COMPLEXING OF GUANYLIC ACID WITH SILVER

Chemistry 80 Thesis

by

Gary F. Mitchell

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Summary

The complexing of deoxyguanylic acid with silver ion has been studied by potentiometric determinations of silver binding, pH-stat proton release measurements, and ultraviolet spectrophotometry.

The stepwise equilibria

$$3\ Ag^+ + 2\ GH \rightleftharpoons Ag_3G_2H^{++} + H^+ \qquad K_{32}$$

$$Ag_3G_2H^{++} + Ag^+ \rightleftharpoons Ag_4G^{++} + H^+ \qquad K_{132}$$

fit the data fairly well, and are proposed as a tentative explanation, although the exclusive occurrence of these reactions has not been conclusively proved.

Complexing of Guanylic Acid with Silver

Introduction

Previous work in this laboratory[1-4] has been concerned with the effect of metal ions on deoxyribonucleic acid (DNA), in the hope of achieving a better understanding of nucleic acid macro-structure, and perhaps resulting in a method for fractionating various DNAs. It has been difficult to fully interpret the DNA results, and it was felt that systematic study of metal-nucleotide complexing would be illuminating. We have chosen to start with deoxyguanylic acid and Ag(I).

The methods of investigation employed have been UV spectrophotometry, potentiometric silver binding studies, and proton release measurements. The proton release studies are appropriate because if silver ion were bound at the N-1 site in guanylic acid, the proton originally there should be released.

Experimental

Solutions were made by dissolving deoxyguanylic acid (5')·NH_4^+ salt, A grade, obtained from California Corporation for Biochemical Research, in 0.1M $NaClO_4$. In the interests of brevity, this nucleotide will henceforth be referred to as guanylic acid (GH). (Fig. 1).

As in Jensen's work[4], the concentration of free silver ion in the solution was determined by measuring the potential of a silver wire

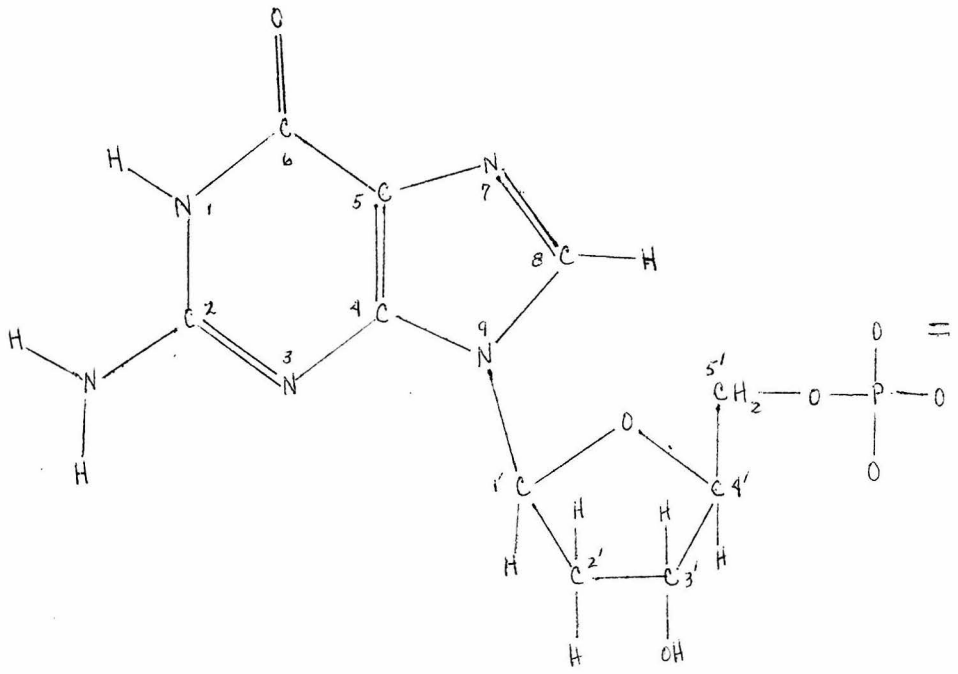


Figure 1

Chemical Structure of Deoxyguanylic Acid Anion

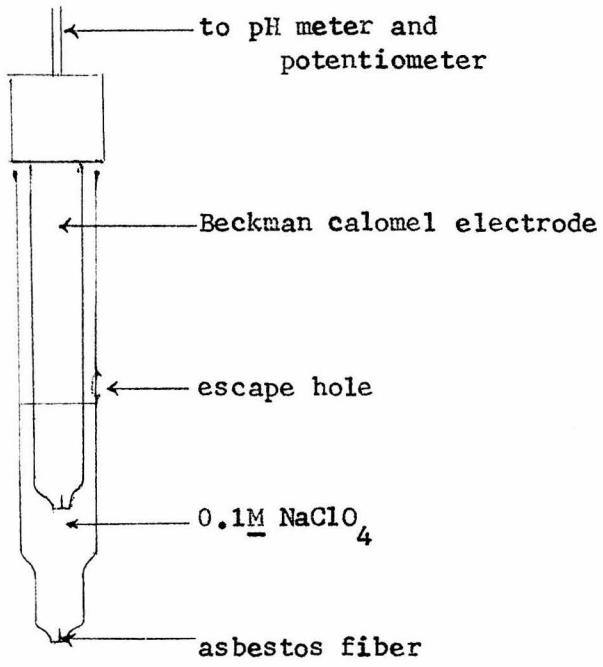


Figure 2

Salt Bridge Assembly

electrode against a reference calomel electrode, using a Leeds and Northrup type K-2 potentiometer, and Keithly 200b dc VTVM as a null indicator. Calibration of the potentiometer was easily accomplished, resulting in the equation

$$E = E^{o} + 59.5 \log (Ag^{+})$$

with E^{o} = 553 millivolts at 25°C in agreement with Yamane[1].

A 0.1M $NaClO_4$ salt bridge was fitted over the calomel electrode to prevent AgCl precipitation. (Fig. 2). Standardization of the pH meter, a Beckman Model 76 expanded scale instrument, was done without the salt bridge.

In a manner similar to Yamane, pH-stat titrations[2] were done simultaneously with potentiometric measurements. In a typical experiment, the three electrodes (silver, calomel, and glass) are inserted in a beaker of 0.001M guanylic acid. All measurements were made at an ionic strength of 0.1M maintained with $NaClO_4$. 0.05M $AgNO_3$, added with a micropipette, causes the pH to decrease. Then 0.1N NaOH is added with a microsyringe of 0.25 ml capacity, the amount of base required to return the pH to its original value being a measure of the proton release. The free silver concentration is determined from the potentiometer reading, and then the next aliquot of $AgNO_3$ added.

Volume corrections (less than 5%) have not been taken into account. Temperature variations were avoided by using a water bath at 25±1°C. The overhead fluorescent lights were turned off to prevent photoreduction of Ag(I).

In one experiment at pH 7.1, 0.25 ml aliquots of solution were removed at intervals and the UV spectra taken, using a Cary Model 14 and 1 mm quartz cells.

Results and Discussion

A few comments concerning the guanylic acid used might be in order here. The A grade material was labelled chromatographically homogeneous, and had a molecular weight given as 382.3, corresponding to the diammonium salt. The given nitrogen analysis (23.15%) agrees more closely with one NH_4^+ group.

Calculated for	$C_{10}H_{12}N_5O_7P(NH_4)_2$	(7x14)/382 = 26.2 %N
"	$C_{10}H_{12}N_5O_7P(H)_2$	(5x14)/348 = 20.1 %N
"	$C_{10}H_{12}N_5O_7P(NH_4)(H)$	(6x14)/365 = 23.0 %N

On the basis of 382.3 as the molecular weight, spectral data show the material to be approximately 90% pure. Molar absorbtivities were obtained from Chargaff and Davidson[5], and experimental absorbancy ratios at various wavelengths agree with those given in the volume[6].

A pH titration of 0.01M guanylic acid with 0.1N NaOH was done to determine the effect of the ammonium ions. (Fig. 3).

The pK_a of NH_4^+ is calculated to be approximately 9.3. The pK_a of the N-1 proton of GH is also about 9.3,[7,8] so the titration curve shows no separation, only an average experimental pK_a value of roughly 9.35. The titration curve also supports the conclusion that the guanylic acid is only 90% pure. The secondary phosphate pK_a is 6.3 experimentally, compared to a literature value of 6.0.[7]

Let us examine the situation of silver ammine complexing.[9]

$$NH_4^+ + Ag^+ \rightleftharpoons Ag(NH_3)^+ + H^+ \qquad K = 10^{-5.8}$$

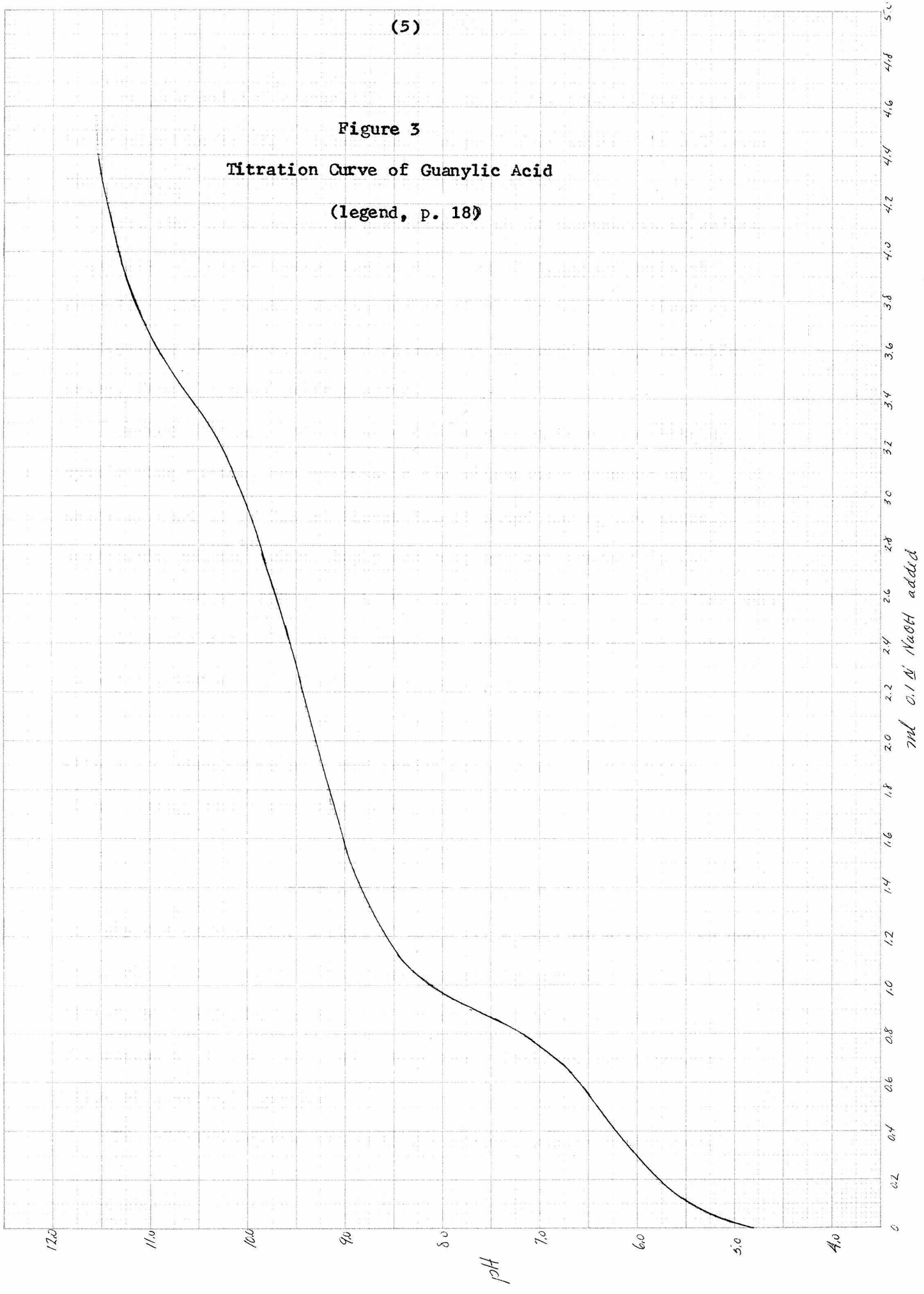
$$(Ag(NH_3)^+)/(Ag^+) = (NH_4^+)K/(H^+) = (2\times10^{-3})(1.6\times10^{-6})/10^{-8}$$

$$= 0.3$$

Figure 3

Titration Curve of Guanylic Acid

(legend, p. 18)



At pH 8 and 10^{-3} M guanylic acid, 30% of the free silver might be involved in $Ag(NH_3)^+$ formation. At pH 7 this is 3%. In all cases the $Ag(NH_3)_2^+$ concentration should be negligible.

For the presentation of the results, it is convenient to define a quantity r_b which equals the moles of Ag(I) bound per mole of guanylic acid present. The amount of silver bound is obtained by subtracting the amount of free silver, as measured by the potentiometer, from the total silver added.

Marked changes, indicating electronic effects on the heterocyclic ring system, are evident in the ultraviolet spectra of guanylic acid at pH 7.1 as silver ion is added and r_b increases. Isosbestic points,[10] which imply one complex, are present in the spectra at 238 and 288 mμ up to $r_b \sim 1.5$. For higher r_b they disappear.

If there are only two UV absorbing species, and one is converted into the other as Ag^+ is added, we can write

$$A = \epsilon_1 c_1 + \epsilon_2 c_2$$

with A the absorbance at a particular wavelength, ϵ the molar absorbtivity, and c the concentration.

$$\text{Let } c_1 + c_2 = c_0$$

$$\text{then } A = \epsilon_1 c_0 + c_2(\epsilon_2 - \epsilon_1)$$

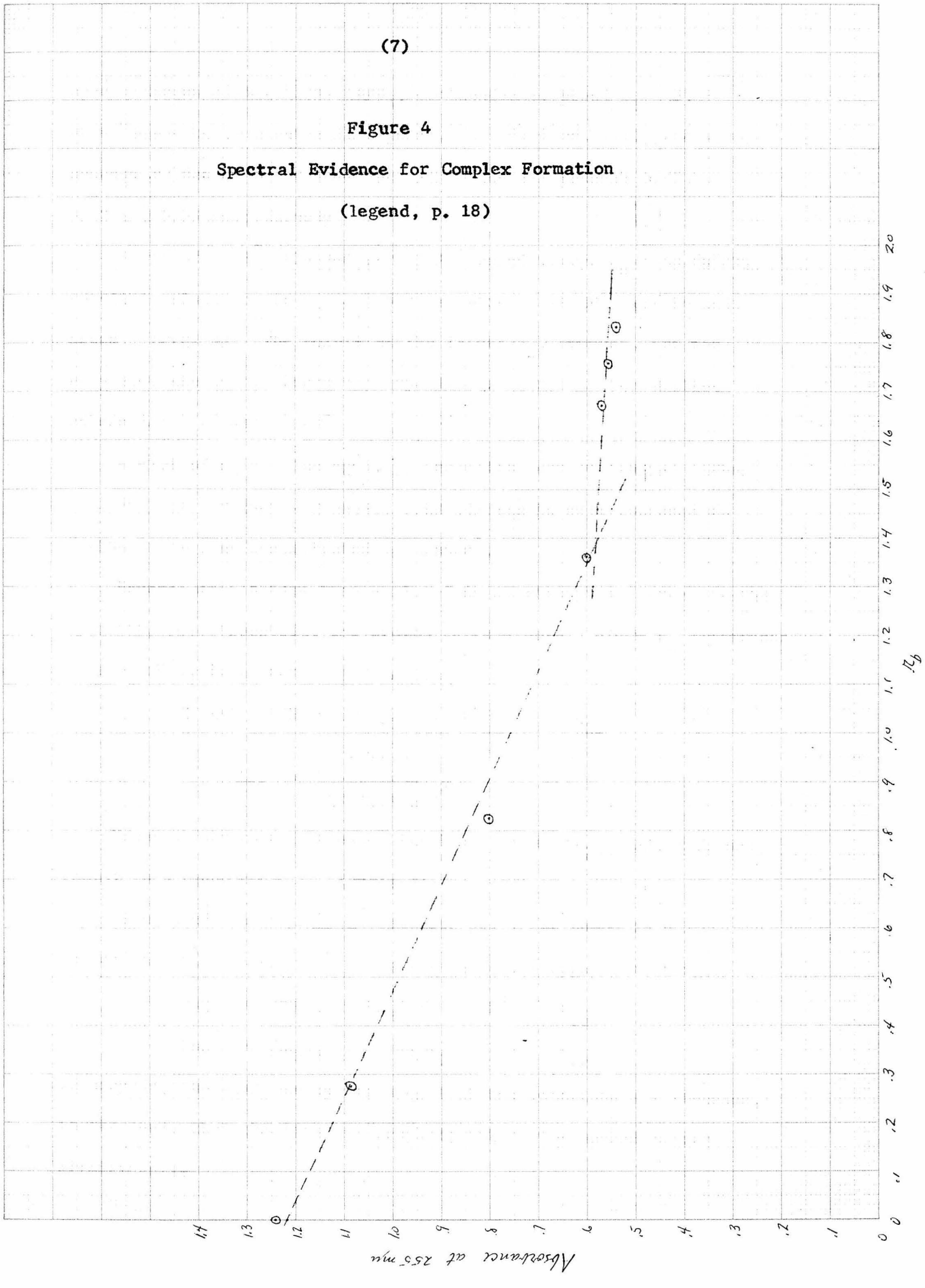
For any one wavelength, a plot of A/c_0 vs. c_2/c_0, or A vs. r_b in our case, should be linear. The experimental plot for 255 mμ is not linear, but might be broken into two straight lines intersecting in the region $r_b = 1.4$ (Fig. 4). This would indicate two separate pairs of absorbing species.

The pH-stat experiments indicate proton release immediately

Figure 4

Spectral Evidence for Complex Formation

(legend, p. 18)



upon addition of the first $AgNO_3$, continuing to about $r_b = 2$, which seems to be a saturation point. At pHs 5.05, 7.10, and 7.80, average values of H^+ released per Ag^+ bound are roughly 0.65, 0.42 and 0.53 respectively.

Plots of $\triangle H^+$ released per $\triangle Ag^+$ bound versus r_b show fairly constant release up to $r_b \sim 1.5$ after which a slight rise is indicated. (Fig. 5). The points at the larger r_b, however, are not very accurate due to increased experimental error in the smaller values for $\triangle H^+$ and $\triangle Ag^+$.

A plot of r_b vs. $\log(Ag^+)_{free}$ indicates more binding at higher pH. (Fig. 6). Silver - guanylic acid binding is weak compared to Ag-DNA binding as investigated by Jensen[4].

Several models have been employed in an attempt to correlate the spectral, potentiometric, and proton release data. Since r_b approaches a value of 2, it is tempting to write:

$$2\,Ag^+ + GH \rightleftharpoons Ag_2G + H^+ \qquad K_{21}$$

$$K_{21} = \frac{r_b(H^+)}{(2-r_b)(Ag^+)^2}$$

However, data calculated on the basis of an Ag_2G complex do not result in a constant K_{21}. (Table I)

If we could assume $\triangle H^+$ released per $\triangle Ag^+$ bound is 0.5, rather than 0.4 or 0.6, the following equations appear inviting.

$$2Ag^+ + 2GH \rightleftharpoons Ag_2G_2H^+ + H^+ \qquad K_{22}$$

$$2Ag^+ + Ag_2G_2H^+ \rightleftharpoons Ag_4G_2^{++} + H^+ \qquad K_{222}$$

We simplify the analysis by assuming that the reactions are stepwise, in the sense that the first is complete before the second occurs significantly.

Figure 5

Proton Release with Increasing r_b

(legend, p.18)

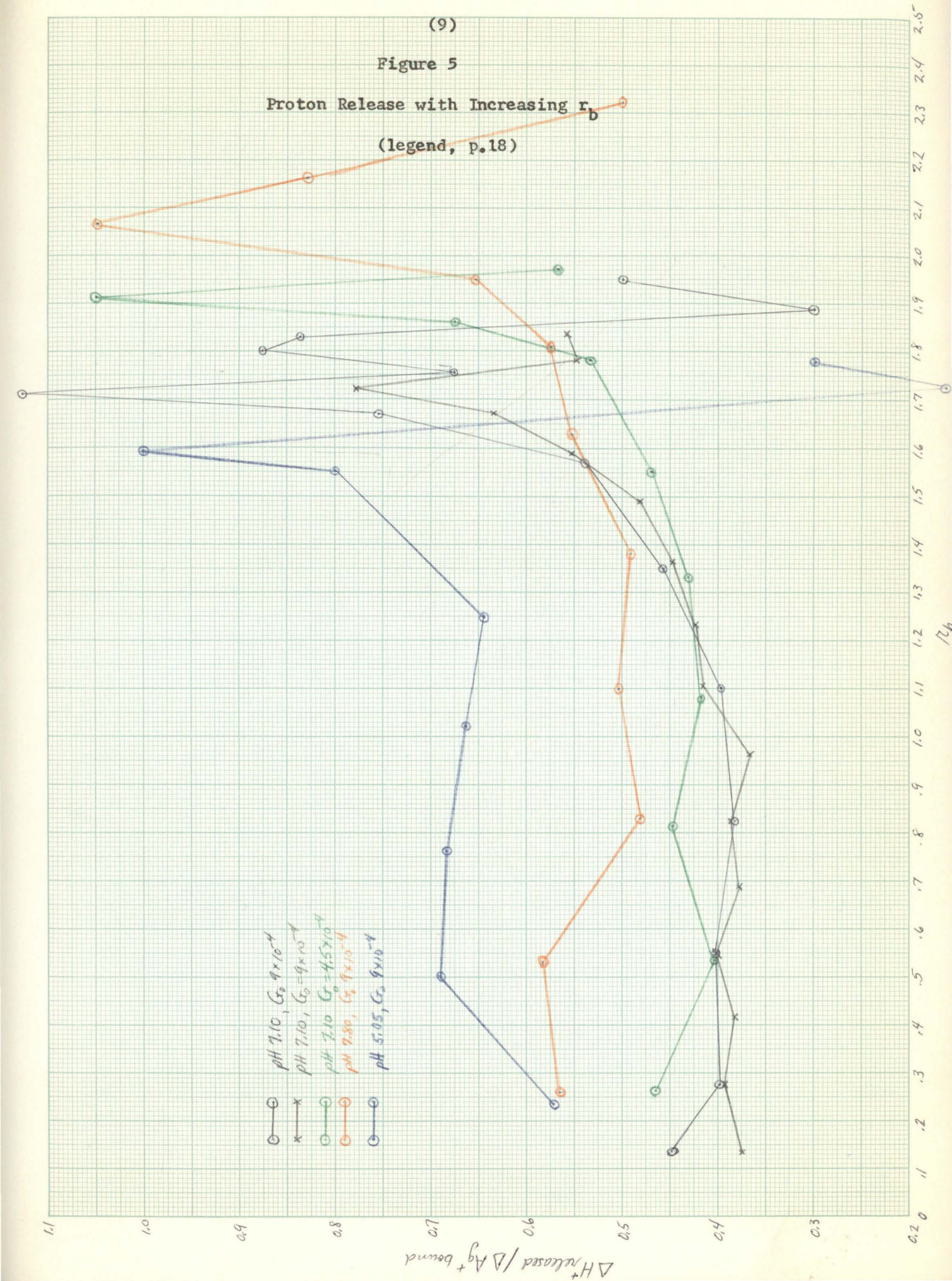
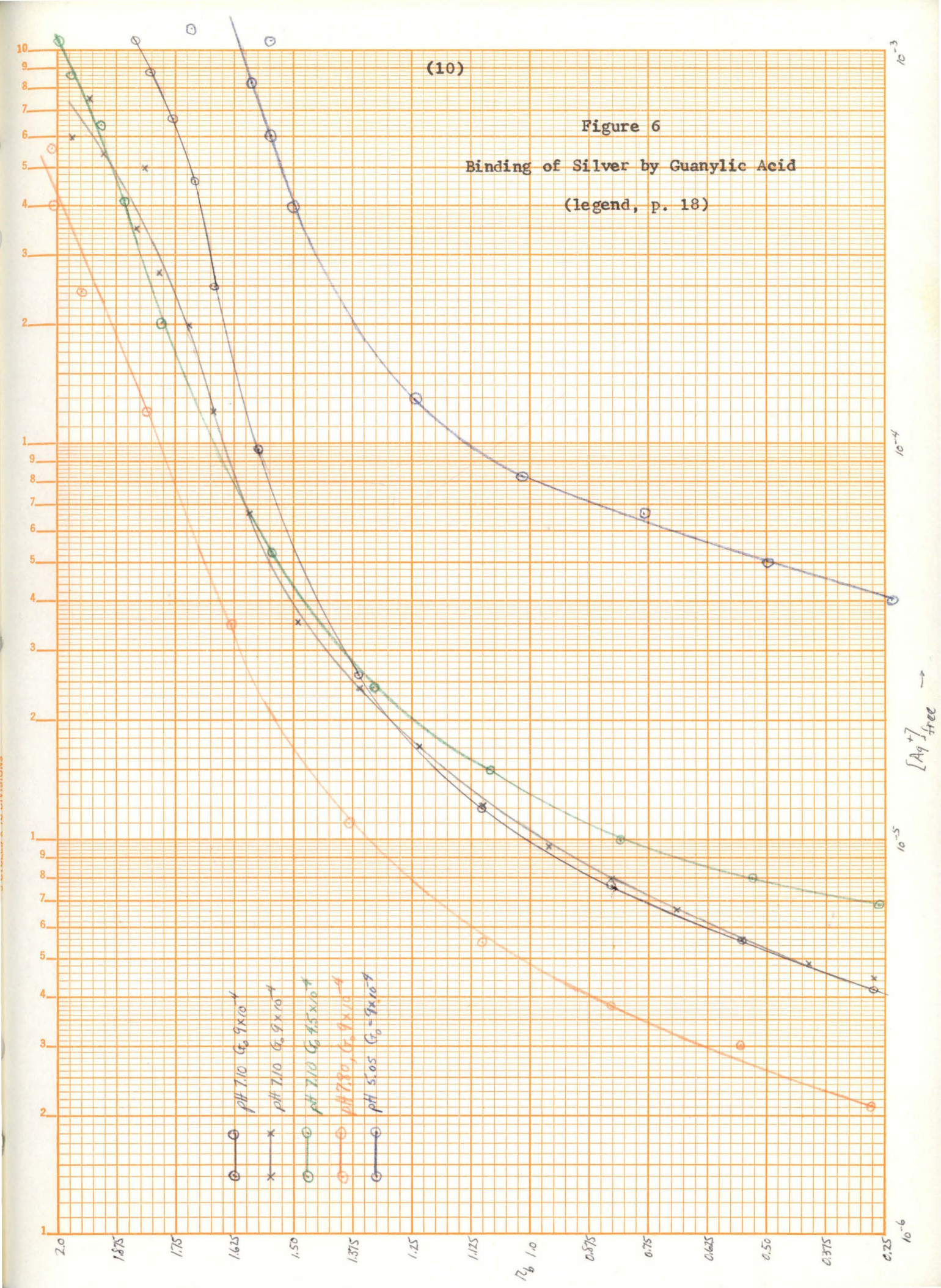


Figure 6

Binding of Silver by Guanylic Acid

(legend, p. 18)



The equilibria represented by K_{22} and K_{222} might explain the spectra and proton release, but again fail to be supported by the potentiometric data. (TableI). The expressions used to calculate these constants are:

$$K_{22} = \frac{r_b(H^+)}{2G_o(1-r_b)^2(Ag^+)^2}$$

$$K_{222} = \frac{(r_b-1)(H^+)}{(2-r_b)(Ag^+)^2}$$

The most successful model to date can be represented by:

$$3\ Ag^+ + 2\ GH \rightleftharpoons Ag_3G_2H^{++} + H^+ \qquad K_{32}$$

$$Ag_3G_2H^{++} + Ag^+ \rightleftharpoons Ag_4G_2^{++} + H^+ \qquad K_{132}$$

These give qualitative agreement with the spectral data, and account for continuous proton release, although experimental values for H^+ released per Ag^+ bound differ from the theoretical values of 0.33 and 1.0. An increase in $\triangle H^+$ released/$\triangle Ag^+$ bound after $r_b = 1.5$ is supported. Potentiometric data for experiments at pH 7.80 and 7.10, and for two G_o concentrations at the latter pH, provide quantitative support for the scheme presented. For $0 < r_b < 1.5$, the quantity $K_{32} = r_b(H^+)/3(1-2/3r_b)^2(Ag^+)^3G_o$ should be constant, and for $1.5 < r_b < 2$, the quantity $K_{132} = (r_b-1.5)(H^+)/(2-r_b)(Ag^+)$ should be constant. (Table I). The calculated formation constant values are questionable in the regions near $r_b = 0$ and $r_b = 2$, and also where the transition to the second complex occurs, i.e., $r_b = 1.0$ or $r_b = 1.5$.

As can be seen from Table I, the results at pH 5.05 are the same order of magnitude, but do not agree as well quantitatively as do the results at the higher pH's.

Table I

Analysis of Potentiometric Data

Experiment I: pH 7.10, $G_o^* = 9 \times 10^{-4}$

$(Ag^+)_{free}$	r_b	$K_{21} \times 10^2$	$K_{22} \times 10^6$	$K_{22_2} \times 10^1$	$K_{32} \times 10^{12}$	$K_{132} \times 10^{-4}$
3.3×10^{-6}	0.136	5.27	0.732		1.35	
4.4	0.274	6.50	1.19		1.42	
4.8	0.412	8.99	2.28		2.06	
5.6	0.550	,9.61	3.83		2.30	
6.6	0.688	9.61	7.15		2.40	
8.0	0.825	8.66	18.6		2.35	
9.6	0.961	7.95	305.		2.46	
1.2×10^{-5}	1.10	6.75		6.15	2.64	
1.7	1.23	4.40		8.25	2.28	
2.4	1.36	2.94		7.77	3.22	
3.5	1.49	1.90		6.24		
6.6	1.59	0.71		2.63		2.66
1.2×10^{-4}	1.67	0.28		1.12		3.41
2.0	1.72	0.122		0.511		3.12
2.7	1.78	0.089		0.39		3.75
3.5	1.83	0.073		0.319		4.41
5.0	1.81	0.030		0.135		2.59
5.4	1.9	0.051		0.243		5.89
6.0	1.97	0.146		0.72		20.8
7.6	1.93	0.037		0.179		

* G_o is the initial guanylic acid concentration

Table I, continued

Experiment II: pH 7.10, $G_o = 9 \times 10^{-4}$

$(Ag^+)_{free}$	r_b	$K_{21} \times 10^2$	$K_{22} \times 10^6$	$K_{222} \times 10^1$	$K_{32} \times 10^{12}$	$K_{132} \times 10^{-4}$
3.6×10^{-6}	0.136	4.48	0.621		1.04	
4.2	0.273	7.11	1.30		1.83	
5.6	0.550	9.70	3.86		2.28	
7.7	0.825	9.45	20.1		2.62	
1.2×10^{-5}	1.10	6.75		6.15	2.58	
2.6	1.36	2.51		6.62	2.69	
9.6	1.57	0.313		1.14		1.34
2.5×10^{-4}	1.67	0.0644		0.259		1.64
4.6	1.71	0.022		0.092		1.26
6.7	1.755	0.0127		0.0545		1.23
8.8	1.80	0.0093		0.0413		1.36
1.1×10^{-3}	1.83	0.007				1.41
1.3	1.89	0.008		0.038		1.88
1.55	1.89	0.0056		0.027		
1.75	1.95	0.0102		0.05		

Table I, continued

Experiment III: pH 7.10, $G_o = 4.5 \times 10^{-4}$

$(Ag^+)_{free}$	r_b	$K_{21} \times 10^2$	$K_{22} \times 10^6$	$K_{222} \times 10^1$	$K_{32} \times 10^{12}$	$K_{132} \times 10^{-4}$
6.8×10^{-6}	0.262	2.58	0.92		0.72	
8.0	0.538	4.56	3.47		1.50	
1.0×10^{-5}	0.812	5.45	20.3		2.28	
1.5	1.08	4.15		3.07	2.39	
2.4	1.33	2.73		6.78	4.3	
5.3	1.55	0.985		3.50		1.67
2.0×10^{-4}	1.78	0.161		0.705		5.06
4.1	1.86	0.063		0.290		4.97
6.4	1.91	0.041		0.196		5.68
8.6	1.97	0.071		0.350		14.5
1.1×10^{-3}	2.0					

Experiment IV: pH 7.80, $G_o = 9 \times 10^{-4}$

2.1×10^{-6}	0.276	5.76	1.055		2.60	
3.0	0.553	6.75	2.67		3.02	
3.8	0.830	7.78	17.5		7.6	
5.5	1.10	6.45		5.87	5.5	
1.1×10^{-5}	1.38	2.92		8.06	9.6	
3.5	1.63	0.574		2.23		1.6
1.2×10^{-4}	1.81	0.105		0.471		2.16
2.4	1.95	0.107		0.523		6.11
4.0	2.06					
5.6	2.16					
6.6	2.32					

Table I, continued

Experiment V: pH 5.05, $G_o = 9 \times 10^{-4}$

$(Ag^+)_{free}$	r_b	$K_{21} \times 10^2$	$K_{22} \times 10^6$	$K_{222} \times 10^1$	$K_{32} \times 10^{12}$	$K_{132} \times 10^{-4}$
4.0×10^{-5}	0.234	7.43	1.23		0.173	
5.0	0.500	11.84	3.95		0.297	
6.6	0.76	12.6	14.7		0.36	
8.2	1.02	14.0		2.75	0.36	
1.3×10^{-4}	1.245	8.72		17.2	0.65	
4.0	1.50	1.67		5.57		[illegible]
6.0	1.55	0.855		3.03		166.
8.2	1.59	0.516		1.91		239.
1.1×10^{-3}	1.55	0.258		0.90		90.
1.2	1.72	0.383		1.60		58.5
1.4	1.78	0.365		1.62		81.1

Perhaps a slightly different interpretation is called for at the lower pH, since the phosphate group should be singly protonated.

The diagram in Figure 7 might be invoked as a possible explanation for the first complex. It is difficult to rationalize the loss of only one proton, but perhaps the other is involved in tautomeric transfer to an oxygen. Two of the silvers would be bound to N-3 or N-7. With the addition of further silver, binding might occur on an unoccupied nitrogen. Calculations do not support the dissociation scheme:

$$Ag_3G_2H^{++} + Ag \rightleftharpoons 2\ Ag_2G^{+} + H^{+}$$

One item as yet unmentioned is polymer formation. This might provide a plausible explanation, since qualitatively satisfactory polymer structures can be drawn. Deoxyguanosine precipitates with Ag(I), as does guanylic acid at pH 3.5. These two facts lend some support to the hypothesis that extensive polymer formation is prevented in guanylic acid at higher pH's due to electrostatic repulsion of the phosphate groups. A quantitative explanation of possible complex polymer formation is not presented here, because of the difficulty involved in writing multiple equilibria.

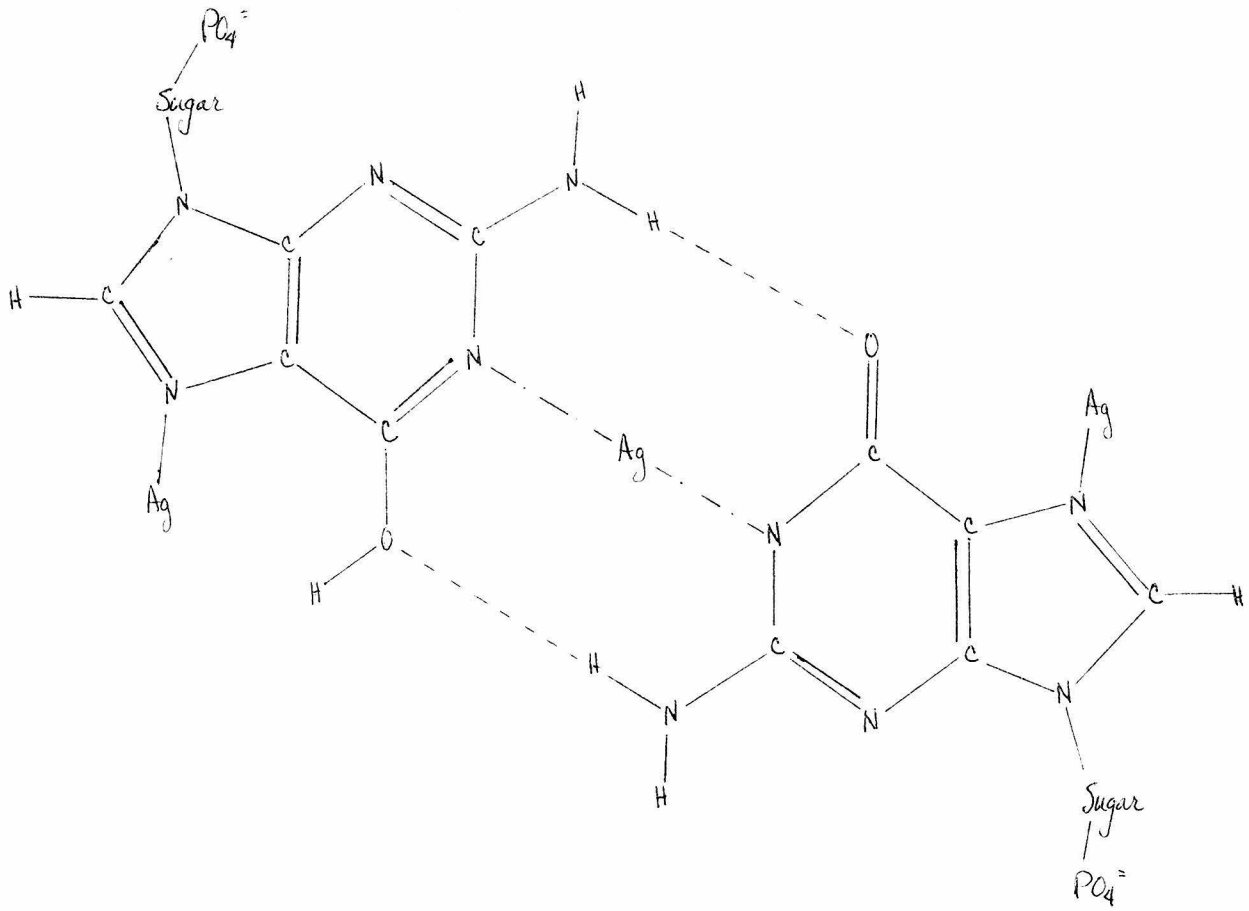


Figure 7

Possible Structure for the $Ag_3G_2H^{++}$ Complex

(legend, p. 18)

Legends for Figures

Figure 3: Titration Curve of Guanylic Acid

Results of a pH titration of 10.0 ml of 0.01M guanylic acid with 0.1N NaOH. The nucleotide concentration of 0.01M is on the basis of 382.3 as a molecular weight.

Figure 4: Spectral Evidence for Complex Formation

For a plot of absorbance at 255 mμ versus r_b for experiment 2, intersecting straight lines have been drawn rather than a curve, to indicate possible stepwise complex formation.

Figure 5: Proton Release with Increasing r_b

In the $\triangle H^+$ released per $\triangle Ag^+$ bound versus r_b plot, experimental error increases as $r_b = 2$ is approached.

Figure 6: Binding of Silver by Guanylic Acid

r_b versus $\log(Ag^+)_{free}$ shows more binding at higher pH.

Figure 7: Possible Structure for the $Ag_3G_2H^{++}$ Complex

Hydrogen bonding seems plausible in this formulation, but tautomeric shift of a proton to oxygen is not so reasonable. Two silver ions have arbitrarily been put on the N-7 positions, but might also have been placed on N-3.

Prospectus

The situation of Ag-guanylic acid complexing is more complicated than we had hoped.

Future investigation will be directed toward further variation of pH and G_o. Before doing this, conversion of the ammonium guanylate to the sodium salt should be carried out by passing the nucleotide through a cation exchange resin loaded with Na^+.

A logical continuation of this guanylic acid work, of course, is the extension to investigation of Ag(I) binding with other nucleotides.

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