Chem. Pharm. Bull.
30(1) 288—295 (1982)

Determination of Stability Constants of Complexes by the Nonlinear Optimization Method and Analysis of Solubility Curves[1]

MASAOKI MIYAHARA* and TOMOO TAKAHASHI[2]

Shionogi Research Laboratories, Shionogi and Co., Ltd., Sagisu 5-12-4 Fukushima-ku, Osaka 553, Japan

(Received June 18, 1981)

Solubilization of the slightly soluble drug 3-benzimidoyl-2-hydroxy-1,4-naphthoquinone was studied by the solubility method using sodium benzoate and several phenol derivatives as solubilizing agents. Their solubilizing mechanism is believed to involve the formation of soluble complexes between drugs and solubilizing agents. Solubility curves observed in cases where complexes of high order were formed at the same time were analyzed by using the nonlinear optimization technique, *i.e.*, the Gauss-Newton method and the conjugate gradient method. Further study is needed to check the accuracy of the estimated parameters.

Keywords——stability constants of complexes; nonlinear optimization method; analysis of solubility curves; solubilizing agent; Gauss-Newton method; conjugate gradient method; optimal parameter; solubility method; solubilization

In order to dissolve slightly soluble drugs, solubilizing agents have been frequently used and in many cases, their solubilizing mechanism is believed to involve the formation of soluble complexes between the drugs and the solubilizing agents. The analysis of solubility curves, that is, the relationship between total amount of drug dissolved and the concentration of complexing agents added, has been done for comparatively simple cases where the formation of a 1:1 complex[3] or of both 1:1 and 1:2 complexes[4] occurred. However, cases in which complexes of higher order occur at the same time are not as simple. We tried to analyze the solubility curves observed in such cases. A 1,4-naphthoquinone derivative, 3-benzimidoyl-2-hydroxy-1,4-naphthoquinone, was used as a solubilizate. This compound was synthesized by the staff at our research laboratory and has been under investigation as a plant growth-regulating substance. It has relatively low solubility in water. The chemical structure of this compound is shown in Chart 1.

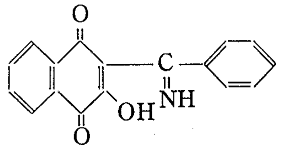
3-benzimidoyl-2-hydroxy-1,4-naphthoquinone

Chart 1

Hereafter this compound will be referred to as "bendroquinone."

Sodium benzoate and several phenol derivatives possessing one or more hydroxyl groups in the molecule were used as solubilizing agents. The solubilization of bendroquinone with solubilizing agents in aqueous and nonaqueous solutions was studied by the solubility method. A method of data analysis similar to that employed by Blau, Klimpel and Steiner[5] was used to estimate the parameter values. This article presents a method for determining the stability constants of complexes and for analyzing solubility curves in cases where high order complexes as well as a 1:1 complex are present together in the system.

Method of Analysis

(Case 1) Consider first the case where only a 1:1 complex is formed. Such a case was discussed by Connors and Mollica.[3]

If the formation of a 1:1 complex is assumed, Eq. 1 is obtained on the basis of the law of mass action.

$$A + B \overset{K_{11}}{\rightleftarrows} AB, \quad K_{11} = \frac{C_{AB}\cdot}{C_A^\circ(C_{Bt}-C_{AB})} \tag{1}$$

where C_A°, C_{Bt}, C_{AB} and K_{11} are the solubility of A in the absence of B, the total concentration of B, the equilibrium concentration of the complex species AB and the stability constant of the 1 : 1 complex, respectively. We may rewrite Eq. 1 as

$$C_{AB} = \frac{K_{11}\cdot C_A^\circ\cdot C_{Bt}}{1+K_{11}\cdot C_A^\circ} \tag{2}$$

The apparent total solubility of A, C_{At}, is given by Eq. 3 from the mass balance equation.

$$C_{At} = C_A^\circ + C_{AB}$$

$$C_{At} = C_A^\circ + \frac{K_{11}\cdot C_A^\circ\cdot C_{Bt}}{1+K_{11}\cdot C_A^\circ} \tag{3}$$

Eq. 3 indicates that plots of C_{At} against the total amount of the complexing agent added, C_{Bt}, should give a straight line relation.

The stability constant, K_{11}, is evaluated with Eq. 4, from the slope of the line.

$$K_{11} = \frac{\text{slope}}{\text{INTERCEPT}(1-\text{SLOPE})} \tag{4}$$

(Case 2) Consider now the case where the formation of both 1 : 1 and 1 : 2 complexes occurs. This case was studied by several investigators.[4)]

If the equilibrium reactions are assumed to be described by Eqs. 5 and 6, the stability constants K_{11} and K_{12} are as follows:

$$A + B \overset{K_{11}}{\rightleftarrows} AB \qquad K_{11} = \frac{C_{AB}}{C_A^\circ(C_{Bt}-C_{AB}-2C_{AB_2})} \tag{5}$$

$$AB + B \overset{K_{12}}{\rightleftarrows} AB_2 \qquad K_{12} = \frac{C_{AB_2}}{C_{AB}(C_{Bt}-C_{AB}-2C_{AB_2})} \tag{6}$$

Since the apparent solubility of A, C_{At}, is given by Eq. 7, Eq. 8 can be derived by combining Eqs. 5, 6 and 7. Eq. 8 indicates that the total solubility of A, C_{At}, can be expressed as a quadratic function with respect to C_{Bt}.

$$C_{At} = C_A + C_{AB} + C_{AB_2} \tag{7}$$

$$C_{At} = C_A^\circ + K_{11}\cdot C_A^\circ\,(C_{Bt}-C_{AB}-2C_{AB_2}) + K_{11}\cdot K_{12}\cdot C_A^\circ\,(C_{Bt}-C_{AB}-C_{AB_2})^2 \tag{8}$$

Rewriting Eq. 8, we obtain Eq. 9.

$$\frac{C_{At}-C_A^\circ}{C_A^\circ(C_{Bt}-C_{AB}-2C_{AB_2})} = K_{11} + K_{11}\cdot K_{12}(C_{Bt}-C_{AB}-2C_{AB_2}) \tag{9}$$

Since it is evident that a plot of the left-hand term of Eq. 9 versus $(C_{Bt}-C_{AB}-2C_{AB_2})$ should give a straight line, stability constants, K_{11} and K_{12}, can be obtained from the slope and the intercept, using the method described by Kakemi, Sezaki, Mitsunaga and Nakano.[4)] Their method is as follows. The initial values for K_{11} and K_{12} are obtained from Eq. 9 by ignoring the C_{AB} and C_{AB_2} terms.

The first approximate values for C_{AB} and C_{AB_2} are obtained from Eqs. 10 and 11:

$$C_{AB} \fallingdotseq K_{11}\cdot C_A^\circ\cdot C_{Bt} \tag{10}$$

$$C_{AB_2} \fallingdotseq K_{11}\cdot K_{12}\cdot C_A^\circ\cdot C_{Bt}^2 \tag{11}$$

The C_{AB} and C_{AB_2} values are put into Eq. 9 to obtain better values for K_{11} and K_{12}. These steps are repeated until a converged straight line is obtained. Hereafter this method will be referred to as the regression line method (R. L. method). The same approach might be applicable to a more general case.

(Case 3) The final case is a more general one where the formation of higher order complexes occurs. Assume that a drug may bind up to m ligands in a sequence of m stepwise reactions (of course, this model contains cases 1 and 2 as special cases). Then the following set of equations is obtained on the basis of the law of mass action.

$$\begin{array}{lll} \mathrm{A} + \mathrm{B} \underset{}{\overset{K_{11}}{\rightleftarrows}} \mathrm{AB}, & K_{11} = \dfrac{C_{AB}}{C_A^{\circ}\cdot C_B}, & C_{AB} = K_{11}\cdot C_A^{\circ}\cdot C_B \\ \mathrm{AB} + \mathrm{B} \overset{K_{12}}{\rightleftarrows} \mathrm{AB_2}, & K_{12} = \dfrac{C_{AB_2}}{C_{AB}\cdot C_B}, & C_{AB_2} = K_{11}\cdot K_{12}\cdot C_A^{\circ}\cdot C_B{}^2 \\ \vdots & & \vdots \\ \mathrm{AB_{m-1}} + \mathrm{B} \overset{K_{1.m}}{\rightleftarrows} \mathrm{AB_m}, & K_{1.m} = \dfrac{C_{AB_m}}{C_{AB_{m-1}}\cdot C_B}, & C_{AB_m} = K_{11}\cdot K_{12}\cdot \cdots K_{1.m}\cdot C_A^{\circ}\cdot C_B{}^{m} \end{array} \tag{12}$$

The apparent total solubility of A in the solution is the sum of the concentration of A which does form complexes, that is, the solubility of A in the absence of B at equilibrium, and the concentration of each complex species formed. Accordingly, Eq 13 is derived:

$$C_{At} = C_A^{\circ} + C_{AB} + C_{AB_2} + \cdot \cdots\cdots + C_{AB_m} \tag{13}$$

By combining Eqs. 12 and 13, we obtain:

$$C_{At} = C_A^{\circ}\{1+ \sum_{i=1}^{m} \overset{i}{\underset{j}{\pi}}={}_1K_j(C_B)^i\} \tag{14}$$

$$C_B = C_{Bt} - \{\sum_{i=1}^{m}(i)C_{AB_i}\} \tag{15}$$

where π denotes the consecutive product of each factor and $\sum$ is the sum notation. Eqs 14 and 15 are general equations which indicate the relationship between the total concentration of the drug dissolved, C_{At}, the total concentration of complexing agents added, C_{Bt}, and the stability constants, K_{1i} (i=1, 2,......m).

The apparent total solubility of A, C_{At}, can be expressed by the polynomial equation of Mth order with respect to C_{Bt}. Consequently, the best values for parameters C_A° and K_{1i} can be determined by the usual least-squares method. However, cases where the parameters had negative values occurred in applying this method. Therefore, we adopted the method of estimating the parameters under the conditions shown in Eq. 16, by the nonlinear optimization method. Choosing the sum of squares of residuals between experimental data and theoretically calculated values as an objective function, J, the transformation of variables as shown by Eq. 18 was carried out for Eq. 14 to find the optimal values of C_A° and K_{1i} (i=1, 2,......m) which minimize J subject to the positivity conditions shown in Eq. 16.

$$K_{1i} > 0\,(i=1,\cdots\cdots m),\ C_{Bt} > 0,\ C_A^{\circ} > 0 \tag{16}$$

$$J = \sum_{k=1}^{n} (C_{A\mathrm{obs}}^{(k)} - C_{A\mathrm{calc}}^{(k)})^2 \tag{17}$$

$$\ln C_{Bt} = X,\ \ln C_A^{\circ} = Z_1,\ \ln K_{1i} = Z_{i+1}(i=1,\cdots m) \tag{18}$$

After the positivity constraints were eliminated by this transformation of variables, the optimal parameters could be found by using the Gauss-Newton method[6)] and the conjugate gradient method.[7)] With these methods, we calculated the theoretical values of apparent total solubility for the solubilization data of bendroquinone with sodium benzoate and several phenol derivatives and compared the calculated and experimental results.

Experimental

Material——Bendroquinone was synthesized by the staff of the Synthesis Division of Shionogi Research Laboratory. All other chemicals (chemicals) were obtained from commercial sources.

Solubility Studies——Excess quantities of bendroquinone were placed into 5-ml ampuls, and 4 ml of solution of various concentrations of complexing agents was added. The ampuls were sealed and shaken vigorously for 5 h at room temperature and then agitated in a constant temperature water bath at 25°C for at least 20 h. After equilibration, a portion of the supernatant solution was withdrawn by means of a pipet fitted with a piece of rubber tubing plugged with cotton.

For xylene or carbon tetrachloride solutions, the clear portions were immediately diluted to appropriate volumes with 1,2-dichloroethane for spectrophotometric measurement. For aqueous solutions, the following procedure was carried out before dilution. The portions were extracted with 1,2-dichloroethane and each organic phase was separated by the use of Whatman liquid separating filter paper[8] (1 ps) from the aqueous solution.

The concentration of bendroquinone in 1,2-dichloroethane was determined spectrophotometrically using a Hitachi EPU-2A spectrophotometer at a wavelength of 340 nm.

Results and Discussion

(1) The solubility diagrams of bendroquinone in the presence of butyl-*p*-hydroxybenzoate(butylparaben), phenol, *p-tert*-butylphenol, butylated hydroxyanisole (BHA) and thymol in carbon tetrachloride are shown in Fig. 1.

The apparent solubility of bendroquinone showed a tendency to increase with increase in the concentration of complexing agent. When the apparent total solubility of bendroquinone, C_{At}, was plotted against the concentration of the complexing agents added, C_{Bt}, upward curves were obtained in all cases. Therefore, the formation of complexes of order higher than the 1: 1 complex seems to have occurred, as discussed in the previous section. The solid lines in Fig. 1 show the calculated curves obtained on the assumption both 1: 1 and 1: 2 complexes were formed. For all examples without phenol, experimental values of the apparent total solubility agreed approximately with the calculated results. Phenol is known to form dimers in carbon tetrachloride,[9] which may be responsible for the difference. We did not examine this point further.

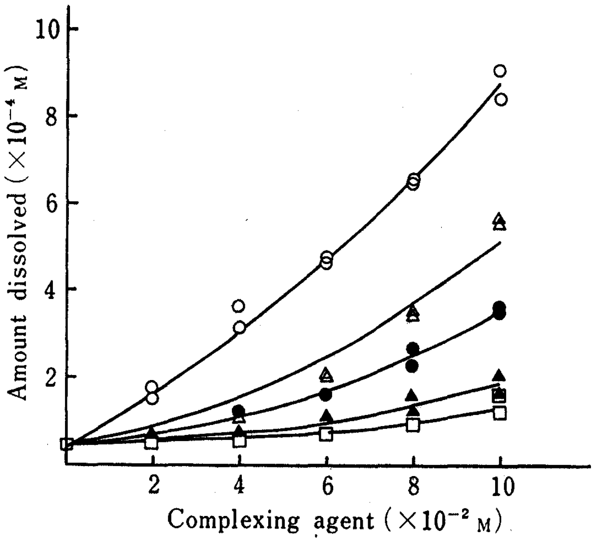

Fig. 1. Solubility Diagrams of bendroquinone in the Presence of Complexing Agents in Carbon Tetrachloride at 25°C

—○—: butyl *p*-hydroxybenzoate (butylparaben).
—●—: *p-tert*-butylphenol.
—△—: phenol.
—▲—: butylated hydroxyanisole (BHA).
—□—: thymol.
The solid lines are calculated curves.

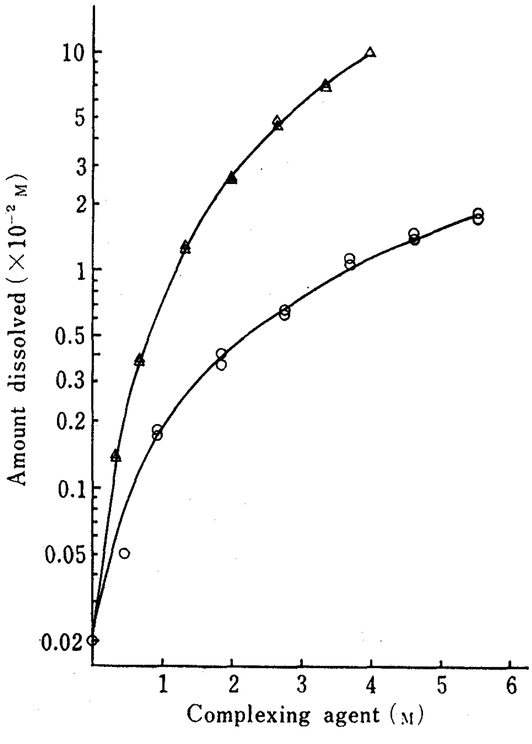

Fig. 2. Solubility Diagrams of bendroquinone in the Presence of High Concentrations of Complexing Agents in Xylene at 25°C

—△—: *m*-cresol.
—○—: thymol.
The solid lines are calculated curves.

(2) The solubility diagrams of bendroquinone in the presence of high concentrations of *m*-cresol and thymol in xylene are shown in Fig. 2.

The solid lines show the calculated curves obtained on the assumption that both 1 : 1 and 1 : 2 complexes were formed. Since the experimental values of the apparent total solubility agreed with the calculated results, it appeared reasonable to assume that the formation of complexes up to the second order did take place.

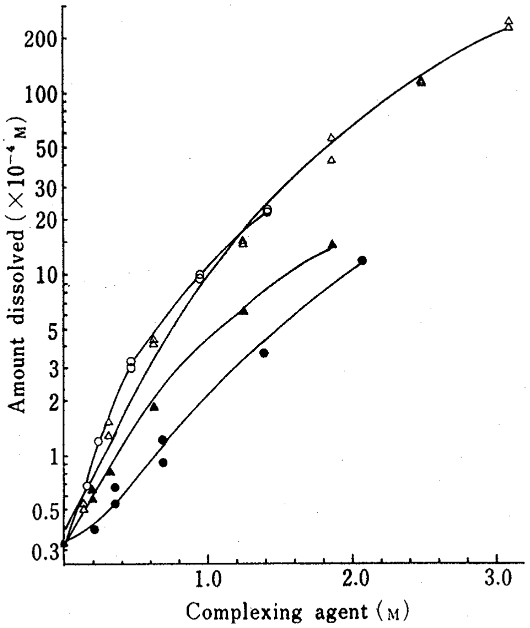

Fig. 3. Solubility Diagrams of bendroquinone in the Presence of High Concentrations of Complexing Agents in Water at 25°C

—△—: Na salicylate.
—○—: Na *p*-NH_2 salicylate.
—▲—: Na *p*-OH benzoate.
—●—: Na benzoate.
The solid lines are calculated curves.

(3) The solubility diagrams of bendroquinone in the presence of high concentrations of sodium salicylate, sodium *p*-aminosalicylate, sodium *p*-hydroxybenzoate and sodium benzoate in aqueous solutions are shown in Fig. 3.

The apparent total solubility of bendroquinone was increased significantly by the addition of these solubilizing agents. A relatively good agreement was found between the experimental values of the apparent total solubility and the calculated results on the assumption that both 1:1 and 1:2 complexes were formed with sodium *p*-aminosalicylate and *p*-hydroxybenzoate and that 1: 1, 1: 2 and 1: 3 complexes were formed with sodium salicylate and sodium benzoate. These assumptions seem to be reasonable on the basis of the above discussion.

(4) When the apparent total solubility of bendroquinone was plotted against the concentration of complexing agents added, upward curves were obtained in all experiments. Accordingly, it appeared to be essential to consider the formation of 1: 2 or higher order complexes, as discussed in the previous section.

Therefore, we first estimated the parameters on the assumption that both 1: 1 and 1: 2 complexes were formed in all cases using the regression line, Gauss-Newton and conjugate gradient methods.

In the case of the regression line method, we considered only the stability constants, K_{11} and K_{12}, as the unknown parameters.

In the latter two cases, we also considered the original solubility, C_{A}°, as an unknown parameter. The results obtained are summarized in Table I.

Though there were unsatisfactory cases where the parameters gave negative values with sodium benzoate, sodium salicylate and phenol in carbon tetrachloride when the regression line method was applied, we obtained reasonable values for the remaining 17 cases. In addition, the calculated concentrations of bendroquinone in the presence of complexing agents almost coincided with the experimental results.

Using the Gauss-Newton method, the converged parameters could not be obtained for 11 among 20 experiments, while for seven other examples, reasonable parameter values were found. However, with higher concentrations of thymol, differences of two to three times were found between calculated and experimental values of the original solubility C_{A}°. thus raising some doubts about the estimated parameters.

The conjugate gradient method gave converged parameters in all cases and the estimates appeared reasonable, except for those for sodium benzoate and sodium salicylate.

TABLE I. Parameters estimated by the Regression Line (R. L.), Gauss-Newton (G. N.) and Conjugate Gradient (C. G.) Methods

Complexing Agent	Solvent	R. L. Method			G. N. Method			C. G. Method		
		$C_{\dot{\mathrm{A}}}$[a] ($\times 10^{-5}$ M)	K_{11}	K_{12}	$C_{\dot{\mathrm{A}}}$[b] ($\times 10^{-5}$ M)	K_{11}	K_{12}	$C_{\dot{\mathrm{A}}}$[b] ($\times 10^{-5}$ M)	K_{11}	K_{12}
1. Na benzoate	Water	3.18	−1.20[c]	−6.55[c]	—	—	—	1.67[c]	1.74[c]	7.52[c]
2. Na *p*-OH benzoate	Water	3.18	1.68	6.48	—	—	—	3.39	2.05	4.93
3. Na salicylate	Water	2.25	−30.6[c]	−3.19[c]	—	—	—	6.20[c]	3.71[c]	8.58[c]
4. Na *p*-NH$_2$ salicylate	Water	3.10	4.21	7.31	—	—	—	3.34	4.19	6.81
5. Thymol	Xylene	20.3	5.46	5.81	68.9[c]	0.07[c]	136[c]	22.7	5.29	5.37
6. Thymol	Xylene	19.8	4.87	6.70	44.2[c]	0.52[c]	29.6[c]	22.6	5.28	5.37
7. *m*-Cresol	Xylene	19.8	62.5	0.35	—	—	—	21.7	75.4	0.20
8. Phenol	CCl$_4$	4.29	−11.6[c]	−112[c]	—	—	—	4.51	27.5	28.2
9. *p*-*tert*-Butylphenol	CCl$_4$	4.62	20.2	21.6	—	—	—	3.79	23.5	25.2
10. Thymol	CCl$_4$	3.70	8.18	19.2	—	—	—	3.32	7.90	27.3
11. Butylparaben	CCl$_4$	4.91	111	5.27	5.15	107	5.01	3.68	149	5.46
12. BHA	CCl$_4$	3.88	12.5	19.7	3.41	21.5	9.93	2.95	15.4	24.9
13. Butylparaben	Xylene	19.7	47.8	4.05	18.6	52.7	3.57	18.9	47.7	4.93
14. BHA	Xylene	19.5	16.4	0.77	—	—	—	19.9	15.7	0.79
15. Phenol	Xylene	19.2	19.6	5.12	21.2	12.7	10.8	18.4	20.7	5.33
16. *p*-*tert*-Butylphenol	Xylene	19.2	14.8	4.01	20.8	9.77	9.29	18.9	14.8	4.59
17. *m*-Cresol	Xylene	19.2	13.3	3.41	21.0	7.95	9.84	18.1	14.4	3.70
18. Thymol	Xylene	20.4	7.8	4.49	19.0	12.2	0.22	20.9	6.82	5.70
19. *β*-Naphthol	Xylene	22.1	12.2	9.74	—	—		19.9	15.3	8.99
20. *α*-Naphthol	Xylene	22.1	27.8	0.30	—	—		23.3	25.6	0.30

a) Experimental value of the original solubility.
b) Calculated value of the original solubility.
c) The estimates are assumed to be incorrect.
d) Dimension of K_{11}, K_{12}, K_{13}: (M^{-}).

The validity and reliability of the parameters evaluated were examined by comparing estimates of C_A° with the experimental values, by investigating whether the calculated total solubility of bendroquinone in various concentrations of complexing agents agreed with the experimental results or not, and also by checking the magnitude of the sum of the squares of the residuals. The parameters obtained with these three different estimation methods agreed well with each other.

(5) Next, we estimated the parameters by the conjugate gradient method on the assumption that 1: 1, 1: 2, and 1: 3 complexes were formed in all cases. We initially considered the stability constants, K_{11}, K_{12}, K_{13} and the original solubility, C_A°, as the unknown parameters then considered only the stability constants as the unknown parameters.

The results obtained are summarized in Table II.

TABLE II. Parameters estimated by the Conjugate Gradient Method on the Assumption that 1: 1, 1: 2 and 1: 3 Complexes were Formed

Complexing agent	Solvent	(A)				(B)		
		C_A°[a] ($\times 10^{-5}$ M)	K_{11}	K_{12}	K_{13}	K_{11}	K_{12}	K_{13}
1. Na benzoate	Water	1.79[b]	1.20[b]	3.03[b]	1.37[b]	1.03	2.09	1.19
2. Na *p*-OH benzoate	Water	3.33	2.10	2.02	0.87	1.79	1.82	1.35
3. Na salicylate	Water	4.11 3.33	2.06 1.75	3.15 3.07	2.44 3.88	2.81	3.74	2.83
4. Na *p*-NH_2 salicylate	Water	3.79	3.75	2.15	1.67	3.49	2.64	1.82
5. Thymol	Xylene	15.7	15.6	2.05	0.06	12.3	1.95	0.08
6. Thymol	Xylene	14.8	14.8	2.29	0.08	12.1	2.05	0.08
7. *m*-Cresol	Xylene	24.8	74.9	0.10	0.10	92.2	0.12	0.07
8. Phenol	CCl_4	5.01	34.2	9.11	9.59	38.8	9.86	9.95
9. *p*-*tert*-Butylphenol	CCl_4	4.26	26.2	9.20	9.62	24.5	8.82	9.43
10. Thymol	CCl_4	4.02	7.87	9.23	9.64	8.36	10.3	10.2
11. Butylparaben	CCl_4	3.55	144	2.64	18.5	107	2.35	17.2
12. BHA	CCl_4	3.30	17.1	9.07	9.55	14.7	8.23	9.12
13. Butylparaben	Xylene	21.4	45.2	2.83	1.98	48.8	29.8	30.0
14. BHA	Xylene	20.1	15.8	0.50	0.30	16.6	0.59	0.40
15. Phenol	Xylene	19.3	21.8	2.80	1.98	21.7	2.78	2.95
16. *p*-*tert*-Butylphenol	Xylene	19.4	15.2	2.83	1.98	15.3	2.82	2.96
17. *m*-Cresol	Xylene	18.7	15.0	1.91	1.99	13.3	2.72	2.94
18. Thymol	Xylene	10.3[b]	28.0[b]	2.47[b]	1.95[b]	8.58	2.24	2.82
19. *β*-Naphthol	Xylene	21.0	16.1	4.70	2.97	14.5	4.53	3.91
20. *α*-Naphthol	Xylene	23.4	25.7	0.10	0.10	28.1	0.10	0.20

a) Calculated value of the original solubility.
b) The estimates are assumed to be incorrect.
c) For (B), we considered only stability constants K_{11}, K_{12} and K_{13} as the unknown parameters and for (A), both the stability constants and the original solubility as unknowns.
d) Dimension of K_{11}, K_{12}, K_{13} : (M^{-1}).

By assuming the presence of the 1: 3 complex besides the 1: 1 and 1: 2 complexes, the relatively significant discrepancy observed with sodium salicylate was reduced, and better parameter values were obtained. With sodium *p*-hydroxybenzoate, *p*-*tert*-butylphenol, thymol, butyl *p*-hydroxybenzoate, butylated hydroxyanisole, phenol, *m*-cresol, *α*-naphthol and *β*-naphthol, good parameter values were obtained if the formation of up to 1: 3 complexes was assumed to take place, and a good agreement was obtained between the experimental values of the apparent total solubility and the calculated results. These results are nearly equal in magnitude in the case of the stability constant K_{11} but slightly different in that of K_{12} as compared with the case where the formation of only 1: 1 and 1: 2 complexes was assumed to occur.

Whether or not we must consider the presence of the 1: 3 complex as well as the 1: 1 and 1: 2 complexes is an interesting question. Bendroquinone possesses four functional groups, *e.g.*, two carbonyl groups, one hydroxyl group and one imino group, in a single molecule as shown in Chart 1, and these groups may be interaction sites. Consequently, if all these functional groups participate in the interaction, the formation of complexes up to the fourth order can be assumed to occur. If we can not prove the formation of 1: 3 or higher order complexes by any other method, it is not convincing to demonstrate their presence only by fitting data numerically to a model.

Furthermore, it seems inappropriate to increase the number of parameters and make the model more complex if estimation of correct values of stability constants of these higher order complexes is of no significance. On the other hand, with sodium *p*-aminosalicylate, thymol, *m*-cresol and phenol, greater differences were found between the experimental and estimated values of C_{A}°, when we assumed the formation of the 1: 3 complex. Also, with sodium benzoate, the difference between the experimental and calculated C_{A}° values was large even when we took account of the formation of the 1:3 complex, considering K_{11}, K_{12}, K_{13} and C_{A}° as the unknown parameters.

Therefore, the following method was used. We assumed the experimental value of C_{A}° to be correct and set it constant, then estimated the parameters under the assumption that up to 1: 3 complexes were formed. Comparison of these two methods showed that approximately the same estimates were obtained for all examples without thymol. Consequently, if we have confidence in the experimental C_{A}° values, it seems reasonable to set C_{A}° constant, consider only the stability constants as the unknown parameters, and then estimate the parameter values. Thus, for sodium benzoate, we considered estimates of stability constants obtained by such a method to be correct. The criterion for convergency was chosen as $\left|\frac{J_{i+1}-J_i}{J_i}\right| \leq 10^{-5}$ in the conjugate gradient method, and as $\left|\frac{\theta_{i+1}-\theta_i}{\theta_i}\right| \leq 10^{-4}$ in the Gauss-Newton method. Initial estimates were evaluated by trial and error.

Acknowledgement The authors are greatly indebted to Mr. Naohiro Yoshitani and Mr. Kazumasa Hayashi of the Computer Center of Shionogi Co., Ltd. for their help with the computer programming.

References and Notes

1) A part of this paper was presented at the 96th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April 1976.
2) Present address: *Kyoto College of Pharmacy, Misasagi-Nakauchi-cho 5, Yamashina-ku, Kyoto 607, Japan.*
3) K.A. Connors and J.A. Mollica, Jr., *J. Pharm. Sci.*, **59**, 1597 (1970).
4) T. Higuchi and H. Kristiansen, *J. Pharm. Sci.*, **59**, 1601 (1970); K. Kakemi, H. Sezaki, T. Mitsunaga, and M. Nakano, *ibid.*, **59**, 1597 (1970).
5) G.E. Blau, R.R. Klimpel, and E.C. Steiner, *Ind. Eng. Chem. Fundam.*, **9**, 334 (1970).
6) W.E. Deming, "Statistical Adjustment of Data," John Wiley and Sons, Inc., New York, 1946.
7) R. Fletcher and M.J.D. Powell, *The Computer Journal*, **6**, 163 (1963).
8) W. & R. Balston Ltd., England.
9) J. Meier and T. Higuchi, *J. Pharm. Sci.*, **54**, 1183 (1965).