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pK DETERMINATION OF SPARINGLY SOLUBLE COMPOUNDS BY DIFFERENCE POTENTIOMETRY

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ABSTRACT

A differential potentiometric titration technique is described which allows the determination of dissociation constants of sparingly soluble and/or labile compounds. The dissociation constants of a series of beta-blockers were determined by this technique. Dissociation constants determined by routine potentiometric titration techniques were found to be equivalent to those determined by the differential potentiometric titration method.

By using a computer to accumulate the titration data, it is shown that the dissociation constants of compounds which degrade due to added titrant base can be accurately determined. This is accomplished by shortening the duration of the experiment (total time $\leq$ 10 minutes) such that minimal degradation occurs during the course of the titration. By combining the computer technique with a differential potentiometric titration technique, it is possible

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to determine the dissociation constants of sparingly soluble compounds which are not stable in solution.

In the determination of dissociation constants, if two pKs are separated by less than 4 pH units, then these constants are said to overlap. To accurately determine a pK, both dissociation constants must be solved for simultaneously. A method is described which corrects for overlapping pKs in a differential potentiometric titration, which then allows the unambiguous determination of the dissociation constants. Also described is a method to correct the differential titration when the amount of overlap is small and the pK of one of the overlapping constants is known.

INTRODUCTION

Potentiometry is a widely used technique to determine dissociation constants. A volume of titrant is added to a known volume and concentration of drug, and the dissociation constant calculated from the resulting volume/pH profile. Application of this technique to soluble, stable compounds is straightforward, even in the cases of multiple and/or overlapping pKs. However, if the solubility of either the neutral or ionized form of the compound is limited then routine potentiometry may not be used.[1] This is due to the fact that in a potentiometric titration the total concentration of the compound must be large enough so that the volume of titrant needed to titrate the solvent (H_2O) is

negligibly small in comparison with the volume needed to titrate the compound. This requires that millimolar concentrations of the drug be present in solution at all points in the titration.

A second consideration in potentiometric titrations is the stability of the compound being titrated. It is essential that over the time-course of the titration the compound remain unchanged. In the case where a compound is reactive in water (solvolysis), or due to the added titrant (acid or base hydrolysis), potentiometry may not be possible. Various other techniques (solubility, partition coefficient, etc.) can be used to determine the dissociation constant of these labile compounds. However, potentiometry is the easiest method and is the least time and manpower intensive.

In order to bypass problems of solubility and/or stability a rapid differential potentiometric titration technique can be used. This technique involves titrating a blank (solvent only) and then a sample (solvent plus drug). The difference in the volume of titrant (sample minus blank) to reach the same pH can be related to the ionization constant of the drug. The concentration of drug in the sample can be 10 to 100-fold less than in conventional potentiometry. For this reason differential potentiometry allows the determination of pKs of sparingly soluble compounds. Also, by using a computer to read the pH values in a rapid fashion, the total time necessary to titrate a labile compound is minimized, and accurate pK values can be determined.

Beta-blockers are a class of compounds whose mode of action is directed at heart arrythmias and glaucoma. At physiologic pH they are typically cationic and relatively soluble and stable in water. However, in the neutral form these compounds are relatively insoluble in water and routine potentiometric titrations are difficult. Also, the class of beta-blockers of interest at ACC (designated USABB) are synthesizd to be short acting and therfore unstable. These compounds are base-labile and rapidly degrade in solution[2] at elevated pHs. In order to accurately determine the pKs of USABB beta-blockers a differential potentiometric titration system was devised and tested. This system is rapid and accurate and allows the determination of pKs of sparingly soluble and/or labile compounds.

EXPERIMENTAL

To circumvent the problems of solubility and stability, a differential potentiometric titration can be done. This technique requires a highly accurate titration apparatus, since it is necessary to know accurately at any point in the titration the exact volume of titrant added. Concentrations of drug being titrated were kept well below the solubility limit of the neutral molecule. In order to prevent problems due to compound stability in solution the times for the potentiometric titration were kept short (10 minutes or less). The pH of the solution was never allowed to exceed 10.5. Also, the potentiometric titrations were

run such that base was added to the solution, thus insuring that the time during which the solution was appreciably basic was very short compared to the total run time.

Potentiometric titrations were done with a Fischer Automatic Titrator. Volumes were delivered by a Fischer Burette Model 390 and pH values read by a Fischer Electrometer Model 380. A Hewlett-Packard computer was used to read the pH values directly from the electrometer. The volume of titrant added at any point was calculated by dividing the total volume dispensed during the course of the titration by the total time of the potentiometric run, and then extrapolating to the point in question. In this way accurate values for pH and volume of titrant added were achievable at any point .

The output of the Electrometer was calibrated, and found to be linear from pH 3 to 12. In all titrations, no buffer was employed. The calculated dissociation constants are thermodynamic values, excluding buffering effects from the drug itself which were taken to be minimal due to its low concentration. It is imperative in these titrations to know accurately the concentration of the drug ($\pm$ 2%) being titrated. For each of the compounds done, the concentration was determined by UV-visible spectroscopy.

Sodium hydroxide solutions were prepared by dilution of a Dilut-it (Baker) with distilled, deionized water. Concentration of the base was checked by dilution of the base into water, determining the pH by the electrometer, and then back-calculating

the molarity of the titrant. The instrumentation and the technique was tested using phenol as a primary standard.

CALCULATIONS

Determination of Single pK Values by Difference Potentiometry

A typical difference titration involves titrating a sample of known concentration and subtracting the volume of titrant needed to reach the same pH when a blank (same volume) was titrated. In the case of a monoprotic drug, the difference in the volume of titrant needed to reach equivalent pHs will be proportional to the degree of ionization and concentration of the drug. The pK of a compound can be calculated by,

$$pK = pH - \log \frac{@}{1-@} \qquad (1)$$

where @ is the degree of ionization of the drug at the pH in question. The degree of ionization at any pH is;

$$@ = V_d/T_b \qquad (2)$$

where T_b is the total volume of titrant necessary to completely titrate the drug and V_d is,

$$V_d=V_s - V_t \qquad (3)$$

where V_s is the sample titrant volume and V_t is the reference

titrant volume. The value of T_b is found by;

$$T_b = C_t/M \qquad (4)$$

where M is the molar concentration of the titrant, and C_t is the concentration of the drug. Equation 2 and 4 show the need to accurately know the concentration of the drug being titrated and the molarity of the titrant. Also, the volume of the blank and sample need to be known as accurately as possible. Substituting equations 2 and into equation 1 gives;

$$pK = pH - \log \frac{V_d*M/C_t}{1-V_d*M/C_t} \qquad (5)$$

which allows the calculation of a pK at any pH.

A computer was used to read the pH at any point in the titration, and from the time-rate of delivery of the titrant the exact volume added at any point in the titration was calculated. To determine a pK, the computer sequentially stepped through the values of pH of the sample (and related volume of added titrant) and then scanned the file of the reference titration for an equal pH value. In the event that an equal pH value was not found in the reference file, the program then interpolated between the two nearest pH values and calculated the volume of base which would have been used to reach the pH value of the sample. In the searching process, if the difference in pH values between the sample and reference was less than or equal to 0.005 pH units, then they were considered equal.

Correction for Multiple pK Values by Difference Potentiometry

Routine potentiometry measures the amount of titrant used in the course of titrating a sample, and from equation 1 calculates the related pK. If a compound has two pKs then the problem of determining an unambiguous pK by potentiometry is more difficult. In potentiometry, measurement is made only on the amount of titrant consumed, and does not directly point to the ionizing group. Other techniques (i.e. UV-Vis spectroscopy, NMR, IR, etc.) may give an indication of the group which is ionizing in the pH range of interest. If the compound of interest has a single dissociating group of interest, and in solution there is also a second dissociating group which is different from the pK of the compound of interest by less than 4 pH units, then both pKs must be solved for simultaneously.

It is possible to solve this problem in two ways. First, both pKs can be solved for simultaneously by conventional equations.[1,3,4] However, if the degree of overlap of the two pKs is minimal then it may be difficult to accurately calculate both pKs.[5] Secondly, if the pK of the secondary sample is known, then it is possible to correct for the volume of titrant being consumed by the secondary ionizing group. The amount of titrant necessary to titrate the secondary ionizing group at any point will be;

$$V_m = (C_m * I_m)/M \qquad (6)$$

where V_m is the volume of titrant needed at a certain pH to

titrate the secondary pK, C_m is the concentration of the sample with the secondary pK, and I_m is the percent C_m ionized at a particular pH. In the experiments described in this paper C_m and C_t are equal; although for the purposes of calculating a pK this is not a requirement. The percent ionized at any pH is calculated as;

$$I_m = \frac{K_{am}}{K_{am} + [H^+]} \qquad (7)$$

where K_{am} is the secondary dissociation constant and $[H^+]$ is the hydrogen ion concentration. Subtracting equation 6 from equation 3 gives V_f, the corrected differential volume (volume sample minus volume reference) of titrant used, corrected for the volume of titrant required to titrate the secondary pK

$$V_f = V_d - V_m \qquad (8)$$

Combining equation 8 with equation 5 gives;

$$pK = pH - \log \frac{V_f * M/C_t}{1 - V_f * M/C_t} \qquad (9)$$

The SEM (standard error of measurement) of a potentiometric titration should be pK $\pm$ 0.05 or less.[1] In all cases shown in Table I the SEM was less than this value. Sources of error in a difference potentiometric titration are; 1) knowing the exact concentration of the compound being titrated, and 2) reading an exact pH for a certain volume of titrant added.

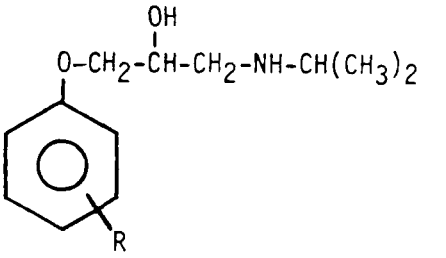
TABLE I

Compound	$pK^{\#}$ (n)*	pK^{+} (n)*
1	9.31 ± 0.022 (1030)	9.35 ± 0.033 (390)
2	9.50 ± 0.032 (2070)	-------
3	9.43 ± 0.027 (1008)	-------
4	9.31 ± 0.048 (1036)	-------

#pKs determined by difference
+pKs determined by routine potentiometry.
*n is the number of values from which the pKs were determined.

RESULTS AND DISCUSSION

Figure I shows the structure of four beta-blockers used in this study. Compounds 2 thru 4 are monoprotic weak bases, whose neutral form has limited solubility in water. Compound 1 is diprotic at pH 7, with a cationic charge on the aliphatic nitrogen, and an anionic charge on the aliphatic carbonyl group. The pK of the carbonyl group is approximately four[6]. Compound 1 is soluble enough for a potentiometric titration due to its charged nature in the pH range of 6 to 12, and is used as a control to show the equivalence of values calculated by difference potentiometry and routine potentiometric titrations. Results of the potentiometric titrations are shown in Table I. Note, that



COMPOUND	R	POSITION
1	CH_2-CH_2-COOH	Para
2	CH_2-CH_2-CO_2CH_3	Para
3	CH_2-CH_2-$CO_2CH_2CH_3$	Para
4	CH_2-CH_2-$CO_2CH_2CH_3$	Ortho

Figure 1

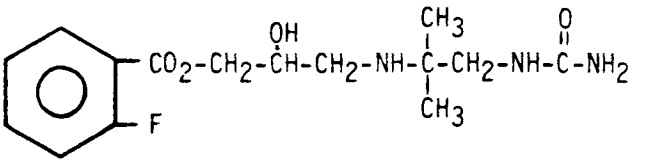
the pK found for the aliphatic nitrogen of compound 1, determined by the two titration methods, is the same within experimental error.

The ionization constants of compounds 2 thru 4 were determined by differential potentiometry exclusively, due to the limited solubility of these drugs in water as the neutral molecule. The exact solubility of the neutral molecule of compounds 2 to 4 could not be determined exactly due to the fast base-hydrolysis of these compounds in solution.[2] The maximum solubility of the neutral drug was approximated to be $5*10^{-4}$ M. This means that a routine potentiometric titration could not be done on these compounds. To minimize the degradation of the sample being titrated, due to hydrolysis and/or solvolysis, the total time of the potentiometric titration was 10 minutes or less. The

compounds being analyzed were solubilized in water (pH 5) and titrated immediately. In this way the pH was always increasing, limiting the total time that the compound was in an appreciably basic media. Subsequent to the titration, the amount of degradation was checked (HPLC), and at no time did it surpass 3% of the total initial concentration of the drug.

Compounds 2 thru 4 degrade by hydrolysis of the aliphatic ester group, forming compound 1. Although the degradation point of compounds 2 to 4 is well removed from the ionization center, the degradation consumes base and will alter the pH of the solution and bias the pK calculation. For this reason the pK of these compounds could not be done unless the total degradation of the sample was kept at a minimum.

As can be seen in Table I, the pK values for compounds 1 thru 4 are all in the range of 9.3 to 9.5. These values compare well with literature values for beta-blockers.[7] The utility of using a computer to record the titration data can be seen from the number of points used to calculate the pKs of all the compounds (See Table I), in that a typical ten minute run gave 500 to 600 points with which to calculate the ionization constant. If appreciable degradation of the sample was occuring during the titration, then the standard error of the pK would be larger than is acceptable. Also, if degradation was occuring, then in calculating the pK the value would appear to progress and not be a random value. Compound 5 (Figure 2) has a single pK in the pH region of interest



COMPOUND

5

Figure 2

(pH 5-10). The solubility of the neutral species of this compound is high enough to allow a routine potentiometric titration to be done. The dissociation constant was calculated in this manner and was found to be comparable to a value determined by differential potentiometry (Table II). Compound 5 is extremely labile in solution[8] in the mid-pH range and for this reason the total run time for the potentiometric titrations was lowered to 5 minutes. The small standard error in both types of potentiometric titration indicates that minimal degradation occured during the run-time of these experiment. This was checked by UV spectroscopy and 2nd

TABLE II

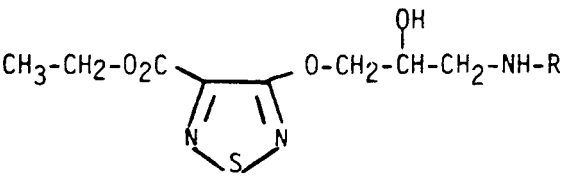
Compound	pK# (n)*	pK+ (n)*
5 (9089)	8.09 + 0.039 ($\bar{9}$94)	8.17 + 0.005 ($\bar{2}$000)

pKs determined by difference potentiometry.
+ pKs determined by routine potentiometry.
* n is the number of values from which the pKs were determined.

derivative UV spectroscopy. As noted before, if degradation was occuring during the titration run a progression would be noted in the calculated pK of the sample. No progression in the calculated pKs for either compound is noted.

In the neutral pH range (pH 6-8) small amounts of added titrant will cause large fluctuations in the pH of the solution being titrated. In doing a differential potentiometric titration in the mid-pH range it was found that using a less concentrated titrant was desirable. The use of a less concentrated titrant caused smaller pH changes resulting in the volume added to the reference or the sample being larger and easier to measure. Irrespective of the normality of the titrant, the difference in the volume added to titrate the sample versus the amount necessary to titrate the reference will be comparable to the drug concentration.

Compounds 6 thru 8 are USABB compounds which are unstable and minimally soluble in water. Compound 6 (Figure 3) has a single dissociation constant which relates to the deprotonation of the aliphatic nitrogen and two dissociation constants which relate to the salt of recrystallization (maleate: pKs of 2.00 and 6.26).[9] Note that the second pK of maleate is within 4 pH units of the anticipated pK of compound 6, thus preventing an unambiguous calculation of this pK. It is possible to calculate for the overlapping pKs simultaneously from titration data,[1,3,4] however the degree of overlap of the two pKs is minimal causing difficulty



COMPOUND	R	Salt Form
6	$CH(CH_3)_2$	Maleate
7	$CH(CH_3)_2$	Oxalate
8	$C(CH_3)_3$	Oxalate

Figure 3

in calculating both pKs.[5] Attempts to experimentally determine the pK of compound 6 and the secondary pK of maleate simultaneously, by calculation, were unsuccessful.

However, since the pK and concentration (equimolar with compound 6) of maleate are known, it should be possible to directly calculate the pK of compound 6 by correcting for titrant uptake due to the secondary pK. Since maleate has two dissociation constants, it is necessary to define the starting pH for the corrected differential potentiometric titration. The starting pH should be at least 2 pH units below the pK of interest and two pH units above any other pK. The starting pH must be less than pH=4.26 (pK_2 maleate=6.26) and greater than pH=4.00 (pK_1=2.00). If the starting pH is appreciably lower than 4 then it would be necessary to correct equation 7 for the percent maleate ionized at the beginning of the titration, due to the

TABLE III

COMPOUND	$pK_{(n)}^{+}$
6*	9.47 ± 0.04 (3̄92)
7*	9.41 ± 0.04 (6̄26)
8#	9.41 ± 0.04 (6̄26)

*Determined by corrected difference potentiometry (eq. 8).
#Determined by difference potentiometry.
+n is the number of points used to calculate the pK.

first pK of maleate. However, by setting the initial pH between 4.0 and 4.2 this problem can be circumvented.

The pK of compound 6 determined by corrected difference potentiometry is shown in Table III. The acceptable standard error of the pK shows that the program and titration method worked. This method allows the titration of a slightly soluble, labile compound in the prescence of a minimally overlapping pK.

To corroborate the calculated value for compound 6, the pKs of compounds 7, (Figure 3) and 8, (Figure 3) were determined by difference potentiometry. Oxalic acid has two pKs,[9] but both are low enough (pK_1=1.19, pK_2=4.21) not to present a problem in

determining the pK of compound 7. Results for compounds 7 and 8 are also reported in Table III.

The agreement of the pK values for compound 6 and 7 shows the viability of this technique. Compound 8 is a homologue of compound 7 and shows that the terminal end of the side chain does not affect the pK of the aliphatic amino group. The pKs shown in Table III are in good agreement with the pKs presented in Table I. It appears that neither the type of aromatic ring (benzyl vs thiadiazole) nor the placement of the aliphatic side chain on the ring (ortho vs para) affects the pK of the aliphatic amino nitrogen.

It should be emphasized that a corrected differential titration technique requires that two parameters be known exactly. First, the molar concentration of the sample with the secondary pK must be known accurately. Although the concentration of this sample does not have to be equimolar with the compound of interest (see equation 6), the exact concentration of the secondary pK sample has to be known. Secondly, the exact pK or pKs of the secondary sample need to be known so that they can be corrected for. By initiating the titration at a defined pH, it is possible to circumvent many problems due to the secondary pK sample.

If it is difficult to accurately set the initial pH of the solution such that the pH is two units below the interfering pK, then equation 6 can be modified such that the corrected differential pK titration could start at any pH. In order to do this, a correction factor would be added to equation 6 which would

correct for the initial amount of secondary sample ionized at the outset of the experiment. This correction would be beneficial for compounds which may be labile in solution, and the time cannot be taken to preset the pH, or if there is a solubility problem in setting the pH at the outset of the titration.

Correcting for an overlapping pK of a secondary sample can be done in another fashion, namely by adding an equal concentration of the secondary sample to the reference, such that the volume of base necessary to titrate the secondary pK will be subtracted out directly. However, this method is tedious, since an exactly equal concentration of secondary sample would need to be titrated in the reference and sample. Measuring the concentration of secondary sample by HPLC, or other analytical means, would allow a titration of this type, but would be manpower intensive.

CONCLUSIONS

A differential potentiometric titration method is described in this paper which allows for the accurate determination of pKs of slightly soluble and/or stable drugs. By using a computer to monitor the change in pH, these titrations can be done in a rapid and accurate manner. The speed of these titrations means that compounds which degrade in solution, or due to added acid or base, can be titrated before appreciable degradation can occur. Also, in the case where a secondary sample pK interfers in determining the dissociation constant of interest, a computer fitting method

is described and shown to give reliable values for the pK of the compound of interest.

The pKs of 7 beta-blockers are determined using a differential or corrected differential titration. The values all are in good agreement and show that neither the aromatic ring nor the placement of a secondary aliphatic side chain affects the pK of the aliphatic nitrogen. All pKs are in the pH range of 9.3 to 9.5.

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