Kinetic and Equilibrium Studies of Formation of N-Salicylideneglycinato Complexes. The Promnastic[1] Effect of the Divalent Ions of Magnesium, Manganese, Zinc, Cadmium, and Lead[2]

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Abstract: The kinetics of formation of several N-salicylideneglycinatometal(II) complexes at 25°, 0.5 *M* ionic strength, are reported. These systems exhibit reaction paths which are both dependent and independent of the metal ion concentration. The latter paths are identical for all metal ion systems and also to rates measured in the absence of metal. These rates are described by the equation: $d[\text{Schiff base}]/dt = (k_a a_H^2 + k_b a_H + k_c)[\text{Sal}^-]\cdot[\text{Gly}^-]$. This rate law probably arises from rate-determining attack of glycinate anion on salicylaldehyde anion (k_c), and on neutral salicylaldehyde (k_b), with the latter reaction exhibiting an acid-catalyzed path (k_a). A maximum of three metal-dependent paths was observed. These are considered to arise from rapid preequilibrium between the reactants to give ternary complexes followed by rate-determining reaction of ligands. $d[\text{Schiff base}]_{M^{2+}}/dt = \{k_0 + k_1\alpha_H + k_2[\text{Gly}^-]\}[M^{2+}][\text{Sal}^-][\text{Gly}^-] = (k_{P1} + k_{PH}a_H)[M(\text{Sal})(\text{Gly})] + k_{P2}[M(\text{Sal})(\text{Gly})_2^-]$. The order of catalytic efficiency of divalent metal ions, as measured by k_{P1}, is Pb $\gg$ Cd $>$ Mn $\sim$ Mg $>$ Zn $\gg$ Co, Ni, Cu (negligibly small). No metal ions with partially filled d orbitals exhibit reactivity. This is presumably due to ligand field effects which cause rigid metal–ligand geometries. The order of increasing catalytic efficacy of the other metal ions is essentially that of increasing weakness of the glycine amine group bond to the metal. This effect results in a greater probability of nucleophilic attack on the carbonyl group of salicylaldehyde ligand. Activation of the coordinated carbonyl group by the metal ion appears to be unimportant, but it is indicated that the larger cations are better catalysts than the smaller. The function of the metal ion seems to be better described by the term promnastic[1] rather than template. The systems of Mn, Zn Cd, and Pb exhibit an acid-catalyzed promnastic path. It appears that similar acid catalyst mechanisms may obtain for both the promnastic and metal-independent reactions because the values of k_{PH}/k_{P1} are similar and are comparable to the value of k_a/k_b. Values of k_{P2}, observed for Zn and Cd systems, were greater by a factor of about 50 than their respective values of k_{P1}. The equilibrium studies showed that the gain in stability resulting from formation of a Schiff base complex from the ternary complex containing independently bound ligands is not dependent on the nature of the metal ion. The formation constant for this process is about 100. Similar behavior is exhibited by analogous ternary systems containing carbonyl compounds other than salicylaldehyde.

Several types of enzymes involved in amino acid metabolism, notably aminotransferases, decarboxylases, and various lyases and synthetases, require pyridoxal phosphate (PLP) as cofactor.[3] It was postulated independently by Metzler, *et al.*,[4] and by Braunstein and Shemyakin[5] that all such reactions involve the formation of Schiff base between the amino acid and PLP as the first stage. Simple systems containing metal ion, amino acid, and an analog of PLP duplicate many of these enzymic reactions.[6] The first stage in these model reactions is formation of a Schiff base complex. Schiff base formation in the absence of metal ions involves reaction between a carbonyl compound and an amine to form a carbinolamine which subsequently dehydrates. Both stages are reversible and subject to general acid–base catalysis.[7,8]

Certain metal ions promote Schiff base formation by forming stable complexes with them and thereby providing a more favorable free energy of reaction.[9] Not all of these metal ions appear to be kinetically active, however. It has been found in a study of the rate of formation of N-salicylideneglycinate[10] that Cu(II) and Ni(II) are kinetically inactive with the highly stable Schiff base complexes being formed through proton-catalyzed paths. With Zn(II) reaction proceeds not only through these proton paths, but a parallel path, rate = $k[\text{Zn}^{2+}][\text{Sal}^-][\text{Gly}^-]$, was also found.[10] Discounting the unlikely possibility of a ternary collision between these three species in aqueous media, the rate law indicates a preequilibrium step. Glycinate attack on complexed salicylaldehyde is ruled out because both Cu(II) and Ni(II) form more stable complexes with salicylaldehyde anion than does Zn(II), and therefore should show activity if salicylaldehyde polarization is required. Salicylaldehyde attack on complexed glycinate is implausible because the amine electron pair bound to the metal ion is not free to react with the carbonyl carbon. The possibility of a preequilibrium between salicylaldehyde and glycinate to give carbinolamine also does not seem

(1) προμνασθαι—to be a matchmaker.

(2) Presented at the Symposium on the Reactions of Coordinated Ligands, Central Regional Meeting of the American Chemical Society, Akron, Ohio, May 1968. This work was kindly supported by the National Science Foundation.

(3) "Chemical and Biological Aspects of Pyridoxal Catalysis," E. E. Snell, P. M. Fasella, A. Braunstein, and A. Rossi Fannelli, Ed. The Macmillan Co., New York, N. Y., 1963.

(4) D. E. Metzler, M. Ikawa, and E. E. Snell, *J. Am. Chem. Soc.*, **76**, 648 (1954).

(5) A. E. Braunstein and M. M. Shemyakin, *Biokhimiya*, **18**, 393 (1953).

(6) E. E. Snell, ref 3, p 1.

(7) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. 2, W. A. Benjamin, Inc., New York, N. Y., 1966, pp 226–300.

(8) W. P. Jencks, *Progr. Phys. Org. Chem.*, 2, 63 (1964).

(9) D. L. Leussing and K. S. Bai, *Anal. Chem.*, 40, 575 (1968), and references cited therein.

(10) K. S. Bai and D. L. Leussing, *J. Am. Chem. Soc.*, **89**, 6126 (1967).

likely because of the observed inactivities of Cu(II) and Ni(II). This point is discussed further below. The remaining plausible possibility involves the formation of a ternary complex, Zn(Sal)(Gly), in which the ligands are independently bound and are in rapid equilibria with uncomplexed ligands. The rate-determining step is the condensation of these complexed ligands within the primary coordination sphere of the metal ion.

Other work[11] concerning metal ion effects on the rates of ketimine formation with methanolic α-ketovaleric acid–pyridoxamine appear to qualitatively support the same order of reactivities with these metal ions although the rate laws governing the reactions have not been established. Metal ions may influence the kinetics of the interacting ligands through one or more of three levels of activity: by complexing the reactant ligands as inert complexes and therefore slowing the reaction; by increasing the acidity of the solution through complexation of basic ligands and, thereby, increasing the rate of proton catalyzed paths; by forming kinetically active ternary species as was found with Zn(II). The last two effects are easily confused. In order to delineate these various modes of activity, it is necessary to vary the reaction conditions as widely as possible and also to take into account the side equilibria involving both the reactants and products. Changes in equilibrium distribution as the reaction proceeds will, of course, influence the relative contributions of the various paths and perturb the rate.

This study extends the earlier investigation to a wider range of divalent metal ions.

Experimental Section

Salicylaldehyde and glycine were purified and used as previously described.[9,10] Standard solutions of the chloride salts of Mg(II) and Co(II) and of the nitrate salts of Zn(II), Cd(II), and Pb(II) were prepared and standardized using accepted procedures. Reaction solutions were adjusted to give an ionic strength of 0.5 *M* by using either KCl or KNO_3 in correspondence to the use of divalent metal chloride or nitrate salts, respectively.

All measurements were made on solutions maintained at 25.0° under an atmosphere of nitrogen.

Equilibrium Measurements. Binary formation constants of both glycine and salicylaldehyde with the various metal ions were calculated using the Bjerrum $\bar{n}$ method from data obtained by potentiometric titration of standardized metal–ligand solutions with standard NaOH solution.

Data from the ternary systems were obtained by the batchwise titration procedure previously described[9] except for the systems containing either Cd(II) or Pb(II) which equilibrated rapidly (<3 min). Data from these latter systems were collected by direct titration of metal(II)–salicylaldehyde solutions with sodium glycinate solution. The formation constants of the ternary complexes were computed using the least-squares procedures previously described.[9]

The formation constants for the ternary system of Co(II) were not measured as they were not needed for the kinetic analysis. Binary and ternary constants of Mn(II) were reported previously,[9] but are in small error due to titrations being carried out only at low pH. Data were obtained in the present study over a greater pH range by titrating under more rigorous conditions of air exclusion to prevent precipitation of higher valence state hydrated oxides.

Kinetic Measurements. Most of the reaction rates were obtained by using the previously described pH-Stat technique.[10,12] However, the fast rates exhibited by the Cd(II) and Pb(II) systems precluded the use of this method and so a spectrophotometric method was used. This method was also used to extend the pH range originally investigated for the Zn(II) rates into more alkaline regions.

(1) pH-Stat Measurements. An aliquot of a solution containing metal ion and salicylaldehyde was brought to the desired reaction pH by rapid addition of 0.1 *M* sodium glycinate solution. As Schiff base formation proceeded, the reaction pH was maintained by automatic addition of the titrant. The volume of added titrant with respect to time was recorded and analyzed to obtain reaction rates. The kinetics are complicated by changes in the distribution of the labile species as the reaction proceeds. The method of data analysis is discussed in detail in Appendix I.

The following conditions were used.

$[Mg^{2+}]_t$ 0.01–0.05 *M*, pH 8.0–9.6

$[Mn^{2+}]_t$ 0.01–0.25 *M*, pH 6.8–8.0

$[Co^{2+}]_t$ 0.005–0.05 *M*, pH 5.0–6.4

Salicylaldehyde concentration was varied from 0.01 to 0.03 *M*. In the cobalt series of experiments it was necessary to lower the reaction pH by the addition of extra glycine to the initial solution to prevent precipitation of hydroxides. A suitable aliquot of 0.3 *M* glycine solution was added immediately prior to titration.

(2) Spectrophotometric Measurements. The uv absorption bands of salicylaldehyde and species derived from it have extinction coefficients of the order of 10^4.[9] Rates of Schiff base formation were obtained under pseudo-first-order conditions of large excess of metal ion and glycine concentrations. An aliquot of a solution containing known concentrations of metal ion and glycine was brought to the desired reaction pH by the addition of standard NaOH solution. A volume of 10–30 μl of a 0.02 *M* salicylaldehyde solution was added to the thermostated solution which was contained in a 1-cm path length stoppered uv cell. The cell was shaken rapidly to effect mixing of the reactants and the increase in absorbance with respect to time at the wavelength (~350 mμ) of the absorption maximum of the Schiff base metal complex was recorded. The following conditions were used.

$[Zn^{2+}]_t$ 0.01–0.10 *M*, $[Gly]_t$ = 0.04–0.40 *M*, pH 7.6–9.2

$[Cd^{2+}]_t$ 0.01–0.05 *M*, $[Gly]_t$ = 0.024–0.12 *M*, pH 7.4–9.2

$[Pb^{2+}]_t$ 0.0025–0.02 *M*, $[Gly]_t$ = 0.0125–0.05 *M*, pH 6.2–7.8

The salicylaldehyde concentrations were varied from 3×10^{-5} to 10×10^{-5} *M*.

The spectral changes which occurred in the course of a representative run are depicted in Figure 1. The conditions were $[Zn^{2+}]_t$ = 0.0103 *M*, $[Gly]_t$ = 0.040 *M*, and $[Sal]_t$ = 7.03×10^{-5} *M* at pH 8.00. The initial spectrum is composed of bands at 254 (1.2) and 323 mμ (0.31) due to neutral salicylaldehyde and bands at 265 (0.70) and 377 mμ (0.63) due to salicylaldehyde anion.[9] (The extinction coefficients in parentheses have been reduced by a factor of 10^4.) The reaction proceeds to equilibrium exhibiting spectra with isosbestic points at 244, 259, 288, 310, and 369 mμ. The equilibrium spectrum is principally due to N-salicylideneglycinatozinc(II) bands at 267 (0.97) and 351 mμ (0.58).[9] No carbinolamine intermediate species were detected either in this run or in others carried out under various conditions. A careful proton resonance study of the analogous system of Zn(II)–pyridoxalanine shows that detectable carbinolamine concentrations are not formed under conditions of high concentration (~0.1 *M*) of all the reactants.[13]

A series of runs in the absence of metal ion was similarly carried out. A 10–30-μl portion of a 0.02 *M* salicylaldehyde solution was added to an aliquot of a glycine solution adjusted to reaction pH with NaOH solution. The rates were determined by recording the absorbance change with respect to time either at 254 mμ, band maximum of neutral salicylaldehyde (pH 6.7–8.7), or at 287 mμ, a wavelength near to a band maximum of the protonated Schiff base (pH 9.2–11.2). These wavelengths afforded near-maximal absorbance changes in their respective pH regions. Below pH 7.9 the solutions were not adequately buffered, and so initial rates of Schiff base formation were calculated from initial rates of absorbance change. However, the runs in the absence of metal above pH 7.9 and all the runs in which metal ion was present gave excellent linear plots of log ($A_\infty - A_t$) *vs.* time (where A_∞ and A_t are the absorbances at the completion of reaction and at any time *t*, respectively). The quantity $(d[SG]_t/dt)/[Sal^-][Gly^-]$ (where $d[SG]_t/dt$

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[11] A. E. Martell and Y. Matsushima, "Pyridoxal Catalysis," E. E. Snell, A. E. Braunstein, E. S. Severin, and Yu. M. Torchinsky, Ed., John Wiley and Sons, Inc., New York, N. Y., 1968, p 33; Proceedings of the 2nd International Symposium on Pyridoxal Catalysis, Moscow, Sept 1966.

[12] D. L. Leussing and C. K. Stanfield, *J. Am. Chem. Soc.*, **88**, 5726 (1966).

[13] O. A. Gansow and R. H. Holm, *ibid.*, **90**, 5629 (1968).

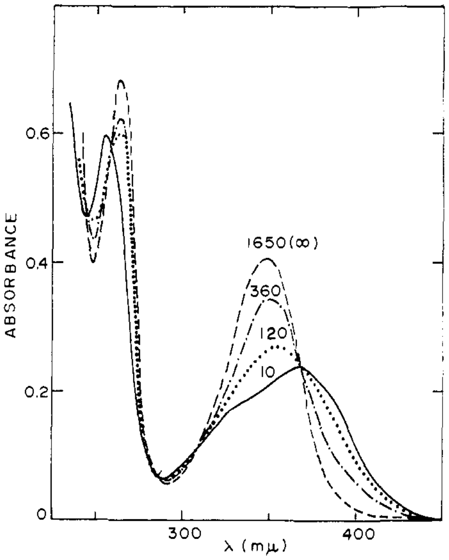

Figure 1. Uv spectra of a kinetic run under conditions of $[Zn^{2+}]_t$ = 0.0103 *M*, $[Gly]_t$ = 0.040 *M*, and $[Sal]_t$ = 7.03 × 10^{-5} *M* at pH 8.00. The numerals give the time after mixing, in seconds, at the start of each scan.

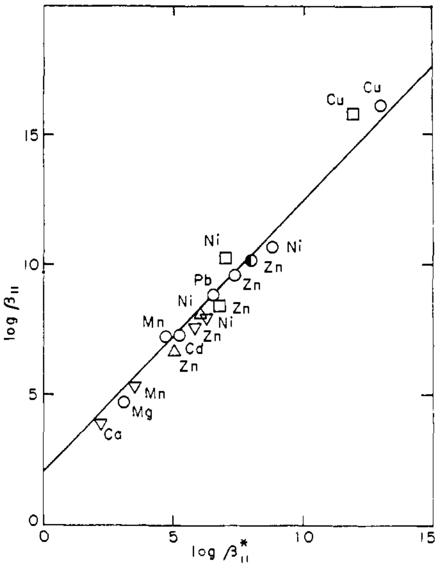

Figure 2. The enhancement of stability on forming a Schiff base complex from the complex containing independently bound ligands—systems containing divalent metal ion, glycine, and the following carbonyl compounds: ○, salicylaldehyde; △, glyoxylic acid; ▽, pyruvic acid; □, pyridoxal; and ◐, pyridoxal phosphate.

is the initial rate of formation of Schiff base) was calculated from each pseudo-first-order rate constant, k_{obsd}, using equations given in Appendix II. It was not possible to record spectra at various times during the course of a reaction due to the rapidity of the rates. Nevertheless carbinolamine intermediates were shown to be in undetectable concentrations because under identical run conditions the rate of reaction of salicylaldehyde species was identical with the rate of appearance of Schiff base species. Furthermore, a rapidly established equilibrium between glycinate and salicylaldehyde with *appreciable* levels of carbinolamine is ruled out because the salicylaldehyde spectra do not show the sharp initial decreases in intensities which are observed in such cases.[8]

Results

The cumulative formation constants, β_{ij}, for reactions

$$M^{2+} + i\text{Sal}^- + j\text{Gly}^- \underset{}{\overset{\beta_{ij}}{\rightleftharpoons}} \text{MSal}_i\text{Gly}_j{}^{2-i-j}$$

are given in Table I. Previously[9] evidence was presented that in the ternary complexes formed between salicylaldehyde, glycine, and M(II) (M = Mn, Ni, Cu, Zn) the ligands are condensed as the N-salicylideneglycinate species at equilibrium. The magnitude of the constants of the ternary complexes of Mg(II), Cd(II), and Pb(II) (Table I) shows that identical behavior obtains.

Table I. Log Formation Constants of Salicylaldehyde–Glycinate Complexes at 25°, *I* = 0.50 *M*[a]

$$M^{2+} + i\text{Sal}^- + j\text{Gly}^- \rightleftharpoons \text{MSal}_i\text{Gly}_j{}^{2-i-j}$$

	β_{01}	β_{02}	β_{03}	β_{10}	β_{21}	β_{11}	β_{12}	$\beta_{11}{}^{*b}$	$\beta_{12}{}^{*b}$
Mg	1.34			1.72		4.77		3.08	
Mn	2.56	4.27	4.87	2.10		7.29	9.95	4.66	
Co	4.51	8.16	10.45	3.22	5.32			6.94	
Zn[c]	4.88	9.11	11.56	2.87	5.00	9.65	13.42	7.36	9.51
Cd	4.18	7.50	9.76	1.60		7.31		5.20	7.18
Pb	4.36	7.62		3.04	4.9	8.86		6.58	

[a] $\beta_{ij} = [\text{MSal}_i\text{Gly}_j{}^{2-i-j}]/[M^{2+}][\text{Sal}^-]^i[\text{Gly}^-]^j$. [b] Independently bound ligands, see text. [c] Reference 9.

The enhanced stability of the Schiff base complexes, M(Sal·Gly), compared to the complexes M(Sal)(Gly), in which the ligands are independently bound, can be estimated. The formation constant $\beta_{11}{}^*$ for the reaction

$$M^{2+} + \text{Sal}^- + \text{Gly}^- \overset{\beta_{11}{}^*}{\rightleftharpoons} \text{M(Sal)(Gly)}$$

is estimated, as previously,[10] from the stepwise formation constants of the binary complexes

$$\beta_{11}{}^* = K_{01}K_{20} + K_{10}K_{02}$$

The two terms, $K_{01}K_{20}$ and $K_{10}K_{02}$, should ideally be equal to each other and the closeness of approach to this condition is an indication of the reliability of the estimated constant. In the cases where K_{20} values were not measurable, $\beta_{11}{}^*$ was estimated as $K_{01}K_{10}$. The values of $\beta_{11}{}^*$ thus calculated are given in Table I and appear to be reliable.[14]

The $\beta_{12}{}^*$ value of the Zn system was estimated as the average of the terms $K_{10}K_{02}K_{03}$ and $K_{01}K_{20}K_{03}$. The value of the Cd constant was estimated from only the first term. Both values are given in Table I.

Figure 2 depicts a plot of log β_{11} *vs.* log $\beta_{11}{}^*$ values from all of the systems investigated in this and the

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(14) The equilibrium data in themselves yield only the number of ligands of each type in each complex as well as its stability. The formation constants have been defined so as to recognize this mathematical restriction. Extrathermodynamic arguments such as enhanced stabilities, spectral properties, and rates of formation[9,10] are strong evidence that the highly stable, slowly forming species are indeed Schiff base complexes. The β_{11} values are the sum of the formation constants for the Schiff base complexes, M(Sal·Gly), and the rapidly equilibrated species, M(Sal)(Gly), where the ligands are independently coordinated. Since the $\beta_{11}{}^*$ values for these latter species are relatively small in most cases, the β_{11} values are usually equal to the formation constants of the Schiff bases within their experimental error. For more details concerning the estimation of the $\beta_{11}{}^*$ values and comparison with experimental values, see ref 15–17.

(15) Y. Kanemura and J. I. Watters, *J. Inorg. Nucl. Chem.*, **29**, 1701 (1967).

(16) Y. Marcus, *J. Phys. Chem.*, **66**, 1661 (1962); Y. Marcus, I. Eliezer, and M. Zangen, Symposium on Coordination Chemistry, Tihany, Hungary, Sept 1964.

(17) J. Schubert, V. S. Sharma, E. R. White, and L. S. Bergelson, *J. Am. Chem. Soc.*, 90, 4476 (1968); V. S. Sharma, and J. Schubert, private communication of a paper to be published in *J. Chem. Educ.*; D. D. Perrin and V. S. Sharma, *J. Chem. Soc.*, *A*, 446 (1968).

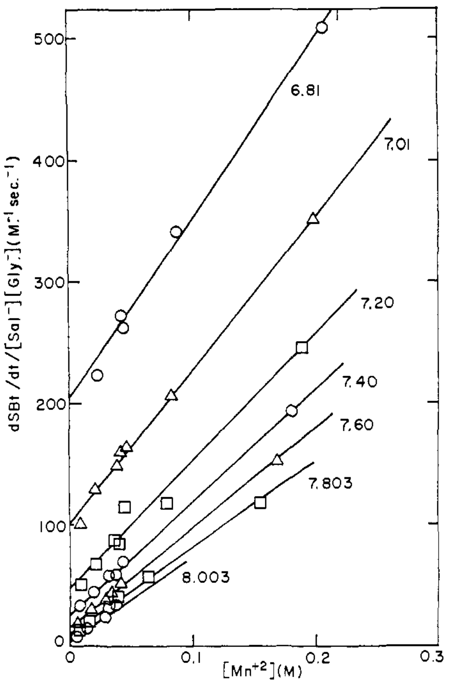


Figure 3. Mn(II) rates as a function of free [Mn^{2+}].

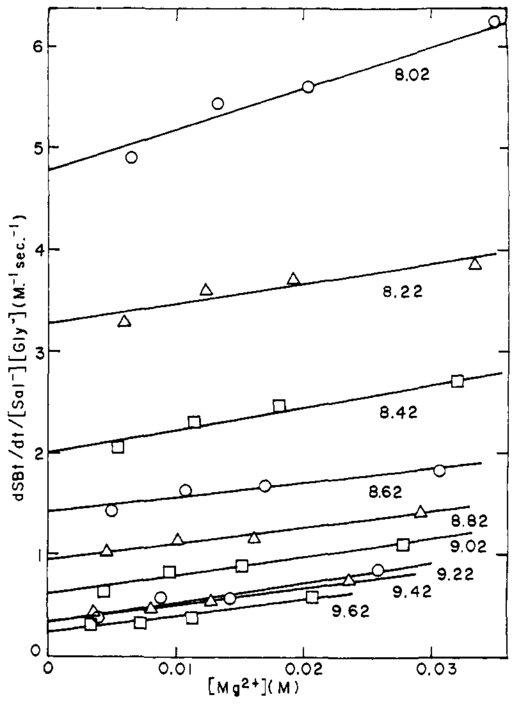
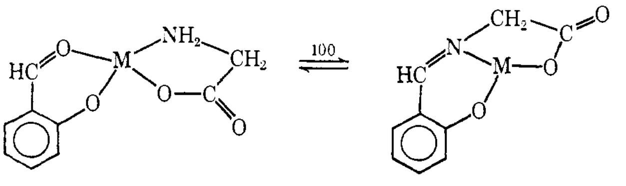


Figure 4. Mg(II) rates as a function of free [Mg^{2+}].

previous work.[9] The plot is linear having a slope of about unity and an intercept of about 2.0. This shows that within experimental error the ratio β_{11}/β_{11}^* is independent of the nature of the metal ion and is of approximate value 100. This ratio is the constant for the reaction



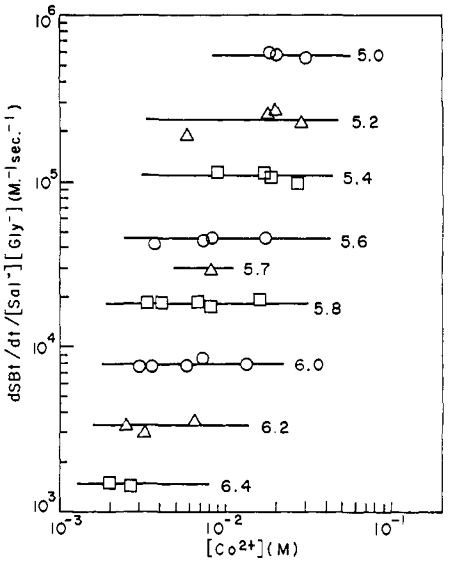


Figure 5. Co(II) rates as a function of free [Co^{2+}].

Thus it appears that the increased stability of the Schiff base complex is due mainly to an entropy increase caused by formation of a mono complex of a tridentate ligand from a bis complex of bidentate ligands. This follows because enthalpy effects are much more dependent on the nature of the metal ion than entropy effects.

It is of great interest that the log β_{11} and log β_{11}^* values from other ternary Schiff base systems based on glycine obey the same relationship as the N-salicylideneglycinatometal(II) series. Shown in Figure 2 are the formation constants from the ternary glycine systems of Ni(II) and Zn(II) with glyoxylate,[18] Cu(II),[19] Mn(II),[19] Ni(II),[19,20] and Zn(II)[19,20] with pyruvate, Ni(II),[21] Cu(II),[21,22] and Zn(II)[21] with pyridoxal, and Zn(II) with pyridoxal phosphate.[23] The independence of the data from the nature of the carbonyl compound reinforces the opinion that the enhancement in stability on forming a Schiff base complex from the independently bound ligands is mainly an entropy effect. The heat and entropy contributions reported for the formation of N-pyruvylideneglycinate complexes[19] also indicate that the entropy increase is the predominant factor.

(i) Metal-Independent Rates. Bai and Leussing[10] showed that the rates of formation of Cu(II) and Ni(II) N-salicylideneglycinato complexes are first order in both [Gly^-] and [Sal^-] and that the rate expression $(d[SB]_t/dt)/[Sal^-][Gly^-]$ is independent of free metal ion concentration under acidic conditions. In addition to this path, the analogous Zn(II) system exhibits a path which is first order dependent on [Zn^{2+}].

In Figures 3 and 4 are plots of $(d[SB]_t/dt)/[Sal^-][Gly^-]$ *vs.* [M^{2+}], where M^{2+} is Mn^{2+} and Mg^{2+}, respectively. These plots indicate both metal-ion-dependent and -independent paths. The Co^{2+} system yielded values of $(d[SB]_t/dt)/[Sal^-][Gly^-]$ which are independent of [Co^{2+}] (Figure 5). A least-squares analysis was per-

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(18) D. L. Leussing and E. M. Hanna, *J. Am. Chem. Soc.*, **88**, 696 (1966).

(19) D. L. Leussing and D. C. Schultz, *ibid.*, **86**, 4846 (1964).

(20) D. L. Leussing and E. M. Hanna, *ibid.*, **88**, 693 (1966).

(21) D. L. Leussing and N. Huq, *Anal. Chem.*, **38**, 1388 (1966).

(22) (a) H. N. Christensen, *J. Am. Chem. Soc.*, **80**, 2305 (1958); (b) L. Davis, F. Roddy, and D. E. Metzler, *ibid.*, **83**, 127 (1961).

(23) D. L. Leussing, C. J. Ekstrom, and W. Felty, unpublished work.

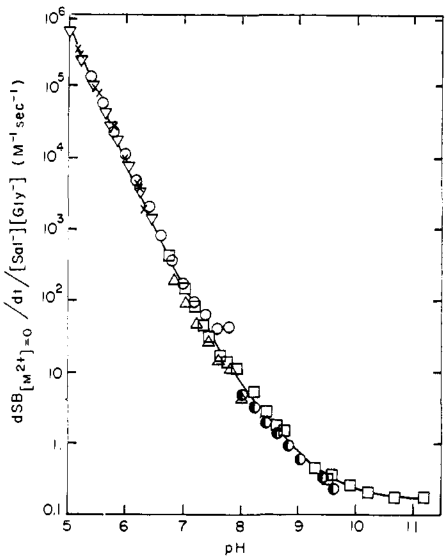

Figure 6. The pH dependence of the rates in the absence of metal, □, and of the metal-independent rates: ◐, Mg(II); △, Mn(II); ▽, Co(II); ×, Ni(II); and ○, Zn(II).

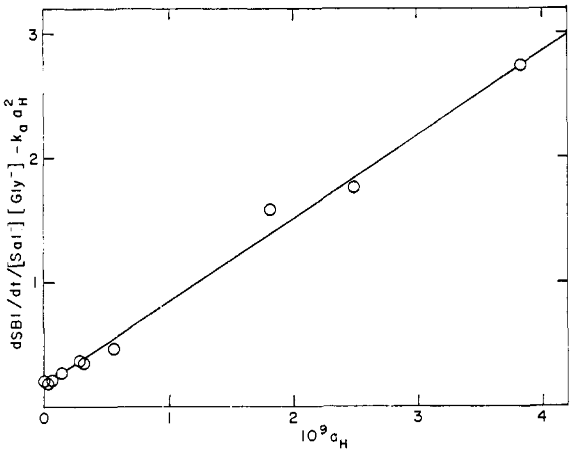

Figure 7. The pH dependence of the rates in the absence of metal above pH 8.4 after subtraction of the term $k_a a_H^2$.

formed on each set of data at a given pH value to determine the best values of k and k' in the rate law

$$d[SB]_t/dt = \{k + k'[M^{2+}]\}[Sal^-][Gly^-] \qquad (1)$$

Least-square values of k and k' were also recalculated from the earlier Zn(II) data of Bai and Leussing.[10] The k' values, which are discussed later, are slightly but significantly pH dependent and not pH independent as previously reported.

All of the metal independent rate constants, k, are plotted *vs.* pH in Figure 6 and within experimental error they lie along a single curve. Below pH 7 this curve is identical with that previously reported,[10] but for reasons of space the Cu and Ni rates below pH 5 are not shown. Also depicted in Figure 5 are values of $(d[SB]_t/dt)/[Sal^-][Gly^-]$ derived from the pseudo-first-order rate constants found from the runs performed in the absence of metal ions, and these are also seen to lie along the same curve. These latter rates are intrinsically of greater accuracy than those derived from metal ion systems because the total reaction was followed and also the calculations involve a lesser number of species. Thus the metal-ion-independent rate of formation of N-salicylideneglycine over the pH range 2.7–11.2 is given by the same rate law previously reported.[10]

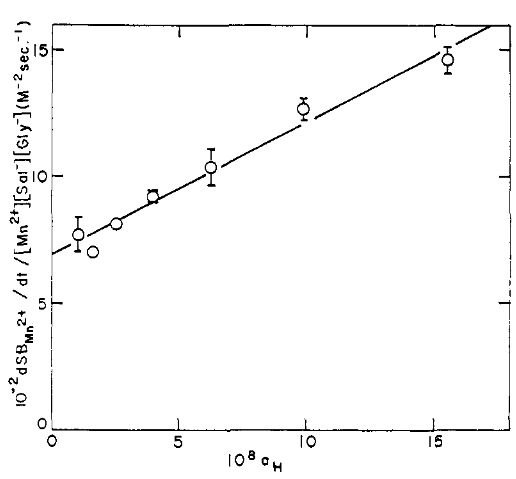

Figure 8. The pH dependence of the $[Mn^{2+}]$-dependent rates.

$$d[SB]_t/dt = (k_a a_H^2 + k_b a_H + k_c)[Sal^-][Gly^-]$$

The value of k_a, $6.9 \times 10^{15}\ M^{-3}\ sec^{-1}$, is identical with the earlier value,[10] but the values of k_b and k_c differ somewhat. The earlier values of k_b and k_c are in error due to the large errors occurring near the upper pH limit at which the pH-Stat is applicable with Zn(II). The values of $6.7 \times 10^8\ M^{-2}\ sec^{-1}$ for k_b and $0.18\ M^{-1}\ sec^{-1}$ for k_c were calculated in this work from the rates obtained in the absence of metal above pH 8.4. The linear plot of $\{(d[SB]_t/dt)/[Sal^-][Gly^-] - k_a a_H^2\}$ *vs.* a_H is depicted in Figure 7. The solid curve drawn through the points in Figure 6 was calculated using the above values of rate constants. The goodness of fit of the metal-independent rates to this curve demonstrates the reliability of these values.

(ii) **Metal-Dependent Rates.** An inspection of Figure 3 shows that the slopes, $k' = (d[SB]_{Mn^{2+}}/dt)/[Mn^{2+}] \cdot [Sal^-][Gly^-]$,[24] increase with respect to decreasing pH and they are plotted *vs.* a_H in Figure 8. The linearity shows that the Mn(II)-dependent rates are resolvable into proton-dependent and -independent paths of the rate law $d[SB]_{Mn^{2+}}/dt = (k_0 + k_1 a_H)[Mn^{2+}][Sal^-][Gly^-]$, where $k_0 = (6.95 \pm 0.30) \times 10^2\ M^{-2}\ sec^{-1}$ and $k_1 = (5.2 \pm 0.4) \times 10^9\ M^{-3}\ sec^{-1}$. A similar plot (Figure 9) of the slopes from earlier Zn(II) data[10] shows that identical behavior obtains, with $k_0 = (5.9 \pm 0.3) \times 10^4\ M^{-2}\ sec^{-1}$ and $k_1 = (3.85 \pm 0.16) \times 10^{10}\ M^{-3}\ sec^{-1}$, rather than the single proton-independent path previously reported.

On the other hand, the slopes from the Mg(II) data (Figure 4) are independent of pH, albeit with a large scatter. The Mg(II)-dependent rate constant, $k_0 = 18 \pm 1\ M^{-2}\ sec^{-1}$, was calculated by plotting $\{(d[SB]_t/dt)/[Sal^-][Gly^-] - k\}$ values from each kinetic run *vs.* $[Mg^{2+}]$ (Figure 10). k is defined in eq 1.

The large scatter of the points shown in Figure 10 is primarily due to imprecision in the values of formation constants.

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[24] $d[SB]_{M^{2+}}/dt$ is the portion of the rate $d[SB]_t/dt$ which is dependent on free metal ion concentration $[M^{2+}]$.

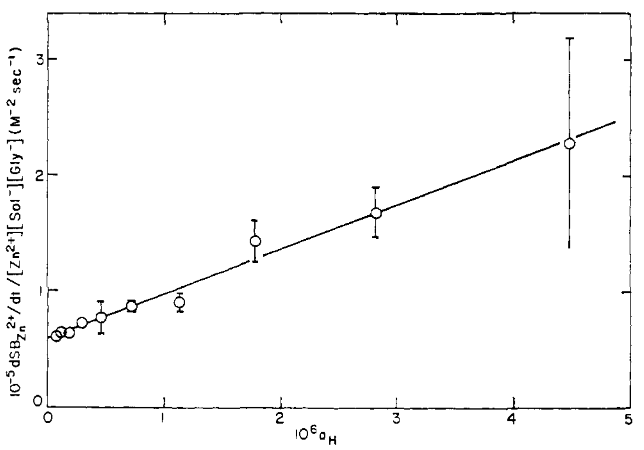

Figure 9. The pH dependence of the $[Zn^{2+}]$-dependent rates from the pH-Stat runs.

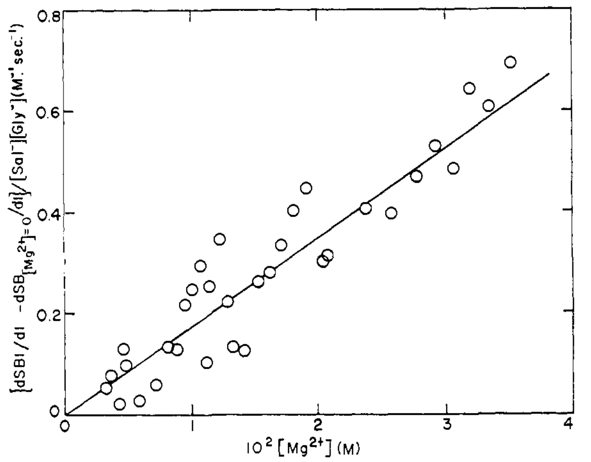

Figure 10. The $[Mg^{2+}]$-dependent rates as a function of free $[Mg^{2+}]$.

The pseudo-first-order rate constants from the Zn(II), Cd(II), and Pb(II) spectrophotometric runs were converted to values of the rate expression $(d[SB]_t/dt)/[Sal^-][Gly^-]$ (Appendix II). Each of these values was corrected for contributions from metal-independent paths by subtraction of $k_a a_H{}^2 + k_b a_H + k_c$ to give values of $(d[SB]_{M^{2+}}/dt)[Sal^-][Gly^-]$. These corrections constitute from 30 to 37% of the Zn(II) rates and from 10 to 20% of the Cd(II) rates but are negligible compared to the Pb(II) rates. In contrast to the pH-Stat runs, the Zn(II) spectrophotometric runs were carried out under conditions where bis- and trisglycinato complexes predominate. The distribution of Zn(II) glycinato complexes is depicted in Figure 11 which also shows that a linear relationship exists between $(d[SB]_{Zn^{2+}}/dt)/[Zn^{2+}][Sal^-][Gly^-]$ and $[Gly^-]$. Hence the Zn(II)-dependent rates are given by the rate law

$$d[SB]_{Zn^{2+}}/dt = \{k_0 + k_1 a_H + k_2[Gly^-]\}[Zn^{2+}][Sal^-][Gly^-]$$

where $k_2 = (1.19 \pm 0.05) \times 10^9\ M^{-3}\ sec^{-1}$. Under the conditions used for the spectrophotometric runs, the k_0 and k_1 paths are almost negligible, contributing only 20% to the smallest rate shown in Figure 11.

The Cd(II)-dependent rates follow a rate law identical with that found with Zn(II). However, the conditions were such that all three paths contributed significantly to the observed rates. A least-squares treatment of the rates yielded values of $k_0 = (1.00 \pm 0.08) \times 10^4\ M^{-2}\ sec^{-1}$, $k_1 = (4.4 \pm 0.09) \times 10^7\ M^{-3}\ sec^{-1}$, and $k_2 = (2.45 \pm 0.09) \times 10^7\ M^{-3}\ sec^{-1}$. The paths k_0 and k_2 and the path k_1 are depicted in plots of $\{(d[SB]_{Cd^{2+}}/dt)/[Cd^{2+}][Sal^-][Gly^-] - k_1 a_H\}$ *vs.* $[Gly^-]$ (Figure 12) and of $\{(d[SB]_{Cd^{2+}}/dt)/[Cd^{2+}][Sal^-][Gly^-] - k_0 - k_2[Gly]\}$ *vs.* a_H (Figure 13), respectively. The distribution of Cd(II) glycinato complexes is also shown in Figure 12.

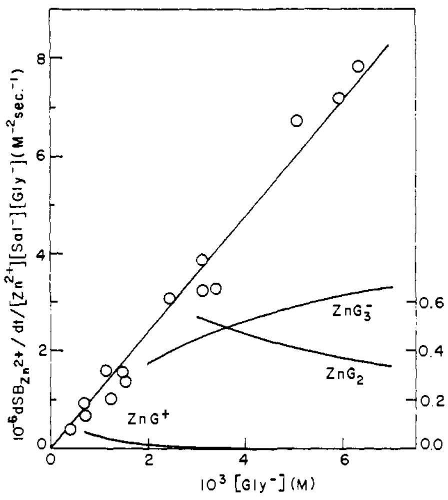

Figure 11. $[Zn^{2+}]$-dependent rate parameters from the spectrophotometric runs as a function of $[Gly^-]$.

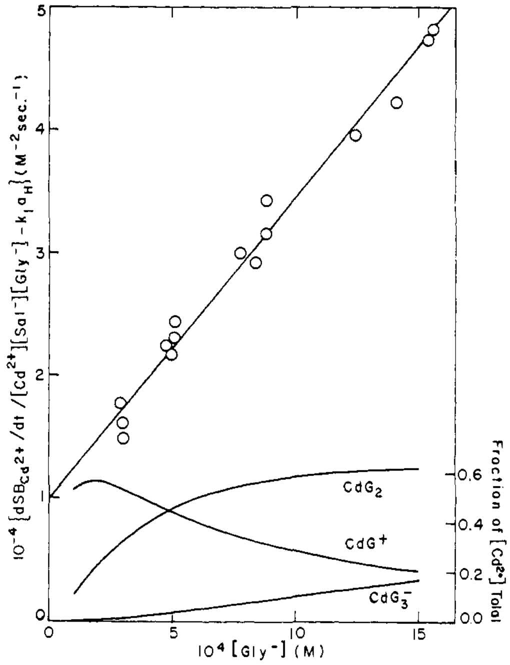

Figure 12. $[Cd^{2+}]$-dependent rate parameters as a function of $[Gly^-]$.

The Pb(II)-dependent rates are also described by an identical rate law except that the k_2 path is not observed. Figure 14 depicts a linear plot of $(d[SB]_{Pb^{2+}}/dt)/[Pb^{2+}][Sal^-][Gly^-]$ *vs.* a_H which gives values of $k_0 = (7.2 \pm 1.3) \times 10^6\ M^{-2}\ sec^{-1}$ and $k_1 = (3.3 \pm 0.5) \times 10^{13}\ M^{-3}\ sec^{-1}$. It was not possible to obtain conditions where significant concentrations of the bisglycinato

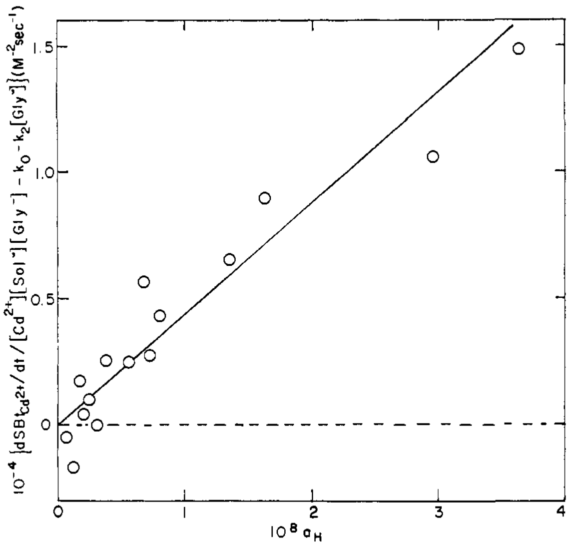

Figure 13. The pH dependence of [Cd^{2+}]-dependent rate parameters.

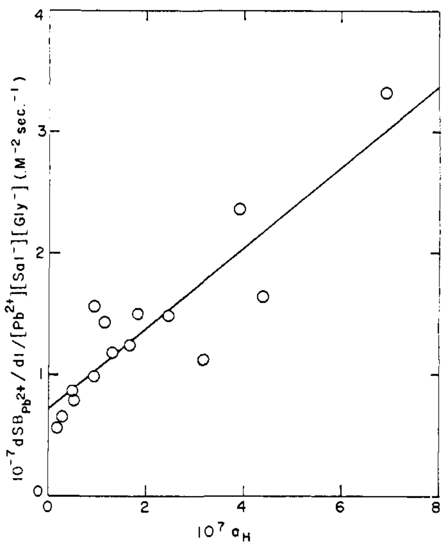

Figure 14. The pH dependence of [Pb^{2+}]-dependent rate parameters.

lead(II) complex were formed due to fast rates and to problems of hydroxo complex formation.

Discussion

The metal-ion-independent rates of formation of N-salicylideneglycine are first order in the concentrations of both aldehyde and amine as has been generally reported for carbonyl addition reactions.[7,8] The relatively simple rate law which is found indicates a single rate-determining step. Under none of the conditions used in this work was the presence of carbinolamine intermediate detected. Two possible mechanisms are apparent, either rate-limiting attack of glycinate anion on neutral or anionic salicylaldehyde followed by rapid dehydration of the carbinolamine intermediate, or formation of carbinolamine intermediate in a preequilibrium step followed by rate-limiting dehydration. Both mechanisms could exhibit general acid–base catalysis. However, if the latter mechanism is correct, one would expect the intermediate to form moderately stable complexes. The stability of such carbinolamine complexes must lie between that of the ternary complex of the uncondensed ligands and that of the Schiff base complex. With a limiting rate of dehydration, it is expected that concentrations of carbinolamine complexes would be formed which are detectable by spectrophotometry. Furthermore, this latter condition would of course result in metal-dependent rate enhancement, but this is not observed in precisely those systems, *i.e.*, Co(II), Ni(II), and Cu(II), which would show the greatest interaction with the carbinolamine. Thus we propose that formation of the intermediate is rate determining. French, Auld, and Bruice[25] proposed the same mechanism for the analogous reactions between 3-hydroxypyridine-4-aldehyde (HPA) and various amino acids. Their arguments, which are based on the magnitude of the rate constants, are also applicable to the salicylaldehydeglycine reactions and reinforce our conclusions.

The kinetic parameters can be variously interpreted except for k_c which is clearly due to reaction of the salicylaldehyde and glycinate anions. Rate constants derived for k_a and k_b for possible reactions involving glycinate anion are given in Table II. Also given in this table are the rates for the analogous reactions between HPA and glycinate anion. In the report describing the latter reactions,[25] the authors argue that the rates obtained by assuming acid-catalyzed paths are unrealistically large and conclude that the paths k_a and k_b and k_c are due to glycinate anion attack on the cationic, neutral (protonated pyridine nitrogen), and anionic forms of HPA, respectively. However, the path k_a from the salicylaldehyde system is very likely due to acid catalysis of reaction of neutral carbonyl with rate constant equal to $4.5 \times 10^7\ M^{-2}\ \mathrm{sec}^{-1}$, a value of magnitude previously considered to be unreasonably large for such a reaction.[25] The only alternative to this mechanism is attack by neutral glycine, in the non-zwitterionic form ($NH_2CH_2CO_2H$), on neutral salicylaldehyde. MacInnes[26] has estimated the dissociation constant, $k_a = [NH_2CH_2CO_2^-]a_H/[NH_2CH_2CO_2H] = 5.0 \times 10^{-5}\ M$. This value leads to a rate constant of $2.1 \times 10^3\ M^{-1}\ \mathrm{sec}^{-1}$ for the reaction, a value which is a factor of 500 greater than the rate constant for reaction of glycinate anion on neutral carbonyl. Thus this reaction is ruled out unless one considers that such an enhancement of rate can result from the possible bifunctional reaction shown below.

Table II. A Comparison of Salicylaldehyde and 3-Hydroxypyridine-4-aldehyde Reaction Rates with Glycinate Anion

Parameter	Reaction	Rate constants, M^n sec^{-1}: Sal	Rate constants, M^n sec^{-1}: HPA[a]
k_c	$^-OArCHO + Gly^-$	0.18	3.3
	$^-OArCHO + Gly^- + H^+$	6.7×10^8	8.2×10^8
k_b	$HOArCHO + Gly^-$	4.1	230
	$HOArCHO + Gly^- + H^+$	4.3×10^7	1.1×10^9
k_a	$HO(ArH^+)CHO + Gly^-$	. . .	9.5×10^4

[a] Reference 25.

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(25) T. C. French, D. S. Auld, and T. C. Bruice, *Biochemistry*, 4, 77 (1965).

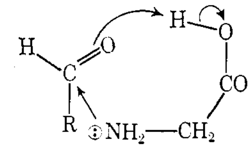
(26) D. A. MacInnes, "The Principles of Electrochemistry," Reinhold Publishing Corp., New York, N. Y., 1939, p 396.

Table III. Metal-Dependent Rates of Schiff Base Formation

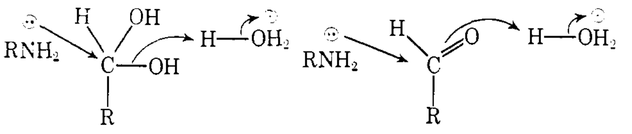
$$d[SB]_{M^{2+}}/dt = \{k_0 + k_1a_H + k_2[Gly^-]\}[M^{2+}][Sal^-][Gly^-]$$

M^{2+}	k_0, M^{-2} sec^{-1}	k_1, M^{-3} sec^{-1}	k_2, M^{-3} sec^{-1}	k_{P1},[a] sec^{-1}	k_{PH},[b] M^{-1} sec^{-1}	k_{P2},[c] sec^{-1}
Mg	18			0.015		
Mn	6.95×10^2	5.2×10^9		0.015	1.1×10^5	
Co	$<7 \times 10^2$			$<10^{-4}$		
Ni[d]	$<7 \times 10^2$			$<10^{-6}$		
Cu[d]	$<1.5 \times 10^6$			$<2 \times 10^{-7}$		
Zn	5.9×10^4 [e]	3.85×10^{10} [e]	1.19×10^9	0.0026	1.6×10^3	0.37
Cd	1.00×10^4	4.4×10^{11}	2.45×10^7	0.062	2.7×10^6	1.6
Pb	7.2×10^6	3.3×10^{13}		1.9	8.7×10^6	
Zn(Pyr)[f]	2.8×10^3	5.6×10^7	3.2×10^7	0.0058	2×10^3	0.32

[a] $k_{P1} = k_0/\beta_{11}^*$. [b] $k_{PH} = k_1/\beta_{11}^*$. [c] $k_{P2} = k_2/\beta_{12}^*$. [d] Reference 10. [e] Recalculated from data in ref 10 and K. S. Bai, Ph.D. Thesis, The Ohio State University, 1967. [f] Reference 32.



These considerations suggest that the path assigned to HPA cation reaction[25] could rather be due to acid catalysis of neutral HPA reaction. This supposition is supported by MO calculations on the various carbonyl species using the Pople and Segal CNDO/2 method.[27] The calculated atomic charge densities indicate that no further activation, *i.e.*, increase of positive charge on carbonyl carbon atom, results from protonation of the neutral species. Acid catalysis of nucleophilic attack on either the hydrated or nonhydrated carbonyl group is easily envisaged.



Some information on the relative effects of the heterocyclic nitrogen atom and the phenolic hydroxyl group may be delineated from these model PLP systems. The anions of salicylaldehyde and HPA differ only in the presence of a heterocyclic nitrogen atom in the latter species. The reactions at high pH (k_c) show that the attack of glycinate anion on HPA$^-$ is faster by a factor of 20 than its attack on salicylaldehyde anion. This is presumably the result of the greater electronegativity of nitrogen compared to carbon. The rates of reaction are enhanced on protonation of the anionic species to give the neutral compounds by factors of 23 and 70 for salicylaldehyde and HPA, respectively. However, the modes of protonation differ. Only the phenolate oxygen is available for protonation in salicylaldehyde in the pH range studied, whereas the predominant form of HPA^0 in aqueous solution is the dipolar ion with protonated ring nitrogen.[28] Thus the relative effects of protonation appear to be that protonation of the ring nitrogen leads to a slightly more reactive species than protonation of the phenolate oxygen atom.

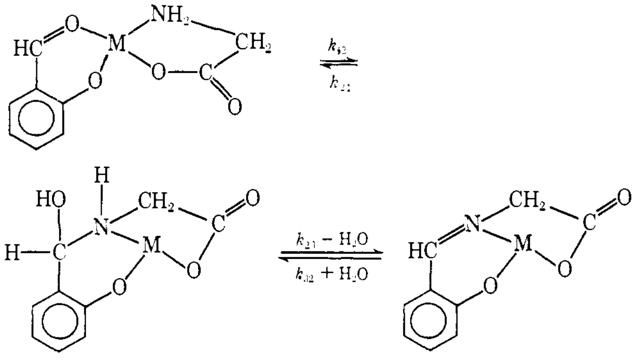
The metal-dependent rates are described by the rate law

$$d[SB]_{M^{2+}}/dt = \{k_0 + k_1a_H + k_2[Gly^-]\}[M^{2+}][Sal^-][Gly^-]$$

and the values of k_0, k_1, and k_2 are given in Table III. Bai and Leussing[10] argued that such a rate law arises from rapid preequilibrium between the reactants to form a ternary complex followed by a rate-determining reaction of the ligands within the coordination sphere of the metal ion. The further data reported here support their proposal. Thus the above rate law reduces to

$$d[SB]_{M^{2+}}/dt = (k_{P1} + k_{PH}a_H)[M(Sal)(Gly)] + k_{P2}[M(Sal)(Gly)_2^-]$$

where $k_{P1} = k_0/\beta_{11}^*$, $k_{PH} = k_1/\beta_{11}^*$, and $k_{P2} = k_2/\beta_{12}^*$. The values of the rate constants given in Table III were calculated using the estimated formation constants β_{11}^* and β_{12}^* given in Table I. Subsequent to the rapid preequilibrium between the reactants, the following type of reaction scheme must be considered in a discussion of the paths.



The over-all equilibrium constant, $K_P = k_{12}k_{23}/k_{21}k_{32} = \beta_{11}/\beta_{11}^*$, is of the order of 100. There are two limiting reaction schemes: either a rapid equilibrium between the intermediate carbinolamine complex and the initial complex followed by rate-determining dehydration to give the Schiff base complex or rate-determining formation of the intermediate followed by rapid dehydration.

For reasons discussed above, the former mechanism is ruled out. Therefore k_{12} is likely to be identical with k_{P1} and the values of this parameter (Table III) give an order of catalytic efficacy of the divalent metal ions of Pb ≫ Cd > Mn ~ Mg > Zn ≫ Co, Ni, Cu (very small).

(27) J. A. Pople and G. A. Segal, *J. Chem. Phys.*, **44**, 3289 (1966). The computor program used was written by G. A. Segal and supplied by Quantum Chemistry Program Exchange, Chemistry Department, Indiana University, Bloomington, Ind.

(28) K. Nakamoto and A. E. Martell, *J. Amer. Chem. Soc.*, **81**, 5863 (1959).

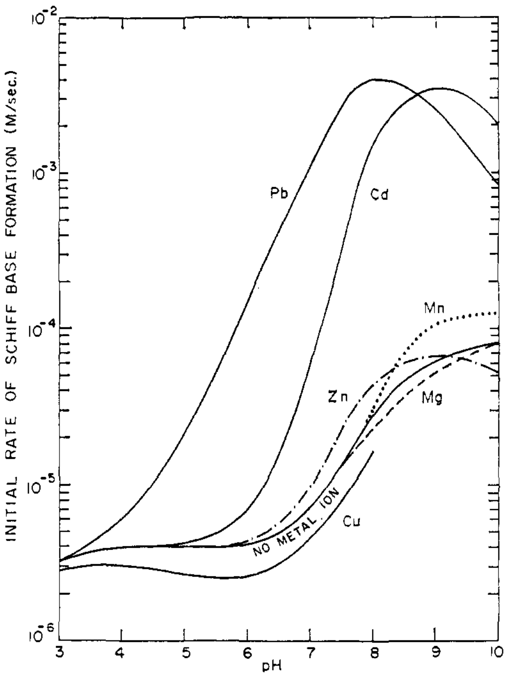


Figure 15. The pH dependence of initial rates of Schiff base formation calculated for several systems under the conditions of $[M^{2+}]_t$ = 0 or 0.01 *M*, $[Sal]_t$ = 0.01 *M*, and $[Gly]_t$ = 0.05 *M*.

Perhaps the most significant trend is that the only metal ions which are effective as catalysts are those which do not exhibit ligand field effects due to partially filled d orbitals. The ligand field effects probably cause the reactants to remain separated by being bound to coordination sites of precise geometry. The effect is appreciable as may be judged by the different behavior of Co(II) compared with Zn(II). These two metal ions otherwise behave very similarly with respect to other properties which are later proposed to be of kinetic significance. A metal ion which catalyzes a bimolecular reaction by forming a ternary complex intermediate with the reactants has previously been considered to act as a template.[10] However, the term template is misleading as it implies a fixed spatial orientation of the reactants to the metal whereas such an effect causes inactivity of the metal ion. Therefore we propose that the new word promnastic[1] be used to describe the effect of a catalytic agent which forms a ternary complex with two reactants but which imposes a minimum steric requirement on them.

The two obvious factors which are involved in determining the degree of catalytic activity of a metal ion are the extent of activation of the coordinated carbonyl group by polarization and the weakness of the bond between the metal and the amine group of glycine. The first effect is related to the energy of the carbonyl oxygen bond to the metal, and this in turn can be shown by thermochemical arguments to follow the order of hydration energies of the metal ions. This order is Zn > Mg > Mn > Cd > Pb[29] which is the reverse of the catalytic order. Thus the predominant factor in catalysis appears to be the weakness of the amine to metal bond which follows an order given by metal–ammonia first formation constants[30] of Pb < Mg < Mn < Zn < Cd. The size of the metal ion also appears to be important (the order of ionic radii[31] is Pb > Cd > Mn > Zn > Mg) as the other factors discussed predict Zn(II) to be a better catalyst than Cd(II). The larger cations appear to permit greater facility in ligand condensation.

The constants, k_{P2}, for condensation within the complexes $M(Sal)(Gly)_2^-$ (M = Zn, Cd) are larger than the corresponding values of k_{P1} by factors of 100 and 30, respectively. The statistical probability of *cis* arrangement of amine and carbonyl groups is greater by 2.14 for the $M(Sal)(Gly)_2^-$ complex as compared with the M(Sal)(Gly). This effect plus the weakening of the metal–amine bonds on forming a coordinatively saturated complex probably explains the catalytic enhancement. The values of K_3 for the stepwise addition of a third bidentate ligand to Zn(II) are considerably lower than those found for K_1 and K_2.[30] This effect is also shown by Cd(II) but to a somewhat lesser degree.[30] These data indicate a considerable weakening of the metal–ligand interaction in the hexacoordinated complexes. The increase in the kinetic activity of the hexacoordinated species, especially of Zn(II), is further evidence that the amine electron pair must not be bound too tightly for activity.

The values of k_{PH}/k_{P1} are fairly similar and are 7.5, 0.65, 52, and 4.6 (in units of $10^6\ M^{-1}$) for the metal ions of Mn, Zn, Cd, and Pb, respectively. These values are comparable to the value of k_a/k_b of $1.0 \times 10^7\ M^{-1}$ which suggests analogous mechanisms of acid catalysis for the promnastic reaction and the bimolecular reaction between neutral salicylaldehyde and glycinate anion. This is in accord with our proposals that the rate-determining step in both types of reactions is formation of the carbinolamine intermediate.

Of great interest is the close similarity between the kinetics of formation of the salicylaldehyde and pyruvic acid[32]-ylideneglycinato complexes of Zn(II). The results of the latter system are given in Table III. Both systems exhibit three metal-ion-dependent paths of identical rate law which yield three pairs of approximately identical promnastic constants. The metal-ion-independent rates are also similar. These striking similarities strongly suggest that the two systems react by identical mechanisms.

It is of interest to compare the calculated initial rates of formation of Schiff bases in the systems investigated here under identical sets of conditions. These are chosen as $[M]_t^{2+}$ = 0.01 *M*, $[Sal]_t$ = 0.01 *M*, $[Gly]_t$ = 0.05 *M* at various pH values. The results are depicted in Figure 15 which shows plots of initial rate *vs.* pH for systems containing the divalent ions of Mg, Mn, Cu, Zn, Cd, and Pb and also that with no metal ion present. The curves for Co and Ni would lie between that for no metal and Cu. Below pH 3 where complexation becomes negligible, all the rates converge to a common curve which is described by the reaction of the free ligands. Above this pH, in comparison with the reaction with no metal ion present, the transition metal ions, exemplified by Cu(II), inhibit the rate of

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(29) D. R. Rosseinsky, *Chem. Rev.*, **65**, 467 (1965).

(30) "Stability Constants," Special Publication No. 17, The Chemical Society, London, 1964.

(31) L. Pauling, "Nature of the Chemical Bond," 3rd ed, Cornell University Press, Ithaca, N. Y., 1960.

(32) D. L. Leussing and L. Anderson, submitted for publication; presented at the 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1968. This work extends and revises the previous report, ref 10.

reaction owing to the complexing of reactants. Even Mg(II) and under some conditions Zn(II) are inhibitors despite their roles as promnastic catalysts. The other metal ions are true catalysts as shown in Figure 15. It should be noted, however, that the Schiff base is unstable in acidic media, and metal ions, whether kinetically active or not, are necessary for the formation of appreciable amounts at equilibrium.

Appendix I

Calculations of the Reaction Rates from pH-Stat Measurements. The following symbols are used: M = metal, S = salicylaldehyde, G = glycine. The cumulative formation constants are defined as

$$\beta_{ij} = [\mathrm{MS}_i\mathrm{G}_j]^{2-i-j}/[\mathrm{M}^{2+}][\mathrm{S}^-]^i[\mathrm{G}^-]^j$$

The constants β_{ij}^* are similarly defined for the uncondensed ligand complexes $[\mathrm{M(S)}_i\mathrm{(G)}_j]$ (see text). Acidity constants are defined as $K_\mathrm{a} = [\mathrm{B}]a_\mathrm{H}/[\mathrm{BH}]$, where a_H is the activity of the hydrogen ion. The following values were used: pK_a of salicylaldehyde = 8.22;[9] for glycine p$K_{1\mathrm{a}}$ = 2.46 and p$K_{2\mathrm{a}}$ = 9.70.[19] Previously determined[9] values of the formation constant of protonated and unprotonated Schiff base were used: $Q_\mathrm{HSG} = [\mathrm{HSG}^-]/[\mathrm{S}^-][\mathrm{G}^-]a_\mathrm{H} = 5.35 \times 10^{11}\ M^{-2}$, and $Q_\mathrm{SG} = [\mathrm{SG}^{2-}]/[\mathrm{S}^-][\mathrm{G}^-] = 3.3\ M^{-1}$.

Calculation of Initial Rates of Schiff Base Formation from pH-Stat Measurements. At any given time during the course of a titration in which NaG is added to a solution of M^{2+}, H^+, and Sal, the composition of the reaction solution is described by the four mass balance equations of the known total concentrations.

$$[\mathrm{M}]_\mathrm{t} = [\mathrm{M}^{2+}] + [\mathrm{MG}^+] + [\mathrm{MG}_2] + [\mathrm{MG}_3^-] + [\mathrm{MS}^+] + [\mathrm{MS}_2] + [\mathrm{M(S)(G)}] + [\mathrm{MSG}]$$

$$[\mathrm{S}]_\mathrm{t} = [\mathrm{S}^-] + [\mathrm{HS}] + [\mathrm{MS}^+] + 2[\mathrm{MS}_2] + [\mathrm{M(S)(G)}] + [\mathrm{MSG}] + [\mathrm{HSG}^-]$$

$$[\mathrm{G}]_\mathrm{t} = [\mathrm{G}^-] + [\mathrm{HG}] + [\mathrm{H_2G}^+] + [\mathrm{MG}^+] + 2[\mathrm{MG}_2] + 3[\mathrm{MG}_3^-] + [\mathrm{M(S)(G)}] + [\mathrm{MSG}] + [\mathrm{HSG}^-]$$

$$[\mathrm{H}]_\mathrm{t} = [\mathrm{H}^+] - [\mathrm{OH}^-] + [\mathrm{HS}] + [\mathrm{HG}] + 2[\mathrm{H_2G}^+] + [\mathrm{HSG}^-]$$

The concentration of the unprotonated Schiff base, SG^{2-}, was negligible under all conditions used. Under some conditions the concentration of protonated Schiff base, HSG^-, could also be ignored as was done in the previous report.[10] Equilibration between the reactants, to give the various binary species and M(S)(G), and also between the various forms of the Schiff base product are rapid compared to the time scale for the Schiff base formation rates. Thus the above equations are reduced to the following four equations containing four unknowns which are chosen as $[\mathrm{M}^{2+}]$, $[\mathrm{S}^-]$, $[\mathrm{G}^-]$, and [MSG].

$$[\mathrm{M}]_\mathrm{t} = [\mathrm{M}^{2+}]\{1 + \beta_{01}[\mathrm{G}^-] + \beta_{02}[\mathrm{G}^-]^2 + \beta_{03}[\mathrm{G}^-]^3 + \beta_{10}[\mathrm{S}^-] + \beta_{20}[\mathrm{S}^-]^2 + \beta_{11}^*[\mathrm{S}^-][\mathrm{G}^-]\} + [\mathrm{MSG}]$$

$$[\mathrm{S}]_\mathrm{t} = [\mathrm{S}^-]\{1 + a_\mathrm{H}/K_\mathrm{aS}\} + [\mathrm{M}^{2+}]\{\beta_{10}[\mathrm{S}^-] + 2\beta_{20}[\mathrm{S}^-]^2 + \beta_{11}^*[\mathrm{S}^-][\mathrm{G}^-]\} + [\mathrm{MSG}]\{1 + Q_\mathrm{HSG}a_\mathrm{H}/[\mathrm{M}^{2+}]\beta_{11}\}$$

$$[\mathrm{G}]_\mathrm{t} = [\mathrm{G}^-]\{1 + a_\mathrm{H}/K_{2\mathrm{aG}} + a_\mathrm{H}{}^2/K_{1\mathrm{aG}}K_{2\mathrm{aG}}\} + [\mathrm{M}^{2+}]\{\beta_{01}[\mathrm{G}^-] + 2\beta_{02}[\mathrm{G}^-]^2 + 3\beta_{03}[\mathrm{G}^-]^3 + \beta_{11}^*[\mathrm{S}^-][\mathrm{G}^-]\} + [\mathrm{MSG}]\{1 + Q_\mathrm{HSG}a_\mathrm{H}/\beta_{11}[\mathrm{M}^{2+}]\}$$

$$[\mathrm{H}]_\mathrm{t} = [\mathrm{H}^+] - [\mathrm{OH}^-] + a_\mathrm{H}[\mathrm{S}^-]/K_\mathrm{aS} + [\mathrm{G}^-]\{a_\mathrm{H}/K_{2\mathrm{aG}} + 2a_\mathrm{H}{}^2/K_{1\mathrm{aG}}K_{2\mathrm{aG}}\} + [\mathrm{MSG}]Q_\mathrm{HSG}a_\mathrm{H}/\beta_{11}[\mathrm{M}^{2+}]$$

It was assumed that no Schiff base had formed at the time of attainment of reaction pH, *i.e.*, $[\mathrm{MSG}]_{t=0} = 0$, and so the initial composition of the reaction solution was calculated in terms of $[\mathrm{M}^{2+}]$, $[\mathrm{S}^-]$, and $[\mathrm{G}^-]$ from the first three equations using the Newton–Raphson method. An independent check on the procedure was made by calculating a value of $[\mathrm{H}]_\mathrm{t}$ using the last equation.

Differentiation of the four mass balance equations with respect to time gives the following set of equations.

$$\mathrm{d}[\mathrm{M}]_\mathrm{t}/\mathrm{d}t = \{1 + \beta_{01}[\mathrm{G}^-] + \beta_{02}[\mathrm{G}^-]^2 + \beta_{03}[\mathrm{G}^-]^3 + \beta_{10}[\mathrm{S}^-] + \beta_{20}[\mathrm{S}^-]^2 + \beta_{11}^*[\mathrm{S}^-][\mathrm{G}^-]\}(\mathrm{d}[\mathrm{M}^{2+}]/\mathrm{d}t) + \{\beta_{10} + \beta_{20}[\mathrm{S}^-] + \beta_{11}^*[\mathrm{G}^-]\}[\mathrm{M}^{2+}](\mathrm{d}[\mathrm{S}^-]/\mathrm{d}t) + \{\beta_{01} + 2\beta_{02}[\mathrm{G}^-] + 3\beta_{03}[\mathrm{G}^-]^2 + \beta_{11}^*[\mathrm{S}^-]\}[\mathrm{M}^{2+}](\mathrm{d}[\mathrm{G}^-]/\mathrm{d}t) + (\mathrm{d}[\mathrm{MSG}]/\mathrm{d}t)$$

$$\mathrm{d}[\mathrm{S}]_\mathrm{t}/\mathrm{d}t = \{\beta_{10}[\mathrm{S}^-] + 2\beta_{20}[\mathrm{S}^-]^2 + \beta_{11}^*[\mathrm{S}^-][\mathrm{G}^-]\} \times (\mathrm{d}[\mathrm{M}^{2+}]/\mathrm{d}t) + \{1 + a_\mathrm{H}/K_\mathrm{aS} + [\mathrm{M}^{2+}](\beta_{10} + 4\beta_{20}[\mathrm{S}^-] + \beta_{11}^*[\mathrm{G}^-])\}(\mathrm{d}[\mathrm{S}^-]/\mathrm{d}t) + \beta_{11}^*[\mathrm{M}^{2+}][\mathrm{S}^-](\mathrm{d}[\mathrm{G}^-]/\mathrm{d}t) + \{1 + Q_\mathrm{HSG}a_\mathrm{H}/\beta_{11}[\mathrm{M}^{2+}]\}(\mathrm{d}[\mathrm{MSG}]/\mathrm{d}t)$$

$$\mathrm{d}[\mathrm{G}]_\mathrm{t}/\mathrm{d}t = \{\beta_{01}[\mathrm{G}^-] + 2\beta_{02}[\mathrm{G}^-]^2 + 3\beta_{03}[\mathrm{G}^-]^3 + \beta_{11}^*[\mathrm{S}^-][\mathrm{G}^-]\}(\mathrm{d}[\mathrm{M}^{2+}]/\mathrm{d}t) + \beta_{11}^*[\mathrm{M}^{2+}][\mathrm{G}^-](\mathrm{d}[\mathrm{S}^-]/\mathrm{d}t) + \{1 + a_\mathrm{H}/K_{2\mathrm{aG}} + a_\mathrm{H}{}^2/K_{1\mathrm{aG}}K_{2\mathrm{aG}} + [\mathrm{M}^{2+}](\beta_{01} + 4\beta_{02}[\mathrm{G}^-] + 9\beta_{03}[\mathrm{G}^-]^2 + \beta_{11}^*[\mathrm{S}^-])\}(\mathrm{d}[\mathrm{G}^-]/\mathrm{d}t) + \{1 + Q_\mathrm{HSG}a_\mathrm{H}/\beta_{11}[\mathrm{M}^{2+}]\}(\mathrm{d}[\mathrm{MSG}]/\mathrm{d}t)$$

$$\mathrm{d}[\mathrm{H}]_\mathrm{t}/\mathrm{d}t = (a_\mathrm{H}/K_\mathrm{aS})(\mathrm{d}[\mathrm{S}^-]/\mathrm{d}t) + \{a_\mathrm{H}/K_{2\mathrm{aG}} + 2a_\mathrm{H}{}^2/K_{1\mathrm{aG}}K_{2\mathrm{aG}}\}(\mathrm{d}[\mathrm{G}^-]/\mathrm{d}t) + \{Q_\mathrm{HSG}a_\mathrm{H}/\beta_{11}[\mathrm{M}^{2+}]\}(\mathrm{d}[\mathrm{MSG}]/\mathrm{d}t)$$

The values of $\mathrm{d}[\mathrm{M}]_\mathrm{t}/\mathrm{d}t$, $\mathrm{d}[\mathrm{S}]_\mathrm{t}/\mathrm{d}t$, and $\mathrm{d}[\mathrm{H}]_\mathrm{t}/\mathrm{d}t$ arise from dilution while $\mathrm{d}[\mathrm{G}]_\mathrm{t}/\mathrm{d}t$ is related to the initial slope ($\mathrm{d}v/\mathrm{d}t$) of the volume–time curve.[10,12] Thus by using the initial values of $[\mathrm{M}^{2+}]$, $[\mathrm{S}^-]$, and $[\mathrm{G}^-]$ previously calculated, we obtain the initial rates, $\mathrm{d}[\mathrm{M}^{2+}]/\mathrm{d}t$, $\mathrm{d}[\mathrm{S}^-]/\mathrm{d}t$, $\mathrm{d}[\mathrm{G}^-]/\mathrm{d}t$, and $\mathrm{d}[\mathrm{MSG}]/\mathrm{d}t$ by solving the above set of linear equations. The initial rate of formation of Schiff base summed over all forms is then calculated from the value of $\mathrm{d}[\mathrm{MSG}]/\mathrm{d}t$.

$$\mathrm{d}[\mathrm{SG}]_\mathrm{t}/\mathrm{d}t = \mathrm{d}([\mathrm{MSG}]/\mathrm{d}t) + (\mathrm{d}[\mathrm{HSG}^-]/\mathrm{d}t) = \left\{1 + \frac{Q_\mathrm{HSG}a_\mathrm{H}}{\beta_{11}[\mathrm{M}^{2+}]}\right\}(\mathrm{d}[\mathrm{MSG}]/\mathrm{d}t)$$

It was possible to estimate the initial slopes of the volume–time curves fairly precisely. The volume–time relationship was approximately linear for a significant time subsequent to the attainment of reaction pH in most of the runs.

Data of initial times were used because there are difficulties of both experiment and calculation in obtaining rate constants from the complete volume–time curves of the reactions followed to equilibrium. The experimental difficulties are due to the wide range of titrant volumes which would be necessary under the range of conditions used. The calculation of rate constants from the curves is complicated due to the redistribution of species concentrations which occurs during the course of the reaction. Coupling these effects with an iterative numerical procedure such as the Runge–Kutta method would markedly increase computation time over the initial slope approach.

Sample Calculation. A solution (20 ml) containing 0.01950 M $MgCl_2$ and 0.02061 M salicylaldehyde was brought to the reaction pH of 9.02 by the rapid addition of 4.68 ml of 0.1 M NaGly. Solution of the first three mass balance equations gives the initial concentrations $[M^{2+}] = 0.009477\ M$, $[S^-] = 0.008696\ M$, and $[G^-] = 0.00310\ M$. Substituted into the fourth equation these concentrations yield a value of $[H]_t = 0.0162\ M$ which is in good agreement with the experimental value of 0.0167 M.

The initial rate of addition of the titrant was 2.54×10^{-3} ml/sec, which gives the following initial rates: $d[M^{2+}]/dt = 3.14 \times 10^{-6}\ M/\text{sec}$, $d[S^-]/dt = -1.014 \times 10^{-5}\ M/\text{sec}$, $d[G^-]/dt = -2.206 \times 10^{-6}\ M/\text{sec}$, and $d[MSG]/dt = 1.208 \times 10^{-5}\ M/\text{sec}$. Thus the initial rate of formation of Schiff base, $d[SB]_t/dt$, is $2.25 \times 10^{-5}\ M/\text{sec}$.

Appendix II

Calculation of Initial Rates of Schiff Base Formation from Pseudo-First-Order Rate Constants (Spectrophotometric Determinations). The formation of Schiff base under pseudo-first-order conditions is treated as a first-order reversible reaction[33]

$$A \underset{k_r}{\overset{k_f}{\rightleftharpoons}} B$$

where the observed rate constant is given by $k_{obsd} = k_f + k_r$. In the present instance A is composed of all species containing salicylaldehyde and B is composed of all species containing Schiff base.

(a) Rates in the Absence of Metal Ion.

$$[A] = [S^-] + [HS] = [S^-](1 + a_H/K_{aS})$$

$$[B] = [HSG^-] + [SG^{2-}] = [HSG^-](1 + K_{aSG}/a_H)$$

where $K_{aSG} = [SG^{2-}]a_H/[HSG^-] = Q_{SG}/Q_{HSG}$.

At equilibrium

$$k_r = k_f[A]/[B] = \frac{k_f[S^-](1 + a_H/K_{aS})}{[HSG^-](1 + K_{aSG}/a_H)}$$

Therefore

$$k_{obsd} = k_f\left[1 + \frac{[S^-](1 + a_H/K_{aS})}{[HSG^-](1 + K_{aSG}/a_H)}\right]$$

The initial rate of formation of Schiff base is given by

$$d[SG]_t/dt = k_f[S^-](1 + a_H/K_{aS})$$

Thus

$$(d[SG]_t/dt)/[S^-][G^-] = k_f(1 + a_H/K_{aS})/[Gly^-]$$

which is the desired rate expression. Substituting the expression for k_{obsd} and rearranging gives the relationship

$$(d[SG]_t/dt)/[S^-][G^-] = \frac{k_{obsd}Q_{HSG}(a_H + K_{aSG})(1 + a_H K_{aS})}{a_H + K_{aS} + Q_{HSG}[G^-](a_H + K_{aSG})}$$

The concentration of glycinate anion under these conditions of pH ≥ 7.9 was calculated from $[G^-] = [G]_t/(1 + a_H/K_{2aG})$.

(b) Rates in the Presence of Metal Ion

$$[A] = [S^-] + [HS] + [MS^+] + [M(S)(G)] + [M(S)(G)_2^-] = [S^-]\{1 + a_H/K_{aS} + \beta_{10}[M^{2+}] + \beta_{11}{}^*[M^{2+}][G^-] + \beta_{12}{}^*[M^{2+}][G^-]^2\} = [S^-]X$$

where X is the term in the right-hand bracket.

$$[B] = [MSG] + [M(SG)G] + [HSG^-] = [MSG] \times \left\{1 + \frac{\beta_{12}[G^-]}{\beta_{11}} + \frac{Q_{HSG}a_H}{\beta_{11}[M^{2+}]}\right\}$$

At equilibrium

$$k_r = \frac{k_f[S^-]X}{[MSG]\left\{1 + \frac{\beta_{12}[G^-]}{\beta_{11}} + \frac{Q_{HSG}a_H}{\beta_{11}[M^{2+}]}\right\}} = \frac{k_f X}{[G^-]\{\beta_{11}[M^{2+}] + \beta_{12}[M^{2+}][G^-] + Q_{HSG}a_H\}} = \frac{k_f X}{[G^-]Y}$$

where Y is the term in the right-hand brackets of the denominator. The initial rate of formation of Schiff base is given by

$$d[SG]_t/dt = k_f[S^-]X$$

Therefore

$$(d[SG]_t/dt)/[S^-][G^-] = k_f X/[G^-] = k_{obsd}X/\{[G^-] + X/Y)\}$$

The concentrations of M^{2+} and G^- were calculated from the following mass balance equations using the Newton–Raphson method

$$[M]_t = [M^{2+}]\{1 + \beta_{01}[G^-] + \beta_{02}[G^-]^2 + \beta_{03}[G^-]^3\}$$

$$[G]_t = [G^-]\{1 + a_H/K_{2aG}\} + [M^{2+}]\{\beta_{01}[G^-] + 2\beta_{02}[G^-]^2 + 3\beta_{03}[G^-]^3\}$$

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[33] A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed, John Wiley and Sons, Inc., New York, N. Y. , 1961, p 186.