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## Nonlogarithmic Linear Titration Curves

*Abstract.* Titration curves can be based on linear nonlogarithmic forms of the equilibrium equation of a dissociation reaction. From such curves, in contrast to those based on logarithmic transformations, both the end point of the titration and the dissociation constant can be derived.

In recent articles in *Science* (*1*), graphic methods, based on a linear logarithmic transformation of the equation $(X) \cdot (Y)/(XY) = K$, have been recommended for the purpose of estimating the kinetic constants of dissociation reactions of the type $X + Y \rightleftharpoons XY$. However, the equilibrium equation can also be written in several nonlogarithmic forms, one of which is:

$$(P) = (X) + (XY) = (XY) + K \cdot (XY)/(Y) \qquad (1)$$

Thus, when $X$ is titrated with $Y$, a plot of $(XY)$ versus $(XY)/(Y)$ gives a straight line. The intercept with one of the coordinates equals $P$ (the end point of the titration), while $K$ is given by the slope. Linear logarithmic plots do not give the end point of the titration, which must be known for the construction of the curves. This is a disadvantage in cases where the concentration of the compound to be titrated is unknown.

Equation 1 is applicable to any dissociation reaction where the (relative) concentrations of $XY$ and that of one of the dissociation products can be measured. For instance, it applies to the "titration" of an enzyme with its substrate, where the "end point" is most often unknown.

For this purpose, three nonlogarithmic linear equations were first suggested by Woolf (see *2*). The equation analogous to Eq. 1 and, for various reasons (*3*) to be preferred to the other two, can be written as:

$$(E_t) = (E) + (ES) = (ES) + K_M \cdot (ES)/(S)$$

or:

$$V_m = v + K_M \cdot v/(S)$$

where $v$ (the initial reaction rate) equals $k \cdot (ES)$ and $V_m$ (the maximal rate for $(S) \rightarrow \infty$ or the "end point of the titration") equals $k \cdot (E_t)$. Since, owing to low enzyme concentration, the concentration of free substrate is practically equal to the total substrate concentration $(S)$, a plot of $v$ versus $v/(S)$ is linear. Extrapolation to $(S) \rightarrow \infty$ gives $V_m$, while $K_M$ (the Michaelis constant) is given by the slope.

A further example is the titration of the salt of a weak acid $(AH)$ with a strong acid. From

$$(A^-) \cdot (H^+)/(AH) = K_H$$

it follows that

$$P = (A^-) + (AH) = (AH) + K_H \cdot (AH)/(H^+)$$

$P$ is found by extrapolation of a plot of $(AH)$ versus $(AH)/(H^+)$ to $(H^+) \rightarrow \infty$. $K_H$ is derived from the slope of the plot.

When the sodium salt of $AH$ is titrated withHCl, one has on the basis of electroneutrality (*4*):

$$(A^-) + (Cl^-) + (OH^-) = (Na^+) + (H^+)$$

Furthermore

$$(A^-) + (AH) = (Na^+)$$

Thus

$$(AH) = (Cl^-) - (H^+) + (OH^-)$$

where $(Cl^-)$ can be expressed in equivalents of added acid.

In Fig. 1 such titration curves are given for 10 ml of $10^{-2}M$ solutions of $Na_2HPO_4$ and of the sodium salt of diethylbarbituric acid (Veronal) titrated with 1$N$ HCl by means of a micrometer and syringe with capillary tip. The $p$H measurements were carried out with a Radiometer $p$H-meter standardized with a buffer based on U.S. National Bureau of Standards certified buffers.

Since the smallest increment of HCl added constituted a concentration of $(Cl^-)$ of $10^{-3}M$ in the reaction mixture, $(H^+)$ and $(OH^-)$ could be neglected over the entire range of $p$H values ($\sim$ 6 to 9) used, and the equivalents $(T)$ of added HCl were simply plotted versus $T/(H^+)$. The slopes of the curves correspond to $pK_H$ values of 7.02 and 7.90 (30°C, 0.01$M$ ionic strength) for phosphate and diethylbarbituric acid, respectively.

When a solution of the acid $AH$ is titrated with a strong base, for example NaOH, one finds that:

$$(AH) = (P) - (Na^+) - (H^+) + (OH^-)$$

that is, for a plot of $(AH)$ versus $(AH)/(H^+)$, the end point must be known. However, since Eq. 1 can be written as:

$$P = (A^-) + (A^-) \cdot (H^+)/K_H$$

a plot of $(A^-)$ versus $(A^-) \cdot (H^+)$, where

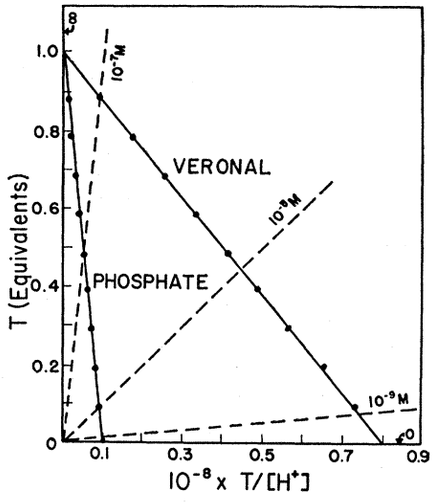


Fig. 1. Nonlogarithmic linear titration curves of phosphate and diethylbarbiturate (Veronal). $T$ represents equivalents of HCl added to a solution of the sodium salt. $[H^+]$ is the hydrogen ion concentration. Points on the curves referring to a particular hydrogen ion concentration lie on a line through the origin. The ordinate represents $[H^+] = \infty$ and the abscissa $[H^+] = 0$. The intercept of the curves with the ordinate gives the end point of the titration, while the (negative) slope is equal to the hydrogen ion dissociation constant.

$(A^-) = (Na^+) + (H^+) - (OH^-)$, gives $P$ as well as $K_H$, also in this case. The equations can readily be adapted to the case of a weak base.

This method of plotting titration data has the further advantage over linear logarithmic plots of being more sensitive to experimental errors and small deviations from theory. This is emphasized by the fact that for $K_H$ values that differ by more than one $pK$ unit, the scale of the plot must be adapted to each constant separately. On the other hand, a more detailed analysis of a composite titration curve, involving two or more $K_H$ values that are less than one unit apart, can be made on the same scale by using the procedure described here (*5*).

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