997. *A Potentiometric and Polarographic Study of Copper–Catechol Complexes.*

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Complex formation between catechol (H_2A) and cupric ions (Cu^{2+}) in aqueous solution at 25° has been studied by potentiometric titration and the stability constants of complexes CuA and CuA_2^{2-} have been determined. The polarographic behaviour of both unbuffered and buffered aqueous systems of copper and catechol at 25° has also been studied. In solutions containing phosphate buffers with pH 7—8, CuA_2^{2-} was reversibly reduced at the dropping mercury electrode and its stability constant, calculated from polarographic measurements, agreed with that obtained potentiometrically. From the results we estimated concentrations of Cu^{2+}, CuA, and CuA_2^{2-} in some copper–catechol systems.

ALTHOUGH complexes of copper and catechol were isolated and described by Weinland and Walter[1] in 1923, it is only recently that a quantitative potentiometric study of their formation has been reported by Näsanen and Markkanen.[2] Our work, started independently and before Näsanen and Markkanen's publication, confirmed their findings: in addition, the determination of the second dissociation constant of catechol has enabled the stability constants of these complexes to be evaluated. Further, the polarographic behaviour of both unbuffered and buffered copper–catechol systems has confirmed the potentiometric results. The interaction of cupric ions with phenols, mainly of the catechol type, in fruit juice buffered with organic acids has been described.[3,4]

RESULTS AND DISCUSSION

Potentiometric Measurements.—Graphs relating the pH of solution to the ratio of concentration of added base to concentration of copper (m_b/m_{Cu}), obtained on titration of catechol solutions containing cupric chloride with sodium hydroxide, showed inflections at values of (m_b/m_{Cu}) of 2 and 4, corresponding to the neutralisation of 2 and 4 hydrogen ions per atom of copper present (Figure). The reactions can be represented by eqns. (1) and (2) where catechol is regarded as a dibasic acid H_2A.

$$Cu^{2+} + H_2A \rightleftharpoons CuA + 2H^+ \quad (1) \;; \qquad CuA + H_2A \rightleftharpoons CuA_2^{2-} + 2H^+ \quad (2)$$

The concentration equilibrium constants for these reactions are

$$K_1^1 = [CuA]\{H^+\}^2/[Cu^{2+}][H_2A] \quad (3) \;; \quad \text{and } K_2^1 = [CuA_2^{2-}]\{H^+\}^2/[CuA][H_2A] \quad (4)$$

The activity equilibrium constants are

$$K_1 = [CuA]\{H^+\}^2/f_2[Cu^{2+}][H_2A] \quad (5) \;; \quad \text{and } K_2 = f_2[CuA_2^{2-}]\{H^+\}^2/[CuA][H_2A] \quad (6)$$

where f_2 represents the ionic activity coefficient of ions of charge ± 2, and the square brackets and braces enclosing the ionic species represent concentrations and activities respectively.

Calculation of K_1. K_1 was calculated from consideration of the ionic species present and (i) the electroneutrality equation, (ii) the total concentration of catechol ($[A_0]$), and (iii) the total concentration of copper ($[Cu_0]$). Over the pH range studied $[HA^-]$, $[A^{2-}]$, and $[OH^-]$ were negligible, so that

$$[H_2A] = [A_0] - [Na^+]/2 - [H^+]/2 \text{ and } [CuA] = [Na^+]/2 + [H^+]/2$$

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[1] Weinland and Walter, *Z. anorg. Chem.*, 1923, **126**, 145.
[2] Näsanen and Markkanen, *Suomen Kem.*, 1956, **29**, 119.
[3] Kieser, Pollard, and Timberlake, *J. Sci. Food Agric.*, 1957, **8**, 151.
[4] Timberlake, *ibid.*, p. 159.

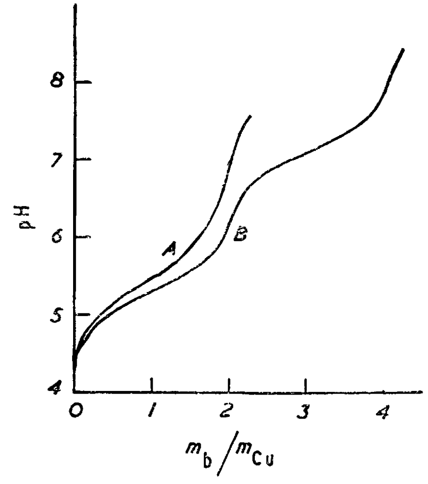
pH was defined as equal to $-\log_{10}\{H^+\}$ and $pK = -\log_{10} K$. The hydrogen-ion concentrations were sufficiently low to permit $\{H^+\}$ to be used for $[H^+]$. Ionic strengths were all calculated from $I = 0{\cdot}5\sum m_i z_i^2$ where m is the molar concentration of ions of type i and valency z_i, and ion-activity coefficients were evaluated from the Davies [5] equation, *viz.*, $\log f_i = -0{\cdot}5\, z_i^2\{\sqrt{I}/(1 + \sqrt{I}) - 0{\cdot}2\, I\}$.

Values of pK_1 calculated from points on the buffer range of the graph obtained by titration of a solution of catechol and cupric chloride in the ratio 1 : 1 are given in Table 1. The relatively constant value obtained (mean 8·10) at pH 5·090—6·052 indicated that CuA was the only complex existing under these conditions. Further pH measurements in solutions containing catechol, cupric chloride, and sodium hydroxide in the ratio 1 : 1 : 1 gave pK_1 values of 8·11 and 8·12. The value of pK_1 was taken as 8·11, in agreement with the previous value [2] of 8·09.

TABLE 1. *Measurement of* K_1.

Titration of 20 ml. of 0·005M-catechol and 4 ml. of 0·025M-$CuCl_2,2H_2O$ with 0·050N-NaOH.)

NaOH (ml.)...	0·20	0·40	0·80	1·24	1·77	2·00	2·27	2·76	3·27	3·51	3·70
pH	4·709	4·886	5·090	5·249	5·422	5·498	5·589	5·782	6·052	6·224	6·422
pK_1^1	8·269	8·286	8·287	8·281	8·278	8·271	8·276	8·279	8·278	8·231	8·183
$10^3 I$	12·2	11·9	11·3	10·7	9·9	9·6	9·3	8·6	8·1	7·7	7·2
pK_1	8·075	8·094	8·099	8·098	8·103	8·105	8·104	8·112	8·117	8·072	8·029



Potentiometric titration. *A*, Titration of 20 ml. of 0·005M-catechol and 4 ml. of 0·025M-$CuCl_2,2H_2O$ with 0·050N-NaOH; *B*, Titration of 40 ml. of 0·005M-catechol and 2 ml. of 0·025M-$CuCl_2,2H_2O$ with 0·050N-NaOH.

Calculation of K_2. K_2 was calculated from consideration of the ionic species present and (i) the electroneutrality equation, (ii) the total concentration of catechol ($[A_0]$), (iii) the total concentration of copper ($[Cu_0]$), and (iv) the first dissociation constant ${}_CK_1$ of catechol, whence

$$[H_2A] = (2[A_0] - [Na^+] - [H^+] + [OH^-])/(2 + {}_CK_1/[H^+])$$

$$[CuA_2^{2-}] = ([Na^+] + [H^+] - [OH^-] - 2[Cu_0] - {}_CK_1[H_2A]/[H^+])/2$$

At pH values over 7, $[HA^-]$ although small was taken into account. The concentrations of H^+ and OH^- were sufficiently small to permit activities to be used for concentrations.

Constant values of K_2 were obtained from points on the second buffer range of the titration graph of a solution of catechol and copper chloride in ratio 4 : 1 (curve *B*, Figure), indicating that CuA_2^{2-} was the only other complex existing with CuA. pK_2 was 11·76, in close agreement with the value of 11·73 found previously.[2]

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[5] Davies, *J.*, 1938, 2093.

The validity of the Davies equation up to ionic strength 0·10 was confirmed for K_1 and K_2 by pH measurements of catechol–cupric chloride–sodium hydroxide solutions to which known amounts of solid potassium nitrate were added. Relative constancy of pK_2 values is shown in the results in Table 2.

TABLE 2. *Effect of ionic strength.*

Additions of solid KNO_3 to 40 ml. of 0·0025M-catechol, 1 ml. of 0·025M-$CuCl_2,2H_2O$, and 3 ml. of 0·025N-NaOH.

10^3I	2·0	11·1	20·3	29·7	47·7	66·0	92·5
pH	7·254	7·201	7·189	7·157	7·131	7·098	7·070
pK_2^1	11·673	11·567	11·543	11·479	11·427	11·361	11·305
pK_2	11·758	11·753	11·783	11·762	11·767	11·743	11·735

Dissociation constants of catechol. The dissociation constants of catechol,

$$ {}_{\mathrm{C}}K_1 = f_1^2[\mathrm{H}^+][\mathrm{HA}^-]/[\mathrm{H}_2\mathrm{A}] \quad . \quad . \quad (7); \quad \text{and } {}_{\mathrm{C}}K_2 = f_2[\mathrm{H}^+][\mathrm{A}^{2-}]/[\mathrm{HA}^-] \quad . \quad . \quad (8)$$

were determined by titration of catechol solutions with potassium hydroxide. The concentrations of the ionic species were found from eqns. (9)—(11).

$$[\mathrm{Na}^+] + [\mathrm{H}^+] = [\mathrm{HA}^-] + 2[\mathrm{A}^{2-}] + [\mathrm{OH}^-] \quad . \quad . \quad . \quad . \quad (9)$$

$$[\mathrm{H}_2\mathrm{A}] + [\mathrm{HA}^-] + [\mathrm{A}^{2-}] = [\mathrm{A}_0] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (10)$$

$${}_{\mathrm{C}}K_1 = f_1^2[\mathrm{H}^+][\mathrm{HA}^-]/[\mathrm{H}_2\mathrm{A}] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (11)$$

Ionic strengths and activity coefficients were obtained by successive approximinations, and pOH was determined from pK_w − pH, where pK_w = 13·9966.[6] Results of a typical titration are shown in Table 3, which includes values of the Bjerrum $\bar{n}$ function and the Speakman [7] parameters X and Y.

TABLE 3. *Dissociation constants of catechol.*

Titration of 40 ml. of 0·01M-catechol with 0·101N-KOH.

KOH (ml.)	pH	$10^3[\mathrm{A}_0]$	$10^3[\mathrm{K}^+]$	10^3I	$10^3[\mathrm{OH}^-]$	$10^3[\mathrm{H}_2\mathrm{A}]$	$10^3[\mathrm{HA}^-]$	$10^3[\mathrm{A}^{2-}]$	$\bar{n}$	$10^{21}X$	$10^{12}Y$
1·5	9·207	9·640	3·651	3·66	0·01730	6·006	3·634	—	1·622	69·05	196·1
2·0	9·424	9·526	4·809	4·83	0·02877	4·746	4·780	—	1·533	35·40	100·4
2·5	9·639	9·412	5·940	5·96	0·04753	3·520	5·892	—	1·374	17·36	49·20
3·7	10·352	9·154	8·551	8·63	0·2495	0·927	8·151	0·076	1·170	1·118	2·835
3·9	10·542	9·111	8·973	9·05	0·4055	0·615	8·416	0·080	1·059	0·494	1·177
4·1	10·741	9·072	9·391	9·49	0·6124	0·397	8·576	0·099	1·033	0·207	0·428
4·3	10·908	9·031	9·797	9·93	0·9016	0·266	8·624	0·141	1·014	0·099	0·125
4·5	11·046	8·989	10·21	10·39	1·242	0·198	8·612	0·179	1·002	0·053	0·014
4·7	11·154	8·950	10·62	10·75	1·596	0·153	8·567	0·230	0·991	0·033	−0·044

The mean value of ${}_{\mathrm{C}}K_1$ was 3·57 × 10^{-10} (p${}_{\mathrm{C}}K_1$ = 9·45), in agreement with that found previously.[2] The value of ${}_{\mathrm{C}}K_1{}_{\mathrm{C}}K_2$ was 0·052 × 10^{-21}, obtained from the intercept of the straight line of slope ${}_{\mathrm{C}}K_1$ according to Speakman's method, and also from ${}_{\mathrm{C}}K_1{}_{\mathrm{C}}K_2 = f_2\{\mathrm{H}^+\}^2$ at $\bar{n} = 1$; hence ${}_{\mathrm{C}}K_2$ = 1·5 × 10^{-13} (p${}_{\mathrm{C}}K_2$ = 12·8). As far as we know, only one value for ${}_{\mathrm{C}}K_2$ has recently been published [8] (p${}_{\mathrm{C}}K_2$ = 11·23 at ionic strength 0·06).

Stability constants of CuA *and* CuA_2^{2-}. The stability constants of CuA and CuA_2^{2-}, *viz.*:

$$K_{s1} = [\mathrm{CuA}]/f_2^2[\mathrm{Cu}^{2+}][\mathrm{A}^{2-}] \quad . \quad (12); \quad \text{and } K_{s2} = [\mathrm{CuA}_2^{2-}]/[\mathrm{CuA}][\mathrm{A}^{2-}] \quad . \quad (13)$$

can be calculated from

$$K_{s1} = K_1/{}_{\mathrm{C}}K_1{}_{\mathrm{C}}K_2 \quad . \quad . \quad . \quad . \quad (14); \quad \text{and } K_{s2} = K_2/{}_{\mathrm{C}}K_1{}_{\mathrm{C}}K_2 \quad . \quad . \quad . \quad (15)$$

[6] Harned and Hamer, *J. Amer. Chem. Soc.*, 1933, **55**, 2194.

[7] Speakman, *J.*, 1940, 855.

[8] Trujillo, Brito, and Cabrera, *Anales real Soc. españ. Fís. Quím.*, 1956, **52**, *B*, 589.

giving $\log_{10} K_{s1} = 14{\cdot}1$ and $\log_{10} K_{s2} = 10{\cdot}5$. The overall stability constant of the reaction $Cu^{2+} + 2A^{2-} \rightleftharpoons CuA_2{}^{2-}$ is

$$K_s = [CuA_2{}^{2-}]/f_2{}^2[Cu^{2+}][A^{2-}]^2 = K_{s1}K_{s2} \quad . \quad . \quad . \quad . \quad (16)$$

Therefore $\log_{10} K_s = 24{\cdot}6$.

Polarography.—Initially, buffer-free solutions of catechol and cupric ions similar to those studied potentiometrically were investigated in which the distributions of cupric ions and cupric complexes were known from pH measurements. The results are in Table 4.

TABLE 4. *Polarography of unbuffered solutions.*

Additions of NaOH (0·025N) to a solution (5 ml.) of catechol (0·004M), $Cu(NO_3)_2,3H_2O$ (0·001M), KNO_3 (0·05M), and methyl-red (0·0004%).

Potentiometric titration						
Soln.	NaOH (ml.)	pH	Cu^{2+} (%)	CuA (%)	$CuA_2{}^{2-}$ (%)	$[Cu^{2+}]$ (10^4M)
A	0	4·60	98	2	—	9·8
B	0·1	5·17	75	25	—	7·3
C	0·2	5·48	50	50	—	4·8
D	0·3	5·76	25	75	—	2·4
E	0·4	6·27	—	100	—	—
F	0·5	6·80	—	75	25	—
G	0·6	7·07	—	50	50	—
H	0·7	—	—	25	75	—

Polarography					
Soln.	No. of waves	First wave $E_{\frac{1}{2}}$ (uncorr.)	Second wave $E_{\frac{1}{2}}$ (uncorr.)	Relative wave-heights	$[Cu^{2+}]$ from wave-height (10^4M)
A	2	+0·03	−0·27 to −0·28	6·8 : 0·2	9·7—9·9
B	2	+0·03	−0·27 to −0·29	4·6 : 1·0	6·6—7·3
C	2	+0·03	−0·28 to −0·31	2·9 : 1·6	4·0—4·4
D	2	+0·03	−0·30 to −0·32	variable	1·7—3·3
E	2	−0·03	−0·31 to −0·36	1·6 : 2·7	—
F	2	−0·06	−0·35 to −0·36	1·1 : 3·1	—
G	2	−0·08	−0·36	0·1 : 3·5	—
H	1	−0·38	—	—	—

In each solution the wave caused by reduction of cupric ions was well defined, except at low Cu^{2+} concentrations, and represented fairly accurately the bulk concentrations of these ions. The diffusion-controlled nature of this wave was shown by measurements of the diffusion current (i_d) at various heights of the mercury reservoir (h). Constant values of $i_d/\sqrt{h}$ were obtained on polarography of solution *C* (Table 4) at head heights ranging from 25 to 75 cm. (drop times 3·05—9·08 sec.). The other waves in the solutions were ill-defined and bore no relation to the bulk concentrations of CuA and $CuA_2{}^{2-}$ because of the increase in pH produced at the electrode surface by liberation of A^{2-} ions during discharge of the complexes.

The use of acetate buffers over pH range 5—6 in attempts to maintain constant pH at the electrode surface increased the height of the first wave and shifted the half-wave potential slightly in a negative direction. The wave-height of the second wave was considerably reduced and the half-wave potential became more positive. Apparently at these pH values, acetate had also formed a complex with copper, and the first wave represented the reduction of both cupric ions and the cupric–acetate complex. The absence of any kinetic contribution to the height of this wave was confirmed by the constancy of the ratio $i_d/\sqrt{h}$ at different heights of the mercury reservoir (drop times 3·69—7·53 sec.). The behaviour of phthalate buffer (0·1M, pH 5·8) was similar to that of acetate. Phosphate buffer (0·05M, pH 5·8) similarly interfered and some copper was precipitated.

Buffer solutions should compete with catechol for cupric ions over the pH range described since the values of K_1^1 and K_2^1 indicate that the apparent stabilities of the catechol complexes are low at these pH values. With increasing pH, however, the cupric–catechol complexes become more stable rapidly (the apparent stability of CuA_2^{2-} increases with $[H^+]^{-4}$), and at high pH values no interference by the usual buffer systems would be expected. This was confirmed by the polarography of solutions containing cupric nitrate, phosphate buffers of pH range 7—8, a large excess of catechol and methylcellulose as maximum suppressor. Well-defined single waves were obtained at each pH. Reversibility was demonstrated by plotting $\log_{10} i/(i_d - i)$ against potential for each wave. Straight lines were obtained with slopes varying from 0·030 to 0·034 (Table 5), in close agreement with the theoretical slope of 0·0296 at 25° for a reversible two-electron reduction. Half-wave potentials were obtained from the intercepts of the lines on the potential scale.

TABLE 5. *Polarographic measurements at high catechol concentration.*

Solutions of catechol (0·781M), cupric nitrate (0·000409M), and methylcellulose (0·0125%) in phosphate–NaOH buffers (0·0469M-KH_2PO_4); $(E_{\frac{1}{2}})_s = +0·031$ v.

pH	6·90	7·21	7·48	7·74	8·07	
Slope	0·034	0·032	0·031	0·030	0·032	
$(E_{\frac{1}{2}})_c$ (v)	−0·193	−0·233	−0·261	−0·289	−0·327	
ΔE (v)	−0·224	−0·264	−0·292	−0·320	−0·358	
$\log K_s$	24·6	24·7	24·6	24·5	24·5	Mean 24·6

The concentration of A^{2-}, although low relative to that of copper, may be assumed to be kept constant at the electrode surface, and equal to that in the bulk of the solution, through rapid establishment of equilibrium between the various catechol species, present in large excess. A similar assumption was made in the polarographic investigation of copper in solutions of ethylenediaminetetra-acetic acid.[9] The shift in half-wave potential is then given by

$$\Delta E = (E_{\frac{1}{2}})_c - (E_{\frac{1}{2}})_s = -(\boldsymbol{R}T/n\boldsymbol{F}) \ln (K_s f_c k_s / f_s k_c) - (p\boldsymbol{R}T/n\boldsymbol{F}) \ln [A^{2-}] f_2 \quad . \quad (17)$$

where the subscripts *c* and *s* refer to complex and simple cupric ions respectively, k_c and k_s are constants proportional to the square-roots of the diffusion coefficients, n is the number of electrons involved in the reduction process, p is the number of A^{2-} ions associated with each Cu^{2+} ion, and K_s is the stability constant of the complex formed from Cu^{2+} and A^{2-}. Substitution in eqn. (17) from eqns. (7) and (8) gives

$$-\Delta E = (\boldsymbol{R}T/n\boldsymbol{F}) \ln (K_s f_c k_s / f_s k_c) + (p\boldsymbol{R}T/n\boldsymbol{F})(2\text{pH} - \text{p}_\text{C}K_1 - \text{p}_\text{C}K_2) + (p\boldsymbol{R}T/n\boldsymbol{F}) \ln [H_2A] \quad . \quad (18)$$

Equation (18) predicts that if $[H_2A]$ is maintained constant and if $n = 2$ and $p = 2$, a plot of $-\Delta E$ against pH will give a straight line of slope 0·118. A linear relation was found between ΔE and pH (Table 5), the slope being 0·112, in sufficient agreement with 0·118 to justify our regarding the complex present in solution as preponderantly CuA_2^{2-}.

$(E_{\frac{1}{2}})_s$ was measured in a solution containing cupric nitrate (0·000409M), methylcellulose (0·0125%), and potassium nitrate (0·0469M). From observation of the relative wave-heights, k_s/k_c was 1·2; the value of $\log K_s$ (Table 5) was then calculated from equation (18) and agreed with that found potentiometrically.

The temperature coefficient of the diffusion current measured in the solution of pH 7·21, at 14—55°, varied from 1·6 to 1·8% per degree, *i.e.*, of the order of magnitude associated with a diffusion-controlled electrode process. The diffusion-controlled nature of the electrode reaction was further confirmed by the constancy of the values of $i_d/\sqrt{h}$, measured

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[9] Pecsok, *Analyt. Chem.*, 1953, **25**, 561.

in the solution of pH 7·21 at different heights of the mercury reservoir. The results are shown in Table 6, which also includes values of the diffusion-current constant I calculated from the Ilkovič equation. Using the mean value of I (2·55), we calculate the diffusion coefficient (D) of the CuA_2^{2-} ion to be **4·41** $\times 10^{-6}$ cm.^{2} sec.$^{-1}$.

TABLE 6. *Effect of height of mercury column.*

Solution pH 7·21, Table 5; m and t measured at $-0{\cdot}24$ v.

	Height of nercury column		
	30 cm.	60 cm.	80 cm.
h (corr.) (cm.)	28·4	58·4	78·4
m (mg. sec.$^{-1}$)	0·963	1·986	2·689
t (sec.)	7·70	3·76	2·76
i_d (μA)	1·45	2·05	2·35
$i_d/\sqrt{h}$ (corr.)	0·272	0·268	0·266
I	2·59	2·55	2·51

Distribution of Cupric Ions and Complexes.—A knowledge of the stability constants of CuA and CuA_2^{2-} enables the distribution of copper in the forms Cu^{2+}, CuA, and CuA_2^{2-} to be calculated for solutions of known pH containing copper and catechol of known concentrations. In such solutions the three species Cu^{2+}, CuA, and CuA_2^{2-} must exist in the following proportions:

$$Cu^{2+} : CuA : CuA_2^{2-} = 1 : f_2^2K_{s1}[A^{2-}] : f_2^2K_{s1}K_{s2}[A^{2-}]^2 \quad . \ . \quad (19)$$

where $[A^{2-}]$ is the equilibrium concentration of bivalent catechol anions existing in solution. $[A^{2-}]$ can be evaluated from eqn. (20):

$$f_2^2\alpha K_{s1}K_{s2}[A^{2-}]^3 - f_2^2(X-2)[Cu_o)K_{s1}K_{s2}[A^{2-}]^2 + f_2^2\,\alpha K_{s1}[A^{2-}]^2 \\ -f_2^2(X-1)[Cu_o]K_{s1}[A^{2-}] + \alpha[A^{2-}] = X[Cu_o] \quad . \quad (20)$$

where

$$X = [A_o]/[Cu_o]$$

and

$$\alpha = (1 + f_2[H^+]/_CK_2 + f_1^2f_2[H^+]^2/_CK_{1C}K_2)$$

Equation (20) is similar to that used for complexes of copper and glycine.[10]

The magnitudes of the stability constants K_{s1} and K_{s2} are such that the three species cannot exist in appreciable quantities at the same time in solution. Cu^{2+} and CuA can exist together, with CuA_2^{2-} negligible, and CuA and CuA_2^{2-} can exist together, with Cu^{2+} negligible. At concentrations of A^{2-} smaller than 10^{-13} only Cu^{2+} and CuA need be considered in the theoretical treatment of the system and at pH values below 6, the first two terms in α are negligible compared with the third term. Under these conditions, the cubic equation (20) can be replaced by a simpler quadratic and the proportions of Cu^{2+} and CuA calculated from eqns. (21)—(23):

$$[Cu^{2+}] = [Cu_c]/(1 + y) \quad . \ . \ . \ . \ . \ . \ . \quad (21)$$

where

$$y = K_1\{-b \pm \sqrt{}(b^2 + 4f_1^2f_2^3[H^+]^2[A]_o/K_1)\}/(2f_1^2f_2[H^+]^2) \quad . \ . \quad (22)$$

where

$$b = (f_1^2f_2[H^+]^2/K_1) - f_2^2([A_o] - [Cu_o]) \quad . \ . \ . \ . \ . \quad (23)$$

Equations (21)—(23) require only a knowledge of the equilibrium constant K_1 and are independent of a knowledge of the dissociation constants of catechol. Table 7 shows that

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[10] Dobbie, Kermack, and Lees, *Biochem. J.*, 1955, **59**, 240.

TABLE 7. *Distributions of cupric ions and complexes.*

Solutions of 10 ml. of 0·010M-catechol, 1 ml. of 0·01047M-$Cu(NO_3)_2,3H_2O$, 1 ml. of 0·50M-KNO_3, and 0·2 ml. of 0·2% gelatin, with (*a*) no addition; (*b*) addition of 0·4 ml. of NaOH (0·01N); (*c*) addition of 1·0 ml. of NaOH (0·01N); (*d*) addition of 1·6 ml. of NaOH (0·01N).

						Eqns. (19)—(20)			Eqns. (21)—(23)		Plrgrphy
	$10^3[A_0]$	$10^3[Cu_0]$	10^3I	pH	$[A^{2-}]$	Cu^{2+} (%)	CuA (%)	CuA_2^{2-} (%)	Cu^{2+} (%)	CuA (%)	Cu^{2+} (%)
(*a*)	8·196	0·858	41·0	4·53	$10^{-14\cdot97}$	96·6	3·4	<0·001	96·6	3·4	98
(*b*)	7·936	0·831	40·0	5·06	$10^{-13\cdot94}$	72·7	27·3	0·01	72·7	27·3	78
(*c*)	7·575	0·793	37·8	5·35	$10^{-13\cdot41}$	43·2	56·7	0·08	42·9	57·1	44
(*d*)	7·246	0·759	36·3	5·71	$10^{-12\cdot72}$	13·1	86·4	0·5	13·3	86·7	16

the percentages of Cu^{2+} and CuA, in a number of solutions, given by these equations are in excellent agreement with those given by equations (19)—(20). Moreover, the percentages of Cu^{2+} calculated are in good agreement with those found polarographically.

EXPERIMENTAL

The potentiometric measurements were made with a Doran Universal pH meter and D.C. potentiometer with Doran micro-calomel and " Alkacid " glass electrodes set at 0·05M-potassium phthalate = pH 4·005 at 25°. The linearity of the glass electrode over the pH range measured was confirmed with the hydrogen electrode and also with the following buffer solutions: 0·025M-KH_2PO_4–0·025M-Na_2HPO_4, pH 6·85; 0·05M-borax, pH 9·18; and the Britton–Welford buffer 0·2N-KH_2PO_4–0·2N-NaOH, pH 10·74—11·56. At the end of each titration the pH meter invariably returned to within 0·01 pH unit of the pH of the phthalate buffer. All pH measurements were made at 25° ± 0·1°. A stream of purified nitrogen was used to stir the solutions and maintain an inert atmosphere.

The polarographic measurements were made with a Tinsley pen recording polarograph and dropping mercury electrode. For most determinations the voltage span of the instrument was reduced from 3·0 v to 0·9 v so that when the potentiometer was run slowly, potentials could be measured to ±1·5 mv. The Doran potentiometer, reading to 0·1 mv, was used to check the applied potential and, in conjunction with a standard resistance, the current indicated by the pen deflections. Current measurements were made on damped waves except in the experiment with phosphate buffers pH 7—8, when values were taken from the tops of the undamped fluctuations of the recorder.[11] The Tinsley polarographic cell was connected by a saturated potassium nitrate agar bridge to a saturated calomel electrode and all potentials quoted refer to this electrode. In the experiment with phosphate buffers pH 7—8, the total internal resistance was 1400 ohms and corrections for the iR drop through the cell were made when plotting $\log i/(i_d - i)$ against potential. The cell temperatures were maintained at 25° ± 0·1°. Purified nitrogen was used for degassing the solutions.

" AnalaR " salts were used, and cupric nitrate solution was analysed iodometrically for copper. Catechol was resublimed (m. p. 104°). The alkali solutions were carbonate-free and boiled-out glass-distilled water was used throughout.

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[11] Gelles and Nancollas, *J.*, 1956, 4847.