_{2}HPO_{4}buffer with final mobile phase pH= 6.54.