Automated Potentiometric Titration Method for Determination of pKa Values: An Application to Hydroxy Chloroquine Sulphate

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ABSTRACT

The acid-base behavior of hydroxy chloroquine sulphate (HCQS) was studied by using a new technique GLpKa in different ethanol-water [up to 50 % (v/v) ethanol] and methanol-water [up to 50 % (v/v) methanol] mixtures at different temperatures. The ionic strength was maintained using potassium chloride. The primary goal of this work was to study the effect of different factors like dielectric constant, temperature, and ionic strength on the dissociation constant of HCQS in detail as this drug is widely used in every day medical practice and clinical investigations. This technique was found to be convenient, accurate and easily applicable. The pKa values obtained were in good agreement with literature values within experimental error.

Key words: Hydroxy Chloroquine Sulphate, Potentiometric Titration, pKa values

1.0 INTRODUCTION:

Hydroxy chloroquine (HQC) (Figure 1) is a potent antimalarial drug [1,2] also being used frequently as an ant rheumatic substance [3,4]. Determination of this compound includes extraction followed by nonaqueous titrimetric [5] or spectrophotometric methods based on reactions with iodide [6], quinones [7,8] and cobalt thiocyanate [9]. Because of the nonspecificity of most of these reactions, prior extraction of HQC is commonly involved in the assay methods. Besides, a number of different HPLC methods have been proposed for the determination of HCQS in biological fluids using spectrophotometric [10] or fluorescence [11, 12] detection. Current USP methods [13] utilizes a simple sample preparation analyzed using an isocratic HPLC system with UV detection. Few electrochemical methods have been used for determination of aminooquinolines. Among them can be mentioned the potentiometric determination of the chloroquine (CQ) in various pharmaceutical preparations using an ion selective membrane electrode [14]. However no reports are available on automated titration methods of Hydroxy chloroquine sulphate. Therefore it is decided to study on the automated titration method for the determination of pKa values of Hydroxy chloroquine sulphate (HCQS).

Due to their wide range of pharmacological activity in synthetic and industrial applications, the determination of pKa values of these compounds have recently received a great deal of attention for the discovery of improved protocol towards milder and high yielding approaches. Knowledge of the pKa values of a substance plays an important role in the pharmaceutical industry for drug design and understanding the mechanism of action, in the new chemical manufacturing industry (environmental impact compliance), and in the environmental field (environmental fates of toxic substances). These values are constructive in determining the extent to which a drug is absorbed by the body’s organs. The determination of the pKa values, in aqueous media, is difficult and problematic for many new compounds which are very poorly soluble in water. Therefore it was decided to determine the pKa values of HCQS (Antimalarial drug) in different mixed solvent (aqua-organic) systems. In these mixed solvent systems, some solvent properties like dielectric constant, solvent polarity, etc. can be changed and have certain advantages over aqueous as well as organic solvent systems. The effect of different factors like dielectric constant, ionic strength, and temperature were also studied. [15].

An Application to Hydroxy Chloroquine Sulphate

1.0 INTRODUCTION:

Hydroxy chloroquine (HQC) (Figure 1) is a potent antimalarial drug [1,2] also being used frequently as an ant rheumatic substance [3,4]. Determination of this compound includes extraction followed by nonaqueous titrimetric [5] or spectrophotometric methods based on reactions with iodide [6], quinones [7,8] and cobalt thiocyanate [9]. Because of the nonspecificity of most of these reactions, prior extraction of HQC is commonly involved in the assay methods. Besides, a number of different HPLC methods have been proposed for the determination of HCQS in biological fluids using spectrophotometric [10] or fluorescence [11, 12] detection. Current USP methods [13] utilizes a simple sample preparation analyzed using an isocratic HPLC system with UV detection. Few electrochemical methods have been used for determination of aminooquinolines. Among them can be mentioned the potentiometric determination of the chloroquine (CQ) in various pharmaceutical preparations using an ion selective membrane electrode [14]. However no reports are available on automated titration methods of Hydroxy chloroquine sulphate. Therefore it is decided to study on the automated titration method for the determination of pKa values of Hydroxy chloroquine sulphate (HCQS).

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2.0 MATERIALS AND METHODS:

2.0.1 Reagents and Solutions.

Of the chemicals used for pKa determination, methanol, ethanol, and acetonitrile were of HPLC grade from Merck. Solutions and solvent mixtures were made up of distilled water obtained from Millipore, Milli-Q (Bedford, MA, USA) purification system. Readymade 0.5 M of potassium hydroxide and hydrochloric acid reagents were obtained from Merck. Potassium hydroxide is standardized against primary standard using potassium hydrogen phthalate. Potassium hydrogen phthalate is purchased from Sigma. Di-potassium hydrogen phosphate and potassium chloride were of analytical grade from Sigma. Hydroxy chloroquine sulphate (HCQS) was obtained from the IPCA Laboratory Ltd, (Mumbai) and is used as received.

A number of methods are available for processing the pH metric data to evaluate the proton ligand stability constants. Some of the widely used methods of computing stability constants from pH titration data are Calvin-Bjerrum’s method and its modified version that are Calvin and Wilson method, Martell and Chaberek method and Irving and Rossoitti method.

2.0.2 Irving and Rossoitti method:

The Irving and Rossoitti method of pH titration is most important and widely used method for determination of stability constant. This method is modification of Calvin-Bjerrum’s technique and offers simpler, generally applicable treatment for the calculation of ñ and pH directly from pH meter readings.

In order to determine stability constants by this method, one must perform three pH metric titrations against carbonate free standard base. The experimental part of these titrations is given below;

Titrating A: Nitric acid.

Titrating A+R: Nitric acid + ligand.

2.0.3 Evaluation of Proton-Ligand stability constant (ñA):

The proton ligand formation number (ñA) is also given by the formula.

\[ \hat{n}_A = \frac{\text{Total concentration of proton bound to ligand}}{\text{total concentration ligand}} \]  

Where;

\[ V_T = \text{initial volume of test solution} \]

\[ iT = \text{initial concentration of nitric acid} \]

\[ V_A = \text{the volumes of alkali required for free acid (A)} \]

\[ V_A+V_L = \text{the volumes of alkali required for free acid (A) and acid + ligand (A+R) titration respectively at a given pH} \]

\[ \gamma = \text{number of replaceable hydrogen ions from the ligand} \]

From the horizontal distance of the titration curves (pH vs. volume of standard alkali added), the value of proton ligand formation number (ñA) can be evaluated by using following expression;

\[ \hat{n}_A = ? \frac{(V_T-V_A)-(N+?)\gamma}{(V_A+V_L)T} \]
In order to get accurate pK values method of point wise calculation was used. The values of pH at ñA= 0.5 and 1.5 corresponds to pK, where carried out in at least in three different wt% at constant ionic strength (solvents independently. At each selected co-solvent mixture, measurements were titrated with 0.5 M KOH to an appropriately high pH, usually 12 in the aqueous solutions, was pre acidified to pH 1.8–2.0 with 0.5 M HCl and then. About 2 mg of selected drug compound, containing 6-10 mL of 0.15 M semi-HPLC for the separation of ionizable compounds [18]. HCQS can be de protonated at higher pH due to the presence of a hydroxyl group (-OH). These have pKa values in the range 8.2 to 9.5. Table 1 and Table 2. represents the values of pKa which are observed and calculated from typical pH metric titrations, which are very much correlates with the literature values [19,20].

**Results and Discussion:**

pKa values are useful physiochemical parameters describing the extent of ionization of functional groups with respect to pH. These are a function of solvent composition and are also useful in the application of reversed-phase HPLC for the separation of ionizable compounds [18]. HCQS can be de protonated at higher pH due to the presence of a hydroxyl group (-OH). These have pKa values in the range 8.2 to 9.5. Table 1 and Table 2. represents the values of pKa which are observed and calculated from typical pH metric titrations, which are very much correlates with the literature values [19,20].

**Table 1** Data for obtaining formation curve and proton – ligand stability constants of HCQS

<table>
<thead>
<tr>
<th>Medium: water</th>
<th>µ = 0.1M (KNO3)</th>
<th>T = 298 °K</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>V1</td>
<td>V2</td>
</tr>
<tr>
<td>7.20</td>
<td>5.04</td>
<td>5.07</td>
</tr>
<tr>
<td>7.60</td>
<td>5.06</td>
<td>5.10</td>
</tr>
<tr>
<td>8.00</td>
<td>5.07</td>
<td>5.16</td>
</tr>
<tr>
<td>8.40</td>
<td>5.08</td>
<td>5.23</td>
</tr>
<tr>
<td>8.80</td>
<td>5.09</td>
<td>5.30</td>
</tr>
</tbody>
</table>

Mean 8.276

**Table 2** Data for obtaining formation curve and proton – ligand stability constants of HCQS

<table>
<thead>
<tr>
<th>Medium: water</th>
<th>µ = 0.1M (KNO3)</th>
<th>T = 298 °K</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>V1</td>
<td>V2</td>
</tr>
<tr>
<td>9.20</td>
<td>5.11</td>
<td>5.49</td>
</tr>
<tr>
<td>9.60</td>
<td>5.17</td>
<td>5.62</td>
</tr>
<tr>
<td>9.80</td>
<td>5.24</td>
<td>5.72</td>
</tr>
<tr>
<td>10.00</td>
<td>5.29</td>
<td>5.79</td>
</tr>
<tr>
<td>10.20</td>
<td>5.34</td>
<td>5.85</td>
</tr>
</tbody>
</table>

Mean 9.376

**Table 3:** pKa values by Yasuda-Shedlovsky extrapolation in various co-solvents.

<table>
<thead>
<tr>
<th>Co solvent</th>
<th>% Dielectric</th>
<th>1000/e</th>
<th>pKa + log (H&lt;sub&gt;2&lt;/sub&gt;O)</th>
<th>pKa</th>
<th>0% pKa</th>
<th>Slope</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol 35.55</td>
<td>64.9</td>
<td>15.4</td>
<td>10.55</td>
<td>9.01</td>
<td>9.07</td>
<td>0.0350</td>
<td>0.9833</td>
</tr>
<tr>
<td>Ethanol 31.37</td>
<td>60.3</td>
<td>16.6</td>
<td>10.42</td>
<td>8.93</td>
<td>8.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetonitrile 48.55</td>
<td>56.2</td>
<td>18.2</td>
<td>10.00</td>
<td>8.41</td>
<td>8.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>49.32</td>
<td>49.7</td>
<td>20.1</td>
<td>9.68</td>
<td>8.29</td>
<td>8.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>54.47</td>
<td>53.4</td>
<td>18.7</td>
<td>10.62</td>
<td>9.28</td>
<td>9.28</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 4** Effect of temperature and ionic strength

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>µ = 1.5 M HCQS</th>
<th>Ionic strength</th>
<th>µ (M)</th>
<th>HCQS</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>8.932</td>
<td>0.15</td>
<td>8.932</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>8.75</td>
<td>0.3</td>
<td>8.82</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>8.57</td>
<td>0.5</td>
<td>8.701</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2 represents the Yasuda-Shedlovsky trend charts for HCQS drug compound selected in this study. It can be seen that basic functional groups have negative slopes and produce straight lines in the total interval. The linearity of the plots is characterized by the regression coefficients values which indicate significant linear correlation for the molecules examined. The average of the R² values is 0.9941.

**Fig 2:** Trend charts for HCQS pKa in various co solvents

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**2.0.4 Half integral method:**

The pK<sub>a</sub> and pK<sub>3</sub> values are obtained by plotting ñA<sub>1</sub> vs pH (formation curves). The value of pH at ñA= 0.5 and 1.5 corresponds to pK<sub>a</sub> and pK<sub>3</sub>, respectively. These are tentative pK values of the ligands. In order to get accurate pK values method of point wise calculation was used.

**2.0.5 Method of Point wise calculations:**

In order to get accurate pK<sub>a</sub> and pK<sub>3</sub>, the following expressions are used.

\[ \text{pK}_1 = - \log \frac{ñA}{1 - ñA} \]  

This expression was solved, when the values of ñA are in between 1.8 and 1.2 for pK.<br>

\[ \text{pK}_1 = - \log \frac{ñA}{1 - ñA} \]  

For pK<sub>a</sub>, when values of ñA are in the range 0.8 - 0.2

**2.0.6 Sirius GLpKa Potentiometric Method.**

The Sirius GLpKa instrument is an excellent example of engineering science useful for chemical analysis. It is an easy, rapid, and convenient method for determination of pKa. It is equipped with a automatic titrator which is useful for accurate pH determination. This method is universal and takes (30 to 60) min/titration (i.e., 10 to 30 samples/day) which is far better than what we could perform by manual titration. It requires an extremely small concentration of substance (10 mm), whereas traditional methods require very large concentrations of substance.

**2.0.7 Potentiometric pKa determination:**

GLpKa automated pKa analyzer (Sirius Analytical Instruments Ltd., Forest Row, UK) fitted with combination Ag/AgCl pH electrode was used for determination of dissociation constants. The pKa and psKa values were calculated by RefinementPro<sup>TM</sup> software (Sirius Analytical Instruments Ltd., Forest Row, UK).

**2.0.8 Preparation of co-solvent mixtures:**

For pKa determination of selected drugs, a 80% (v/v) methanol, 60% (v/v) ethanol and 50% (v/v) of acetonitrile in 0.15 M KCl adjusted water is prepared and used throughout our investigation.

**2.0.9 Titration in co-solvent–water mixtures:**

About 2 mg of selected drug compound, containing 6-10 mL of 0.15 M semi-aqueous solutions, was pre acidified to pH 1.8–2.0 with 0.5 M HCl and then titrated with 0.5 M KOH to an appropriately high pH, usually 12 in the presence of co-solvents containing 6 and 60 wt% of methanol, ethanol and acetonitrile. The selected compound was measured in all the three selected co-solvents independently. At each selected co-solvent mixture, measurements were carried out in at least in three different wt% at constant ionic strength (I = 0.15M using KCl) and temperature (25±0.5 °C) under argon atmosphere. The effect of ionic strength (I = 0.15 M, 0.3 M and 0.5 M using KCl) at constant temperature (25±0.5 °C) and the effect of temperature (25°, 35° and 45 °C) at constant ionic strength (I = 0.15 M KCl) using methanol as co solvent were also carried out under argon atmosphere. The apparent ionization constants in the mixed solvent (psKa) were calculated from the difference (Bjerrum) plot. To obtain the best aqueous pKa value from psKa data the Yasuda-Shedlovsky (YS) procedure was applied to estimate the aqueous pKa value. The following equation has been adopted.

Where log [H<sub>2</sub>O] is the molar water concentration of the given solvent mixture, ‘c’ is the dielectric constant of the mixture and ‘a’ and ‘b’ are the slope and intercept, respectively. This method is the most widely used procedure in co-solvent techniques [16, 17].

**3.0 RESULTS AND DISCUSSION:**

pKa values are useful physiochemical parameters describing the extent of ionization of functional groups with respect to pH. These are a function of solvent composition and are also useful in the application of reversed-phase HPLC for the separation of ionizable compounds [18]. HCQS can be de protonated at higher pH due to the presence of a hydroxyl group (-OH). These have pKa values in the range 8.2 to 9.5. Table 1 and Table 2. represents the values of pKa which are observed and calculated from typical pH metric titrations, which are very much correlates with the literature values [19,20].
3.0.1 Determination of pKa in Methanol-Water mixtures
Methanol is widely accepted as a co-solvent, and its effect on pKa has been investigated extensively [19]. The pKa values of HCQS were determined in three different methanol-water proportions between 31 and 51% at constant ionic strength (0.15M) and temperature (25°C). The pKa values obtained at constant ionic strength and constant temperature are summarized in Table 3. With an increase in the percentage of methanol (decrease in dielectric constant), the pKa values of HCQS tend to decrease as these are weak bases which is confirmed by the negative slope of -103. A representative distribution of species, difference curve and titration curve for HCQS in 40% methanol-water system at 0.15 M (KCl) as ionic strength and T = 25 °C is shown in Figure 3. The linearity of the plots is characterized by the regression coefficients values which indicate significant linear correlation for the molecules examined.

3.0.2 Determination of pKa in Ethanol-Water mixtures
The pKa values of HCQS were determined in three different ethanol-water proportions between 31 and 49% at constant ionic strength (0.15M) and temperature (25°C). The pKa values obtained at constant ionic strength and constant temperature are summarized in Table 3. With an increase in the percentage of ethanol (decrease in dielectric constant), the pKa values of HCQS tend to decrease as these are weak bases which is confirmed by negative slope of -0.17.

3.0.3 Determination of pKa in Acetonitrile-Water mixtures
The pKa values of HCQS were determined in three different acetonitrile-water proportions between 39 and 54% at constant ionic strength (0.15M) and temperature (25°C). The pKa values obtained at constant ionic strength and constant temperature are summarized in Table 3. Contrary to findings in methanol and ethanol media, the pKa values shown increasing trend with increasing acetonitrile (decreasing in dielectric constant) which is confirmed by the positive slope of 0.05. This can be due to the fact that, a number of changes are observed by an addition of solvent to aqueous systems. Main effect of this is decrease in activity coefficient of water, change in dielectric constant, the gradual break down of the structure of water and solvation of protons at a high concentration of solvent. Earlier studies show that the stability constant is linearly related to mole fraction of the solvent and varies with solvent. The addition of organic solvent to water leads to a gradual break down of the tetrahedral lattice and formation of a hydrogen bond. The stability of the hydroxonium ion increases with decreases in proton donating ability [20].

Irving and Rossotti [21] observed that the stability constant of compounds containing a O-H bond increases, and a N-H bond decreases with an increase in the content of solvent. An increase in the content of organic solvent in an aqueous system leads to the interaction between protons and negatively charged oxygen atoms to a greater extent than the ion-dipole interaction between protons and the solvent. An increase in the solvent content leads to an increase in the ion-dipole forces between protons and nitrogen atoms in the ligand to a lesser extent than that of more electronegative donor oxygen atoms of the solvent [22].

3.0.4 Effect of Temperature and Ionic strength on pKa Values
The effect of temperature and ionic strength on HCQS was examined and the pKa values showed a decrease with an increase in temperature. Figure 4 represents the trend charts on the effect of temperature and ionic strength. The pH measurements were made in 30% methanol-water systems. The results are presented in Table 4. The pKa values of HCQS decreased with increasing temperature and ionic strength.
and for the evaluation of biopharmaceutical properties. These are also used in
the determination of the degree at which these drugs are absorbed by body
organs. A decrease in dielectric constant, decrease in pKa values of HCQS was
observed with a exception of acetonitrile as co solvent. With increasing
temperature and ionic strength, a decrease in pKa values was observed for the
selected compound in the present study. The data obtained will be utilized in
the development of different chromatographic methods by means of an ap-
propriate selection of buffer and solvent system.

5.0 ACKNOWLEDGMENT
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