The response of ion-selective electrodes that are sensitive to the protonated species of sparingly soluble organic bases is examined theoretically at various pH values. It is shown that for such bases the potential versus pH plots are unsuitable for the accurate determination of the pKₐ. A new method is proposed that can be used to measure the aqueous ionization constants of organic compounds with low solubilities in water. The method was applied to the organic basic drugs chlorpromazine and amitriptyline. The results obtained were in close agreement with previously reported values.

Keywords: Ion-selective electrodes; ionisation constants; organic bases; chlorpromazine; amitriptyline

The value of the ionisation constant of a substance that behaves as a weak acid or base in aqueous solution is of considerable importance as at certain pH values, it indicates the degree of dissociation of the substance into its ionic species. Usually, the different ionic species formed have different physicochemical and biological properties. For drug substances, the percentage of the ionised and non-ionised species in solution determines the solubility of the drug in the gastrointestinal fluids, the rate of absorption, the extent of binding and explains the mode of action and the kinetics of their degradation.

The methods most commonly used for the determination of ionisation constants are based on potentiometric titrations and spectrophotometric measurements.¹⁴ In potentiometric titration methods a solution of the acid (or protonated base) is titrated with a solution of a strong base. The pH that corresponds to the volume equal to 0.5V (where V is the volume at the equivalence point) is an estimate of the pKₐ. Refined calculation techniques, which are based on the potentiometric titration principle, have also been described.¹

However, for many basic compounds, including important drugs, the uncharged species are frequently sparingly soluble in water and are precipitated during potentiometric titration at pH values lower than the pKₐ, value, thus prohibiting an accurate determination of the pKₐ. To circumvent this problem, it has been suggested that the ionisation constant be determined in water - ethanol mixtures of various proportions and by plotting the pKₐ versus the percentage of ethanol. The graph can then be extrapolated to give the pKₐ at 0% ethanol.¹ This approach has been criticised by many workers¹⁴ because the presence of the organic solvent decreases the dielectric constant of the mixture and causes an overestimation of pKₐ.

Ion-selective electrodes (ISE) that respond selectively to the protonated species of organic bases and are unaffected by the presence of the non-ionised molecule have been constructed.⁵⁷¹²⁰ With these electrodes, electrode potential (E) versus pH graphs have been plotted and used for the determination of the pKₐ values of various water-soluble organic bases such as creatinine,² atropine³ and meperidine.² However, it can be shown, theoretically (see below), that when a base is sparingly soluble in water and precipitates at a pH < pKₐ, the E versus pH graphs are unsuitable for an accurate determination of the pKₐ.

In this paper we have treated theoretically the response of an ISE that is sensitive to the protonated species of a sparingly soluble base at various pH values and have proposed a method for the determination of the pKₐ value. The validity of the proposed method was tested for the organic basic drugs chlorpromazine and amitriptyline. The results of the pKₐ determinations were found to be in close agreement with previously reported values.

Theory

The ionisation constant of an acid - base equilibrium

\[ BH^+ \rightleftharpoons B + H^+ \]  (1)

is given by

\[ K_a = \frac{[B][H^+]}{[BH^+]} \]  (2)

where \( K_a \) is the ionisation constant of the acid BH⁺, [B] and [BH⁺] are the concentrations of the non-ionised base and acid, respectively, and [H⁺] is the hydrogen ion concentration.

The potential, \( E \), of an electrochemical cell, which consists of an ISE sensitive to the BH⁺ cation and a reference electrode, is given by the Nernst equation:

\[ E = E^\circ + \frac{S}{2.303} \log([BH^+] \div [B]) \]  (3)

where \( E^\circ \) is a constant term if the measurements are carried out in dilute solutions (i.e., at concentrations <10⁻³ M) or at constant ionic strength and \( S \) is the electrode slope.

Consider an aqueous solution containing only the protonated base BH⁺, of concentration \( C \). If the pH is increased by adding small volumes of a concentrated base solution, so as to obtain a solution containing both BH⁺ and B, the following possibilities exist:

(a) The non-ionised base B is sufficiently soluble so that it remains in solution until the pH becomes equal to the pKₐ. In other words, if \( S \) is the solubility of B, then \( [B]<S \) when \( \text{pH}<pK_a \).

Conservation of the weak acid in solution requires that at any time

\[ [B] = C - [BH^+] \]  (4)

Substitution from equation (4) into equation (2) and rearranging yields

\[ [BH^+] = \frac{C[H^+]}{K_a + [H^+] \times C} \]  (5)

Substitution from equation (5) into equation (3) yields

\[ E = E^\circ + S \log \left( \frac{[H^+]}{K_a + [H^+] \times C} \right) \]  (6)

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When the value of E is plotted as a function of pH at four different values of C, the graphs shown in Fig. 1 are obtained. At hydrogen ion concentrations much greater than \( K_a \) ([H\(^+\)] \gg [H\(^+\)]) equation (6) becomes \( E = E^0 + S' \log C \), and E is independent of pH. When [H\(^+\)] = \( K_a \) (or pH = \( pK_a \)) equation (6) becomes \( E = E^0 + S' \log (C/2) \), i.e., the \( pK_a \) of the acid is equal to the pH where the initial concentration C of the acid is halved, owing to the gradual loss of the proton as the pH increases. In this instance, if the E versus pH graph is constructed experimentally, the \( pK_a \) of the acid H\(^+\) can be calculated and is equal to the pH value where the potential of the respective electrode decreases by \( S' \log 2 \) mV (Fig. 1).

(b) The non-ionised base, B, is sparingly soluble in water and precipitates before the p\( K_a \) is reached. In this instance, equation (6) still applies at any pH value, until the solution is saturated with B. At higher pH values the excess of B precipitates and pK\(_a\) of the B ion is constant and equal to its solubility S. Under such conditions and after rearrangement equation (2) becomes

\[
[BH^+] = (S[H^+])/K_a
\]  

This graphical technique for determining the \( pK_a \) of B is illustrated in Fig. 1.

Substituting from equation (7) into equation (3) yields

\[
E = E^0 + S' \log \left( \frac{(S[H^+])}{K_a} \right)
\]

and, therefore

\[
E = E^0 + S' \log S + S'pK_a - pH
\]

When pH = \( pK_a \)

\[
E = E^0 + S' \log S
\]

It is shown that when pH = \( pK_a \), and the solution is saturated with B, the value of E is constant and independent of the initial concentration C. This situation is different from case (a) where if pH = \( pK_a \) the value of E is a function of C/(2C). Hence the graphical technique for \( pK_a \) determination shown in Fig. 1 is not applicable for case (b).

For case (b), the graph of E as a function of pH is shown in Fig. 2. At low pH values where no precipitation of B occurs, the graph is the same as in (a) and equation (6) is valid. After the saturation point (indicated by the arrow) the graph of E versus pH is linear with a slope of -S' and is described by equation (9).

The calibration graph (E versus log C) of the electrode at constant pH is linear with a slope of S' (equation (6)) until the point of saturation with B. At higher concentrations, the potential E is independent of C and remains constant (equation (9)).

Fig. 3 presents a group of calibration graphs that would be expected for an electrode responding to BH\(^+\). Each graph is constructed at constant pH. Before the saturation point (indicated by the arrow) the graph is linear. After saturation, the potential remains constant. The breaks appear earlier if the calibration graphs are constructed at higher pH values.

At the saturation point, conservation of the weak acid in solution requires that

\[
S + [BH^+] = C
\]

Substituting the [BH\(^+\)] from equation (7) into equation (11) and rearranging yields

\[
[H^+] = (K_a/S)C - K_a
\]  

A graph of [H\(^+\)] versus C at the saturation point gives a straight line with a slope of \( K_a/S \) and an intercept of \(-K_a\).
Experimental

Apparatus

The electrodes, the reaction cell and the recording system were as previously reported for chlorpromazine and amitriptyline. All measurements were carried out at room temperature (24 ± 1 °C).

Reagents

All solutions were prepared in de-ionised, distilled water from analytical-reagent grade materials. Stock solutions (0.1000 mm) of the hydrochloride salts of chlorpromazine (CPM.HCl) and amitriptyline (AMT.HCl) were prepared in water from the highest quality salts available, and were gifts from pharmaceutical companies. The solutions were stored in amber bottles at 4 °C.

Procedure

Calibration graphs at different pH values were obtained as follows. A 20.00-mI volume of a 4 × 10⁻⁵ m CPM.HCl or 6 × 10⁻³ m AMT.HCl solution (lower limits of linear response of the electrodes) were pipetted into a 50-mI beaker. The ISE, the external reference electrode and the combination glass electrode were immersed in the solution and stirring was started. The pH of the solution was adjusted to the desired value by adding small volumes of 6 m NaOH solution. After the potential of the ISE was stabilised, the pH was recorded with an accuracy of at least 0.01 pH unit. Various small increments of a 0.1 m solution of the cation of interest (CPM.HCl or AMT.HCl) were then added and the electrode potential was recorded after stabilisation (± 0.1 mV) in each instance. The experiment was terminated when the total concentration of CPM.HCl or AMT.HCl was 6 × 10⁻³ m (upper limit of linear response of the two electrodes). The pH was monitored continuously during the experiment and it was adjusted, if necessary, to the initial value (± 0.01 pH unit) by adding small aliquots of 6 m NaOH solution.

A graph of E versus log C was constructed. The point of saturation was found graphically as the intersection of the two linear parts of the calibration graph.

The experiments were performed at pH values (pKₐ - 3) < pH < pKₐ because in this region the calibration graph consists of two linear parts, which describe the electrode response before and after the saturation point. The graph of [H⁺] versus C at each saturation point yields a straight line with a slope of Kₛ/S and an intercept of −Kₛ.

Results and Discussion

A buffer was not included in the experiments because it was observed that the presence of a buffer such as phosphate increased the response time of the electrodes. The pH was kept constant by adding small volumes of a concentrated base solution as necessary.

Calibration graphs at various pH values for the chlorpromazine and amitriptyline electrodes are shown in Fig. 4. The point of saturation of the solution with the non-ionised base B was found graphically as shown in Fig. 4. The graphs of [H⁺] versus the concentration (C) at the saturation point are shown in Fig. 5. From this graph the Kₛ of the protonated base can be found directly from the y-intercept (which is −Kₛ). The solubility S of the base can be calculated because it is equal to the x-intercept of the graph. However, owing to the extrapolation of the calibration graph to the point where it intersects the y-axis, relatively small experimental errors in the points plotted can lead to great uncertainty in the estimation of the y-intercept. For example, the y-intercept of the graphs in Fig. 6 were −2.01 × 10⁻¹⁰ ± 1.68 × 10⁻⁹ (standard deviation) for chlorpromazine and −1.83 × 10⁻¹⁰ ± 7.88 × 10⁻¹⁰ for amitriptyline. Such uncertainties in the determination of Kₛ are unsatisfactory. A far better calculation of the Kₛ of the protonated base is achieved by the use of the slope of the
graphs in Fig. 6 and known values of the solubilities of the bases in water. Solubility data are readily available from the literature and can be calculated with greater accuracy using simple spectrophotometric techniques.\textsuperscript{8}

When the solubility of chlorpromazine was taken as $8 \times 10^{-6}$ M at 24°C and an ionic strength ($\mu$) of ca. 0.01 M the calculated $\text{pK}_a$ value was 9.38 ± 0.08 (mean of three experiments, $\mu \leq 6 \times 10^{-3}$ M). When the solubility of amitriptyline was taken as $3.5 \times 10^{-5}$ M at 24°C the calculated $\text{pK}_a$ value was 9.46 ± 0.05 (three experiments, $\mu \leq 6 \times 10^{-3}$ M). $\text{pK}_a$ values of 9.3 and 9.4 have previously been reported for chlorpromazine and amitriptyline, respectively.\textsuperscript{8}

Recently, the $\text{pK}_a$ of chlorpromazine has been determined to be 7.24 by use of a chlorpromazine cation-selective electrode.\textsuperscript{10} This large negative error in $\text{pK}_a$ value can be explained by the theoretical treatment described above. Chlorpromazine precipitates at pH values lower than the $\text{pK}_a$ and, consequently, the $\text{pK}_a$ cannot be calculated by $E$ versus pH graphs.

In conclusion, the proposed method is theoretically sound, rapid, simple and reliable. It extends the capabilities of ISEs and offers a means of measuring aqueous ionisation constants of organic compounds that have low solubilities in water.

References