Notes

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Considerations on the Colorimetric Determination with Dye as a Weak Acid-Weak Base Reaction.

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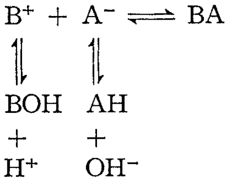
In recent years, colorimetric determination with dye has been widely employed in pharmaceutical fields. An acidic dye is usually added to organic base to form a complex, which is extracted with an organic solvent, and its solution colorimetrically determined.

Determination of organic bases with sulfophthalein dyes has especially been studied extensively by Horioka1) who described the procedures and conditions in detail.

On the other hand, Epton method has been employed in the determination of anionic surface-active agents with basic dyes,2,3,4) but its mechanism is scarcely known.

It is considered that these dye methods are based on a weak acid-weak base reaction and the equations which conveniently explain the mechanism are derived. Further, these considerations will be useful in applying to other pharmaceutical problems involving weak acids and weak bases; for example, determination of organic bases with reineckate, precipitation of oxytetracycline by arylsulfonic acid dye, precipitation of some tinctures by dehydroacetic acid, incompatibilities of some organic bases and mercurochrome solution, absorption of medicines into living body, and so on.

To make the problems simpler, the reaction of monobasic acid, AH, and monoacidic base, BOH, will be considered at first. The equilibrium may be expressed by the following equation.



$$\begin{array}{ccccc} B^+ & + & A^- & \rightleftharpoons & BA \\ \updownarrows & & \updownarrows & & \\ BOH & & AH & & \\ + & & + & & \\ H^+ & & OH^- & & \end{array}$$

Salts are generally believed to be in complete dissociation, but in this case, undissociated salts must be treated. In past works the terms "complex" and "addition product" have been used to denote substances formed from a sample and a dye, but they are usually weak acids and weak bases, and term "salt" is preferable to "complex." Therefore, it is natural that molar ratio of the combination of a sample and a dye is in a ratio of integers, for example, 1:1.

If AH and BOH are organic substances, they are usually soluble in organic solvents and it is not strange that BA is more soluble in organic solvent than in water from which it will precipitate out.

In the case of extraction with organic solvent, which is the usual procedure employed in the dye method, BA (occasionally a part of AH or BOH, too) is in equilibrium between aqueous phase and an organic solvent phase, with a definite partition coefficient.

First, in the case of aqueous phase alone, with the organic solvent layer, there will be four equilibrium constants:

$$[B^+][OH^-]/[BOH]=k_b,\quad [A^-][H^+]/[AH]=k_a,\quad [B^+][A^-]/[BA]=k_{ab},\quad [OH^-][H^+]=k_w$$

Constants, k_a, k_b, k_{ab}, and k_w are dissociation constants of the acid, base, salt, and water,

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1) M. Horioka: Yakugaku Zasshi, **77**, 200 (1957).

2) S. R. Epton: Trans. Faraday Soc., **44**, 226 (1948).

3) W. A. Moore: Anal. Chem., **28**, 161 (1956).

4) P. Mukerjee: *Ibid.*, **28**, 870 (1956).

respectively. Taking C_A and C_B as total acid and total base concentrations, respectively, there will be the following five equations.

$$[A^-]=k_a[AH]/[H^+] \cdots\cdots\cdots\cdots\cdots\cdots (1)$$

$$[B^+]=k_b[H^+][BOH]/k_w \cdots\cdots\cdots\cdots\cdots\cdots (2)$$

$$C_A=[A^-]+[AH]+[BA] \cdots\cdots\cdots\cdots\cdots\cdots (3)$$

$$C_B=[B^+]+[BOH]+[BA] \cdots\cdots\cdots\cdots\cdots\cdots (4)$$

$$[B^+][A^-]=k_{ab}[BA] \cdots\cdots\cdots\cdots\cdots\cdots (5)$$

Number of independent variables are six, namely $[A^-]$, $[AH]$, $[BA]$, $[B^+]$, $[BOH]$, and $[H^+]$. Once $[H^+]$ is limited all the residual values will be fixed. The value of $[BA]$ is obtained from the above five equations:

$$[BA]^2-\{(C_A+C_B)+([H^+]+k_a)(k_w/[H^+]+k_b)k_{ab}/k_ak_b\}[BA]+C_AC_B=0 \cdots\cdots (6)$$

Next, the presence of two layers of aqueous and organic solvent phases will be considered. As long as the transition of AH and BOH from water to organic solvent phase is negligible in comparison to that of BA, the problem will become simple and, for practical purposes, determinations with dye method will be feasible.

Provided that the BA has a partition coefficient, and AH and BOH do not transit to the organic solvent phase, the equations (3) and (4) can be written as equations (3′) and (4′), from which (6′) can be calculated:

$$C_A=[A^-]+[AH]+[BA](1+dR) \cdots\cdots\cdots\cdots\cdots\cdots (3')$$

$$C_B=[B^+]+[BOH]+[BA](1+dR) \cdots\cdots\cdots\cdots\cdots\cdots (4')$$

$$[BA]^2-\{(1+dR)(C_A+C_B)+([H^+]+k_a)(k_w/[H^+]+k_b)k_{ab}/k_ak_b\}[BA]/(1+dR)^2+C_AC_B/(1+dR)^2=0 \cdots\cdots\cdots\cdots\cdots\cdots (6')$$

where $[BA]$: Concentration of BA in aqueous phase
(BA): Concentration of BA in organic solvent phase
d: Partition coefficient of BA
R: Volume ratio of organic solvent phase to water

The (BA) is given directly from the equation, $(BA)=[BA]\,d$, so, only $[BA]$ will be treated here.

When organic solvent phase is not present, it will be the case where $R=0$. Then considerations were made with equation (6′).

When $\partial[BA]/\partial k_a=0$, equation (7) is obtained.

$$[BA]^2-\{(1+dR)(C_A+C_B)+(k_w/[H^+]+k_b)k_{ab}/K_b\}[BA]/(1+dR)^2+C_AC_B/(1+dR)^2=0 \cdots\cdots\cdots\cdots\cdots\cdots (7)$$

Since the equations (6′) and (7) can be established at the same time, the value of $[BA]$ will be the maximum when k_a is infinite. It is also estimated from equation (6′) that the value of $\partial[BA]/\partial k_a$ decreases from a constant value to zero as the value of k_a increases from zero to infinite.

The consideration of k_b is symmetrically the same as that for k_a, i.e. the larger the dissociation constants of AH and BOH, the larger will be the concentration of undissociated salt BA formed. In other words, in colorimetric determination with a dye, the larger the dissociation constants of a sample and a dye are, the more successful it will be.

This fact agrees with many examples, but the effect of partition coefficient is so large that the effect of dissociation constant sometimes becomes obscure.

Now, it is necessary to consider the reaction of a polybasic acid and a polyacidic base, since samples and dyes are often polybasic acid and polyacidic base. The equation will be analogous to equation (6′), but it will be too complicated for practical application. It will be more convenient to treat the polybasic acid (or polyacidic base) as a mixture of monobasic acids (or bases) with different dissociation constants.

The afore-mentioned results suggest that the radical with a larger dissociation constant combines more readily than the radical with a smaller dissociation constant, and the latter is usually negligible. This is reasonable since the ratio of sample and dye is usually approximately 1 : 1. However, when the two dissociation constants are very close the smaller one cannot be neglected.

Now $[BA]$ in connection with $[H^+]$ will be considered. When $\partial[BA]/\partial[H^+]=0$, equation (8) is obtained.

$$[BA]^2-\{(1+dR)(C_A+C_B)+(k_w+2k_b[H^+]+k_ak_b)k_{ab}/k_ak_b\}[BA]/(1+dR)^2+C_AC_B/(1+dR)^2=0 \qquad \cdots\cdots(8)$$

When equation (6′) and (8) are established at the same time,

$$[H^+]=\sqrt{k_wk_a/k_b} \qquad \cdots\cdots(9)$$

$[BA]$ will reach the maximum value when equation (9) is established.

In the dye method, the amount of the dye which transits to the organic solvent layer minus the blank value is regarded as the amount of the dye combined, and its value generally has the maximum point at a certain pH value. In an experiment$^{5)}$ on the determination of penicillin V as the acid and Methylgreen as the basic dye, there was a maximum value of the combined dye at a certain pH value.

However, the practical value did not agree exactly with the theoretical value, and the degree of disagreement will be around 1 or 2 in pH value. This disagreement can be ascribed to many factors such as: 1) Association of the dye, 2) activity coefficient is not a unit in actual practice, 3) transition of substances other than BA into organic solvent to some degree, and 4) partition coefficient is not necessarily constant, etc.

For practical purposes, determination is usually performed at such a pH value where $[BA]$ is not the maximum but sufficient for determination and where blank value is negligible.

Differentiation of equation (6′) with respect to C_B gives the case where base is the sample and the acid is dye, in the dye method, and equation (10) is obtained.

$$\partial[BA]/\partial C_B=\frac{(1+dR)[BA]-C_A}{2(1+dR)^2[BA]-(1+dR)(C_A+C_B)-([H^+]+k_a)(k_w/[H^+]+k_b)k_{ab}/k_ak_b} \qquad \cdots\cdots(10)$$

Since $[BA]<C_B$ and $C_B \ll C_A$ in the dye method, or a large excess of the dye is present against the amount of the sample,

$$\partial[BA]/\partial C_B=C_A/\{(1+dR)C_A+([H^+]+k_a)(k_w/[H^+]+K_b)k_{ab}/k_ak_b\} \qquad \cdots\cdots(11)$$

Namely, such a differential coefficient becomes constant at a certain pH value. In other words the calibration curve of absorbance to a sample is linear.

Equation (11) also endorses the above-mentioned facts that if k_a and k_b are larger, $[BA]$ is larger, and $[BA]$ reaches maximum value at $[H^+]=\sqrt{k_wk_a/k_b}$.

The consideration of C_A is symmetrically the same as that for C_B.

These considerations indicate that colorimetric determination with a dye is based on a weak acid-weak base reaction.

The authors wish to thank Mr. Horioka of the Tokyo University Hospital Pharmacy for his valuable advices on colorimetric determination with a dye.

Summary

Considerations on the colorimetric determination with dye as a weak acid-weak base reaction have been described. Other applications in the pharmaceutical fields are suggested. Several equations were derived on which are based the following five items:

1) Some of organic acids may be determined by colorimetry with a basic dye as well as with organic bases.

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5) H. Nogami, S. Kanazawa: Paper read before the Kanto Local Meeting of the Pharmaceutical Society of Japan, Tokyo, November 2, 1957.

2) The dye which combined with sample reaches maximum value at a certain pH value.

3) Dissociation constants of a sample and of a dye should preferably be as large as possible.

4) The calibration curve is linear.

5) In general, the ratio of combination of a sample and a dye is nearly in integral ratio, such as 1:1.

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Yoshio Ban and Takeshi Oishi: The Preparation of 2-(Substituted phenyl)ethanols.

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As the 2-(substituted phenyl)ethanols (Va, b, c in Chart 1) are very useful starting compounds for the syntheses of benzoquinolizine derivatives, several routes for the preparation of these alcohols have been described.[1~3]

Of these, the best one has been to reduce the (substituted phenyl)acetic acid ester with lithium aluminum hydride, but this process involves many steps and the reagent is rather expensive to use on a preparative scale.

Now it was found that veratraldehyde (Ia) can be converted to 3,4-dimethoxyphenethyl alcohol (Va) in a four-step process involving Darzens reaction with an over-all yield of about 45~50%.

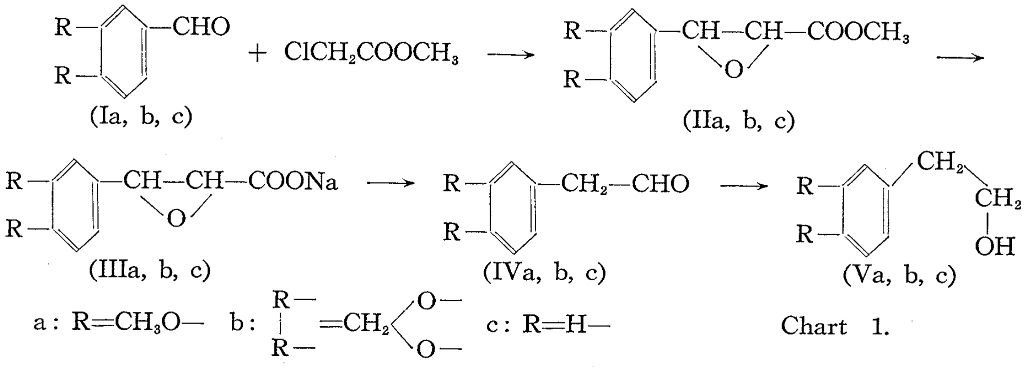

Chart 1.

As illustrated in Chart 1, veratraldehyde (Ia) was condensed with methyl chloroacetate, giving methyl 3-(3,4-dimethoxyphenyl)glycidate (IIa) in a yield of about 85% by applying Henecka's conditions,[4] which used sodium methoxide in methanolic solution as a condensing agent.

Methyl glycidate (IIa) thus obtained was hydrolysed to give its sodium salt (IIIa), which was converted to the corresponding aldehyde (IVa) very smoothly by carrying out the decarboxylation under anhydrous condition. Sodium glycidate (IIIa) suspended in a solution of just one molar equivalent of acetic acid in benzene was stirred and heated gently until the evolution of carbon dioxide ceased. Although the aldehyde (IVa) could be isolated in a pure state in 70% yield by this procedure, it was more convenient in most instances to subject the crude reaction product directly to hydrogenation without distillation.

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[1] C. Schöpf, G. Göttmann, E. Meisel, L. Neuroth: Ann., **563**, 86(1949).

[2] S. Sugasawa, Y. Suzuta: Yakugaku Zasshi, **71**, 1159(1951).

[3] M. Pailer, K. Schneglberger, W. Reifschneider: Monatsh., **83**, 513(1952).

[4] H. Henecka: Ann., **583**, 110(1953).