Spectrophotometric Study of the Reactions of Proton and Copper(II) with Arsenazo I † in Aqueous Solution

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The systems of proton and/or copper(II) ion with Arsenazo I (H_6L) have been characterized at 25 °C and I = 1.0 mol dm^{-3} (KCl) using u.v.–visible spectrophotometry. The cumulative mixed protonation constants of the ligand L^{6-} are $\log \beta_{011} = 12.114(19)$, $\log \beta_{021} = 21.871(18)$, $\log \beta_{031} = 29.506(16)$, $\log \beta_{041} = 32.155(6)$, and $\log \beta_{051} = 34.298(6)$. Complexing reactions and equilibrium constants of the copper(II)–Arsenazo I system are given by (i)—(v) below. Both mixed and mononuclear

$$Cu^{2+} + H^+ + L^{6-} \rightleftharpoons [Cu(HL)]^{3-} \qquad \log \beta_{111} = 23.529(29) \qquad (i)$$

$$Cu^{2+} + L^{6-} \rightleftharpoons [CuL]^{4-} \qquad \log \beta_{101} = 16.797(58) \qquad (ii)$$

$$2Cu^{2+} + 2H^+ + L^{6-} \rightleftharpoons [Cu_2(H_2L)] \qquad \log \beta_{221} = 31.878(31) \qquad (iii)$$

$$Cu^{2+} + HL^{5-} \rightleftharpoons [Cu(HL)]^{3-} \qquad \log K^{011}_{111} = 11.415(35) \qquad (iv)$$

$$2Cu^{2+} + H_2L^{4-} \rightleftharpoons [Cu_2(H_2L)] \qquad \log K^{021}_{221} = 10.007(36) \qquad (v)$$

complexes have been found, the former includes a diprotonated dinuclear species in solutions at higher metal-to-ligand ratios and hydrogen ion concentrations, the latter a monoprotonated mononuclear species prevailing at lower metal-to-ligand ratios and at higher pH values. Data have been analysed by using two computer programs: the least-squares program LETAGROPVRID, version SPEFO, and the program SQUAD. The experimental spectra are compared with the calculated ones and the constants are discussed in connection with those of similar compounds reported in the literature. Accurate molar absorption coefficients are computed and the resolved spectral bands are discussed and assigned. Tentative structures of the proposed complexes are given.

As a part of a study carried out in our Institute on systems containing hydrogen, metal, and organic dye ions, the results of a systematic investigation on the hydrogen–copper(II)–Arsenazo I system are reported.

The chemical properties of Arsenazo I, 3-[(2′-arsonophenyl)azo]-4,5-dihydroxy-2,7-naphthalenedisulphonic acid (H_6L), in aqueous media are little known.[1–9] In order to establish an appropriate chemical model to describe aqueous solutions containing Arsenazo I, hydrogen, and copper(II) ions, we have collected a large number of as precise data as possible by means of spectrophotometry and pH measurements.

Table 1. Initial concentration (C/mol dm^{-3}) and the measured pH of the solutions

Arsenazo I

C_L	pH	C_L	pH	C_L	pH
0.000 087 7	2.013	0.000 087 7	2.367	0.000 109 6	2.850
0.000 109 6	3.385	0.000 109 6	3.856	0.000 109 6	4.304
0.000 109 6	5.328	0.000 087 7	5.381	0.000 065 8	5.390
0.000 043 9	5.440	0.000 021 9	5.793	0.000 021 9	5.793
0.000 109 6	6.409	0.000 109 6	7.580	0.000 109 6	7.890
0.000 109 6	8.346	0.000 109 6	9.978	0.000 109 6	10.415
0.000 109 6	11.159	0.000 109 6	11.508	0.000 109 6	12.018
0.000 109 6	12.455	0.000 109 6	2.523		

Cu^{2+}–Arsenazo I

	C_M	C_L	pH
1	0.003 432 4	0.000 109 6	3.221
2	0.006 864 9	0.000 109 6	3.824
3	0.004 290 5	0.000 109 6	4.644
4	0.000 429 1	0.000 109 6	4.665
5	0.013 729 7	0.000 109 6	4.713
6	0.002 145 3	0.000 109 6	4.714
7	0.004 290 5	0.000 111 4	5.310
8	0.001 716 2	0.000 109 6	5.890
9	0.002 145 3	0.000 109 6	6.058
10	0.000 858 1	0.000 021 9	6.274
11	0.000 858 1	0.000 065 8	6.367
12	0.000 858 1	0.000 043 9	6.374
13	0.000 858 1	0.000 109 6	6.680
14	0.000 858 1	0.000 109 6	6.680
15	0.000 858 1	0.000 087 7	6.718
16	0.000 086 1	0.000 109 6	8.925
17	0.000 043 1	0.000 093 0	10.179
18	0.000 086 1	0.000 093 0	10.942
19	0.000 129 1	0.000 093 0	11.045

Experimental

Arsenazo I (Carlo Erba, RPE product), controlled by elemental (C,H,N) analyses, was dried. Stock solutions of copper(II) chloride were prepared by dissolving $CuCl_2 \cdot 2H_2O$ (BDH, AnalaR product) in doubly distilled water and the copper content was electrogravimetrically determined. All the other reagents and standard solutions (KCl, HCl, and KOH) were prepared, purified, and analysed as previously described.[10,11] The pH measurements, using a digital Radiometer PHM84 Research pH meter, were carried out as previously described[10] at 25 °C, I = 1.0 mol dm^{-3} (KCl). The pH and ionic strength values were maintained by addition of suitable amounts of KCl, HCl, and KOH. U.v.–visible spectra were measured on a Jasco spectrophotometer, model UVIDEC–505, equipped with a digital printer DP–101. Matched quartz cells of pathlength 1.0 cm, calibrated before use, were employed. Sample and reference compartments were thermostatted to 25.0 ± 0.1 °C by a Paratherm II (Julabo) electronic circulating water-bath. Al

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† 3-[(2′-Arsonophenyl)azo]-4,5-dihydroxy-2,7-naphthalenedisulphonic acid.

Supplementary data available (No. SUP 23360; 15 pp.): complete list of experimental and theoretical absorbance data, molar absorption coefficients calculated by the SQUAD program.

the spectrophotometric measurements were performed on solutions having both different pH and metal-to-ligand ratios. Spectra were measured for 23 different solutions of Arsenazo I at pH values in the range 2.013—12.455 and for 19 different solutions of the ligand–copper(II) system in the range 3.221—11.045. For each solution 55 absorbance data (Arsenazo I, 260—800 nm) and 37 absorbance data [Arsenazo I–copper(II) system, 340—700 nm], to the third decimal place, were collected on a digital printer with a stepping of 10.0 nm. Initial concentrations and pH values of the solutions employed at the chosen ionic strength are reported in Table 1.

Results and Refinement

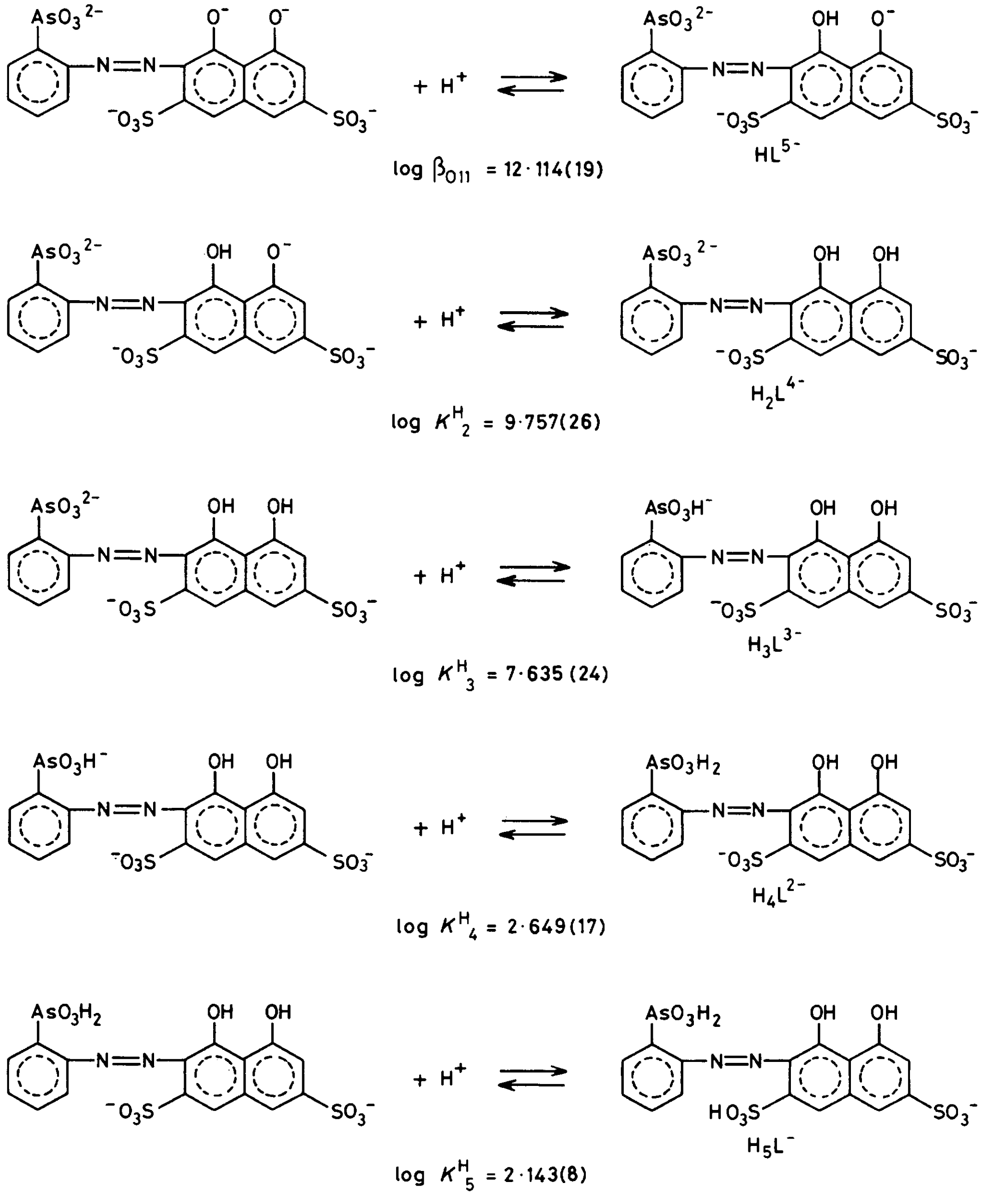
The mixed protonation constants of Arsenazo I at 25 °C and $I = 1.0$ mol dm^{-3} (KCl) are given below.

On the basis of the results and by analogy with the known values for some azo-derivatives[12–15] log β_{011} and log K_2^H can be assigned to the protonated species HL^{5-} and H_2L^{4-} respectively (see below). Moreover, the stepwise protonation equilibria and corresponding log K_3^H and log K_4^H can be referred to the H_3L^{3-} and H_4L^{2-} species, because the respective values are comparable with those of the AsO_4^{3-} ion. Log K_5^H refers to the protonation of the sulphonic group. The mixed protonation constants have been obtained through the refinement of several measured sets of absorbance data with two different computer programs, LETAGROPVRID–SPEFO[16] and SQUAD.[17]

The mass-balance equations to be solved for each solution at known pH are equation (2) for only protonation and equations (1) and (2) for complex formation. The best procedure of refinement was the selected one: first, starting from trial values of β_{pqr}, calculated by graphical methods, it was possible to obtain approximate values for the molar absorption coefficient ε_{pqr} in the range 260—800 nm; second, all the ε_i values were fixed and the stability constants (β_{011}, β_{021}, β_{031},

$$T_M = [M] + \Sigma p\beta_{pqr}[M]^p[H]^q[L]^r \quad (1)$$

$$T_L = [L] + \Sigma r\beta_{pqr}[M]^p[H]^q[L]^r \quad (2)$$



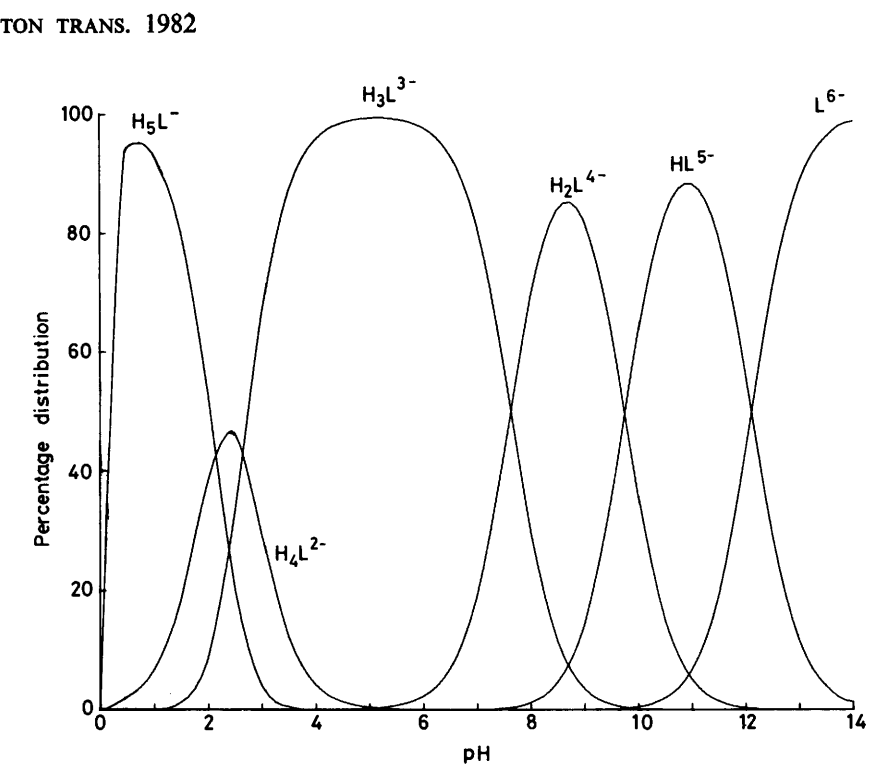


Figure 1. Typical distribution graph for the Arsenazo I system. The percentage of each species has been calculated from a hypothetical solution (C_L = 0.000 109 6 mol dm^{-3}) in the range pH 0—14 by the HALTAFALL program (N. Ingri, W. Kakalowicz, L. G. Sillén, and B. Warnqvist, *Talanta*, 1967, **14**, 1261), equipped with the PLOTTER Calcomp 936

Table 2. Stepwise and total mixed protonation and formation constants for the Cu^{2+}–Arsenazo I system at 25 °C and I = 1.0 mol dm^{-3} (KCl). Results with different refinement programs; estimated standard deviations (σ) are given in parentheses [a]

	LETAGROPVRID–SPEFO	SQUAD
$\log \beta_{011}$ [b]	12.114(18)	12.114(19)
$\log \beta_{021}$	21.870(17)	21.871(18)
$\log \beta_{031}$	29.506(16)	29.506(16)
$\log \beta_{041}$	32.155(6)	32.155(6)
$\log \beta_{051}$	34.298(6)	34.298(6)
$\log K_2^H$	9.756(25)	9.757(26)
$\log K_3^H$	7.636(23)	7.635(24)
$\log K_4^H$	2.649(17)	2.649(17)
$\log K_5^H$	2.143(8)	2.143(8)
$\log \beta_{221}$ [b]	31.878(30)	31.878(31)
$\log \beta_{111}$	23.530(29)	23.529(29)
$\log \beta_{101}$	16.795(68)	16.797(58)
$\log K_{111}^{011}$ [c]	11.416(34)	11.415(35)
$\log K_{221}^{021}$ [d]	10.008(34)	10.007(36)

[a] $\sigma(\log K_q^H) = \sqrt{\sigma^2(\log \beta_{o,q,1}) + \sigma^2(\log \beta_{o,q-1,1})}$. [b] $\beta_{pqr} = [M_pH_qL_r]/[M]^p[H]^q[L]^r$. [c] $\log K_{111}^{011} = \log \beta_{111} - \log \beta_{011}$. [d] $\log K_{221}^{021} = \log \beta_{221} - \log \beta_{021}$.

β_{041}) were refined with both programs. This scheme of calculation with the new set of refined constants was also repeated until the minimum value of the error square sum [10]U was obtained; $\log \beta_{051}$ was refined separately. The procedure can be repeated as long as the new set of trial parameters gives a smaller value of U than the minimum value previously obtained. The best set of complexes was $[Cu_2(H_2L)]$, $[Cu(HL)]^{3-}$, and $[CuL]^{4-}$. The different ε_{pqr} values for the complexes have been also refined with both programs. The errors for the protonation constants are probably underestimated since it was necessary to hold the ε_{pqr} values constant during the calculation. Values of β_{pqr} were refined by LETAGROPVRID–SPEFO, and $\log \beta_{pqr}$ values by SQUAD. All the calculations were carried out on the CDC CYBER 70/76 computer of the ' Consorzio per la Gestione del Centro di Calcolo Elettronico Interuniversitario dell'Italia Nord-Orientale, Casalecchio, Bologna ' with the financial support from the University of Parma.

Discussion

Protonation Equilibria.—The refinement of the mixed protonation constants gives the values reported in Table 2. In most cases it is possible to assign the $\log \beta_{0qr}$ values to particular ionizable groups on the basis of the spectral changes. The value $\log \beta_{011}$ = 12.114(19) is in fairly good agreement with those of analogous compounds (Eriochrome Blue SE,[12] $\log \beta_{011}$ = 11.900; Acid Chrome Dark Blue,[14] $\log \beta_{011}$ = 12.400); the stepwise protonation constant of the second hydroxy group, $\log K_2^H$ = 9.757(26), is in agreement with those of Acid Chrome Dark Blue [14] (9.300), Eriochrome Blue SE [12] (10.50), and Acid Chrome Blue K [15] (10.200). The little differences agree with the effects of substituent groups on the benzene ring.

The spectra in acid and weak basic media show three bands: a more intense overlapping one in the range 503—523 nm, a less intense composite one in the range 282—297 nm, and a weak one at 363 nm. These bands are usually associated with $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$, or charge-transfer transitions. In strong alkaline media (pH > 9.757) pH increases induce weak different wavelength and intensity variations in the peaks; in particular the proton ionization of HL^{5-} causes the shifts 522 $\longrightarrow$ 526 and 302 $\longrightarrow$ 310 nm, as well as a split of the band at 271 nm and a shoulder at 363 nm. When the pH increases, small combined batho-hyperchromic, batho-hypochromic, and hypso-hyperchromic shifts are observed from 522 to 526 nm,

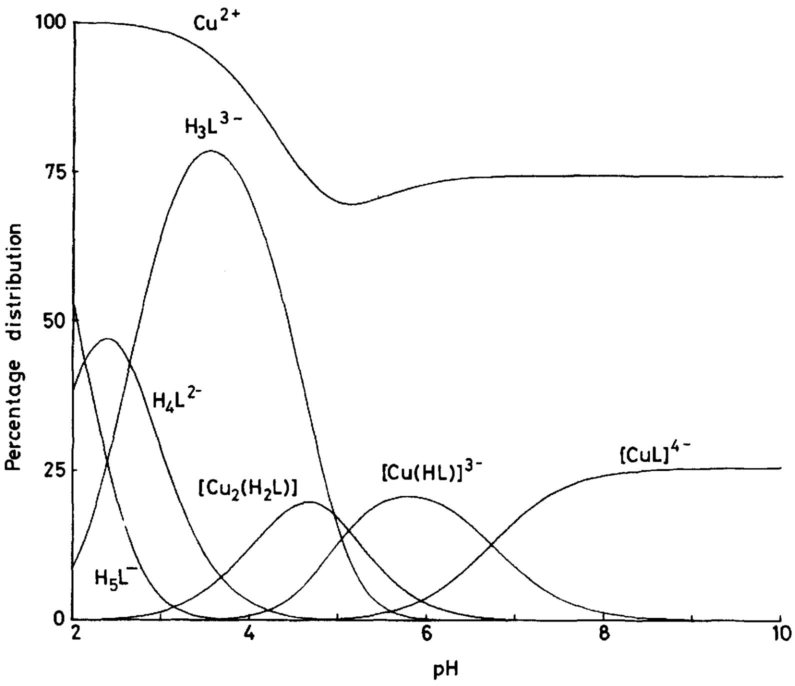


Figure 2. Representative distribution diagram for the Cu^{2+}–Arsenazo I system. The percentages have been calculated from the data of a hypothetical solution of Cu^{2+} (0.000 429 1 mol dm^{-3}) and Arsenazo I (0.000 109 6 mol dm^{-3}) by the HALTAFALL program (see Figure 1), equipped with the PLOTTER Calcomp 936

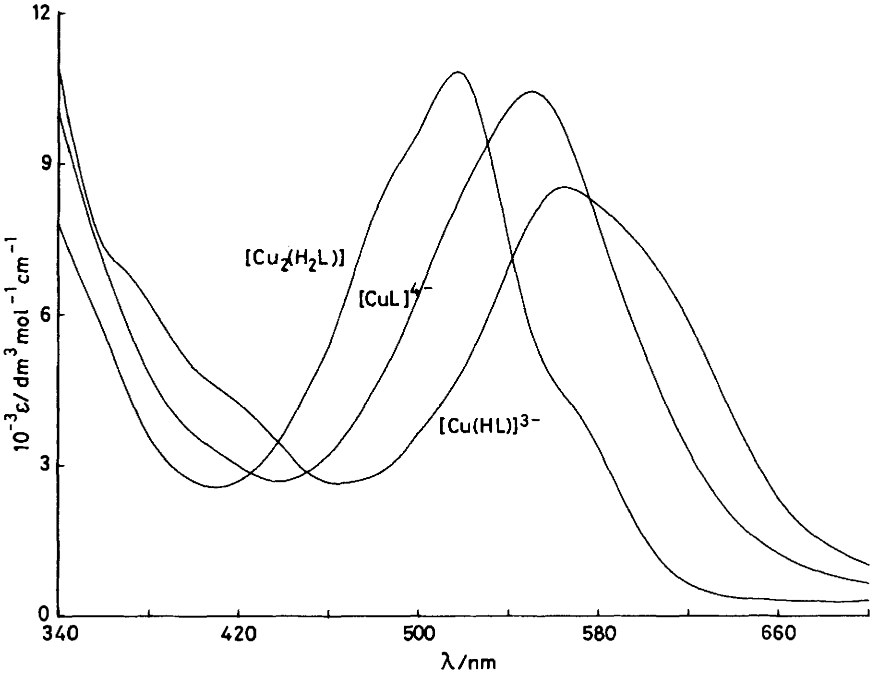


Figure 3. Plots of molar absorption coefficients (ε) of the three complexed species of the Cu^{2+}–Arsenazo I system at 25 °C by the program SQUAD with the PLOTTER Calcomp 936

from 302 to 310 nm, and at 271 nm respectively. With the shift of the band in the 503—523 nm region is associated the change of colour from red-orange (acid, neutral, and weak basic media) to intense red-purple for pH > 9.757 (log K_2^H). Isosbestic points (281, 380, and 430 nm) suggest that the simultaneous equilibria are altered by the pH changes, and moreover indicate the coexistence of several species in solution. The refined mixed protonation constants (Table 2) together with the calculated molar absorptivities of the corresponding species of Arsenazo I have been used to plot the spectra of the different solutions by the program SQUAD with PLOTTER Calcomp 936. The plots comparing computed and observed absorbances show a nearly perfect fit, the standard deviation (*s*) amounting to only 0.0075 absorbance units [$s = (U/N_{d.f.})^{\frac{1}{2}}$ where $N_{d.f.}$ are the degrees of freedom of all data]. The sum of squares of deviations for 23 solutions and 55 λ values was 0.052 582, *i.e.* an excellent fit.

During early attempts to analyse the data, another possible species, H_6L, was included in the chemical model. The amount of such a species was found to be immeasurably small. The mixed protonation constant log β_{061} has not been introduced in the calculation because it gave rise sometimes to non-convergence of the refinement procedure; this was attributed to the low contribution of H_6L to the total absorbance. In fact this species is chemically insignificant in nearly all the solutions and only in few of them does it contribute more than 1—2% of the absorbance. A typical species distribution diagram for the Arsenazo I-hydrogen ion mixture is shown in Figure 1.

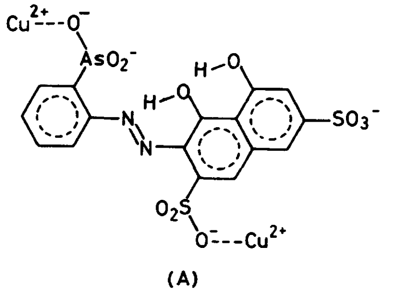
Copper(II) *Complexes.*—Three copper(II) complexes were indicated by the refinement in the range $3.0 < pH < 8.0$: $[Cu_2(H_2L)]$, $[Cu(HL)]^{3-}$, and $[CuL]^{4-}$ (Figure 2). After a wide range of research (both programs), including hydrogen-containing and mono- and poly-nuclear species, the choice was restricted to the equilibria given below. Refined formation

$$2\,Cu^{2+} + 2H^+ + L^{6-} \rightleftharpoons [Cu_2(H_2L)] \quad \log \beta_{221} = 31.878(31)$$
$$Cu^{2+} + H^+ + L^{6-} \rightleftharpoons [Cu(HL)]^{3-} \quad \log \beta_{111} = 23.529(29)$$
$$Cu^{2+} + L^{6-} \rightleftharpoons [CuL]^{4-} \quad \log \beta_{101} = 16.797(58)$$

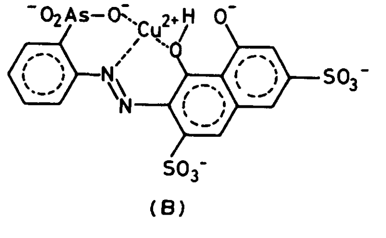
Additional equilibria

$$Cu^{2+} + HL^{5-} \rightleftharpoons [Cu(HL)]^{3-} \quad \log K^{011}_{111} = 11.415(35)$$
$$2\,Cu^{2+} + H_2L^{4-} \rightleftharpoons [Cu_2(H_2L)] \quad \log K^{021}_{221} = 10.007(36)$$

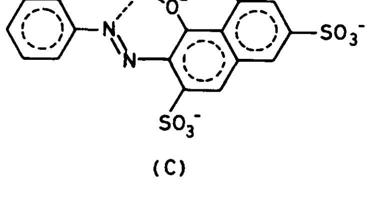
constants are given in Table 2. A typical species distribution graph for the Arsenazo I–copper(II) system ([M] : [L] = 4 : 1) is shown in Figure 2. $[Cu_2(H_2L)]$ is supposed to be formed by Cu^{2+} and $[H_3L]^{3-}$, the latter species being prevalent in the range pH 4—5. For this polynuclear complex, which reaches a maximum concentration of 19.6% total metal at pH 4.7, the structure (A) is proposed.



(A)



(B)



(C)

By increasing the pH, only above *ca.* pH 5.25 does the hydrogen complex $[Cu(HL)]^{3-}$ become important; it reaches a peak of 20.7% total metal concentration at pH 5.8. Structure (B) can be proposed for the monoprotonated complex on the basis of the results and by analogy with other copper(II)–azo-complexes.[18] The above schemes show that in the *trans* configuration of the azo-bridge for compounds of the *o,o'*-disubstituted azo-type, only *one* nitrogen atom co-ordinates to the metal ion, except for the polynuclear complex $[Cu_2(H_2L)]$ [structure (A)]. Finally, $[CuL]^{4-}$ reaches a maximum concentration of 24.2% total metal at pH 8.0. Taking into account the high stability and the complete deprotonation of the mononuclear complex $[CuL]^{4-}$ a double chelate may be suggested [structure (C)].

The constants (Table 2) and the molar absorptivities were used to predict, by the program SQUAD with the PLOTTER Calcomp 936, the spectra of additional solutions. The individual spectra of three complexes are shown in Figure 3. The standard deviation (*s*) was 0.0302 absorbance units. The spectra consist always of a single band differing in the wavelength (517—564 nm) of its maximum according to the experimental conditions; the changes with pH involve a displacement toward longer λ. In addition, the relative intensity of this one band changes by varying the reagent concentrations. At pH < 5.4, it seems reasonable to assume that the spectrum contains a stronger peak at 517 nm, corresponding to $[Cu_2(H_2L)]$. In weak acid solutions (pH *ca.* 6.0) the spectrum of $[Cu(HL)]^{3-}$ presents a lower single peak at 564 nm, with a +47 nm shift from its position in strong acid solutions. For pH > 7.0 the anionic form $[CuL]^{4-}$ displays a stronger peak at 550 nm. In acid, neutral, and weak basic media the spectra show that the colour corresponds to a single band in the range 517—564 nm, while by increasing the pH the colour changes from reddish purple to violet.

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