

CHAPTER 3

CHARGE STATE

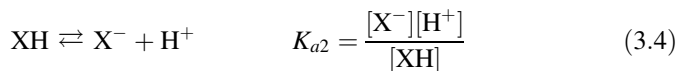
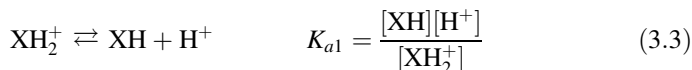
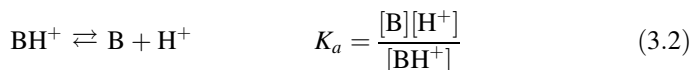
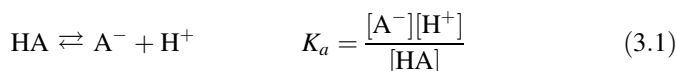
Weak acids and bases ionize in solutions to varying extent, depending on pH. This, in turn, affects the distribution of the chemicals in solution and affects their availability to enter biological reactions. The characteristic thermodynamic parameter relating the pH to the charge state of a molecule is the ionization constant, pK_a [34,35]. Knowledge of the pK_a of a substance is widely useful. It can predict the absorption, distribution, and excretion of medicinal substances. For example, urine pH (normally 5.7–5.8) can be altered (with oral doses of NH_4Cl or $NaHCO_3$) to satisfy reabsorption of uncharged species for therapeutic reasons, or to ease excretion of ionized species in toxicological emergencies [111]. Weak acids may be excreted in alkaline urine and weak bases may be eliminated in acidic urine, a principle that may be lifesaving with overdoses of barbiturates, amphetamines, and narcotics, for example. Knowledge of the pK_a of a substance can be used in maximizing chemical reaction or synthesis yields. For example, solvent extraction can be best applied in a pH region where the synthesized molecule is uncharged. Interpretations of kinetic measurements can depend on the pK_a of a reactant.

The method of choice for the measurement of ionization constants is potentiometry [35,112–119]. Special circumstances warrant the determination of the pK_a by UV spectrophotometry [120–143], capillary electrophoresis (CE) [144–147], and a chromatographic technique [148]. In principle, UV and CE methods are more sensitive and less sample-demanding than is the pH-metric method. That notwithstanding, the latter method is preferred because it is so much better developed,

and is very strongly supported commercially [Sirius]. Currently, the UV method is under vigorous development, and is also supported commercially [131–143]. The CE method is in the orphan stage, with apparently little interest shown by the manufacturers of CE equipment, although that may soon change. A small and enthusiastic user base exists, however. Many other techniques have been used, but the methods described above are best suited for pharmaceutical applications.

3.1 CONSTANT IONIC MEDIUM REFERENCE STATE

The ionization reactions for acids, bases, and ampholytes (diprotic) may be represented by the generic forms



Listed after the reactions are the corresponding equilibrium quotients. The law of mass action sets the concentration relations of the reactants and products in a reversible chemical reaction. The negative log (logarithm, base 10) of the quotients in Eqs. (3.1)–(3.4) yields the familiar Henderson–Hasselbalch equations, where “p” represents the operator “–log:”

$$\text{p}K_a = \text{pH} + \log \frac{[\text{HA}]}{[\text{A}^-]} \quad (3.5)$$

$$\text{p}K_a = \text{pH} + \log \frac{[\text{BH}^+]}{[\text{B}]} \quad (3.6)$$

$$\text{p}K_{a1} = \text{pH} + \log \frac{[\text{XH}_2^+]}{[\text{XH}]} \quad (3.7)$$

$$\text{p}K_{a2} = \text{pH} + \log \frac{[\text{XH}]}{[\text{X}^-]} \quad (3.8)$$

Equations (3.5)–(3.8) indicate that when the concentration of the free acid, HA (or conjugate acid, BH⁺), equals that of the conjugate base, A[−] (or free base, B), the pH has the special designation, pK_a. If the pH is two units lower than the pK_a for an acid, Eq. (3.5), [HA]/[A[−]] = 100, and the uncharged species accounts for 100/101

(99%) of the total substance in solution. If the pH is two units higher than the pK_a , then it is the anion that accounts for 99% of the total.

Ibuprofen (HA) has a pK_a 4.45 ± 0.04 [149] determined at 25°C and ionic strength I 0.15 M (fixed by KCl). Chlorpromazine (B) has a pK_a 9.24 ± 0.01 at 25°C, I 0.15 M (NaCl) [150]. Morphine (XH) has pK_{a1} 8.17 ± 0.01 and pK_{a2} 9.26 ± 0.01 at 25°C, I 0.15 M (NaCl) [151].

All equilibrium constants in the present discussion are based on the *concentration* (not activity) scale. This is a perfectly fine thermodynamic scale, provided the ionic strength of the solvent medium is kept fixed at a “reference” level (and therefore sufficiently higher than the concentration of the species assayed). This is known as the “constant ionic medium” thermodynamic state. Most of the results reported these days are determined in 0.15 M KCl or NaCl, the physiological level, because of standardization in the available commercial instruments. If the ionic strength is changed, the ionization constant may be affected. For example, at ionic strength of 0.001 M, morphine pK_a values were determined to be 8.13 ± 0.01 and 9.46 ± 0.01 [151]. The change in the second constant illustrates the need to report the ionic strength (and the temperature, since constants are also temperature-dependent) [34,35].

The ionic strength dependence of ionization constants can be predicted by the Debye–Hückel theory [34,35]. In the older literature, values were reported most often at “zero sample and ionic strength” and were called the “thermodynamic” constants. The constants reported at 0.15 M ionic medium are no less thermodynamic. Nevertheless, a result determined at 0.15 M KCl background, can be corrected to another background salt concentration, provided the ionic strength is within the limitations of the theory (<0.3 M for the Davies [152] variant of the Debye–Hückel expression). It is sometimes convenient to convert constants to “zero ionic strength” to compare values to those reported in older literature. A general ionic strength correction equation is described in the literature [112,118,153].

3.2 pK_a DATABASES

The “blue book” compilations [154–158] are probably the most comprehensive sources of ionization constants collected from the literature (up to the end of 1970s). These are recommended for experts in the field. On the other hand, the “red books” contain critically selected values [159]. The six-volume set has been put into electronic form in cooperation with NIST (National Institute of Standards and Technology), and is available at a very reasonable price [160]. A two-volume set of critically determined constants is available from Sirius Analytical Instruments Ltd., and covers molecules of particular interest to the pharmaceutical community [161,162]. In Section 3.8 at the end of this chapter, a list of “gold standard” pK_a values of mostly drug-like molecules is presented (see Table 3.1), with many of the values determined by the author since the early 1970s.

3.3 POTENTIOMETRIC MEASUREMENTS

In pH-metric titration, precisely known volumes of a standardized strong acid (e.g., HCl) or base (e.g., KOH or NaOH) are added to a vigorously-stirred solution of a protogenic substance, during which pH is continuously measured with a precision combination glass electrode, in a procedure confined to the interval pH 1.5–12.5. The substance (50–500 μM or higher) being assayed is dissolved in 2–20 mL of water or in a mixed solvent consisting of water plus an organic water-miscible cosolvent [e.g., methanol, dimethylsulfoxide (DMSO), acetonitrile, or 1,4-dioxane]. An inert water-soluble salt (0.15 M KCl or NaCl) is added to the solution to improve the measurement precision, and to mimic the physiological state. Usually, the reaction vessel is thermostated at 25°C and a blanket of a heavy inert gas (argon, but *not* helium) bathes the solution surface.

The plot of pH against titrant volume added is called a *potentiometric titration curve*. Figure 3.1a shows two examples. The shape of such a curve can suggest the amount of substance present and its characteristic acid–base ionization properties. The left curve in Fig. 3.1a represents a strong acid–base titration, containing no sample species. The curve on the right side of Fig. 3.1a is that of morphine-6-glucuronide (M6G), which has three $\text{p}K_a$ values ($\text{XH}_3^+ \rightleftharpoons \text{XH}_2^\pm \rightleftharpoons \text{XH}^- \rightleftharpoons \text{X}^{2-}$) [151]. The inflection points corresponding to where the slope in such plots is maximum in size are called endpoints (pH 7 in the left curve, pH 5.5 and 10 in the right curve). At the endpoint the sample is almost completely in one state of ionization (e.g., XH_2^\pm zwitterion at pH 5.5). The inflection points where the slope is at a minimum size designate regions of maximum buffering (pH 8.8 in the morphine metabolite curve). At such a point the molecule is present in two states of protonation of equal concentration ($\text{pH} = \text{p}K_a$), *unless two or more overlapping* $\text{p}K_a$ values are in the buffer region. So by inspection of Fig. 3.1a, one can say that a $\text{p}K_a$ of M6G may be ~ 8.8 . (We will see in the next section that such a simple interpretation of the titration curve can lead to the wrong conclusion, because M6G has two overlapping $\text{p}K_a$ values centered about pH 8.8.) Where are the other $\text{p}K_a$ values of M6G? Unfortunately, a titration curve does not always reveal all the $\text{p}K_a$ values that a molecule may have. To reveal the other two $\text{p}K_a$ values of M6G and to test for overlapping $\text{p}K_a$ values, it is necessary to transform the titration curves into Bjerrum plots [112,116,118,153,163–165].

3.3.1 Bjerrum Plots

The Bjerrum plots are probably the most important graphical tools in the initial stages of titration data analysis. Since one knows how much strong acid and strong base have been added to the solution at any point and also how many dissociable protons the sample substance brings to the solution, one knows the *total* hydrogen ion concentration in solution, despite what equilibrium reactions are taking place. By measuring the pH (and after converting it into $\text{p}_c\text{H} = -\log[\text{H}^+]$), one knows the *free* hydrogen ion concentration $[\text{H}^+]$. The difference between the total and the free concentrations is equal to the concentration of the *bound* hydrogen ions. The latter

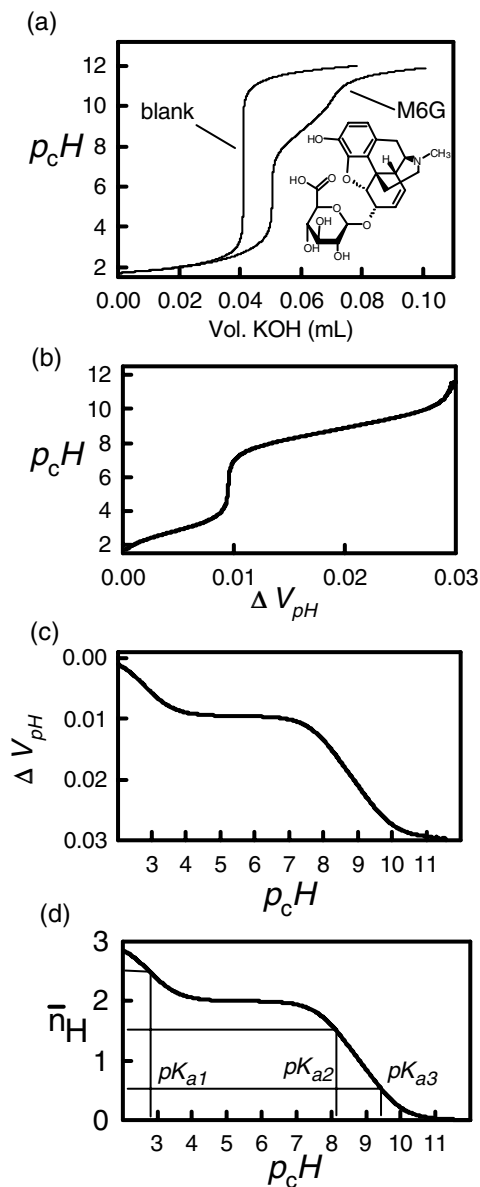


Figure 3.1 Four-step construction of the Bjerrum difference plot for a three- pK_a molecule, whose constants are obscured in the simple titration curve (see text): (a) titration curves; (b) isohydric volume differences; (c) rotated difference plot; (d) Bjerrum plot. [Avdeef, A., *Curr. Topics Med. Chem.*, **1**, 277–351 (2001). Reproduced with permission from Bentham Science Publishers, Ltd.]

concentration divided by that of the sample gives the average number of bound hydrogen atoms per molecule of substance \bar{n}_H . The Bjerrum curve is a plot of \bar{n}_H versus p_cH .

Operationally, such a plot can be obtained by subtracting a titration curve containing no sample (“blank” titration; left curve in Fig. 3.1a) from a titration curve with sample (right curve in Fig. 3.1a) at fixed values of pH. The resultant difference plot is shown in Fig. 3.1b. The plot is then rotated (Fig. 3.1d), to emphasize that \bar{n}_H is the dependent variable and pH is the independent variable [163]. The volume differences can be converted to proton counts as described in the preceding paragraph, to obtain the final form, shown in Fig. 3.1d.

The Bjerrum plot in Fig. 3.1d reveals all the pK_a terms as p_cH values at half-integral \bar{n}_H positions. The three pK_a values of M6G are evident: 2.8, 8.2, and 9.4. In contrast to this, deducing the constants by simple inspection of the titration curves is not possible (Fig. 3.1a): (1) the low pK_a is obscured in Fig. 3.1a by the buffering action of water and (2) the apparent pK_a at pH 8.8 is misleading. M6G has two overlapping pK_a terms, whose *average* value is 8.8. M6G nicely illustrates the value of Bjerrum analysis. With Bjerrum analysis, overlapping pK_a s pose no difficulty. Figure 3.2a shows an example of a 6- pK_a molecule, vancomycin [162,166]. Figure 3.2b shows an example of a 30- pK_a molecule, metallothionein, a small heavy-metal-binding protein, rich in sulfhydryl groups [167]. (The reader is challenged to identify the six ionization sites of vancomycin.)

3.3.2 pH Definitions and Electrode Standardization

To establish the *operational* pH scale [168–170], the pH electrode can be calibrated with a single aqueous pH 7 phosphate buffer, with the ideal Nernst slope assumed. Because the \bar{n}_H calculation requires the “free” hydrogen ion concentration (as described in the preceding section) and because the concentration scale is employed for the ionization constants, an additional electrode standardization step is necessary. That is where the operational scale is converted to the *concentration* scale p_cH ($= -\log [H^+]$) using the four-parameter equation [116,119,171,172]

$$pH = \alpha + k_s p_cH + j_H [H^+] + \frac{j_{OH} K_w}{[H^+]} \quad (3.9)$$

where K_w is the ionization constant of water [173]. The four parameters are empirically estimated by a weighted nonlinear least-squares procedure using data from alkalimetric titrations of known concentrations of HCl (from pH 1.7 to 12.3) or standard buffers [116,174–180]. Typical aqueous values of the adjustable parameters at 25°C and 0.15 M ionic strength are $\alpha = 0.08 \pm 0.01$, $k_s = 1.001 \pm 0.001$, $j_H = 1.0 \pm 0.2$, and $j_{OH} = -0.6 \pm 0.2$. Such a scheme extends the range of accurate pH measurements and allows pK_a values to be assessed as low as 0.6 (caffeine [161]) and as high as 13.0 (debrisoquine [162]).

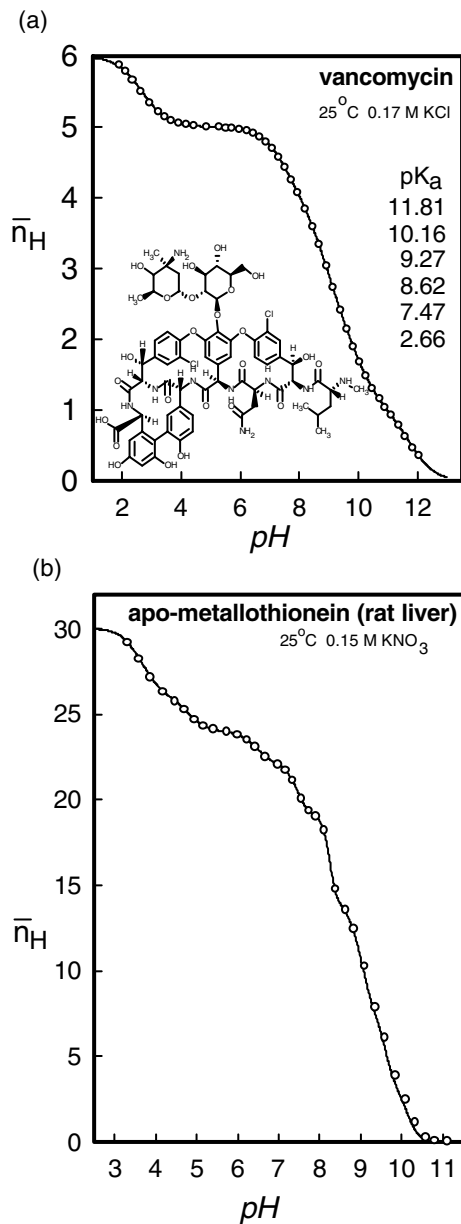


Figure 3.2 Example of (a) 6- pK_a molecule Bjerrum plot (vancomycin [166]) and (b) 30- pK_a molecule plot (apometallothionein [167]). [Avdeef, A., *Curr. Topics Med. Chem.*, **1**, 277–351 (2001). Reproduced with permission from Bentham Science Publishers, Ltd.]

3.3.3 The “Solubility Problem” and Cosolvent Methods

Since many new substances of interest are very poorly soluble in water, the assessment of the pK_a in aqueous solution can be difficult and problematic. Potentiometry can be a quick technique for such assessment, provided the solubility of the substance is at least 100 μM . (Solutions as dilute as 10 μM can still be analyzed, but special attention must be given to electrode calibration, and ambient carbon dioxide must be excluded.) If the substance is soluble to only 1–10 μM and possesses a pH-sensitive UV chromophore, then spectrophotometry can be applied. CE methods may also be useful since very small sample quantities are required, and detection methods are generally quite sensitive.

If the compound is virtually insoluble ($<1 \mu\text{M}$), then a pH-metric mixed-solvent approach can be tried [112]. For example, the pK_a of the antiarrhythmic amiodarone, 9.06 ± 0.14 , was estimated from water–methanol mixtures, though the intrinsic solubility of the molecule is $\sim 0.008 \mu\text{M}$ (6 ng/mL) [$p\text{ION}$].

The most frequently explored solvent systems are based on water–alcohol mixtures [119,164,166,181–210]. DMSO–water [211–215], dioxane–water [216–220], and other systems [221,222] have been explored. Where possible, methanol is the solvent of choice, because its general effect on pK_a values has been studied so extensively. It is thought to be the least error-prone of the common solvents.

Mixed-solvent solutions of various cosolvent–water proportions are titrated and $p_s K_a$ (the apparent pK_a) is measured in each mixture. The aqueous pK_a is deduced by extrapolation of the $p_s K_a$ values to zero cosolvent. This technique was first used by Mizutani in 1925 [181–183]. Many examples may be cited of pK_a estimated by extrapolation in mixtures of methanol [119,161,162,191,192,196,200], ethanol [184,188–190,193], propanol [209], DMSO [212,215], dimethylformamide [222], acetone [221], and dioxane [216]. Plots of $p_s K_a$ versus weight percent organic solvent, $R_w = 0 - 60 \text{ wt}\%$, at times show either a “hockey-stick” or a “bow” shape [119]. For $R_w > 60 \text{ wt}\%$, S-shaped curves are sometimes observed. (Generally, $p_s K_a$ values from titrations with $R_w > 60 \text{ wt}\%$ are not suitable for extrapolation to zero cosolvent because KCl and other ion pairing interferes significantly in the reduced dielectric medium [223].)

For values of $R_w < 60 \text{ wt}\%$, the nonlinearity in $p_s K_a$ plots can be ascribed partly to electrostatic long-range ion–ion interactions. Extensions of the Born electrostatic model, drawing on Bjerrum’s theory of ion association [223], were introduced by Yasuda [194] and Shedlovsky [201]. It was recognized that equilibrium quotients in mixed solvents of varying proportions ought explicitly to incorporate the concentration of water, since constancy in water activity cannot be expected in cosolvent mixtures. It was thus proposed that the plot of $p_s K_a + \log [\text{H}_2\text{O}]$ versus $1/\epsilon$ should produce a straight line for solutions with dielectric constant $\epsilon, > 50$, which for methanol at 25°C means $R_w < 60 \text{ wt}\%$. The slope in such a plot is expected to be inversely proportional to the average ionic diameter of the solvated molecule [201]. The Yasuda–Shedlovsky procedure is now widely used to assess pK_a values of very sparingly soluble pharmaceutical compounds [119,150,166,172, 224,225].

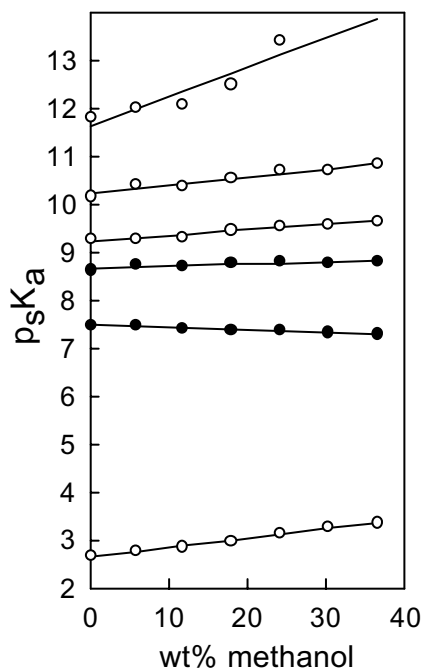


Figure 3.3 The six apparent ionization constants of vancomycin plotted as a function of weight % methanol. Unfilled circles denote acid groups, and filled circles denote basic groups. Acids usually are indicated by positive slopes and bases, by negative slopes. [Avdeef, A., *Curr. Topics Med. Chem.*, **1**, 277–351 (2001). Reproduced with permission from Bentham Science Publishers, Ltd.]

3.3.4 Use of Cosolvents for Water-Soluble Molecules

As the dielectric constant of the solvent mixture decreases, the pK_a of an acid increases and the pK_a of a base decreases. In a multiprotic molecule, this can be a useful property in identifying the ionization groups. Figure 3.3 shows how the pK_a values of vancomycin are affected by changing dielectric constant [62,166]. The p_sK_a/R_w curves with positive slopes were assigned to the carboxylic group and the phenolic residues (structure in Fig. 3.2a), and the two remaining curves, one with a distinct negative slope, were assigned to bases (primary amine on the vancosamine moiety and the secondary amine on the right side of the molecule pictured in Fig. 3.2a). The nonlinear appearance of the highest pK_a in Fig. 3.3 is notably improved in a Yasuda–Shedlovsky plot [162].

It is conceivable that the lowest descending pK_a and the lowest ascending pK_a may cross as R_w approaches 100% [162]. It is interesting that the dielectric constant for pure methanol is about 32, the same value associated with the surface of phospholipid bilayers (in the region of the phosphate groups). This point is further explored later.

3.4 SPECTROPHOTOMETRIC MEASUREMENTS

The most effective spectrophotometric procedures for pK_a determination are based on the processing of whole absorption curves over a broad range of wavelengths, with data collected over a suitable range of pH. Most of the approaches are based on mass balance equations incorporating absorbance data (of solutions adjusted to various pH values) as dependent variables and equilibrium constants as parameters, refined by nonlinear least-squares refinement, using Gauss–Newton, Marquardt, or Simplex procedures [120–126,226].

For an ionizable molecule, the refinement model can be posed as

$$A_{ik} = \sum_j^{\text{species}} c_{ij} \epsilon_{jk} \quad (3.10)$$

where A_{ik} is the calculated absorbance at the k wavelength in the i spectrum. Different values of i denote spectra collected at different pH levels. The molar absorptivity of the j species at the k wavelength is denoted by ϵ_{jk} , and the concentration of the j species at the i pH is c_{ij} . “Species” here refers to the different charge-state forms of a molecule. The values of c_{ij} are functions of the total sample concentration and the ionization constants; these are calculated as in procedures for the pH-metric refinement of constants [118]. One can estimate pK_a values, intelligently guess the values of ϵ_{jk} , and use these to calculate values of A_{ik} . In the calculation, the objective is to minimize the sum of the residuals between the calculated and observed absorbances,

$$S = \sum_k^{\text{species}} \sum_i^{\text{spectra(pH)}} \frac{(A_{ik}^{\text{obs}} - A_{ik}^{\text{calc}})^2}{\sigma_{ik}^2} \quad (3.11)$$

where σ_{ik} are the estimated uncertainties in the measured values of absorbances. Mathematically imposed constraints prevent the calculation of negative values of absorbances [227]. The “best” set of refined pK_a constants are those that minimize S .

In complicated equilibria, uninformed guessing of pK_a values and ϵ_{jk} can be unsettling. Elegant mathematical methods have evolved to help this process of supervised calculation. Since not all species in a multiprotic compound possess detectible UV chromophores or sometimes more than one species have nearly identical molar absorptivity curves, methods had to be devised to assess the number of spectrally active components [121]. With ill-conditioned equations, damping procedures are required [122]. Gampp et al. [127] considered principal-component analysis (PCA) and evolving factor analysis (EFA) methods in deciding the presence and stoichiometries of the absorbing species.

Tam and others [131–135,137,138,140–143,228,229] developed a very effective generalized method for the determination of ionization constants and molar absorptivity curves of individual species, using diode-array UV spectrophotometry,

coupled to an automated pH titrator. Species selection was effected by target factor analysis (TFA), and EFA methods were used. Multiprotic compounds with overlapping pK_a values were investigated. Binary mixtures of ionizable compounds were considered [141]. Assessment of microconstants has been reported [138,140]. The use of cosolvents allowed the deconvolutions of 12 microconstants of cetirizine, a 3- pK_a molecule [142]. Validation studies, comparing the TFA method to the first derivative technique, were reported [132,137].

A 96-well microtiter plate high-throughput method, called *spectral gradient analysis* (SGA), based on a pH gradient flow technique with diode-array UV detection has been reported [135,136,139]. A universal buffer, consisting of citric acid, phosphate, tris(hydroxymethyl)-aminomethane, and *n*-butylamine, was developed in an acidified and an alkaline form [139]. Mixture of the two forms in a flowing stream produced a pH gradient very linear in time. The SGA method was successfully validated using 110 structurally unrelated compounds [135]. Poorly soluble molecules still pose a challenge to the SGA method, although this problem is being vigorously addressed by the manufacturer.

Apparently similar flowstream universal buffers have been developed by Alibrandi and others [128,129] for assessing kinetic parameters, such as the pH-dependent hydrolysis of acetylsalicylic acid. The pH–time curves are not as linear as in the SGA system. Other reports of continuous flow pH gradient spectrophotometric data have been described, with application to rank-deficient resolution of solution species, where the number of components detected by rank analysis is lower than the real number of components of the system [130]. The linear pH–time gradient was established in the flowstream containing 25 mM H_3PO_4 by the continuous addition of 100 mM Na_3PO_4 .

At *p*ION's analytical services laboratory, the pK_a of a molecule (whose structure may not be known beforehand) is first measured by the TFA method, because very little sample is consumed. (Sometimes there is not much more than 1 mg of sample with which to work.) Only when the analysis of the data proves problematic do we repeat the measurement, the second time using potentiometry, where more sample is required. If any indication of precipitation is evident, either DMSO or methanol is added to the titrated solution and the titration is repeated 3 times (using the same sample), with additional water added between the repeats, to obtain different R_w values of the mixed solvent solutions. It has been our experience that if the TFA method fails and more sample is available, the follow-up pH-metric method always works.

3.5 CAPILLARY ELECTROPHORESIS MEASUREMENTS

CE determination of pK_a is new, compared to the other techniques [144–147]. It has the advantage of being a rather universal method since different detection systems can be coupled to CE. Because it is a separation technique, sample impurities seldom are a problem. A fused-silica capillary, with an inner diameter of 50–75 μm and 27–70 cm in length is filled with a dilute aqueous buffer solution (ionic strength

0.01–0.05 M) [144]. About 10 nL of a sample solution, whose concentration is ~50 μM, is gathered at one end of the capillary, and a 20–30-kV potential is applied between the ends of the capillary dipped into each of two beakers. Sample consumption is roughly 0.2 ng per injection. Sample species migrate according to their charge and fluid drag. Apparent electrophoretic mobility is determined, which is related to the migration time, the length of the capillary, and the applied voltage. The mobility of ionizable compounds is dependent on the fraction of the compound in the charged form. This, in turn, depends on the p*K*_a. The plot of the apparent mobility versus pH has a sigmoidal shape, with the midpoint pH equal to the p*K*_a. The practical range for buffer pH in CE is 2–3 at the low end and 11–12 at the high end. When UV detection is used, the limit of detection for a molecule having the molar absorptivity of benzoic acid at 220 nm is ~2 μM [144]. Ishihama et al. [145] were able to determine the p*K*_a of multiprotic molecules by CE, one molecule having seven ionization groups. They reported a 10 μM limit of detection for verapamil. Its reported p*K*_a, 8.89, compares well to that determined by potentiometry, 9.07 [*p*ION].

Ishihama et al. [147] have describe a rapid screening method for determining p*K*_a values of pharmaceutical samples by pressure-assisted CE, coupled with a photodiode array detector. Each CE run was completed in less than 1 min, so a 96-well microtiter plate could be measured in one day. Determinations of the p*K*_a values of 82 drugs illustrated this interesting new method.

Since most drug discovery projects deal with very sparingly soluble compounds, the usual CE sample concentration would lead to precipitation. The handling of “real” drug candidate molecules is poorly developed in CE applications, in comparison to the most robust potentiometric method.

3.6 CHROMATOGRAPHIC p*K*_a MEASUREMENT

Oumada et al. [148] described a new chromatographic method for determining the aqueous p*K*_a of drug compounds that are sparingly soluble in water. The method uses a rigorous intersolvent pH scale in a mobile phase consisting of a mixture of aqueous buffer and methanol. A glass electrode, previously standardized with common aqueous buffers, was used to measure pH online. The apparent ionization constants were corrected to a zero-cosolvent pH scale. Six sparingly soluble non-steroidal antiinflammatory weak acids (diclofenac, flurbiprofen, naproxen, ibuprofen, butibufen, fenbufen) were used successfully to illustrate the new technique.

3.7 p*K*_a MICROCONSTANTS

In certain types of multiprotic molecules it is possible that chemically different species of the same stoichiometric composition are formed [142,230–244]. The pH-metric titration technique cannot distinguish between such tautomeric species. In such cases the determined p*K*_a is a composite constant, a macroconstant. The

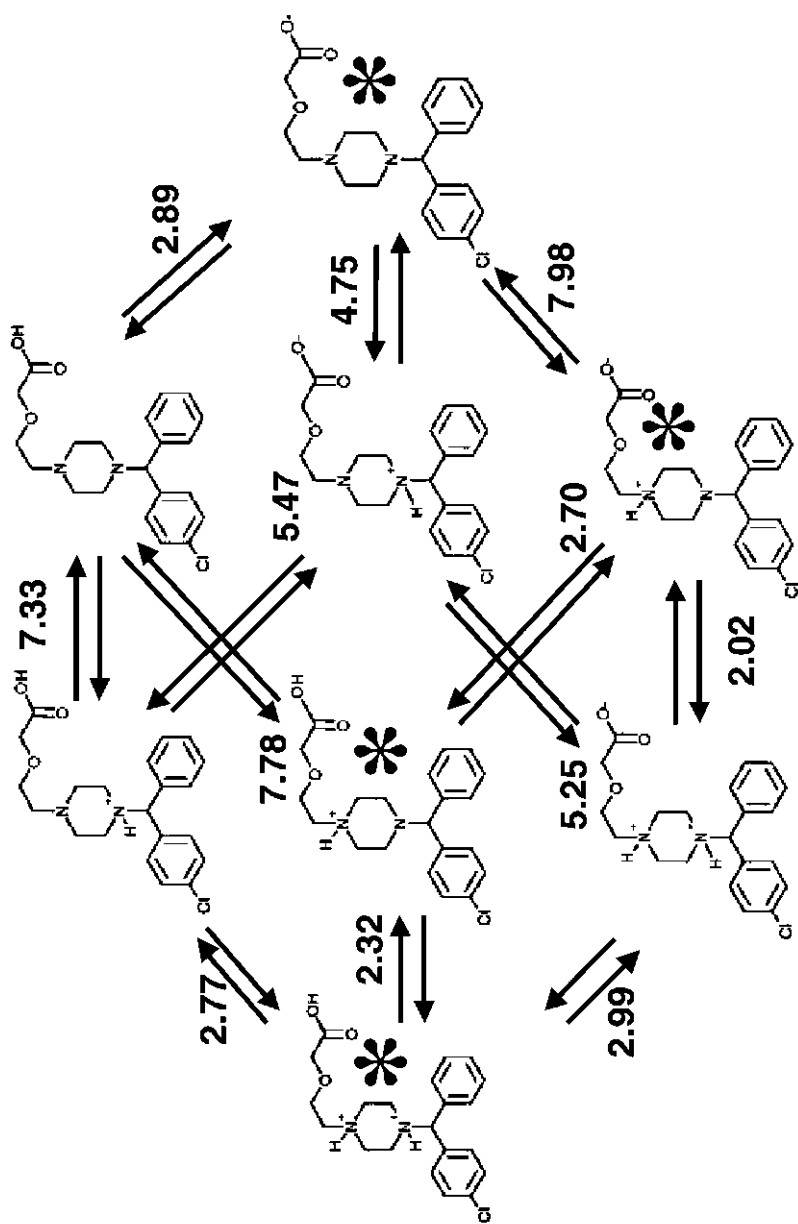


Figure 3.4 Microspeciation of cetirizine, a three- pK_a molecule [142]. The numeric quantities refer to micro- pK_a values. The asterisks denote the principal species at various pH states. [Avdeef, A., *Curr. Topics Med. Chem.*, 1, 277–351 (2001). Reproduced with permission from Bentham Science Publishers, Ltd.]

thermodynamic experiment is a proton-counting technique. It cannot identify the site in the molecule from which the proton comes. It can only be said that a proton emerges from somewhere in the molecule. On the other hand, microconstants are characteristic of individual species, of which there may be more than one with the same composition.

Various relationships between macro- and microconstants have been derived in the cited literature. The microspecies and microconstants of cetirizine (triprotic molecule with macroconstant p*K_a* values 2.12, 2.90, and 7.98) are shown in Fig. 3.4, based on the impressive work of Tam and Qu  r   [142]. The microspecies denoted by an asterisk in Fig. 3.4 are the principal species present in solution. As pH increases, the protonated nitrogen nearest the phenyl groups is the first center to shed charge. The corresponding dication \rightleftharpoons monocation reaction has the micro-p*K_a* 2.32. The next principal center to shed a proton is the carboxylic group, leading to the formation of a zwitterion (micro-p*K_a* 2.70). The highest-pH principal deprotonation consists of the protonated nitrogen nearest the carboxylate group losing its proton (micro-p*K_a* 7.98) to form the anionic species on the right side of Fig. 3.4.

In cetirizine, the carboxylic group has four different micro-p*K_a* values in the range, 2.70–5.47, depending on the neighboring-group charge state. The nitrogen nearest the phenyl groups has the micro-p*K_a* values in the range 2.02–7.33. The other nitrogen has values in the range 2.77–7.98.

3.8 p*K_a* “GOLD STANDARD” FOR DRUG MOLECULES

About 250 experimentally determined p*K_a* values of drugs and some agrochemicals are listed in Table 3.1. These have been critically selected to represent high-quality results. Most of these constants have been determined either at Sirius or *p*ION since 1990, with many personally determined by the author.

TABLE 3.1 Critically Selected Experimental p*K_a* Values of Drug Molecules

Compound	p <i>K_a</i>	<i>t</i> ^a (��C)	<i>I</i> (M)	Ref.
1-Benzylimidazole	6.70	—	0.11	119,153
2-Aminobenzoic acid	4.75, 2.15	—	0.15	161, p. 8
2-Naphthoic acid	4.18	—	0.15	26
2,4-Dichlorophenoxyacetic acid	2.64	—	0.15	161, p. 63
3-Bromoquinoline	2.74	—	0.15	150
3-Chlorophenol	9.11	—	0	150
3-Aminobenzoic acid	4.53, 3.15	—	0.15	161, p. 25
3,4-Dichlorophenol	8.65	—	0	150
3,5-Dichlorophenol	8.22	—	0	150
4-Butoxyphenol	10.26	—	0	119,150
4-Phenylbutylamine	10.50	—	0.15	149

TABLE 3.1 (Continued)

Compound	pK_a	t^a (°C)	I (M)	Ref.
4-Aminobenzoic acid	4.62, 2.46	—	0.15	161, p. 105
4-Chlorophenol	9.46	—	0	150
4-Me-umbiliferyl- β -D-glucuronide	2.82	—	0.15	151
4-Methoxyphenol	10.27	—	0	150
4-Iodophenol	9.45	—	0	150
4-Ethoxyphenol	10.25	—	0	119,150
4-Propoxyphenol	10.27	—	0	150
4-Pentoxyphenol	10.13	—	0	150
5-Phenylvaleric acid	4.56	—	0.15	149
6-Acetylmorphine	9.55, 8.19	—	0.15	151
α -Methyl-DOPA	12.66, 10.11, 8.94, 2.21	—	0.15	56
Acebutolol	9.52	—	0.15	362
Acetaminophen	9.63	—	0.15	166,357
Acetic acid	4.55	—	0.15	119
Acetylsalicylic acid	3.50	—	0.15	161, p. 167
Acyclovir	9.23, 2.34	—	0.15	— ^b
Albendazole sulfoxide	9.93, 3.28	—	0.15	166
Allopurinol	9.00	37	0.15	385
Alprenolol	9.51	—	0.15	362
Amiloride	8.65	—	0.15	26,— ^b
Aminophenazone (aminopyrine)	5.06	—	0.15	357
Amiodarone	9.06	—	0.15	— ^b
Amitriptyline	9.49	—	0.15	— ^b
Amitrole	10.72, 4.19	—	0	265
Amlodipine	9.26	—	0.15	— ^c
Amoxicillin	9.53, 7.31, 2.60	—	0.15	— ^b
Ampicillin	7.14, 2.55	—	0.15	162,p. 133
Amylobarbitone	8.07	—	0	150
Antipyrine (phenazone)	1.44	—	0.15	56
Ascorbic acid	11.62, 4.05	—	0.15	357
Aspartic acid	9.67, 3.66, 1.94	—	0.17	161, p. 120
Atenolol	9.54	—	0.15	362
Atropine	9.84	—	0.15	— ^b
Azithromycin	9.69, 8.65	—	0.17	358,— ^b
Bentazone	2.91	—	0	265
Benzocaine	2.39	—	0.15	162, p. 25
Benzoic acid	3.98	—	0.15	153,474
Benzydamine	9.27	—	0.15	472
Bisoprolol	9.57	—	0.15	362
Bromocriptine	5.40	—	0.15	509
Buprenorphine	9.62, 8.31	—	0.15	151
Buspirone	7.60	—	0.15	357
Butobarbitone	8.00	—	0	150
Caffeine	0.60	—	0.15	161, p. 26
Carazolol	9.52	—	0.15	362

TABLE 3.1 (Continued)

Compound	pK _a	<i>t</i> ^a (°C)	<i>I</i> (M)	Ref.
Carbenicillin	3.25, 2.22	—	0.11	162, p. 109
Carbomycin B	7.55	—	0.17	358
Carbomycin A	7.61	—	0.17	358
Carvedilol	7.97	—	0.15	362
Cefalexin	7.14, 2.53	—	0.15	166
Celiprolol	9.66	—	0.15	150
Chlorpromazine	9.24	—	0.15	26
Chlorsulfuron	3.63	—	0	265
Cimetidine	6.93	—	0.15	474
Ciprofloxacin	8.62, 6.16	—	0.15	— ^b
Citric acid	5.59, 4.28, 2.88	—	0.17	347
Clarithromycin	8.99	—	0.17	358
Clopyralid	2.32	—	0	265
Clozapine	7.90, 4.40	—	0.15	509
Codeine phosphate	8.22	—	0.15	151
Debrisoquine	13.01	—	0.18	161, p. 119
Deprenyl	7.48	—	0.15	162, p. 26
Deramciclane	9.61	—	0.15	166
Desipramine	10.16	—	0.15	— ^b
Desmycarosyl carbomycin A	8.44	—	0.17	358
Desmycosin	8.36	—	0.17	358
Diacetylmorphine	7.96	—	0.15	151,312
Diazepam	3.40	—	—	—
Diclofenac	3.99	—	0.15	26,149
Diltiazem	8.02	—	0.15	474
Diphenhydramine	9.10	—	0.15	— ^b
Disopyramide	10.32	—	0.15	— ^b
DOPA	12.73, 9.81, 8.77, 2.21	—	0.15	56
Doxycycline	11.54, 8.85, 7.56, 3.21	—	0.15	— ^b
Enalapril	5.42, 2.92	—	0.15	474
Enalaprilat	7.84, 3.17, 1.25	—	0.15	56
Ephedrine	9.65	—	0.15	166
Ergonovine	6.91	—	0.15	— ^b
Erythromycin	8.80	—	0.15	— ^b
Erythromyclamine	9.95, 8.96	—	0.17	358
Erythromyclamine-11,12-carbonate	9.21, 8.31	—	0.17	358
Ethirimol	11.06, 5.04	—	0	265
Famotidine	11.19, 6.74	—	0.15	473
Fenpropimorph	7.34	—	0	265
Flamprop	3.73	—	0	265
Fluazifop	3.22	—	0	265
Flufenamic acid	4.09	—	0.15	— ^b
Flumequine	6.27	—	0.15	161, p. 19
Fluoxetine	9.62	37	0.15	385
Flurbiprofen	4.03	—	0.15	472,473

TABLE 3.1 (Continued)

Compound	pK_a	t^a (°C)	I (M)	Ref.
Fluvastatin	4.31	—	0.15	56
Folinic acid	10.15, 4.56, 3.10	—	0.15	— ^b
Fomesafen	3.09	—	0	265
Furosemide	10.63, 3.52	—	0.15	26,473
Gly-Gly-Gly	7.94, 3.23	—	0.16	161, p. 126
Gly-Gly-Gly-Gly	7.88, 3.38	—	0.16	161, p. 127
Glyphosate	10.15, 5.38, 2.22, 0.88	—	0.17	162, p. 46
Haloperidol	8.65	—	0.15	— ^b
Hexachlorophene	11.40, 3.90	—	0.15	161, p. 32
Hydrochlorothiazide	9.95, 8.76	—	0.15	473
Hydroxyzine	7.52, 2.66	—	0.16	161, p. 146
Ibuprofen	4.45	—	0.15	149,172,473
Imazapyr	11.34, 3.64, 1.81	—	0	265
Imazaquin	11.14, 3.74, 2.04	—	0	265
Imazethapyr	3.91, 2.03	—	0	265
Imidacloprid	11.12, 1.56	—	0	265
Imipramine	9.51	—	0.15	— ^b
Indomethacin	4.42	—	0.15	26,— ^b
Ioxynil	4.08	—	0	265
Ketoprofen	3.98	—	0.15	473
Labetalol	9.42, 7.48	—	0.15	473
Leucine	9.61, 2.38	—	0.15	56
Lidocaine	7.95	—	0.15	149
Maleic hydrazide	5.79	—	0	265
Mannitol	13.50	—		312
Mecoprop	3.21	—	0	265
Mefluidide	4.79	—	0	265
Mellitic acid	6.04, 5.05, 4.00, 2.75, 1.69, 1.10	—	0.2	153
Meloxicam	3.43	—	0.15	162, p. 112
Metformin	2.93	—	0.15	— ^b
Methotrexate	5.39, 4.00, 3.31	—	0.15	— ^b
Metipranolol	9.54	—	0.15	362
Metolazone	9.70	—	0.15	509
Metoprolol	9.56	—	0.15	362
Metsulfuron, methyl	3.64	—	0	265
Mexiletine	9.14	—	0.15	166
Miconazole	6.07	—	0.15	26
Morphine-3 β -D-glucuronide	8.21, 2.86	—	0.16	151
Morphine-6 β -D-glucuronide	9.42, 8.22, 2.77	—	0.16	151
Morphine	9.26, 8.18	—	0.15	151,166
Moxonidine	7.36	—	0.15	385
<i>N</i> -Methylaniline	4.86	—	0.15	150
<i>N</i> -Methyl-D-glucamine	9.60	—	0.15	225
Nadolol	9.69	—	0.15	474

TABLE 3.1 (Continued)

Compound	pK _a	t ^a (°C)	I(M)	Ref.
Nalidixic acid	6.01	—	0.15	— ^b
Naloxone	9.44, 7.94	—	0	334
Naproxen	4.18	—	0.15	473
Neomycin B	9.33, 8.78, 8.18, 7.64, 7.05, 5.69	— 23	0.15	— ^b
Nicotine	8.11, 3.17	—	0.15	161, p. 36
Niflumic acid	4.44, 2.26	—	0.15	161, p. 18
Nitrazepam	10.37, 3.02	—	0.15	161, p. 169
Nizatidine	6.75, 2.44	37	0.15	385
Norcodeine	9.23	—	0.15	151
Norfloracin	8.51, 6.23	—	0.15	— ^b
Normorphine	9.80	—	0.15	151
Nortriptyline	10.13	—	0.15	26
Ofloxacin	8.31, 6.09, 0.77	—	0.15	161, p. 9
Olanzapine	7.80, 5.44	37	0.15	385
Oleandomycin	8.84	—	0.17	358
Ontazolast	4.20	—	0.15	— ^b
Oxprenolol	9.57	—	0.15	362
<i>p</i> - <i>F</i> -Deprenyl	7.42	—	0.15	162, p. 28
Papaverine	6.39	—	0.15	166
Paromomycin	8.90, 8.23, 7.57, 7.05, 5.99	37	0.15	385
Penbutolol	9.92	—	0.15	362
Pentachlorophenol	4.69	—	0	265
Pentobarbitone	8.18	—	0	150
Pericyazine	8.76	—	0.15	150
Phe-Phe	7.18, 3.20	—	0.15	162, p. 6
Phe-Phe-Phe	7.04, 3.37	—	0.15	162, p. 12
Phenazopyridine	5.15	—	0.15	26
Phenobarbital	7.49	—	0	166,150
Phenol	10.01	—	0	150
Phenylalanine	9.08, 2.20	—	0.15	161, p. 116
Phenytoin	8.21	—	0.15	473
Potassium phosphate	11.80, 6.81, 2.01	—	0.17	162, p. 162
Phosphoserine	9.75, 5.64, 2.13	—	0.15	162, p. 79
Phthalic acid	4.92, 2.70	—	0.15	347
Pilocarpine	7.08	—	0.15	357
Pindolol	9.54	—	0.15	362
Pirimicarb	4.54	—	0	265
Pirimiphos, methyl	3.71	—	0	265
Piroxicam	5.07, 2.33	—	0.15	26
Prazosin	7.11	—	0.15	— ^b
Primaquine	10.03, 3.55	—	0.15	— ^b
Probenecid	3.01	—	0.15	26
Procaine	9.04, 2.29	—	0.16	149

TABLE 3.1 (Continued)

Compound	pK_a	t^a ($^{\circ}\text{C}$)	$I(\text{M})$	Ref.
Promethazine	9.00	—	0.15	— ^b
Propamocarb	9.48	—	0	265
Propoxyphene	9.06	—	0.15	474
Propranolol	9.53	—	0.15	26,149,362
Prostaglandin E ₁	4.87	—	0.15	161, p. 40
Prostaglandin E ₂	4.77	—	0.15	161, p. 46
Pyridoxine	8.87, 4.84	—	0.16	161, p. 19
Quinalbarbitone	8.09	—	0	150
Quinine	8.55, 4.24	—	0.15	172,474
Quinmerac	3.96	—	0	265
Quinoline	4.97	—	0.15	150
Ranitidine	8.31, 2.11	—	0.15	— ^b
Repromicin	8.83	—	0.17	358
Rifabutine	9.37, 6.90	37	0.15	385
Rivastigmine	8.80	—	0.15	509
Rosaramicin	8.79	—	0.17	358
Roxithromycin	9.27	—	0.15	162, p. 107
Salicylic acid	13.35, 2.88	—	0.15	166
Serotonin	10.91, 9.97	—	0.15	— ^c
Sethoxydim	4.58	—	0	265
Sotalol	9.72, 8.28	—	0.15	162, p. 167
Sucrose	12.60	—	—	312
Sulfamethazine	7.80, 2.45	—	0	150
Sulfanilamide	10.43, 2.00	—	0.17	161, p. 64
Sodium sulfate	1.33	—	0.17	162, p. 136
Sulfasalazine	10.51, 7.95, 2.65	—	0.15	— ^b
Tamoxifen	8.48	—	0.15	— ^b
Terbinafine	7.05	37	0.15	385
Terbutaline	11.02, 9.97, 8.67	—	0.15	162, p. 36
Terfenadine	9.86	—	0.15	26
Tetracaine	8.49, 2.39	—	0.15	149
Theophylline	8.55	—	0.15	162, p. 128
Thiabendazole	4.64, 1.87	—	0	265
Ticarcillin	3.28, 2.89	—	0.11	162, p. 109
Tilmicosin	9.56, 8.18	—	0.17	358
Timolol	9.53	—	0.15	362
Tralkoxydim	4.98	—	0	265
Triazamate acid	3.49	—	0	265
Trimethoprim	7.07	—	0.15	— ^b
Trovaflaxacin	8.11, 5.90	—	0.15	474
TrpPphe	7.30, 3.18	—	0.15	162, p. 2
Trp-Trp	7.27, 3.38	—	0.15	162, p. 8
Tryptophan	9.30, 2.30	—	0.16	162, p. 10
Tylosin	7.73	—	0.17	358
Tyrosine	10.12, 9.06, 2.20	—	0.16	161, p. 112

TABLE 3.1 (Continued)

Compound	pK _a	<i>t</i> ^a (°C)	<i>I</i> (M)	Ref.
Uracil	13.28, 9.21	—	0.16	162, p. 121
Valsartan	4.70, 3.60	—	0.15	509
Vancomycin	11.86, 10.16, 9.26, 8.63, 7.49, 2.66	—	0.17	162, p. 32
Verapamil	9.07	—	0.15	— ^b
Warfarin	4.82	—	0.15	149
Xipamide	10.47, 4.58	37	0.15	385
Zidovudine	9.53	—	0.15	— ^b
Zopiclone	6.76	37	0.15	385

^aTemperature 25°C, unless otherwise noted.^bpION.^cSirius Analytical Instruments.