KINETICS OF THE ACID CATALYSIS OF THE HYDRATION OF FUMARIC ACID TO MALIC ACID[1]

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Received July 1, 1957

Equilibrium constants and rate constants for the hydration of fumaric acid to malic acid have been determined at hydrochloric acid concentrations of one to four molar in the temperature range, 125 to 200°. The logarithms of the pseudo first-order rate constants do not give linear plots of unit slope against either the logarithms of the concentrations of hydrochloric acid or the Hammett acidity function, but the slopes are closer to unity for the plots *versus* log C_{HCl}. The thermodynamic quantities ΔF, ΔH and ΔS are calculated as well as the enthalpy and entropy of activation, $\Delta H\ddagger$ and $\Delta S\ddagger$. The values of $\Delta S\ddagger$ of hydration and dehydration support the hypothesis that the rate-determining step in the hydration reaction is a bimolecular reaction with water.

While much research has been done on the kinetics of the enzyme-catalyzed hydration of fumarate to L-malate,[2] very few data are available on the non-enzymatic reaction

$$\text{fumaric acid} + H_2O \rightleftarrows d,l\text{-malic acid} \qquad (1)$$
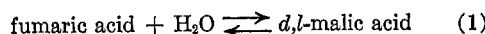
Weiss and Downs in 1922 reported that the hydration of fumaric to malic acid was a "first-order type" reaction.[3] A similar reaction, the hydration of crotonic acid to β-hydroxybutyric acid, has been reported to be first order in organic acid and hydrogen ion.[4] A thorough study of the kinetics of the acid-catalyzed hydration of fumaric acid is of interest because of the hypothesis[2] that the enzymatic mechanism of this reaction involves acidic groups in the enzyme. This paper presents kinetic and thermodynamic data obtained in a study of the hydration of fumaric acid in strong hydrochloric acid solutions.

Materials.—Fumaric acid (from the National Aniline Division of Allied Chemical and Dye Corporation) was purified by decolorizing with Norite and recrystallizing twice from water. It was dried 12 hours at 45° under approximately one millimeter pressure.

dl-Malic acid (from the Coleman and Bell Co.) was decolorized in ethyl acetate solution by use of Norite. The malic acid was then crystallized from ethyl acetate solution by addition of petroleum ether (b.p. 60 to 68°). After the recrystallization had been repeated, the malic acid was dried at 35° under approximately one millimeter pressure. The low temperature was used in order to avoid formation of the anhydride.[5]

The reactions were carried out in 8-ml. Pyrex ampules (Corning Glass Works) constricted near the top for sealing purposes. An aluminum block thermostat was used. The insulated block was four inches in diameter and was drilled with holes to contain eight ampules, one Fenwal Thermoswitch with a 38 to 315° range (Fenwal, Incorporated), one 150 watt cartridge heater and a 250° thermometer. The thermoswitch was connected in series with the cartridge heater. The temperature variation of this thermostat over a period of a day was ±0.5°.

Procedure.—Twenty millimolar (0.020 M) aqueous solutions of fumaric acid were made in small amounts (100 ml.), stored at 5° and used within 72 hours to avoid attack by molds. A hydrochloric acid solution of twice the normality desired was diluted with an equal volume of the 20 mM fumaric acid solution. The exact initial concentration of fumaric acid was then determined by use of a Beckman DU

spectrophotometer. Five-ml. portions of the solution were pipetted into cleaned Pyrex ampules. For every ampule containing the fumaric acid–HCl solution there was one containing HCl solution of the same concentration. It was found necessary to clean the ampules with $K_2Cr_2O_7\cdot H_2SO_4$ solution before using them in order to eliminate impurities that catalyze the reaction. The ampules were sealed and placed in the aluminum block thermostat which had previously been brought to the desired temperature. At specified times, a pair of ampules (reaction and its blank) was removed and cooled in an ice-bath to room temperature. After cooling, the tops of the tubes were carefully broken off, the solutions put into silica cuvettes and the absorbancy determined by use of a Beckman DU spectrophotometer. The value of the equilibrium quotient was determined when the reaction had run for a long enough time so that the ratio of malic acid to fumaric acid was constant. The equilibrium constants were verified by approaching the equilibrium from both sides.

Due to its double bond, fumaric acid strongly absorbs light in the ultraviolet range. The absorbancy index of malic acid in the wave length range from 250 to 300 mμ is 0.1% that of fumaric acid. It is important that the blank tube be subjected to the same conditions as the reaction tube to eliminate errors due to the absorbancy of substances dissolved from the Pyrex ampules. The absorbancy due to these dissolved substances reached 0.021 for 3.85 M HCl in four days at 150°. Lower concentrations of HCl lead to lower blank absorbancies for the same and lower temperatures and shorter periods of time.

The absorbancy indices (mM^{-1}cm.$^{-1}$) of fumaric acid in hydrochloric acid solution were calculated by use of the equation $a_s = A_s/lc$ where A_s (absorbancy) is $\log(I_0/I)$, l is the length of the optical path (in cm.) through the cuvette and c is the millimolar concentration of fumaric acid. The absorbancy indices of fumaric acid in acid solution are plotted *versus* wave length in Fig. 1. The absorbancy index for fumaric acid is independent of the hydrochloric acid concentration in the range of one to four normal, and Beer's law is obeyed.

Data and Discussion

Because of lack of data on the activity of water in hydrochloric acid solutions at these high temperatures, or of the activity coefficients of the organic acids, the equilibrium data for reaction 1 are expressed in terms of the equilibrium quotient K'

$$K' = \frac{(H_2M)_{eq}}{(H_2F)_{eq}} = K\frac{f_{H_2F}a_{H_2O}}{f_{H_2M}} \qquad (2)$$

where K is the thermodynamic equilibrium constant, f_{H_2M}/f_{H_2F} is the ratio of activity coefficients of malic and fumaric acids, and a_{H_2O} is the activity of water. The experimental values of the equilibrium quotients which are accurate to about ±5% are summarized in Table I. With this experimental uncertainty it has not been possible to demonstrate a dependence of the equilibrium quotient on hydrochloric acid concentration in the range of 1–4 M.

(1) This research was supported by the National Science Foundation and by the Research Committee of the University of Wisconsin from funds supplied by the Wisconsin Alumni Research Foundation.

(2) R. A. Alberty, *Adv. Enzymology*, **17**, 1 (1956).

(3) J. M. Weiss and C. R. Downs, *J. Am. Chem. Soc.*, **44**, 1118 (1922).

(4) D. Pressman and H. J. Lucas, *ibid.*, **61**, 2271 (1939).

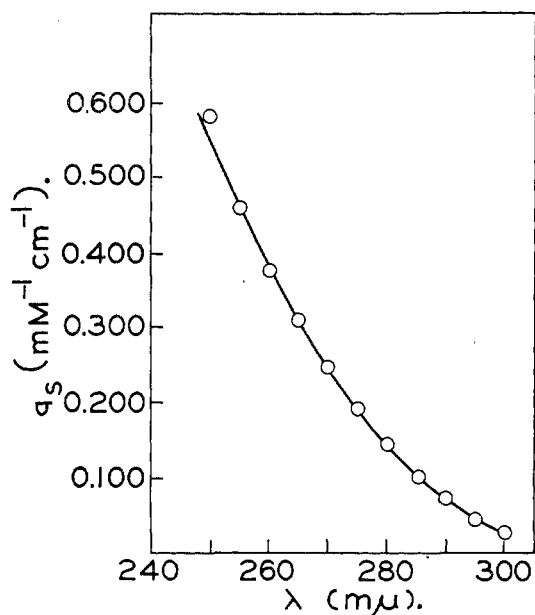(5) R. Descamps, *Bull. soc. chim. Belg.*, **48**, 201 (1939); **49**, 1 (1940).

Fig. 1.—Absorbancy indices of fumaric acid measured in HCl solution.

TABLE I

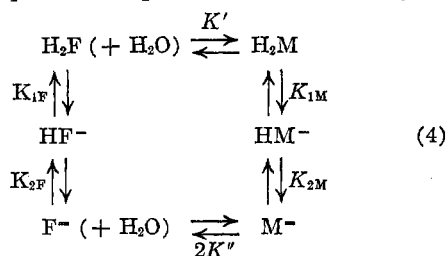EQUILIBRIUM QUOTIENTS FOR THE HYDRATION OF FUMARIC ACID IN HYDROCHLORIC ACID SOLUTIONS

T (°C.)	K'	ΔF^0, kcal. mole^{-1}
125	7.1	−1.55
150	4.8	−1.32
175	3.7	−1.17
200	2.7	−0.935

The average value for the entropy change (ΔS) is −16 ± 1 cal. deg.$^{-1}$ mole^{-1}. The enthalpy change (ΔH) is −4.9 kcal. mole^{-1} over the range from 125 to 200°. The change in enthalpy is independent of the HCl concentrations in the range 1–4 M, as it must be since the equilibrium quotient is independent of the acid concentration.

The equilibrium quotients K' in Table I can be compared only indirectly with those for the enzyme-catalyzed hydration of fumarate to l-malate, K''. Extrapolation of K' values to 25° yields a value of 51. The relationship between K' and K'' is given by

$$K'' = \frac{K'K_{1M}K_{2M}}{2K_{1F}K_{2F}} = \frac{(l\text{-}M^-)}{(F^-)} \qquad (3)$$

where the equilibrium quotients are defined by

$$
\begin{array}{ccc}
 & K' & \\
H_2F \ (+\ H_2O) & \rightleftharpoons & H_2M \\
K_{1F}\Big\updownarrow & & \Big\updownarrow K_{1M} \\
HF^- & & HM^- \qquad (4) \\
K_{2F}\Big\updownarrow & & \Big\updownarrow K_{2M} \\
F^- \ (+\ H_2O) & \rightleftharpoons & M^- \\
 & 2K'' &
\end{array}
$$

The equilibrium quotient for the lower reaction is $2K''$ since M$^-$ represents l-M$^-$ plus d-M$^-$. Substitution of the approximate values[6] which are known

(6) "Handbook of Chemistry and Physics," Chemical Rubber Publ. Co., Cleveland, Ohio, 1954.

for the acid dissociation quotients for fumaric and malic acids into equation 3 yields $K'' = 3$ which is in satisfactory agreement with the more accurate value of 4.3 at zero ionic strength obtained directly from enzymatic experiments.[7] The ΔH value for the enzyme-catalyzed reaction is −3.96 kcal. mole^{-1} at pH 7 in the temperature range 5–40°.

The kinetic data were obtained only in the temperature range where the above experimental technique is satisfactory. Below 125° in one and two molar HCl the reaction is too slow to get data in a reasonable time. Above 150° in the three and four molar HCl, the reaction is too fast for the experimental method used.

The pressure due to water vapor in the reaction ampules at these temperatures varies from 2.4 atmospheres at 125° to 15 atmospheres at 200°.[6] Although there are insufficient data for calculating the effect of pressure on this equilibrium it is apparent from $\partial \ln K/\partial P = -\Delta V/RT$ that if ΔV is less than 5 ml. mole^{-1}, the effect on K will be less than 0.2% at 200° and a pressure of 15 atmospheres.

The hydration–dehydration reaction was found to be reversible first order under the conditions used. Thus, for the expression of rate constants the reaction is written

$$F \underset{k_2}{\overset{k_1}{\rightleftharpoons}} M \qquad (5)$$

where k_1 is perhaps proportional to the concentration of water in the solution. These rate constants were calculated by use of the integrated rate equation

$$k_1 + k_2 = -\frac{1}{t}\ln [(F) - (F)_{eq}]/[(F)_0 - (F)_{eq}] \qquad (6)$$

where t is the time, (F) is the concentration of fumaric acid at time t, (F)$_{eq}$ is the concentration at equilibrium, and (F)$_0$ is the initial concentration. When $\log [(F) - (F)_{eq}]/[(F)_0 - (F)_{eq}]$ is plotted against time, the slope is equal to $-(k_1 + k_2)/2.303$. Using the relation $K' = k_1/k_2$ together with the slope, the individual rate constants for the forward and reverse reactions can be calculated.

TABLE II

FIRST-ORDER RATE CONSTANTS IN SEC.$^{-1}$ FOR THE HYDRATION AND DEHYDRATION REACTIONS

Temp. (°C.)		HCl, M				
		0.970	1.94	2.45	2.96	3.85
125	$k_1 \times 10^6$	9.5	20.6	24.3	34.2	50
	$k_2 \times 10^6$	1.3	2.9	3.6	4.8	6.9
150	$k_1 \times 10^6$	63.9	123	167	213	306
	$k_2 \times 10^6$	13	25	35	42	64
175	$k_1 \times 10^6$	270	550	...	...	...
	$k_2 \times 10^6$	72	150	...	...	...
200	$k_1 \times 10^6$	920	...	...	...	...
	$k_2 \times 10^6$	330	...	...	...	...

Figure 2 is a typical plot for the calculation of the rate constants. This plot along with the many others like it show that the hydration is reversible first order in concentrated HCl. In Table II the first-order rate constants are given for the various temperatures and HCl concentrations. Fumaric

(7) R. M. Bock and R. A. Alberty, J. Am. Chem. Soc., 75, 1920 (1953).

acid concentrations of 3 to 10 mM were used since they give absorbances of 0.2–0.6 in the wave length range of 270–290 mμ. The random error due to the spectrophotometer at the end of an hour of reaction starting with fumaric acid is 1.5% at 290 mμ (at 150° and (HCl) = 2.96 M). Under the same conditions this error is 3.4% when starting with malic acid. A run was made starting with a malic acid–HCl solution in order to check the rate constants. At 150° and 2.96 M HCl the rate constant differed from that given in Table II by only 4%. The value of k_1 is known more accurately than that of k_2 since k_2 has a smaller numerical value. It then follows that the entropies and enthalpies of activation corresponding to k_1 are more accurate than those corresponding to k_2.

TABLE III

ENERGIES OF ACTIVATION AND ENTROPIES OF ACTIVATION FOR THE HYDRATION AND DEHYDRATION REACTIONS

HCl, M	0.970	1.94	2.45	2.96	3.85
$\Delta H_1^{\ddagger}$, kcal. mole^{-1}	22	22	25	24	23
$\Delta H_2^{\ddagger}$, kcal. mole^{-1}	27	27	30	29	28
$\Delta S_1^{\ddagger}$, cal. deg.$^{-1}$ mole^{-1}	−26	−25	−18	−20	−20
$\Delta S_2^{\ddagger}$, cal. deg.$^{-1}$ mole^{-1}	−10	− 9	− 2	− 4	− 4

At 0.970 and 1.94 M HCl linear plots of log k versus $1/T$ are obtained and the Arrhenius activation energies ΔH_a are accurate to about 1 kcal. mole^{-1}. At the higher HCl concentrations the rate constants were determined only at two temperatures and so there is no check on the ΔH_a values. The $\Delta H^{\ddagger}$ values in Table III were calculated using the relation $\Delta H^{\ddagger} = \Delta H_a - RT$, and the $\Delta S^{\ddagger}$ values were calculated by use of[8]

$$k = \frac{RT}{Nh}e^{-\Delta S^{\ddagger}/R} e^{\Delta H^{\ddagger}/RT} \qquad (7)$$

An investigation was made to detect the existence of any side reactions. In order to test for the absence of maleic acid a chromatographic separation of the reaction products near equilibrium was made. In a 51 cm. column packed with 45 g. of Celite and an eluent solution of 65% chloroform and 35% butanol,[9] fumaric acid is eluted in tubes 20 to 30 (3 ml. per tube) whereas maleic acid is eluted in tubes 50 to 60 at a flow rate of 0.46 ml. per minute. Malic acid is not eluted before tube 200. No additional peak was observed between the fumaric acid and malic acid peaks. Hence detectable amounts of maleic acid are not formed under the conditions used. The incorporation of HCl at the double bond of fumaric acid to form chlorosuccinic acid is another possibility. It was found that the chromatographic column used above did not separate fumaric and chlorosuccinic acids. However, a sodium fusion of the reaction mixture indicated that less than 0.013 millimole liter^{-1} chlorine was present. It is thus evident that there are no appreciable side reactions during this hydration.

It is of interest to see whether the logarithms of the reaction rate constants are directly proportional to the Hammett acidity function[10] because this in-

(8) S. Glasstone, K. J. Laidler and H. Eyring, "The Theory of Rate Processes," McGraw–Hill Book Co., Inc., New York, N. Y., 1941.

(9) W. W. Cleland and M. J. Johnson, *J. Biol. Chem.*, **220**, 595 (1956).

(10) L. P. Hammett, *J. Am. Chem. Soc.*, **50**, 2666 (1928).
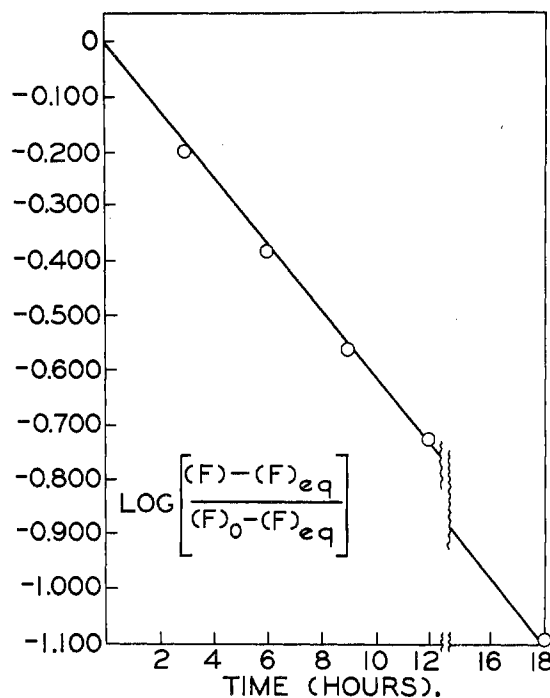
Fig. 2.—First-order plot of the kinetic data at 125° ([F]$_0$ = 10 mM, [HCl] = 2.96 M).
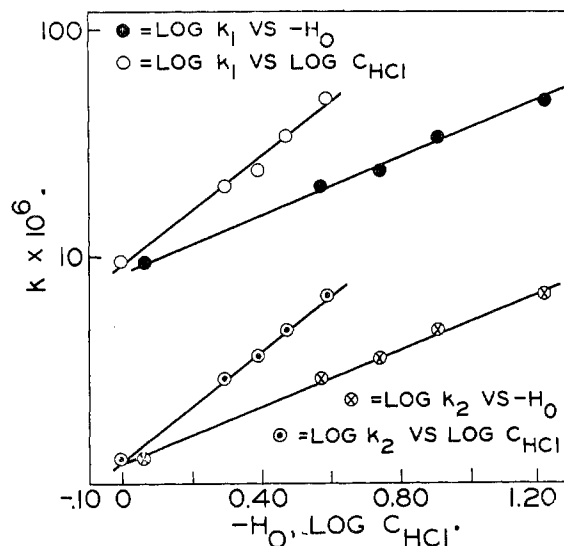

Fig. 3.—Semi-log plot of the rate constants for the hydration and dehydration steps as a function of the logarithm of the hydrochloric acid concentration and the Hammett acidity function (T = 125°).

dicates whether the rate-determining step is unimolecular or bimolecular, that is, whether a water molecule is added in the rate-determining step or after the rate-determining step.[11,12] The logarithms of the reaction rate constants are plotted versus $-H_0$ and log C_{HCl} at 125 and 150° in Figs. 3 and 4, respectively. The H_0 values for HCl were interpolated from the data of Bell and Brown.[13]

(11) L. P. Hammett, "Physical Organic Chemistry," McGraw–Hill Book Co., Inc., New York, N. Y., 1940, pp. 251–290.

(12) For an excellent review on this subject see F. A. Long and M. A. Paul, *Chem. Revs.*, **57**, 1 (1957).

(13) R. P. Bell and A. H. Brown, *J. Chem. Soc.*, 774 (1954).

Although all the plots are linear none of the slopes are unity. Considering k_1 (which is known more accurately than k_2), the slopes at 125 and 150° for the H_0 plots are 0.607 and 0.610, respectively.
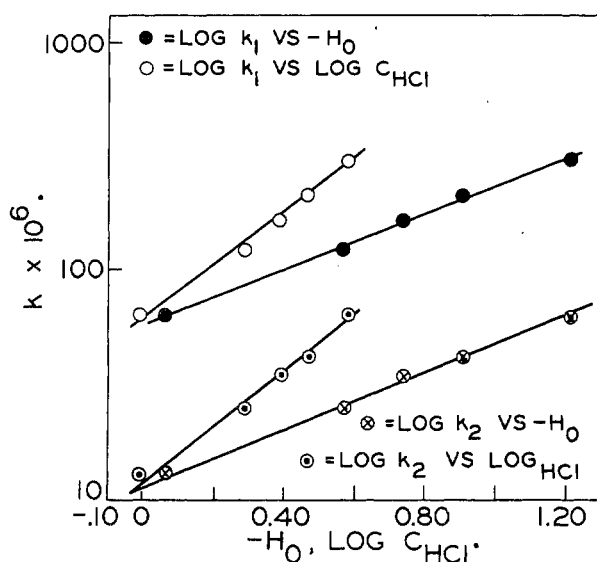

Fig. 4.—Semi-log plot of the rate constants for the hydration and dehydration steps as a function of the logarithm of the hydrochloric acid concentration and the Hammett acidity function ($T = 150°$).

These slopes are considerably lower than any others reported for reactions whose rates are believed to follow the H_0 function. Among the lowest values reported for the slope is 0.86 for the hydrolysis of epi-

bromohydrin in aqueous solutions of perchloric acid.[14]

The slopes of the plots of log k_1 versus log C_{HCl} at 125 and 150° are 1.18 and 1.15, respectively. Since these slopes are closer to unity than those for H_0 it is presumed that the rate-determining step in the hydration reaction is a bimolecular reaction with water. Thus, the mechanism of hydration of fumaric acid is similar to that for the hydration of crotonic acid to β-hydroxybutyric acid[4] and that for the hydration of crotonaldehyde to aldol[15] and different from that for the hydration of olefins to carbinols.[16]

The $\Delta S_1{}^{\ddagger}$ values are also more like those for the hydration of crotonaldehyde[15] (which is -23 cal. deg.$^{-1}$ mole^{-1}) than for the hydration of olefins to carbinols[16] (which are around zero). The $\Delta S_2{}^{\ddagger}$ value is similar to that for the dehydration of the aldol[15] (which is -2 cal. deg.$^{-1}$) rather than the carbinols[16] (which are about 20 cal. deg.$^{-1}$ mole^{-1}). The entropies of activation found for the $H_2F + H_2O = H_2M$ reaction, therefore, support the hypothesis that the rate-determining step of the hydration reaction is a bimolecular reaction with a water molecule.

Acknowledgments.—The authors are indebted to Professor Edward L. King for many helpful suggestions and discussions, and to Mr. Wilmer G. Miller for data and advice concerning the chromatographic experiments.

(14) J. G. Pritchard and F. A. Long, J. Am. Chem. Soc., 78, 2667 (1956).

(15) S. Winstein and H. J. Lucas, ibid., 59, 1461 (1937).

(16) R. W. Taft, Jr., ibid., 74, 5372 (1952).

HEATS OF PRECIPITATION OF SOME METAL 8-HYDROXYQUINOLATES

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Heats of precipitation are reported for the reaction, in aqueous solution, of a number of divalent metal ions with the 8-hydroxyquinolate anion. The reactions are all exothermic. Heats of reaction are discussed in terms of the electronegativity of the bonded metal atoms and of the structures of the solid compounds formed. Entropy changes for the reactions are large and positive. A qualitative correlation is noted between the observed entropy changes and the partial molal entropies of the corresponding metal cations.

Few data are available for the heat content changes accompanying the reaction of chelating agents in solution with metal ions to give solid metal chelates. Such values are of interest both in themselves and as a means of calculating entropy changes from equilibria data for the same reactions. In the present work heats of reaction between a series of divalent metal ions and 8-hydroxyquinolate, in aqueous solution, have been determined calorimetrically. The reaction studied is (1) and this formulation actually represents equilibria. The equilibrium constants associated with the equilibria are in most of the cases studied, however, sufficiently large[1] that the reactions are essentially quantitative.

The experimental procedure involved first form-

(1) R. Näsänen, Suomen Kemistilehti, B2, 11 (1953).
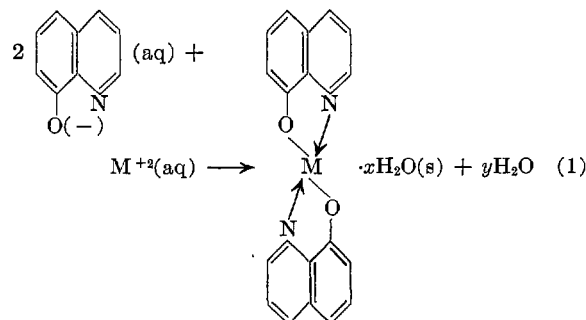
ing the 8-hydroxyquinolate anion in solution by the interaction of 8-hydroxyquinoline with the calculated amount of sodium hydroxide. To this solution was then added a solution containing slightly less than the theoretical amount of metal