

Determination of Distribution Constants by Potentiometric Titration

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By potentiometric titration of a substance with acid-base properties in a medium consisting of an emulsion of two equally large immiscible liquid-phases a titration curve is obtained of normal appearance, but shifted $\log(1 + K_D)$ units along the pH axis, where K_D is the distribution constant for the substance between the two phases. This titration curve can in many instances afford an estimation of the distribution of the substance between the aqueous phase and the lipid phase in a biological system. A method is here suggested to determine the pH required in a counter-current distribution.

Verification of theories on the mechanisms of action of pharmacons will often require knowledge of the distribution constants of a number of substances between an aqueous and a lipid phase with reasonable though not very great accuracy.

In the course of time a number of substances have been used as lipid-phase models, *e.g.* olive oil^{1,2}, chloroform, benzene, heptane³, and oleyl alcohol⁴. In this laboratory decanol is generally employed, *inter alia* because it is a well-defined and easily accessible compound.

Titration of a substance with acid-base properties in aqueous solution will give

$$\text{pH} = \text{p}K_a + \log \frac{[\text{A}^-]}{[\text{A}]}$$

During titration in a two-phase system where the acidic form is so distributed between the two phases that the same species of molecule is found in both phases, the distribution law

$$\frac{[\text{A}_o]}{[\text{A}_{\text{aq}}]} = K_D$$

will apply.

pH in the aqueous phase is determined by

$$\text{pH} = \text{p}K_a + \log \frac{[\text{A}^-]}{[\text{A}_{\text{aq}}]}$$

Assuming that A^- does not occur in the organic phase, the amount of A will be

$$A = \text{vol. water } ([A_{\text{aq}}] + [A^-]) + \text{vol. organic phase } [A_o]$$

The computations below will consider only the case where the two phases are of equal volume, *i.e.*

$$\begin{aligned} A &= \text{phase vol. } ([A_{\text{aq}}] + [A_o] + [A^-]) \\ &= \text{phase vol } ([A] + [A^-]) \end{aligned}$$

where

$$[A] = [A_{\text{aq}}] + [A_o] = [A_{\text{aq}}](1 + K_D)$$

Hence, with pH in terms of a function of $[A^-]$:

$$\text{pH} = \text{p}K_a + \log \frac{[A^-]}{[A]} + \log (1 + K_D)$$

Titration in a two-phase system will thus cause the titration curve to be shifted $\log (1 + K_D)$ units to the basic side, and no other changes.

If the substance is of such a nature that the basic form is distributed between the two phases and the acidic form is found only in the aqueous phase, *e.g.* hydrochlorides of amines, the formula will be the same, but with opposite sign for the term $\log (1 + K_D)$, *i.e.* the titration curve is shifted to the acidic side.

Where the assumption of the same species of molecule in the two phases is untenable, *e.g.* in the case of carboxylic acids in the water/toluene system where the carboxylic acid occurs mostly as double molecules in the toluene phase, K_{D2} , as defined by the ratio of concentration in the two phases, will not be constant, but depend upon the concentration.

The ratio

$$K_{D2} = \frac{[A_2] \text{ (toluene)}}{[A]^2 \text{ (aq)}}$$

is constant, the process being:



The total concentration of non-ionized A is now:

$$\begin{aligned} [A] &= [A_{\text{aq}}] + [A_o] \\ &= [A_{\text{aq}}] + K_{D2} [A_{\text{aq}}]^2 \end{aligned}$$

hence:

$$[A_{\text{aq}}] = \frac{\sqrt{1 + 4 K_{D2} [A]} - 1}{2 K_{D2}}$$

which does not lead to any convenient expression.

From the qualitative point of view, however, a high total concentration means comparatively much material in the organic phase whereas a low total concentration means comparatively little, *i.e.* the shifting of the titration curve is most pronounced while there is but little of the ionized form present. The content of substance in either phase at any pH-value is, moreover, computable from an experimental curve, and the applicability of this distribution law will thus be verifiable.

For the titrations were used Radiometer's titrator TTT1 and titrigraph SBR2/SBU1. Without discussing how the instrument functions we shall merely state that a curve is obtained which presents pH as a function of an added amount of base. Satisfactory working of the instrument requires continuous equilibrium in the system, but even the most effective mechanical stirring fails to maintain sufficient equilibrium in the mixture of water and decanol, and the curves obtained are entirely unsatisfactory. It appears that introduction of an emulsifier to the system improves the curve's appearance, presumably because of the minute droplets then obtained.

1 % Tween 80 * proved very suitable for oil-phases like benzene, toluene, and decanol, but not, however, for chloroform. The application of several other emulsifiers (Tween 20, Span 20, 60 and 80) has been attempted for the water/chloroform system, but without success. It is characteristic of this system that the pH-meter is unsteady and erratic, possibly owing to formation of an emulsion of the water-in-chloroform type, which impairs the conductivity in the emulsion.

In order to enhance the method's applicability, the medium's own protolytic reaction is compensated for by subtracting a blind curve of the medium from the experimental curve so that the amounts of base consumed at the same pH are subtracted as suggested by Grove-Rasmussen ^{5, cf. 6}.

For the purpose of estimating the accuracy of the method, the distribution constants of some substances in the water/decanol system have been determined, in part by distribution without emulsifier and subsequent determination of concentrations in the two phases by titration, in part by recording titration curves in water and in water/decanol emulsion (Table 1).

Table 1.

	C_{decanol} mole/l	C_{water} mole/l	K_D	shifting of the titration curve	K_D acc. to this method
Acetic acid	0.0115	0.0323	0.35	0.13	0.35
Propionic acid	0.0190	0.0141	1.35	0.36	1.30
Butyric acid	0.135	0.0275	4.9	0.78	5.0
4-Aminobenzoic acid	0.044	0.013	3.4	0.73	4.3
Aniline	0.083	0.0125	6.6	0.83	5.8

The criterion for the method's applicability is that the quantities relating to the solubility, the acidity constant and the distribution constant of the substance are such as to permit the determination of a titration curve in the emulsion of the two phases.

If, for instance, 0.2 mmole of substance can be dissolved in 10 ml emulsion and titrated with 1 N acid or base, the apparent pK_a -value of the substance

* Tween 80 = polyoxyethylene sorbitan monooleate.

in the emulsion can be determined, provided the latter is located between 3 and 11, *i.e.* for an acid:

$$3 < \text{p}K_a + \log(1 + K_D) < 11$$

If $\log(1 + K_D)$ is small, K_D will not be very well determined since the method applied will determine $\log(1 + K_D)$ with an accuracy of 0.1 approx., which means that the method is inapplicable where $K_D < 0.5-1$, all according to the accuracy required.

In many cases the $\text{p}K_a$ of the substance cannot be determined by potentiometric titration because of its slight solubility, but the choice of method is inessential.

If $K_D > 10$, the substance is found essentially as A_o and A^- , *i.e.* the titration curve will indicate the concentration in water-phase and oil-phase, respectively, as a function of pH, a function that is often of some importance for the discussion of biological problems.

The titration curve of a substance dissolved in emulsion will likewise prove useful in planning a counter-current distribution for the purpose of determining the pH-value which a water-phase is to have in order to make the distribution of a substance between the water-phase and an organic phase equal to 1.

In the titration curve of the substance in an emulsion the distance from the pH-axis to a point on the curve represents the amount of A^- , and the distance from the point to the equivalence line $A_{aq} + A_o$.

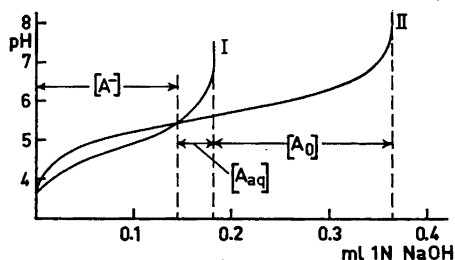


Fig. 1. I: Titration curve of 25 mg of 4-aminobenzoic acid in 10 ml of water.

II: Titration curve of 50 mg of 4-aminobenzoic acid in a mixture of 5 ml of water, 5 ml of decanol and 100 mg of Tween 80.

$[A^-]$: Concentration of ionized 4-aminobenzoic acid in the water phase.

$[A_{aq}]$: Concentration of non-ionized 4-aminobenzoic acid in the water phase.

$[A_o]$: Concentration of 4-aminobenzoic acid in the decanol phase.

What is required is that $[A_o] = [A_{aq}] + [A^-] = \frac{1}{2}[A_{total}]$. The pH-value is found as the point of intersection of this titration curve and a titration curve of half the amount of substance in water.

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