Ion-selective Electrodes for the Determination of Ionisation Constants of Sparingly Soluble Organic Bases in Aqueous Solutions: Applications to Chlorpromazine and Amitriptyline

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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 pK_a. A new method is proposed that can be used to measure the aqueous ionisation 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.[1–4] 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 pK_a. 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 pK_a value, thus prohibiting an accurate determination of the pK_a. 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 pK_a *versus* the percentage of ethanol. The graph can then be extrapolated to give the pK_a at 0% ethanol.[1] This approach has been criticised by many workers[1,4], because the presence of the organic solvent decreases the dielectric constant of the mixture and causes an overestimation of pK_a.

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.[5–7,10] With these electrodes, electrode potential (E) *versus* pH graphs have been plotted and used for the determination of the pK_a values of various water-soluble organic bases such as creatinine,[5] atropine[6] and meperidine.[7] However, it can be shown, theoretically (see below), that when a base is sparingly soluble in water and precipitates at a pH < pK_a, the E *versus* pH graphs are unsuitable for an accurate determination of the pK_a.

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 pK_a value. The validity of the proposed method was tested for the organic basic drugs chlorpromazine and amitriptyline. The results of the pK_a determinations were found to be in close agreement with previously reported values.

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Theory

The ionisation constant of an acid - base equilibrium

$$BH^+ \rightleftharpoons B + H^+ \quad \ldots \quad (1)$$

is given by

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

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:

$$E = E^0 + S'\log[BH^+] \quad \ldots \quad (3)$$

where E^0 is a constant term if the measurements are carried out in dilute solutions (*i.e.*, at concentrations $<10^{-2}$ 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 strong 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 pK_a. In other words, if S is the solubility of B, then $[B]<S$ when pH≤pK_a.

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

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

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

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

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

$$E = E^0 + S'\log\left(\frac{[H^+]}{K_a + [H^+]}.C\right) \quad \ldots \quad (6)$$

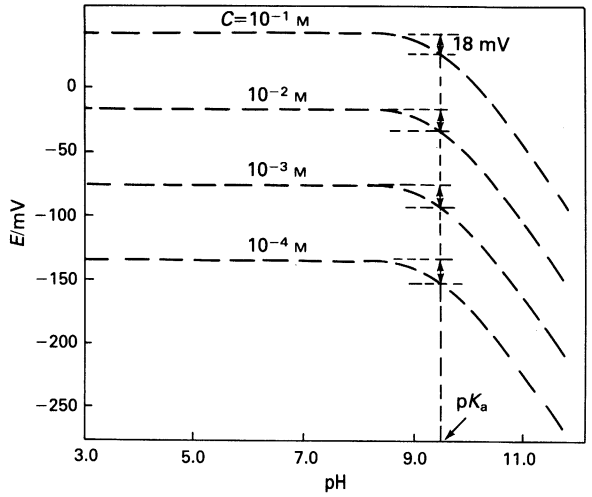

Fig. 1. Simulated graphs of the electrode potential (E) of a BH^+ cation ISE *vs.* pH at concentrations (C) from 10^{-1} to 10^{-4} M. The graphs are described by equation (6). It is assumed that the non-ionised base B is water-soluble, $S' = 59$ mV, $E^0 = 100$ mV and $pK_a = 9.50$. The pK_a of BH^+ is calculated graphically as shown

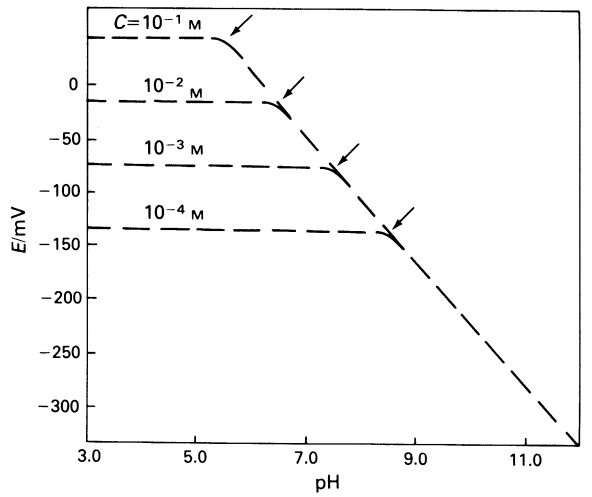

Fig. 2. Simulated plots of the electrode potential (E) of a BH^+ cation ISE *vs.* pH, when the non-ionised base B precipitates before the pK_a is reached. At each concentration, the saturation point is indicated by the arrow. The graphs before and after saturation are described by equations (6) and (9), respectively. It is assumed that the solubility (S) of B is 1.00×10^{-5} M. Other parameters as in Fig. 1

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 ($[H^+] >> K_a$) 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 BH^+ 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 pK_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 the concentration of B in solution is constant and equal to its solubility S. Under such conditions and after rearrangement equation (2) becomes

$$[BH^+] = (S[H^+])/K_a \quad .. \quad .. \quad .. \quad (7)$$

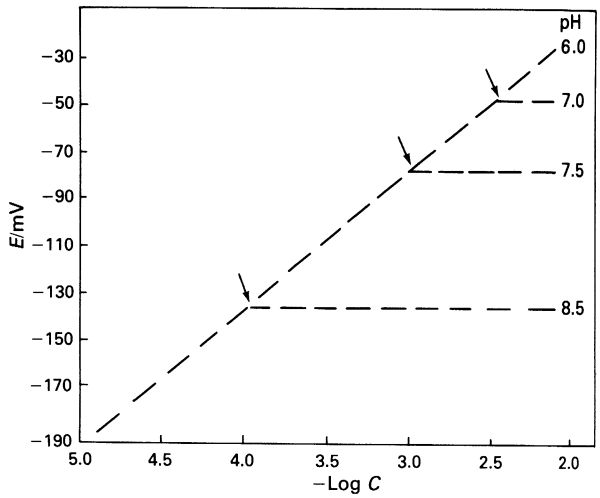

Fig. 3. Simulated calibration graphs of a BH^+ cation ISE when the non-ionised base B precipitates before the pK_a is reached. Each graph was constructed at the constant pH indicated. The saturation point is indicated by the arrows. The graphs before the saturation point are described by equation (6). After saturation, the potential remains constant [equation (9)]. Other parameters as in Fig. 2

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

$$E = E^0 + S' \log \{(S[H^+])/K_a\} \quad .. \quad .. \quad (8)$$

and, therefore

$$E = E^0 + S'(\log S + pK_a - pH)$$
$$= E^0 + S' \log S + S' pK_a - S' pH \quad .. \quad .. \quad (9)$$

When pH = pK_a

$$E = E^0 + S' \log S \quad .. \quad .. \quad (10)$$

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 $C/2$. 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

$$S + [BH^+] = C \quad .. \quad .. \quad .. \quad .. \quad (11)$$

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

$$[H^+] = (K_a/S)C - K_a \quad .. \quad .. \quad .. \quad (12)$$

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 $-K_a$.

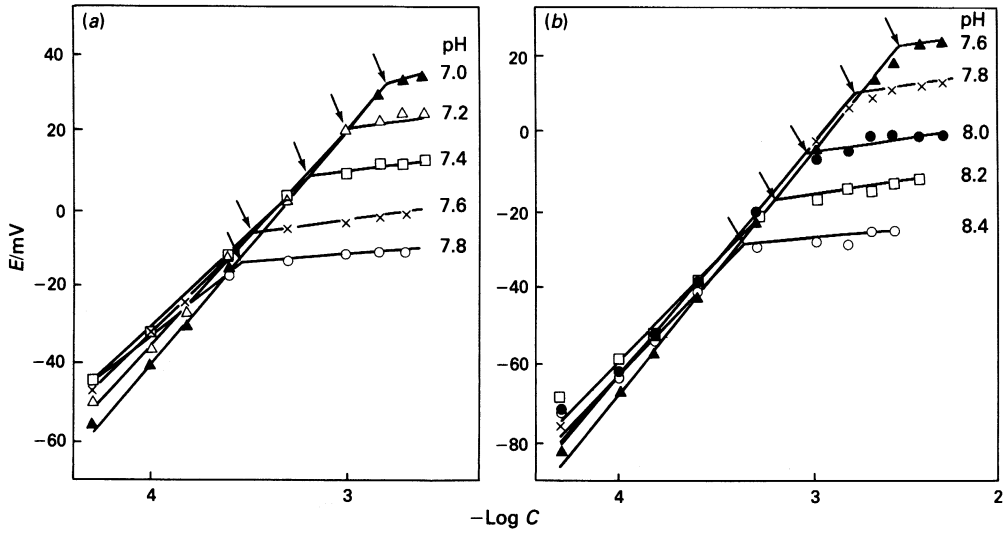

Fig. 4. Experimental calibration graphs of (*a*) the chlorpromazine and (*b*) the amitriptyline cation ISE. Each graph was constructed at the constant pH indicated. The saturation point (indicated by the arrow) is found graphically from the intersection of the two linear parts of each graph

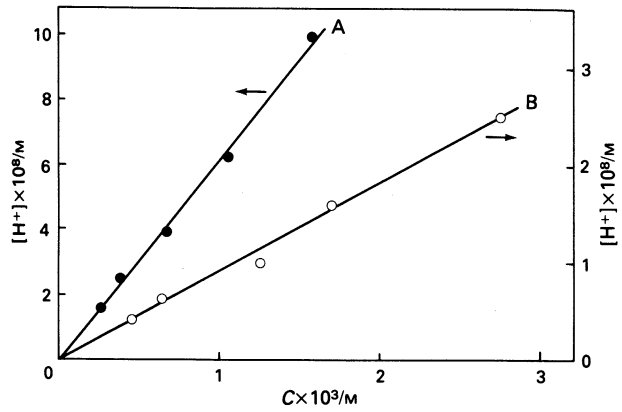

Fig. 5. Graphs of [H+] *vs.* concentration (*C*) at the saturation point for (A) chlorpromazine and (B) amitriptyline. The slope of each graph is K_a/S and the intercept is $-K_a$ [equation (12)].

Experimental

Apparatus

The electrodes, the reaction cell and the recording system were as previously reported for chlorpromazine and amitriptyline.[7] 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 M) 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-ml volume of a 4×10^{-5} M CPM.HCl or 6×10^{-5} M AMT.HCl solution (lower limits of linear response of the electrodes[7]) were pipetted into a 50-ml 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).[7] The pH was monitored continuously during the experiment and it was adjusted, if necessary, to the initial value (to ± 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 $(pK_a - 3) < pH < pK_a$ 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 K_a/S and an intercept of $-K_a$.

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 K_a of the protonated base can be found directly from the *y*-intercept (which is $-K_a$). 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 $-2.01 \times 10^{-10} \pm 1.68 \times 10^{-9}$ (standard deviation) for chlorpromazine and $-1.83 \times 10^{-10} \pm 7.88 \times 10^{-10}$ for amitriptyline. Such uncertainties in the determination of K_a are unsatisfactory. A far better calculation of the K_a 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.[8]

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

Recently, the pK_a of chlorpromazine has been determined to be 7.24 by use of a chlorpromazine cation-selective electrode.[10] This large negative error in pK_a value can be explained by the theoretical treatment described above. Chlorpromazine precipitates at pH values lower than the pK_a and, consequently, the 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.

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