for more than twenty hours (Fig. 3). This unusual phenomenon can be explained by the fact that the stomachs of rabbits emptied very slowly in the fasted state. Since a large amount of this water insoluble drug was given, the stomach acted as a reservoir and released the drug to the intestine, presumably the main absorption site, over a long period of time.

Recently a deconvolution method[22,23] has been proposed to study the *in vivo* dissolution rate of drugs. By this method, the dissolution rate of drugs *in vivo* is compared directly with that given in the solution. Owing to the fact that the rabbit stomach is always full of solid material and that its emptying time is a function of the fasted or unfasted state, the absorption of drugs in rabbits will be greatly influenced not only by the rate of drug dissolution, but also by possible drug–food interactions and by their effects on stomach-emptying time.

From the facts presented above that it is almost impossible to obtain an empty stomach in rabbits by using the conventional method of fasting and the fasted state markedly prolongs the stomach emptying time, it is, therefore, concluded that the rabbit is not a useful animal in which to study drug absorption.

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Spectroscopic Studies on Molecular Interactions. III.[1) Improvement of the Benesi-Hildebrand Method for the Determination of Equilibrium Constants[2)

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There are numerous literatures on the properties of weak complexes such as charge-transfer complexes,[4) and the determination of equilibrium constants for 1:1 complexation is often made by the spectroscopic method proposed by Benesi and Hildebrand[5) (B–H) or some equivalent[6) of it. The B–H method can be employed to the systems where the initial concentration of the one component is in great excess of that of the other.[5) But Drago and Rose[7) indicated the inaccuracy of the complexation constant obtained by applying the B–H equation to the triethylamine–iodine system[8) where the initial concentration of triethylamine

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1) Part II: I. Moriguchi, Y. Araki, and N. Kaneniwa, *Chem. Pharm. Bull.* (Tokyo), 17, 2088 (1969).
2) A part of this work was presented at the 89th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, April, 1969.
3) Location: *Hatanodai, Shinagawa-ku, Tokyo.*
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7) R.S. Drago and N.J. Rose, *J. Am. Chem. Soc.*, 81, 6141 (1959).
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used was from 8 to 50 times as much as that of iodine and the B–H plot of which was entirely linear. Unfortunately the evaluation method developed by Drago and Rose[9] is very laborious though it may give considerably accurate values of equilibrium constants. The present paper will be concerned with the derivation of an improved B–H method and its application to the triethylamine–iodine system.

The equilibrium constant, K, for a 1:1 complexation betweeen A and B can be expressed by

$$K=x/(a-x)(b-x) \tag{1}$$

where a and b are the initial concentrations of A and B, respectively, and x is the concentration of the complex. The alteration of the optical absorption, ΔE, induced by the complexation may be given as

$$\Delta E=l\cdot\Delta\varepsilon\cdot x \tag{2}$$

In this equation l is the length of the optical path, and $\Delta\varepsilon$ is shown as

$$\Delta\varepsilon=\varepsilon_C-(\varepsilon_A+\varepsilon_B) \tag{3}$$

where ε_A, ε_B, and ε_C are the molar absorptivities of A, B, and the complex, respectively. Substituting x from Eq. (2) into Eq. (1), we have

$$\frac{a}{\Delta E}+\frac{\Delta E}{b(l\cdot\Delta\varepsilon)^2}=\frac{1}{b\cdot l\cdot\Delta\varepsilon}\left(\frac{1}{K}+a\right)+\frac{1}{l\cdot\Delta\varepsilon} \tag{4}$$

The B–H equation, including an approximation $a+b-x\doteqdot b$, is shown as

$$\frac{a}{\Delta E}=\frac{1}{b\cdot l\cdot\Delta\varepsilon\cdot K}+\frac{1}{l\cdot\Delta\varepsilon} \tag{5}$$

Equation (4) derived without any omission can easily be employed in the treatment of the absorbance data obtained under the conditions where a is fixed and b is varying. It is not neccessary that b is in great excess of a. The techniques for employing this method are as follows. At the first step, $a/\Delta E$ is plotted against $1/b$. This plot is idendical with the B–H plot, and the first approximate value of $1/\Delta\varepsilon$ is obtained from the intercept on the ordinate of the graph. At the second step, the value of $\Delta E/b(l\cdot\Delta\varepsilon)^2$ is calculated from the approximate value of $1/\Delta\varepsilon$ thus obtained, then $a/\Delta E+\Delta E/b(\Delta l\cdot\varepsilon)^2$ is plotted against $1/b$, and an improved value of $1/\Delta\varepsilon$ is obtained. This step is repeated until the intercept of the plot on the ordinate converges to a point. Usually the consistency is attained by two or three iterations. The equilibrium constant, K, is then calculated from the convergent values of the intercept and the slope by using Eq. (4).

Recently another iterative method including no omission was developed[10] by improving the Rossotti–Rossotti method.[11]

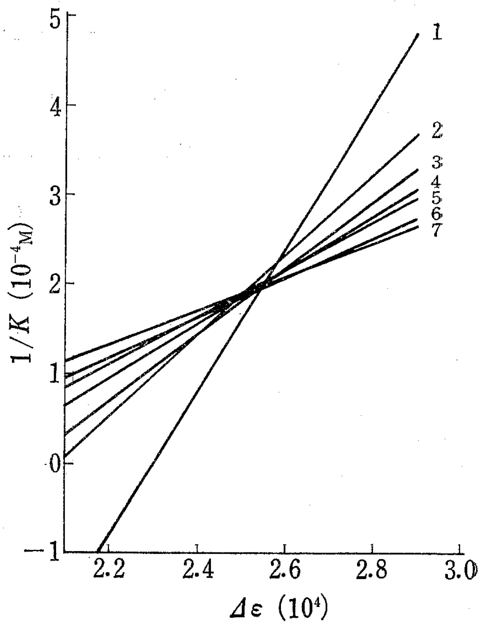

Fig. 1. Drago–Rose Plot for the Triethylamine–Iodine System

initial concentration of triethylamine (M):

1.	18.90×10^{-4}
2.	9.73×10^{-4}
3.	7.74×10^{-4}
4.	5.87×10^{-4}
5.	4.89×10^{-4}
6.	3.89×10^{-4}
7.	2.94×10^{-4}

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[9] N.J. Rose and R.S. Drago, *J. Am. Chem. Soc.*, **81**, 6138 (1959).

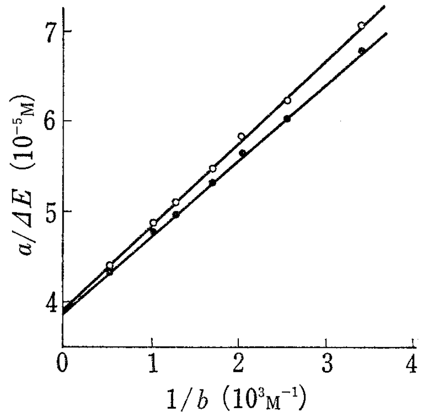

Fig. 2. Original and Improved Benesi–Hildebrand Plots for the Triethylamine–Iodine System

—●—: original —○—: improved ordinate for the improved plot: $a/\Delta E + \Delta E/b(l\cdot\Delta\varepsilon)^2$ (10^{-5}M)

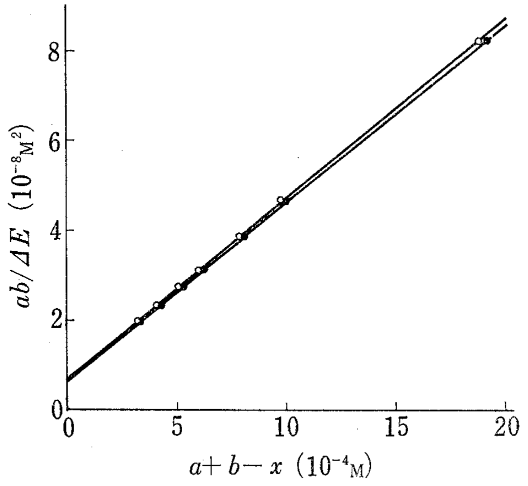

Fig. 3. Original and Improved Rossotti–Rossotti Plots for the Triethylamine–Iodine System

—●—: original —○—: improved

For the purpose of comparing our new method with others, an attempt was made to employ these methods to the triethylamine–iodine system. Absorbance data for this system at 25° in heptane could be obtained for the charge-transfer band at 280 mμ from Fig. 4 of Nagakura's paper.[8] The plots according to these methods are drawn in Fig. 1, 2, and 3. The Drago–Rose plot in Fig. 1 is considerably complicated. The improvement of the B–H plot by the iterations shown in Fig. 2 is clearer than that of the Rossotti–Rossotti plot shown in Fig. 3. The values of $\Delta\varepsilon$ and K obtained from these plots are listed in Table I.

Table I. Values of $\Delta\varepsilon$ and K obtained from the Absorbance Data[8] for Triethylamine–Iodine Complexation at 25° in Heptane

Method	$\Delta\varepsilon$	K (M^{-1})
Drago–Rose[9]	2.53×10^{4}[a]	5.09×10^{3}[a]
Benesi–Hildebrand[5]	2.56×10^{4}	4.71×10^{3}
Improved Benesi–Hildebrand[b]	2.54×10^{4}	5.11×10^{3}
Rossotti–Rossotti[11]	2.57×10^{4}	5.64×10^{3}
Improved Rossotti–Rossotti[10]	2.53×10^{4}	5.27×10^{3}

a) Drago and Rose evaluated $\Delta\varepsilon$ and K being 2.54×10^{4} and 5.13×10^{3}M^{-1}, respectively,[7] from part of the data[8].
b) presented in this work

The Drago–Rose plot is considered to give the most accurate values from the nature of the method. But the treatment is very laborious. On the contrary the improved B–H method presented here is not so troublesome, and seems to give relatively accurate values of $\Delta\varepsilon$ and K. It may be possible to conclude that the new method is comparatively useful for the determination of equilibrium constants.

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