Spectrophotometric Investigation of the Formation of Association Complexes between Alizarin Red S and β-Cyclodextrin

Toshiki TAYA, Nobuko FUKUI, Kunio DOI and Makoto OTOMO

Department of Applied Chemistry, Nagoya Institute of Technology, Gokiso, Showa, Nagoya 466, Japan

The acid-base equilibria of Alizarin Red S (ARS) were investigated spectrophotometrically at 25°C and µ=0.2 in the presence of β-cyclodextrin (β-CD). In the presence of β-CD, the dissociation of the first proton (β-OH) of ARS is slightly enhanced and that of the second one (α-OH) is significantly suppressed. All the ionic species of ARS interact with β-CD to form 1:1 associated complexes, the stabilities of which decrease in the order: HL-HA>H2L-HA>L-HA>L-A, where L and A denote the fully deprotonated form of ARS and the deprotonated form of β-CD, respectively. The distribution curves for all the species present in the ARS-β-CD system are given as a function of the pH of the solution.

Keywords Alizarin Red S, β-cyclodextrin, acid-base equilibria, inclusion complex, spectrophotometry

Alizarin Red S (ARS) is a well-known α,β-dihydroxy anthraquinone dyestuff; it acts as a bidentate ligand in metal complexation. Although the ligand is not selective for metal ions, it has widely been used as a chromogenic reagent in the spectrophotometric determination of multivalent metal ions. The acid-base characteristics of the ligand have also been described.1,2 Cyclodextrins form complexes in aqueous solutions with azo dyes, nitrophenols and many other substances.3 The complexes formed are usually regarded as inclusion compounds in which hydrogen bonding4, van der Waals forces5 and hydrophobic interactions6,7 are the main binding forces. Cramer et al.8 studied thermodynamically the binding of a series of azo dyes with α-cyclodextrin by means of spectrophotometry; they proposed a mechanism in which the dyes are enclosed in the cyclodextrin ring. They also found the stereospecificity of the complex formation which indicates the formation of inclusion compound. It is of interest, especially expecting improved selectivity of ARS in the spectrophotometric determination of metals, to investigate the stability and spectral characteristics of the metal-ARS complexes formed in the presence of cyclodextrins. In this paper, as a starting point, we have studied the acid-base equilibria of ARS spectrophotometrically in the presence and absence of β-cyclodextrin (β-CD). The association constants for all the complex species present in the solution have also been determined.

Experimental

Reagents
ARS (purchased from E. Merck Co.) was purified by recrystallization from 60% (v/v) aqueous ethanol. The purity as a form of monosodium salt-monohydrate was ensured by pH titration with a standard potassium hydroxide solution. Commercial β-cyclodextrin (Wako Pure Chemical Industries, Ltd.) was recrystallized twice from hot water and dried under vacuum at 80°C. Other chemicals (reagent grade) were used without further purification.

All the measurements were made at an ionic strength of 0.2 M (KNO3) and 25.0°C.

Potentiometric measurements
The pH titration of β-CD was carried out with a Toko TP-1000 pH meter. The glass electrode was calibrated by titration with potassium hydroxide. The resulting calibration graph was used to evaluate the −log[H+] values from the observed pH readings. A 1.52×10−2 M β-CD solution (M=mol dm−3) was titrated with 0.2 M KOH. The pKw value was taken as 13.77 in the data analysis.9 The mixed acid dissociation constant of β-CD was evaluated by taking account of the activity coefficient of hydrogen ion (fH+=0.747)10 at µ=0.2.

In spectrophotometric measurements the glass electrode used was standardized against standard JIS buffer solutions: phthalate (pH 4.01), phosphate (pH 6.87), borax (pH 9.18) and carbonate (pH 10.01).

Spectrophotometric measurements
Both the absorption spectra and absorbances of the test solutions were measured by using a Hitachi U-3400 recording spectrophotometer equipped with a thermostated (25.0±0.1°C) cell compartment and matched 1.00-cm quartz cells.

The determination of the proton dissociation constants of ARS in the presence and absence of β-CD was carried out with 8.0×10−3 M ARS solutions at varying pH values. In the determination of the association constants of ARS with β-CD, the solutions containing
ARS at a constant concentration and β-CD at different total concentrations in the range 0–2.3×10^{-2} M were prepared and adjusted to a fixed pH value.

**Calculations**

The proton dissociation constant of β-CD was evaluated by minimizing the sum of the squares of the deviations: \( U=\sum(\log[H^+]_{\text{obs},i}-\log[H^+]_{\text{calc},i})^2 \), using nonlinear regression with the aid of a computer.

In analysis of spectrophotometric data for the proton dissociation of ARS and for the association of ARS with β-CD, the sum of the squares of the deviations was defined as \( U=\sum(A_{\text{obs},i}-A_{\text{calc},i})^2 \). For a comparison, the proton dissociation and association constants were also evaluated by the usual methods of log-log plot and Benesi–Hildebrand plot, respectively. The results obtained graphically were in good agreement with those obtained by the nonlinear regression analysis.

The association constants for the H2L-HA and HL-HA complexes, where H2L and HA denote ARS and β-CD, respectively, were obtained by the analysis of absorbance data for the solutions adjusted at pH 3.2 and 8.9 and with varying concentrations of β-CD. Similarly, the apparent association constant, \( K(L)A \), of ARS with β-CD was determined at different pH values in the range 11.0–13.3. The association constants for the L-HA and L-A complexes were refined from the pH dependence of \( K(L)A \) by using the nonlinear regression analysis, in which \( U=(K(L)A_{\text{obs},i}-K(L)A_{\text{calc},i})^2 \).

**Results**

The equilibrium relationships between various species of ARS in the presence and absence of β-CD are illustrated in Fig. 1. The corresponding equilibrium constants determined in this work are listed in Table 1. Preliminary spectral data clearly showed that all the ionic species of ARS (H2L, HL and L; charges are omitted) associated with β-CD in the form of HA or A, depending on the pH of the solution. The association of the L form of ARS with HA and A forms of β-CD was confirmed by the fact that \( K(L)A \) approached a constant positive value at pH values higher than 13.

![Fig. 1 Scheme for equilibrium relationships between each species of ARS in the presence and absence of β-CD.](image-url)

**Table 1 Equilibrium constants for ARS and β-CD and their association complexes at 25°C**

<table>
<thead>
<tr>
<th>Equilibrium</th>
<th>Constant</th>
<th>( pK_a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2L ⇌ HL + H</td>
<td>( pK_a )</td>
<td>5.57±0.02</td>
</tr>
<tr>
<td>HL ⇌ L + H</td>
<td>( pK_a )</td>
<td>10.67±0.03</td>
</tr>
<tr>
<td>HA ⇌ A + H</td>
<td>( pK_{HA} )</td>
<td>11.83±0.06</td>
</tr>
<tr>
<td>H2L + HA ⇌ (HL)HA</td>
<td>( \log K_{H2LHA} )</td>
<td>2.30±0.08</td>
</tr>
<tr>
<td>HL + HA ⇌ (HL)HA</td>
<td>( \log K_{HLHA} )</td>
<td>2.61±0.04</td>
</tr>
<tr>
<td>L + HA ⇌ (L)HA</td>
<td>( \log K_{LHA} )</td>
<td>1.9±0.1</td>
</tr>
<tr>
<td>L + A ⇌ (L)A</td>
<td>( \log K_{LA} )</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td>(H2L)HA ⇌ (HL)HA + H</td>
<td>( pK_{H2LHA} )</td>
<td>5.26</td>
</tr>
<tr>
<td>(HL)HA ⇌ (L)HA + H</td>
<td>( pK_{HLHA} )</td>
<td>11.36</td>
</tr>
<tr>
<td>(L)HA ⇌ (L)A + H</td>
<td>( pK_{LHA} )</td>
<td>12.27</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>( pK_{HA} ) expression</th>
<th>( pK_{HA} ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Mixed constant including the activity of hydrogen ion.</td>
<td></td>
</tr>
<tr>
<td>b. ( pK_{HA} = pK_a + \log K_{H2LHA} - \log K_{HLHA} )</td>
<td></td>
</tr>
<tr>
<td>c. ( pK_{HA} = pK_a + \log K_{HLHA} - \log K_{LHA} )</td>
<td></td>
</tr>
<tr>
<td>d. ( pK_{HA} = pK_{HA} + \log K_{HLHA} - \log K_{LHA} )</td>
<td></td>
</tr>
</tbody>
</table>

**Determination of \( pK_{HA} \), \( pK_a \) and \( pK_2 \)**

In strongly alkaline solution one of the secondary hydroxyl groups of β-CD molecule is dissociated into an alcololate anion acting as a monoacidic base:

\[ \text{HA} \Leftrightarrow \text{A} + \text{H}^+ \]

In this study, the proton dissociation constant was determined potentiometrically. Combination of the proton dissociation constant of β-CD, defined as \( K_{HA}=[\text{A}][\text{H}^+] / [\text{HA}] \), and the stoichiometric relations leads to Eq. (1):

\[ (C_H - C_{OH} - C_A)K_{HA}[\text{H}^+]^2 + (C_H - C_{OH} + K_aK_{HA})[\text{H}^+] + K_a = 0, \]  

(1)

where \( C_H \), \( C_{OH} \) and \( C_A \) are the analytical concentrations of hydrogen ion, hydroxide ion and β-CD, respectively. The value of \(-\log[H^+]\) of a solution was calculated from Eq. (1). The value of \( pK_{HA} \) was then refined by minimizing the sum of the squares of the deviations. Taking account of the activity coefficient of hydrogen ion, we obtain 11.83 for the value of \( pK_{HA} \) defined in terms of the activity.

The proton dissociation constants of ARS were determined spectrophotometrically (using 8.0×10^{-3} M ARS) and found to be 5.57 for \( pK_1 (\beta-OH) \) and 10.67 for \( pK_2 (\alpha-OH) \). Beer's law was obeyed for aqueous solutions of ARS over the concentration (less than 2×10^{-4} M) and pH ranges examined.

**Determination of \( K_{H2LHA} \) and \( K_{HLHA} \)**

Figures 2 and 3 show the absorption spectra of ARS at pH 3.2 and 8.6 in the absence and presence of β-CD. Although spectral variation is relatively small in both systems, the absorption bands around 420 nm for H2L and around 320 nm for HL shift toward longer wavelengths with increasing concentrations of β-CD.
The isosbestic points appearing at about 426 nm and about 399 and 576 nm, for the H₂L-HA and HL-HA systems, respectively, indicate equilibria:

\[
K(H_L)HA = \frac{[H_nL]HA}{[H_nL] \times [HA]} \quad (n=2 \text{ at pH 3.2 and } n=1 \text{ at pH 8.6}).
\]

The absorbance of the solution, \(A_{\text{cal}}\), at a selected wavelength is calculated by

\[
A_{\text{cal}} = \varepsilon_{H_{n}L} [H_{n}L] + \varepsilon_{HL,HA} [HA] ,
\]

where \(\varepsilon_{H_{n}L}\) and \(\varepsilon_{HL,HA}\) are the molar absorptivities of the species \(H_nL\) and \((HL)_{HA}\), respectively. Since \(\varepsilon_{H_{n}L}\) is a measurable quantity, the values of \(K(\text{HL,HA})\) and \(\varepsilon(\text{HL,HA})\) can be refined to give the minimum error square sum, \(U=(A_{\text{obs,i}}-A_{\text{cal,i}})^2\). The values thus obtained were 2.30 for log \(K(\text{HL,HA})\) and 2.61 for log \(K(\text{HL,HA})\). In Fig. 4, experimental absorbance data are plotted against the logarithmic concentration of \(\beta\)-CD in solution. All the experimental points lay on the calculated curve.
In order to confirm the observed values of $K_{(HL)HA}$, a linear relationship, i.e., Benesi–Hildebrand plot, was also employed which related the observed spectral changes to the added concentrations of $\beta$-CD. The results gave 2.29 for log $K_{(HL)HA}$ and 2.61 for log $K_{(HL)HA}$, which are consistent with the values obtained above.

**Determination of $K_{(L)A}$ and $K_{(L)HA}$**

Figure 5 shows the absorption spectra of ARS (5.34×10^{-5} M) at pH 13.1 in the absence and presence of $\beta$-CD. The appearance of two related isosbestic points (494 and 612 nm), which are observed by increasing concentrations of $\beta$-CD, suggests a 1:1 apparent equilibrium involving the following associations:

$$
H_nL + H_mA \rightleftharpoons (H_nL)H_mA,
$$

where

$$
K_{(HL)HmA} = \left[ (H_nL)H_mA \right] / [H_nL][H_mA],
$$

The 1:1 equilibrium constant, i.e., the apparent association constant of ARS with $\beta$-CD, is defined as

$$
K_{(L)A} = \left[ (L)A' \right] / [L'][A'],
$$

where

$$
[L'] = [L](1 + [H^+]/K_{a2}),
$$

$$
[A'] = [A](1 + [H^+]/K_{aHA}),
$$

The absorbance of the solution is then given by

$$
A_{abs} = \varepsilon'[L'] + \varepsilon_{HL}A'[L']',
$$

where $\varepsilon'$ and $\varepsilon_{HL}'$ are the apparent molar absorptivities of ARS and the associated complex, respectively. As the values of $\varepsilon_{HA}$ and $\varepsilon_{L}A$ are constant at a given pH, the values of the apparent association constant, $K_{(L)A}$, and $K_{(L)HA}$ can be determined by means of a nonlinear regression method. The absorbance measurements were made at eight different pH values with a fixed ARS concentration and varying concentrations of $\beta$-CD. The results (denoted by log $K_{(L)A,obs}$) shown in Table 2 indicate a gradual decrease in the $K_{(L)A}$ value with increasing pH values.

On the other hand, Eq. (10) shows that the values of $K_{(L)A}$ at arbitrary pH values can also be calculated by assuming the $K_{(HL)A}$, $K_{(L)HA}$ and $K_{(L)A}$ values properly. It was suggested, however, that the concentration of $(HL)_A$ formed was so small (less than one tenth that of $(HL)_HA$) under our experimental conditions that the values of $K_{(HL)A}$ and $K_{(L)HA}$ could not be estimated accurately. In this study, therefore, we assumed tentatively log $K_{(HL)A}$ to be 2.30 by taking account of an electrostatic repulsion between negatively charged $HL$ and $A$ ions and then we took $\varepsilon_{HL}A$ as 5770 (at 556 nm), which is identical to the value of $\varepsilon_{HL}A$. The values of $K_{(HL)A}$ and $K_{(L)HA}$ were then refined to give the minimum error square sum. The results gave log $K_{(L)A}=1.48$ and log $K_{(L)HA}=1.92$. As shown in Table 2, the values of $K_{(L)A}$ calculated at different pH values with these constants well reproduced the observed values, implying the validity of the above evaluations for log $K_{(HL)A}$ and $\varepsilon_{HL}A$.

**Proton dissociation of ARS in the presence of $\beta$-CD**

**Determination of $K_{a1}$**

The absorption spectra of ARS (8×10^{-5} M) were obtained in the pH range 3.4–8.0 in the presence of 2.3×10^{-2} M $\beta$-CD, the absorption maxima

![Absorption spectra for ARS-$\beta$-CD system at pH 13.1.](image)

(1) $C_1=5.34\times10^{-5}$ M; (2) $C_1=5.34\times10^{-5}$ M, $C_A=9.97\times10^{-3}$ M; (3) $C_1=5.34\times10^{-5}$ M, $C_A=7.00\times10^{-3}$ M.

**Table 2** log $K_{(L)A}$ values at different pH values

<table>
<thead>
<tr>
<th>pH</th>
<th>log $K_{(L)A,obs}$</th>
<th>log $K_{(L)A,cal}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.63</td>
<td>(2.61)*</td>
<td></td>
</tr>
<tr>
<td>11.05</td>
<td>2.20</td>
<td>2.22</td>
</tr>
<tr>
<td>11.52</td>
<td>1.97</td>
<td>2.00</td>
</tr>
<tr>
<td>11.81</td>
<td>1.91</td>
<td>1.87</td>
</tr>
<tr>
<td>12.10</td>
<td>1.79</td>
<td>1.75</td>
</tr>
<tr>
<td>12.57</td>
<td>1.60</td>
<td>1.60</td>
</tr>
<tr>
<td>12.86</td>
<td>1.56</td>
<td>1.56</td>
</tr>
<tr>
<td>13.06</td>
<td>1.53</td>
<td>1.53</td>
</tr>
<tr>
<td>13.33</td>
<td>1.49</td>
<td>1.50</td>
</tr>
</tbody>
</table>

a. log $K_{(HL)HA}$. 

$$
$$

It is clear from Eqs. (8) – (10) that $K_{(L)A}$ is expressed as a function of pH and is a constant at a fixed pH value.
being located at 425 and 525 nm. The appearance of isosbestic points at 378 and 459 nm indicates an apparent equilibrium. The equilibrium concentrations of diprotonated and monoprotonated forms of ARS are given by

$$[\text{H}_2\text{L}'] = [\text{H}_2\text{L}] + [(\text{H}_2\text{L})\text{HA}],$$

(12)

and

$$[\text{HL}'] = [\text{HL}] + [(\text{HL})\text{HA}],$$

(13)

and the apparent proton dissociation constant, $K_a'$, is represented as

$$K_a' = \frac{[[\text{HL}'][\text{H}^+]]}{[\text{H}_2\text{L}']} = \frac{K_{\text{HL}HA}[\text{HA}]}{1 + K_{\text{HL}HA}[\text{HA}]}.$$  

(14)

Equation (14) shows that the values of $K_a'$ can be computed as a function of the concentration of β-CD by using the known values of $K_{\text{HL}HA}$ and $K_{\text{HL}JA}$; e.g., the value of $K_a'$ is found to be 5.35 in reciprocal logarithmic units in the presence of $2.3 \times 10^{-2}$ M β-CD.

The absorbance of the solution under investigation is given by

$$A_{\text{abs}} = \varepsilon_{\text{HL}}[\text{H}_2\text{L}] + \varepsilon_{\text{HL}}'[\text{H}_2\text{L}'],$$

(15)

where $\varepsilon_{\text{HL}}$ and $\varepsilon_{\text{HL}'}$ are the apparent molar absorptivities of the mixed species designated by subscripts and are expressed as a function of the concentration of β-CD. Thus the value of $K_a'$ can be obtained together with the values of $\varepsilon_{\text{HL}}$ and $\varepsilon_{\text{HL}'}$ from the experimental absorbance data by a nonlinear regression method. The $pK_a'$ value determined was 5.34 at $[\beta$-CD$]=2.3 \times 10^{-2}$ M β-CD.

The absorbance of the solution under investigation is given by

$$A_{\text{abs}} = \varepsilon_{\text{HL}}[\text{H}_2\text{L}] + \varepsilon_{\text{HL}'}[\text{H}_2\text{L}'],$$

(15)

where $\varepsilon_{\text{HL}}$ and $\varepsilon_{\text{HL}'}$ are the apparent molar absorptivities of the mixed species designated by subscripts and are expressed as a function of the concentration of β-CD. Thus the value of $K_a'$ can be obtained together with the values of $\varepsilon_{\text{HL}}$ and $\varepsilon_{\text{HL}'}$ from the experimental absorbance data by a nonlinear regression method. The $pK_a'$ value determined was 5.34 at $[\beta$-CD$]=2.3 \times 10^{-2}$ M β-CD.

The equilibrium concentration of β-CD is large enough and therefore the concentration of unassociated ARS is negligibly small*, Eq. (14) can be simplified into a logarithmic form:

$$pK_a - pK_a' = \log K_{\text{HL}JA} - \log K_{\text{HL}HA}.$$  

(16)

This relationship indicates that the decrease in the $pK_a$ value for H$_2$L, implying an enhancement of the first deprotonation of ARS by the complex formation with β-CD, can be expressed by the difference between logarithmic values of the two association constants, $\log K_{\text{HL}JA}$ and $\log K_{\text{HL}HA}$. The value of $pK_a'$ thus obtained was 5.26, which is smaller than that of $pK_a$ by 0.31.

Calculating $K_a'$ and evaluation of absorbance vs. pH curve. The absorption spectra of ARS were recorded in the pH range 8.0-12.9 in the presence of excess β-CD (1.3×10^{-2} M). The following total equilibrium concentrations were considered:

$$[\text{HL}'] = [\text{HL}] + [(\text{HL})\text{HA}] + [(\text{HL})\lambda],$$

and

$$[\text{L}'] = [\text{L}] + [(\text{L})\text{HA}] + [(\text{L})\lambda].$$

The apparent proton dissociation constant, $K_a'$, can be written as

$$K_a' = \frac{[\text{H}^'[\text{L}']]}{[\text{HL}']} = K_a \cdot \alpha,$$

(17)

where

$$\alpha = \frac{1 + [\text{A}](K_{\text{HL}HA}[\text{H}^+]/K_{\text{HA}A} + K_{\text{HL}JA})}{1 + [\text{A}](K_{\text{HL}JA}[\text{H}^+]/K_{\text{HA}A} + K_{\text{HL}JA})}.$$  

The value of $K_a'$ at a given pH value can easily be obtained from Eq. (17) by calculating the value of $\alpha$. The absorbance of the solution is calculated by the following equation:

$$A_{\text{abs}} = \varepsilon_{\text{HL}}[\text{HL}'] + \varepsilon_{\text{L}'}[\text{L}'],$$

(18)

where $\varepsilon_{\text{HL}}$ and $\varepsilon_{\text{L}'}$ are the apparent molar absorptivities of the mixed species designated by the subscripts. Because of the partial proton dissociation of β-CD in the pH range studied, the expression of Eq. (17), which contains the terms of hydrogen ion concentration, is somewhat more complicated than that of Eq. (14). The absorbance vs. pH curve for the system, therefore, is subject to a slight deviation from the symmetrical sigmoid curve as obtained for Eq. (15). The calculated absorbance curve (solid line in Fig. 6) is in reasonable agreement with the experimental absorbance data (open circles in Fig. 6), strongly suggesting the presence of the species (HL)A and (L)A, assumed in the above equilibria, and the validity of all the constants determined.

* Because of the limited water solubility of β-CD, it was impossible to obtain these conditions experimentally.

![Fig. 6 Absorbance vs. pH plot for ARS-β-CD system.](image)
Discussion

The formation of both 1:1 and 2:2 complexes has been pointed out for the association of naphthalene with β-CD.\(^{14}\) In the present ARS-β-CD system, however, well-defined isosbestic points in the spectral curves as well as linear Benesi-Hildebrand plots did not suggest the existence of any species other than the 1:1 complexes. The distribution curves for the ARS-β-CD association complexes are depicted in Fig. 7 as a function of the pH of the solution. The first proton dissociation of ARS is slightly enhanced, while the second one is considerably suppressed in the presence of β-CD. This means that among the associated complexes formed between ARS and β-CD, the (HL)\(_{HA}\) species is most stable. In fact, the experimentally determined association constants decreased in the order:

\[
K_{(HL)HA} > K_{(H_2L)HA} > K_{(L)HA} > K_{(L)_A}.
\]

It has been pointed out\(^{15-17}\) that the hydrophobic interaction between several organic substrates and the cavity of β-CD is important for the formation of inclusion complexes. In the present ARS-β-CD system, we assumed that the water structure around the quinoid oxygens of ARS is the dominant factor affecting the stability of the inclusion complexes. The formation of the most stable association complex between the HL form of ARS and HA is reasonably explained by assuming the formation of intramolecular hydrogen bond between the adjacent two phenolate oxygens of ARS molecule, which would eliminate unfavorable steric\(^{19,20}\) or electrostatic repulsive interactions possibly involved in the other inclusion systems. The association constants for 1 : 1 complexes formed with ARS and some metal ions including iron(III), aluminum(III) and gallium(III), which form a chelate ring through the adjacent two phenolate oxygens of ARS molecule, were found to be the same order of magnitude as that for the HL form of ARS, reasonably supporting the above interpretation. Detailed results on the formation of the inclusion complexes between metal-ARS chelates and β-CD will be described elsewhere.

References


(Received March 27, 1991)
(Received June 3, 1991)