SILVER BINDING STUDIES WITH ADENYLIC ACID AND RELATED COMPOUNDS

Chemistry 80 Thesis

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Summary

The silver ion complexes of deoxyadenylic acid, deoxyadenosine, and various derivatives of adenine have been studied and compared. The major analytical methods used have been potentiometric determination of silver binding and pH-stat determination of proton release during binding. Spectrophotometric data have been used as supplementary information in some experiments.

The main reaction for adenylic acid appears to be:

$$2n\ Ag^+ + n\ HA \longrightarrow Ag_{2n}A_n^{(+n)} + n\ H^+$$

($Ag_{2n}A_n^{(+n)}$: precipitate or polymeric species in solution)

$$\text{with } (K^{1/n}) \cong \frac{[H^+]}{[Ag^+]^2\left[\frac{2-r_b}{2}\right][HA_o]} \quad \text{where } r_b = \frac{\text{moles Ag bound}}{\text{moles HA present}}$$

The actual binding is probably more complex.

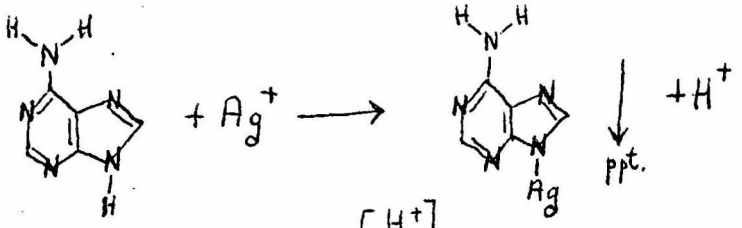
Adenosine also forms polymers with silver. The mechanism may be similar to adenylic acid binding; but a binding scheme that fits the data better is a two-reaction scheme, with (a) occuring twice as often as (b):

$$\text{(a)}\quad Ag^+ + Ads \longrightarrow \frac{1}{n}\left(-Ag-Ads-\right)_n \downarrow + H^+$$

$$\text{(b)}\quad Ag^+ + Ads + (ClO_4^-) \longrightarrow Ads-Ag^+-(ClO_4^-)$$

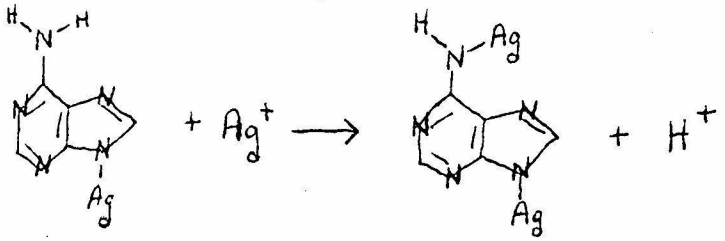
The 9-methyl adenine reaction is also a polymerization reaction; it may well be similar to either the adenylic acid scheme or the adenosine scheme.

With adenine the first reaction is probably:



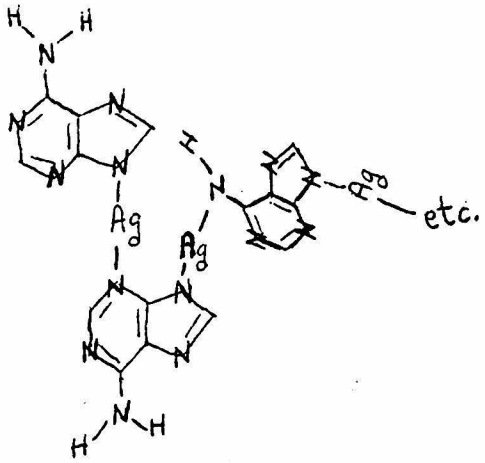
$$\text{with } K = \frac{[H^+]}{[Ag^+][AH_o][1-r_b]}$$

followed by a second reaction:



On the other hand, 6-N,N-dimethyl adenine has the same first step as adenine, but does not have the strong second reaction because of its blocked amino group.

I propose that in all cases part of the driving force of the reaction is the tendency to form polymers with structures of the type illustrated here for adenine.



Introduction

The members of this research group have recently been interested in the study of cation complexing with deoxyribonucleic acid (DNA)[1-4]. It was hoped that these studies would yield valuable insight into DNA secondary structure, reactivity, and binding-site availabilty. The mercury(II)- DNA complex studies were of special interest, since mercury(II) binds much more strongly to the purine and pyrimidine base groups than to the negative phosphates of the chain[1]. For this reason the mercury(II)-DNA studies were extended to include an investigation of mercury complexing with various derivatives of the four naturally-occurring purines and pyrimidines[5,6]. Silver(I) is known to have a complex chemistry and an electronic structure similiar to mercury(II); and it also exhibits a high reactivity with nitrogen bases. Hence, silver-DNA studies were conducted[7]. Unfortunately, the presence of four nucleotides in various arrangements of base-pairs in the DNA molecule has made interpretation of the silver binding studies difficult. In an attempt to remove these difficulties a study of silver binding complexes with the separate purine and pyrimidine bases and with related compounds was undertaken. The silver-deoxyguanylic acid system was the first studied extensively[8]; the results are complicated and are difficult to explain by a simple mechanism. Further work on other silver complexes, especially complexes with deoxyadenylic acid, deoxyadenosine, 9-methyl adenine, 6-N,N-dimethyl adenine, and adenine will be described in this paper.

Experimental Apparatus and Techniques

The compounds studied were:

deoxyadenylic acid(5') disodium salt·$3H_2O$
-Obtained from Calif. Corp. for Bioch. Research
-Henceforth, abbreviated adenylic acid or HAd

deoxyadenosine
-Cal. Biochem.
-Abbreviated adenosine or Ads

adenine
-Cal Biochem.

6-N,N-dimethyl adenine
-Cal Biochem.

9-methyl adenine
-Cyclo Chemical

Each reagent was of the highest grade available.

Solutions of the above were made by dissolving the appropriate compound in 0.1 M $NaClO_4$ solution (to insure constant ionic strength during reactions studied); the concentrations of species studied ranged from 10^{-3} to 3×10^{-3} M.

In the binding study experiments 20,00 ml of the desired solution, which will be referred to by general symbol A, was titrated by the addition of micro-portions of 0.1 M silver nitrate; the pH of the solution was kept constant by the addition of small portions of sodium hydroxide. The silver nitrate solution and the sodium hydroxide solution were delivered most conveniently from Burroughs and Wellcome "Agla" micrometer syringes (accurate to at least 4×10^{-4} ml) The binding was followed by three electrodes in the solution: a Beckman Glass Electrode, a Beckman Saturated KCl-calomel Electrode, and a silver wire electrode. The calomel and glass electrodes were used for pH readings from a Beckman Model 76 Expanded Scale pH Meter. The silver and calomel electrodes were used to find the concentration of unbound silver in solution by balancing potentials with a

Leeds and Northrop Type K-2 Potentiometer. A Keithly Model 200-B VTVM Electrometer fitted with a Keithly 200-B Decade Shunt was used as a null meter on the potentiometer. A sketch of the titration apparatus is shown in Figure 1.

For electrode measurements on the solutions containing Ag^+ it is necessary to separate the saturated calomel electrode from the solution by a salt bridge containing 0.1 M $NaClO_4$. Since this is essentially the ionic medium in the solution being studied, there is no liquid junction.

The glass electrode was zeroed against the calomel electrode at pH 7 in the conventional way without using the special salt bridge on the calomel electrode. If the salt bridge were used in the pH 7 standardization, a liquid junction between 0.1 M $NaClO_4$ and pH 7 Beckman buffer would develop.

In a typical titration experiment approximately 30 ml of the desired A solution was bubbled with argon in a special bubbling tube and adjusted to the desired pH. Meanwhile, the two syringes were filled, and the potentiometer was calibrated against a standard cell. After at least 45 minutes, 20.00 ml of the bubbled solution was transferred to the titration tube and argon was allowed to play slowly over its surface; then the Glass Electrode and the Calomel Electrode with its 0.1 F salt bridge were placed in the solution. After the pH stabilized at the desired reading, its value was noted. A small aliquot of 0.10 N $AgNO_3$ solution was added to the sample (from a syringe). The silver wire electrode was then placed in the solution. The pH was adjusted to the original value with 0.05 F NaOH from the other "Agla" The silver potential was read for the silver electrode vrs. the Calomel reference. Then, the pH value

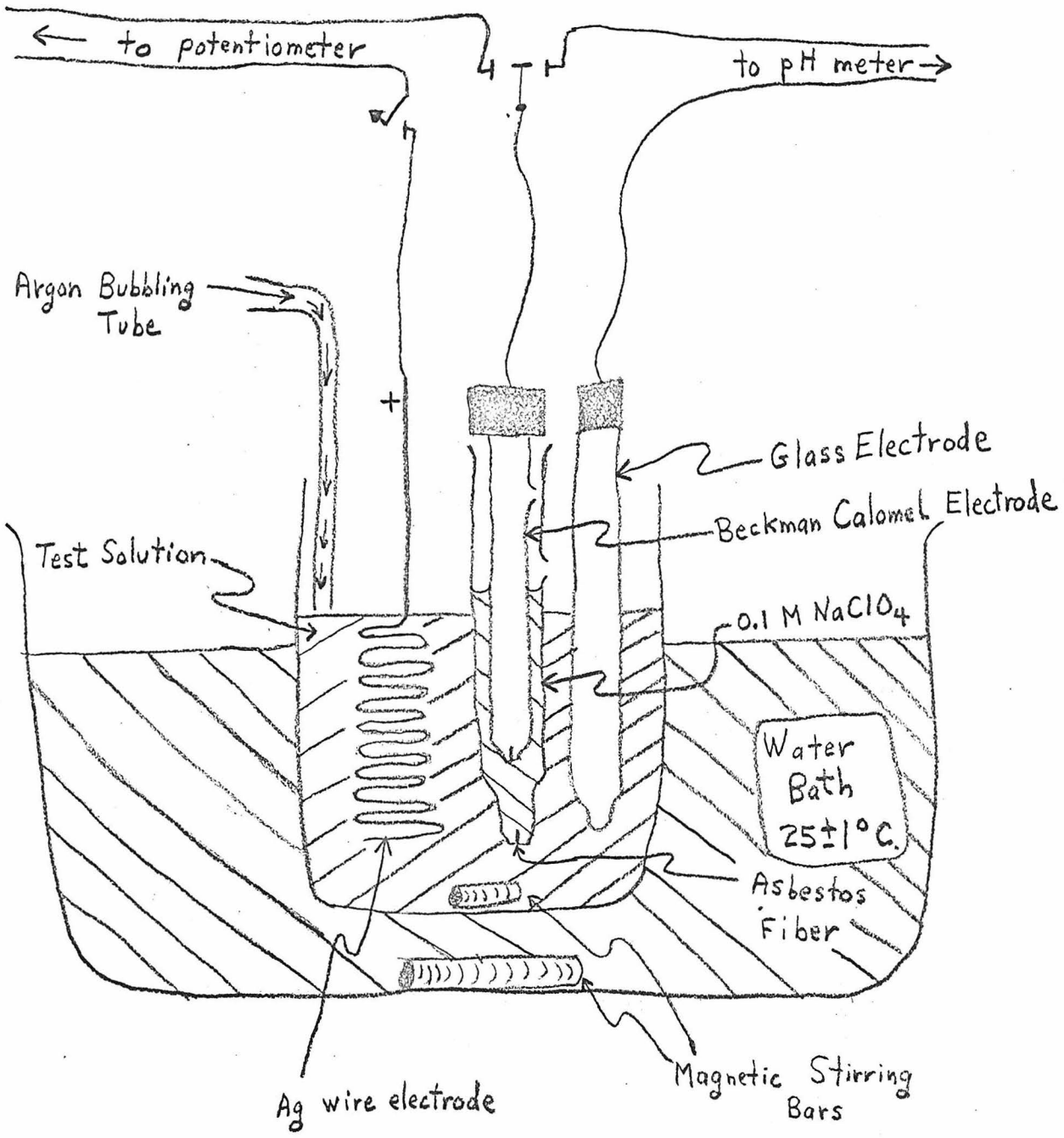


FIGURE 1 TITRATION APPARATUS

was read and recorded. These readings many times were repeated until "equilibration." Then another aliquot of silver nitrate was added for the next determination.

Most titrations were done at pH 7; but some experiments were done at pH 6 or 8. A water bath and magnetic stirring were used to keep the temperature at 25±1° C.

The argon atmosphere was used to prevent CO_2 interference in proton release determinations. Two switches on the electrode leads were used to allow independent reading of potential and pH. Metal shielding of the various components of the system, in conjunction with a grounding system to the "solution ground" connection on the pH Meter, were used to remove a large error in potential and pH readings due to A.C. pickup from the surroundings[9].

In analyzing the data the concentration of free silver in a solution was determined by comparison with Ron Jensen's experimental standard silver-potential graph (in agreement with Yamane and Mitchell).[10]

The fact that many of my silver-potential standards varied tremendously from Jensen's plot was responsible for the eventual grounding and shielding of the apparatus against pickup. Even after the shielding corrections, a number of my standard curves deviated from Jensen's line for an unknown reason (possibly due to non-reproducibility of liquid junction). Hence, in later experiments a quick silver potential standard curve was run on the apparatus just before a binding experiment. If this plot fit Jensen's graph (as it usually did), the experiment was continued.

The amount of silver bound at any point in the titration was determined by subtracting the $[Ag^+_{free}]$ (as measured by the potential) from the total silver added. From this, a useful quantity r_b

was calculated; "r_b" is defined as "the ratio of the moles of silver bound to the moles of total A present."

Next, from the amount of NaOH necessary to raise the pH again to its original value, the proton release per silver bound at any point in the titration $\left(\frac{\Delta H^+}{\Delta Ag_{tot}}\right)$ and the total proton release were calculated. Graphs of r_b vrs. $[Ag^+_{free}]$ and $\left(\frac{\Delta H^+}{\Delta Ag_{tot}}\right)$ vrs. r_b were plotted. Volume corrections in the calculations were made when necessary.

The essential purpose of the work was to compare the titration data under varied conditions in an effort to formulate suitable mechanisms for Ag^+ binding to adenine and the different substituted adenines. In some experiments other techniques (most notably UV spectrophotometry with a Cary Model 14 Spectrophotometer and 1 mm quartz cells) were used and supplementary data were recorded. These will be mentioned later as is necessary or enlightening.

Results

The reactions of silver with the several adenine derivatives studied are somewhat complicated, and the data that were obtained do not always lead to a unique, simple interpretation. I have tried to describe the results in some detail so that the reader can judge the validity of the conclusions for himself. However, in the interest of clarity, each section of these results begins with a presentation of my interpretation of the data of that section.

Adenylic acid

At pH's of 7 and 8 the reaction of silver ion with adenylic acid may be approximately represented by the equation:

$$2n\ Ag^+ + n\ HAd^= \longrightarrow [Ag_2 Ad]_n^{-n} + n\ H^+$$

where the proton involved is from the amino group of each adenylic acid molecule. The polymeric species is soluble for $r_b < 1.6$, but a precipitate forms when r_b approaches 2. At pH 6 precipitates form at lower r_b values; this is probably due to protonation of the phosphate groups.

Adenylic acid is seen to bind far less strongly than guanylic acid at pH 7 (Figure 2); but cytidylic and thymidylic acids have even less binding affinity for silver. This fact would immediately indicate (as expected) that the silver-adenylic acid binding is not involved primarily with the sugar of phosphate groups.

The amount of silver bound is a function of adenylic acid concentration (Figure 3); there is also a definite dependence of

Figure 2 Binding Comparisons

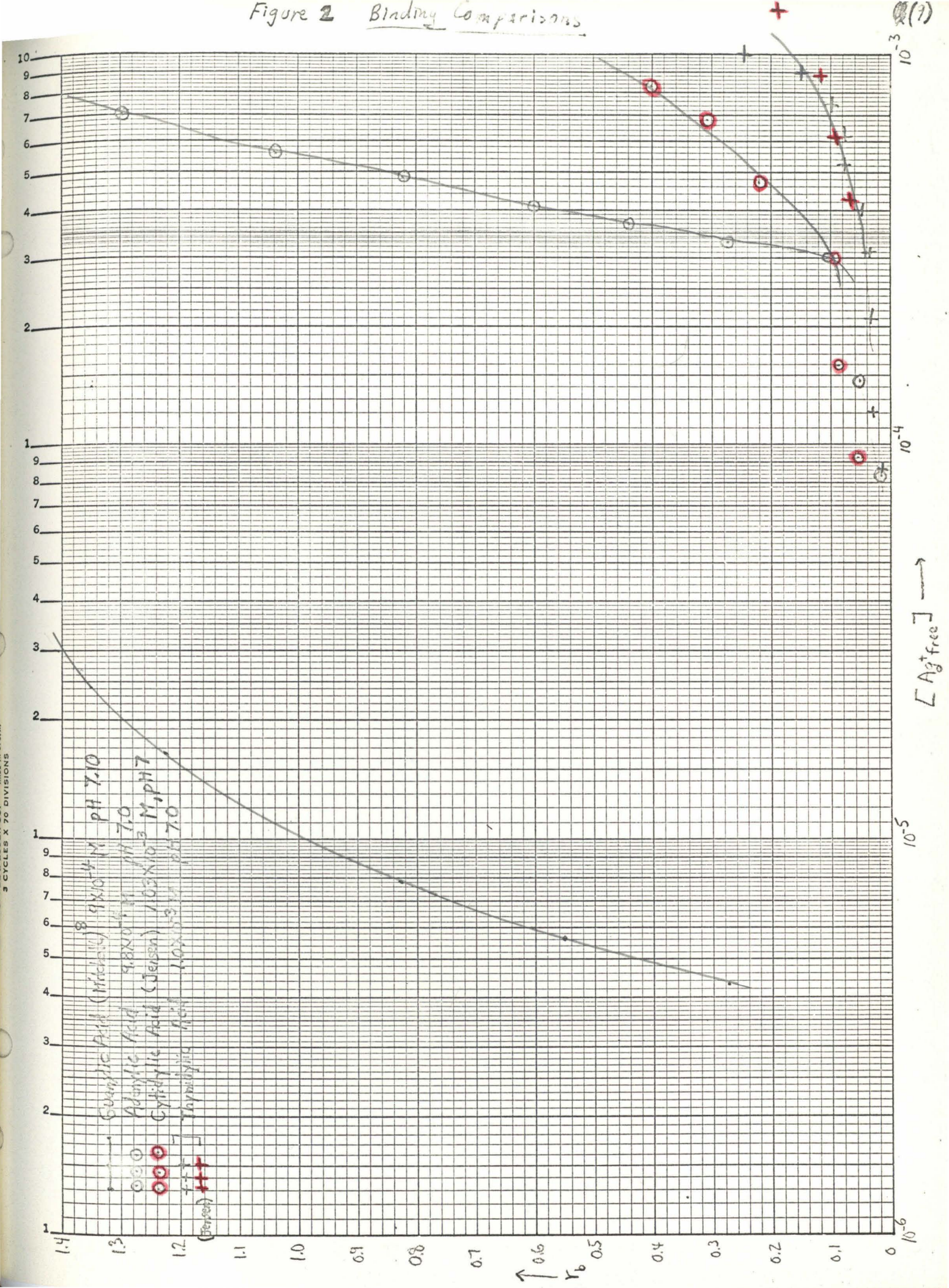
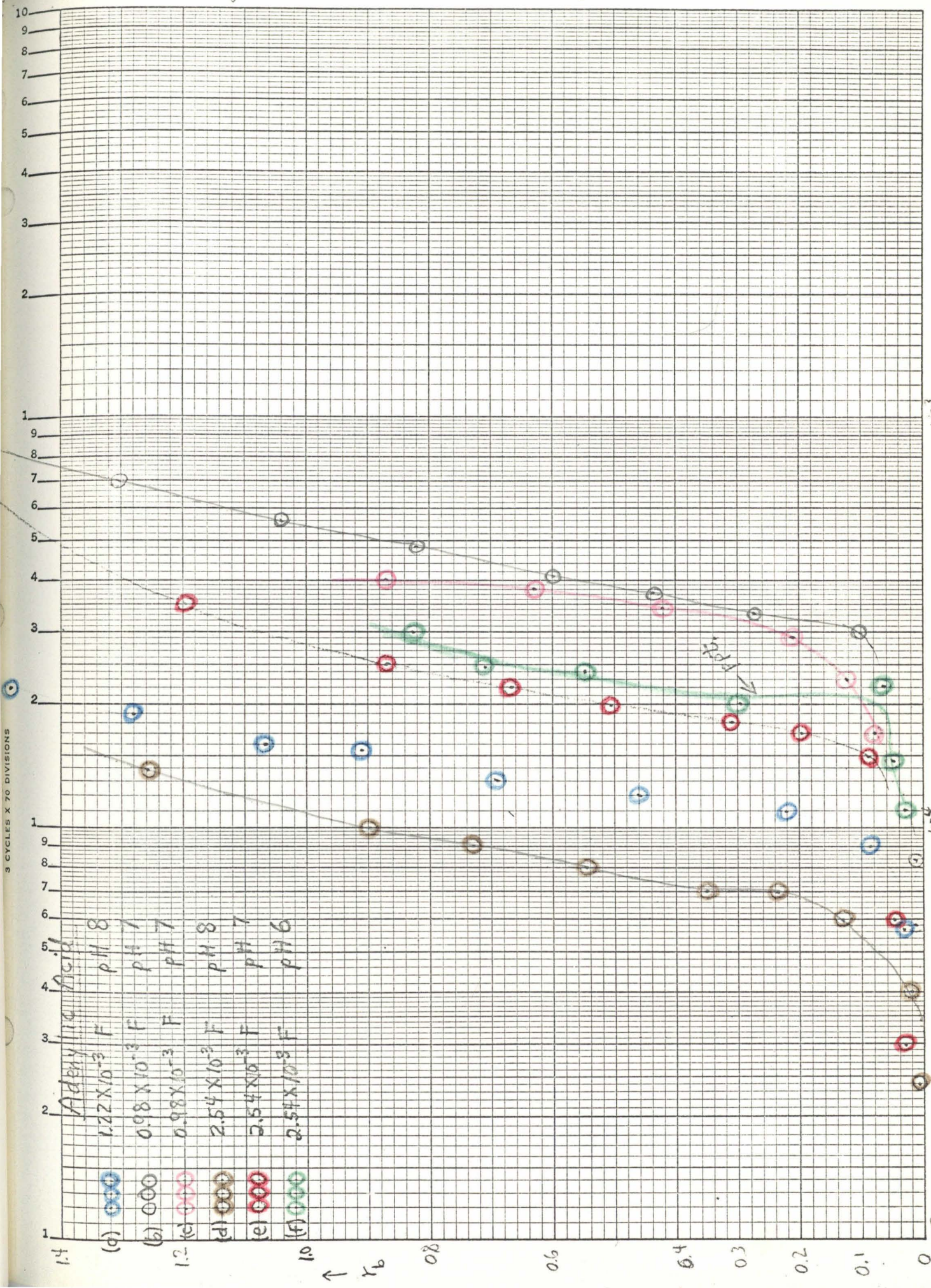


Figure 3 Adenylic Acid Binding Comparisons



silver binding on pH (Figure 3), stronger binding at a lower hydrogen ion concentration (pH range studied: 6 to 8). Yet, the difference in binding curves for pH 7 and pH 6 is much smaller that the pH 8 to pH 7 difference, indicating more than a simple pH dependence.

Proton release data (Figure 4) show continued and relatively constant release of 0.5 protons per silver bound up to $r_b \sim 2$ for adenylic acid. The total proton release per adenylic acid molecule present (taken for experiments that seemed to reach partial saturation at high r_b values) varied from ~0.9 to 1.1 for four different experiments (Table I); this value is probably a good indication of total available protons per adenylic acid molecule.

Of the compounds studied, adenylic acid was the most difficult to precipitate; for four experiments at pH 7 and 8, no precipitate was noticed before $r_b = 1.6$. Yet experiment (f) at pH 6 had a precipitate at $r_b = 0.3$ (Figure 3). This early precipitation can be attributed to a greater degree of protonation of the phosphate groups[11] at pH 6, making less silver binding necessary to neutralize the polymer. This change in phosphate protonation between pH 6 and pH 7 may also be responsible for the non-linear pH dependence (Figure 3) mentioned above.

The ultraviolet absorption spectrum of adenylic acid at pH 8 changes considerably upon addition of silver ions (Figure 5); two isosbestic points develop and remain until $r_b \sim 1$ at 241 mμ and 274 mμ. A plot of absorbance vrs. r_b for any wavelength would be linear if there were only one complexing species[8];

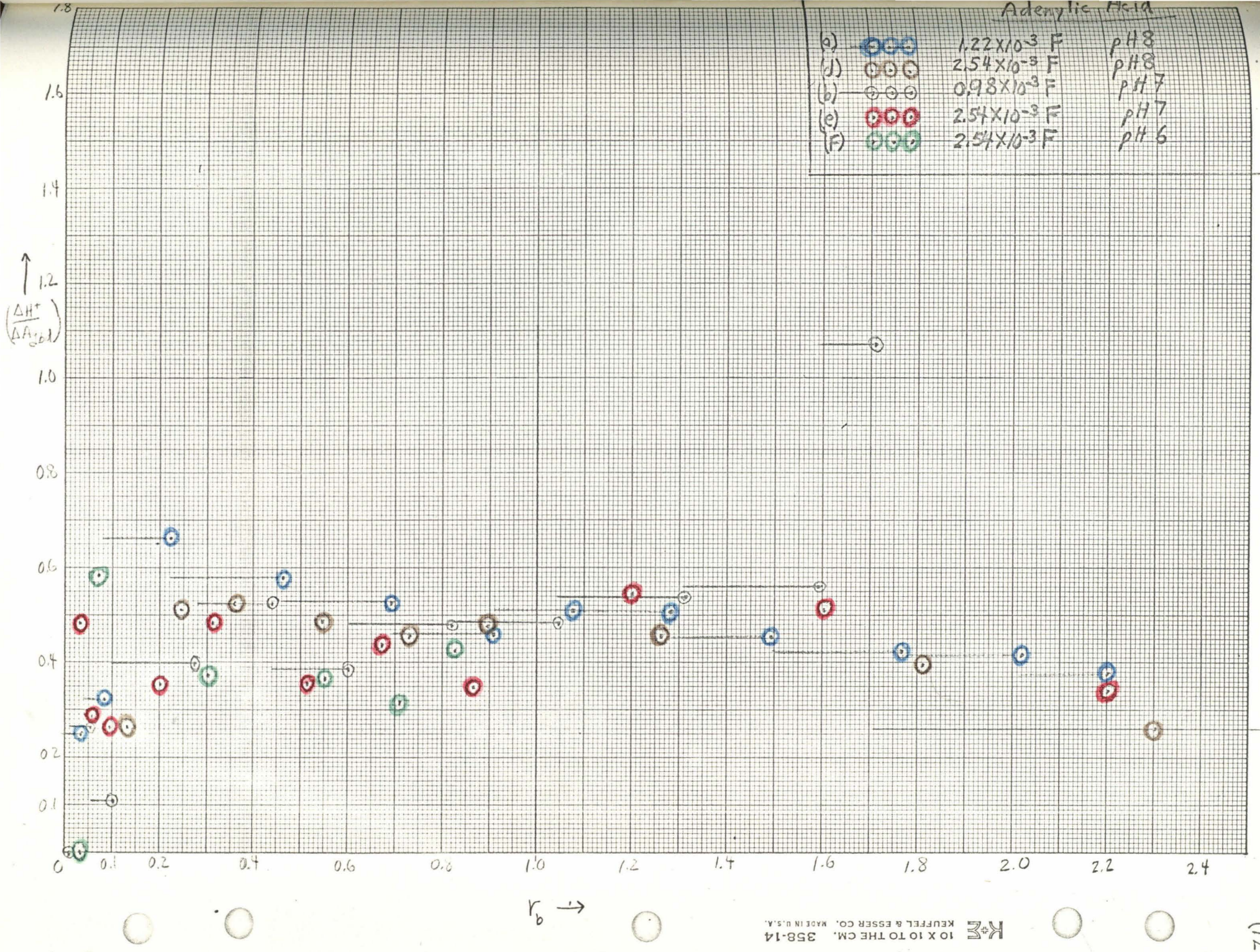


Figure 4 Proton Release for Adenylic Acid Binding

TABLE I

Solution	Species	pH	r_D at end	Total H^+ release / Total A present
a	Adenylic acid	8	2.7	1.13±0.05
b	"	7	2.84	1.0 ±0.05
d	"	8	2.3	0.96±0.2
e	"	7	2.1	0.91±0.10
g	Adenosine	7	>1.5	1.0 ±0.1
k	9-methyl adenine	7	1.6	0.94±0.1
l*	"	7	1.5	1.0 ±0.1
m	"	7	>1.5	0.8 ±0.1
o*	6-N,N-dimethyl adenine	7	1.1	0.9 ±0.1
p	"	7	1.47	0.99±0.02
r*	Adenine	7	1.6	1.5 ±0.2

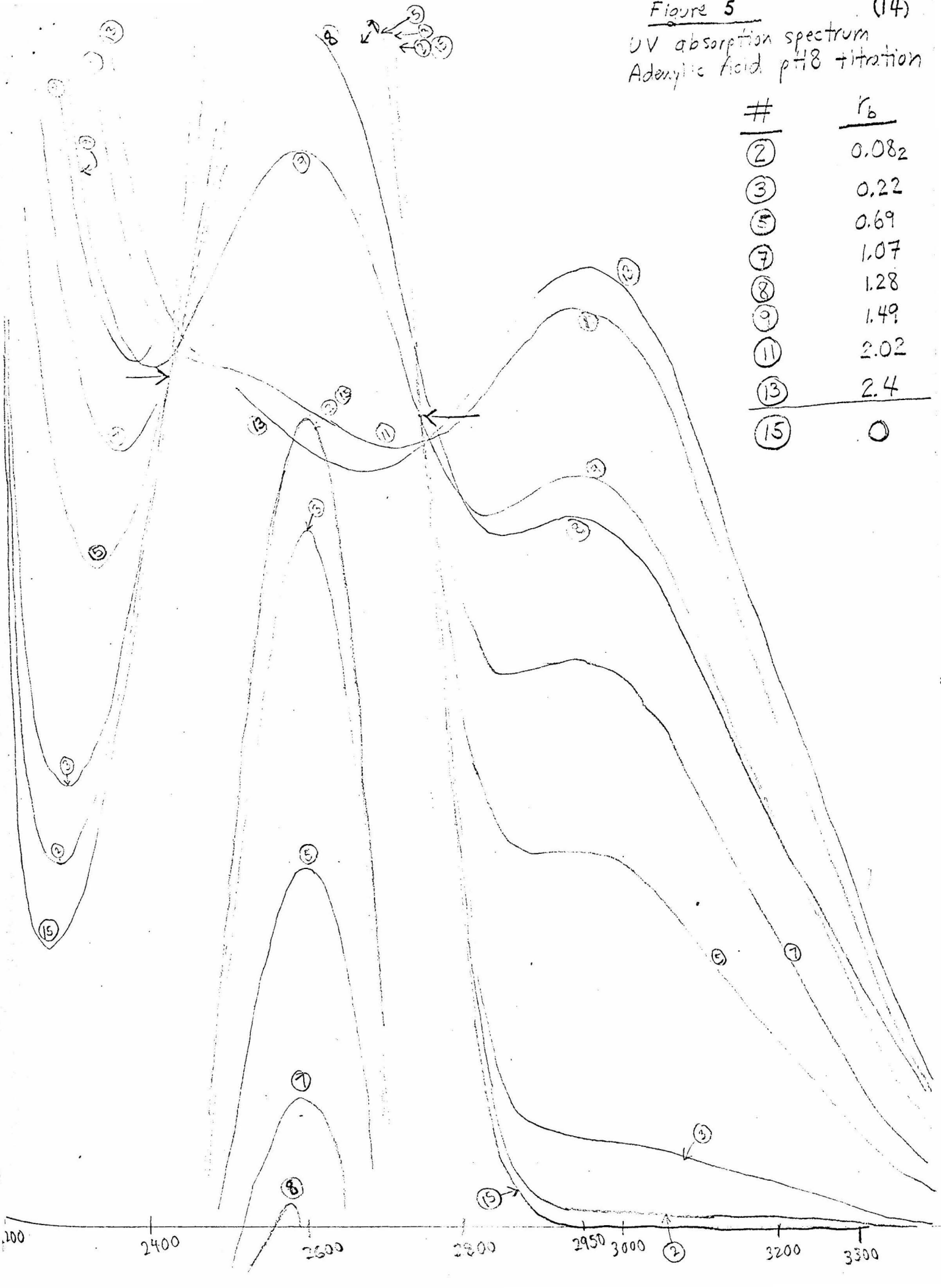
* Experiment done prior to argon bubbling system.

Correction made for CO_2 interference.

Figure 5

UV absorption spectrum
Adenylic acid pH8 titration

#	r_b
(2)	0.08_2
(3)	0.22
(5)	0.69
(7)	1.07
(8)	1.28
(9)	1.49
(11)	2.02
(13)	2.4
(15)	0



suitable plots for 260 mμ and 295 mμ show linearity up to $r_b \sim 1.5$ or 1.7, followed by a distinct break (Figure 6). Hence, at least two complex species are indicated for binding below $r_b \sim 2$. Precipitation evidence indicates that high polymers with two or more binding sites are more likely (even prior to actual precipitation).

Adenosine

The silver-adenosine reaction has not been studied as extensively as the silver-adenylic acid system; hence, the mechanism is not as well understood. At pH 7 silver seems to bind to adenosine on at least two distinct binding sites; one of the binding positions is associated with the release of an amino hydrogen. There is extensive polymerization, and there is early precipitation of the polymers. There is some evidence to support the following two reactions occuring simultaneously, with (a) occuring twice as often as (b):

(a) $Ag^+ + Ads \longrightarrow \frac{1}{n}\underline{(-Ag-Ads-)_n}\downarrow + H^+$ Polymer

(b) $Ag^+ + Ads + (ClO_4^-) \longrightarrow Ads-Ag^+-(ClO_4^-)$

For adenosine the amount of silver bound shows the same concentration dependence as for adenylic acid (Figure 7) but with less reproducibility. All adenosine titrations were done at pH 7, so no pH dependence can be estimated.

The chances of equilibrium in adenosine titrations are probably less than for the comparable adenylic acid solution, for adenosine titrations yield precipitates at much lower r_b values (Figure 7). At similar concentrations

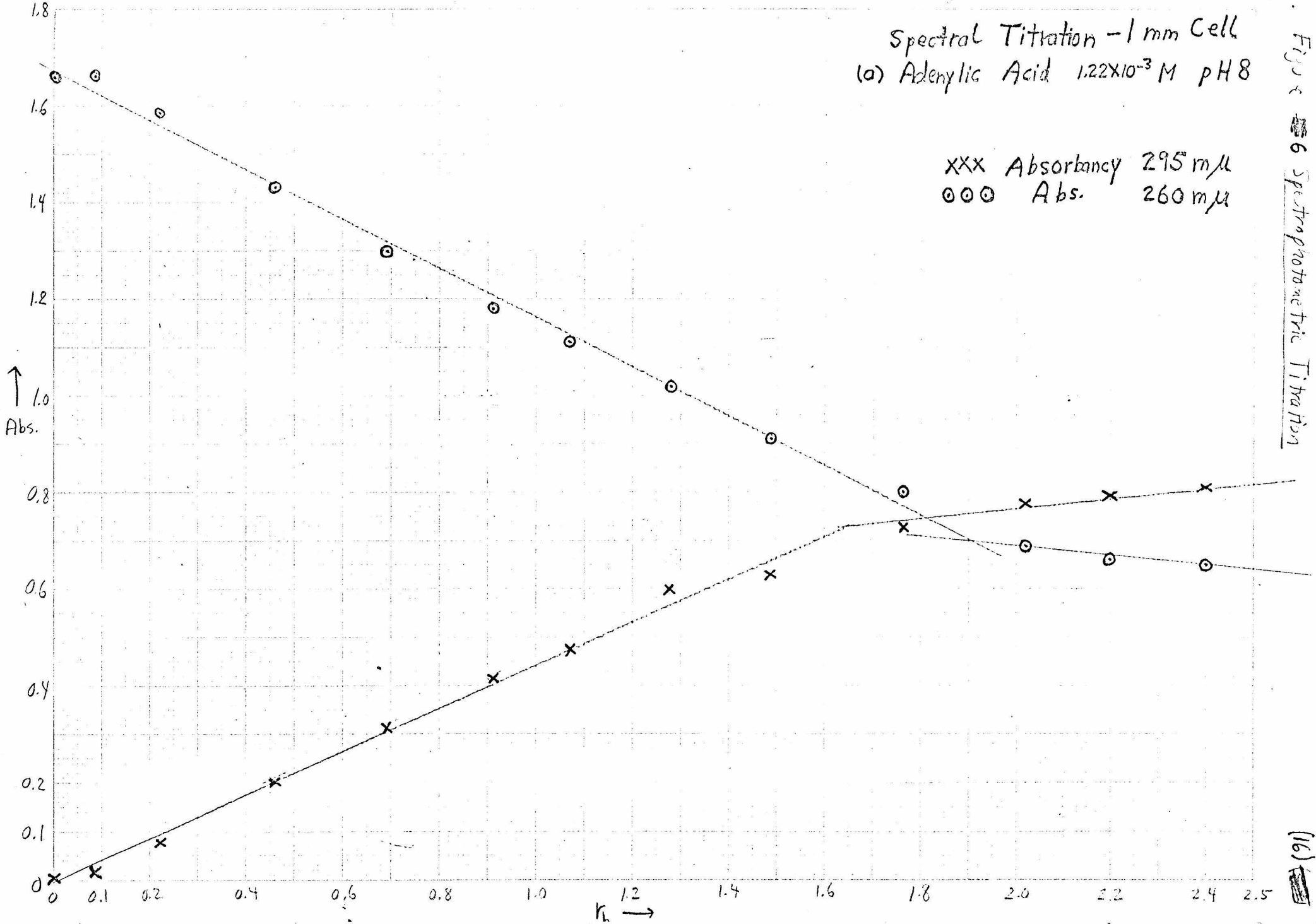
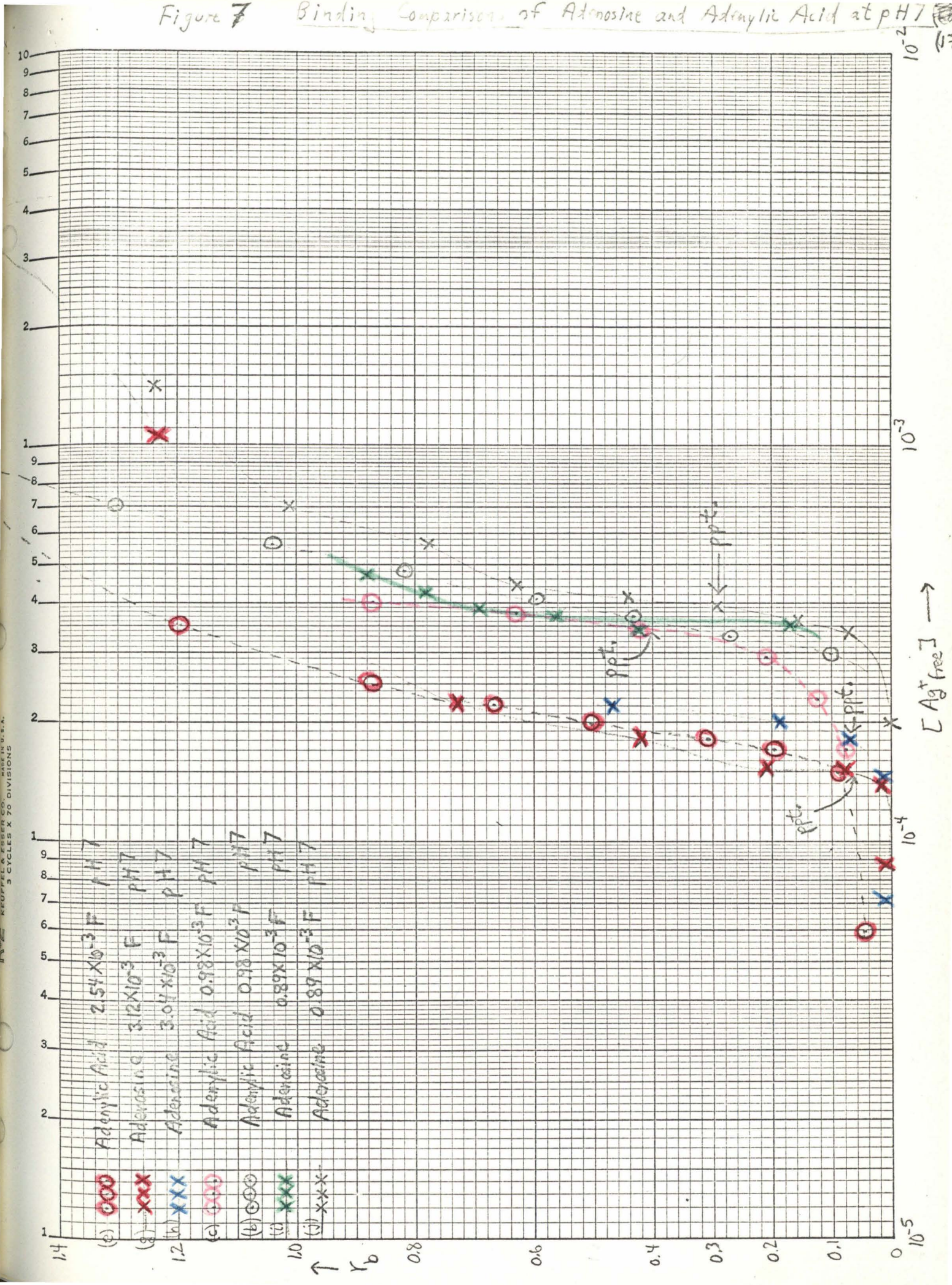


Fig. 6 Spectrophotometric Titration

Figure 7 Binding Comparisons of Adenosine and Adenylic Acid at pH 7



adenosine and adenylic acid have comparable binding curves at low r_b values; yet adenosine binds weaker at $r_b > 1$ (Figure 7), the deviation probably caused by the non-equilibrium and earlier precipitation.

Proton release is higher for adenosine (Figure 8) at low r_b values than for adenylic acid (Figure 4); yet, proton release tends to stop after $r_b = 1$ for adenosine. "Total proton release" data (Table I) for adenosine also shows one available proton per base, even though $r_b \geq 1.5$ (experiment g). This would indicate two different binding positions, the stronger involving release of a proton.

The charge of the phosphate group is probably the main reason for the differences in silver binding by adenylic acid and adenosine. If silver binds the bases together in a polymer, adenylic acid would be expected to precipitate much less readily because of the accumulation of negative charges in the polymer. The differences in proton release could possibly be explained as a charge effect in polymer formation.

Adenosine sample (g) was complexed to $r_b \sim 0.5$ and centrifuged; and the precipitate and supernatant were analyzed separately for adenosine and silver content. The total proton release was $\sim \frac{2}{3} \frac{H^+}{Ag_{bd}}$; and the precipitate contained $\sim \frac{1}{3}$ of the original adenosine and greater than $\frac{2}{3}$ of the silver bound. A mechanism that fits this data will be presented in the discussion section.

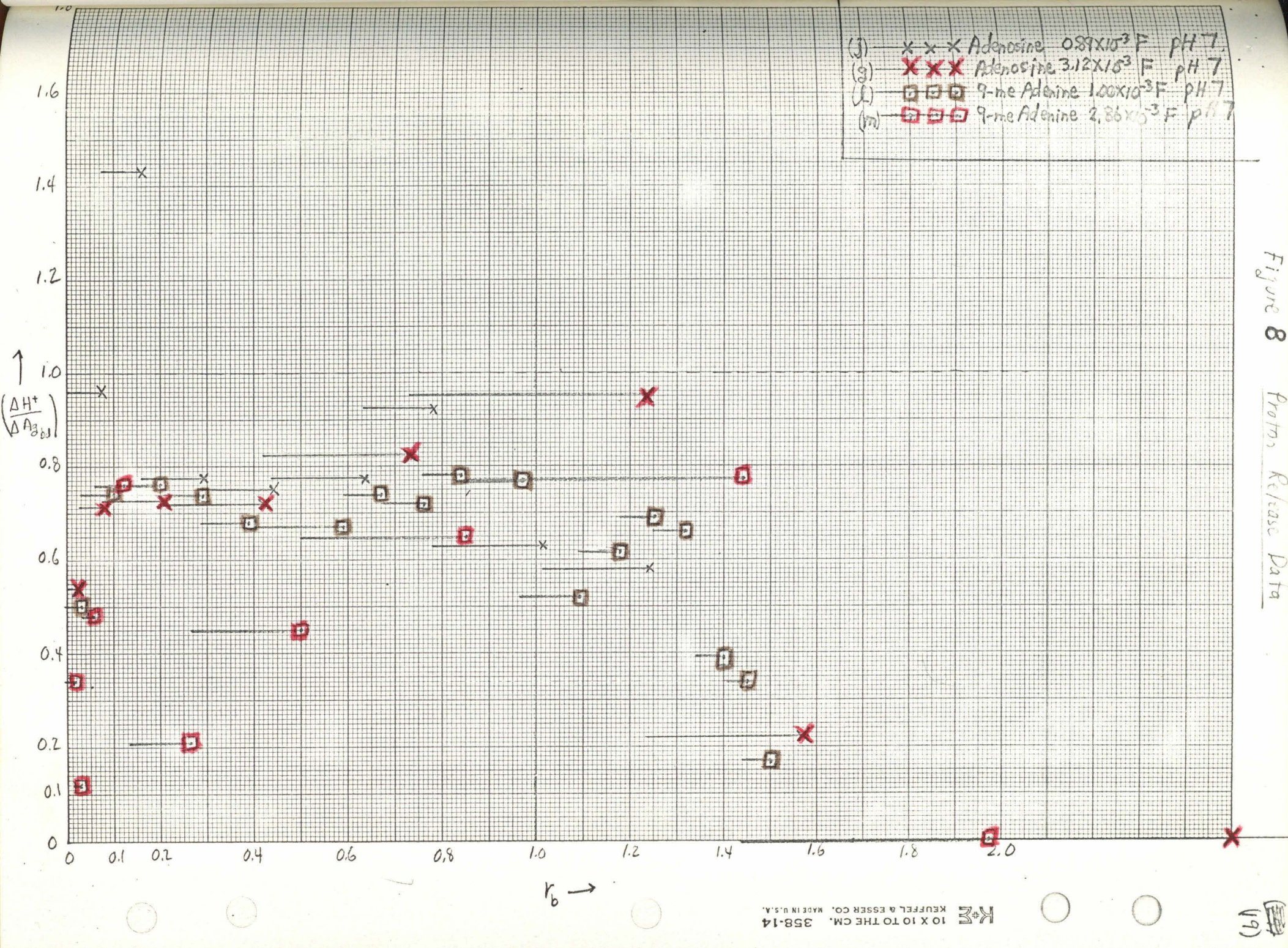


Figure 8 Proton Release Data

9-methyl adenine

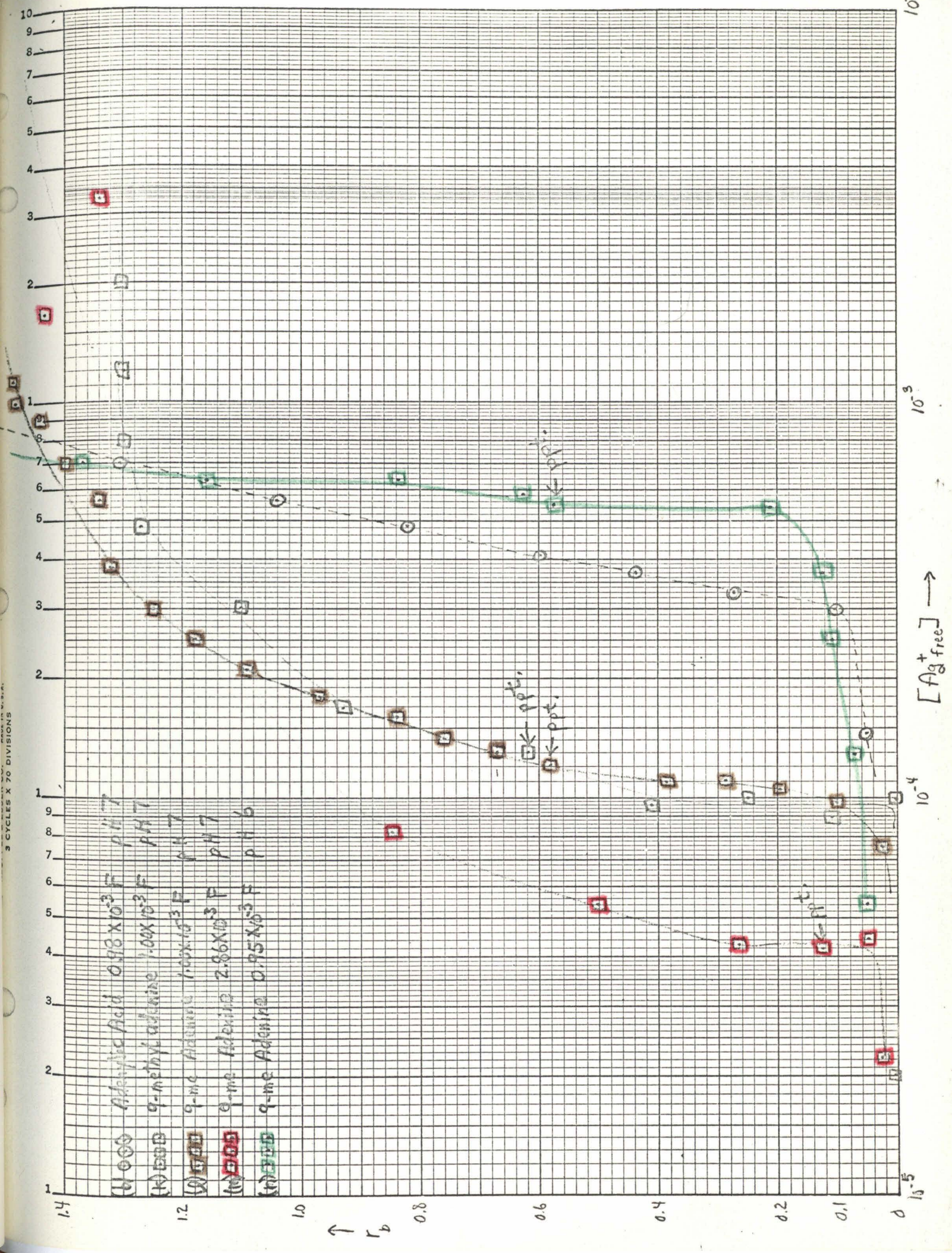
With 9-methyl adenine, extensive polymerization and early precipitation are observed. There are two or more binding sites for silver ions; amino protons are released during binding. No suitable binding mechanism has been found as yet.

The usual dependence of silver binding on concentration and on pH is shown by 9-methyl adenine (Figure 9). Proton release starts out at ~0.7 and tapers off after $r_b \cong 1$ (Figure 8). The binding curve in general tapers off after $r_b \cong 1$; but the binding is not very reproducible at these high r_b values. The binding uncertainties at high r_b values are probably due to early precipitation of the polymers (Figure 9). At low r_b values very reproducible results were obtained for two equivalent binding studies even though one of the experiments (L) was done previous to the electronic grounding system.

"Total proton release" data on three titration runs taken up to $r_b \sim 1.5$ indicated one available proton per base present (Table I); a two-position binding is again suggested, the first involving the replacement of a proton.

The binding of silver by 9-methyl adenine is much stronger than adenosine or adenylic acid binding at pH 7 (Figure 9). There is no easy way to explain these differences; possibly packing effects in polymer precipitates may be important; but polymerization effects are in general very difficult to analyze or to reduce to simple mechanisms. It is reasonable to say that the differences cannot yet be explained, but probably have something to do with precipitation orientations and packing; and 9-methyl adenine must stand out as an anomaly in the analysis.

Figure 9 Binding Comparisons for 9-methyl adenine



6-N,N-dimethyl adenine and adenine

In both adenine and 6-N,N-dimethyl adenine, silver is bound strongly to the 9-position with release of the 9-position proton and with precipitation:

$$Ag^+ + HA \longrightarrow AgA\downarrow + H^+$$ "Primary binding"

Adenine continues to bind past $r_b = 1$, with release of an amino proton ("Secondary binding"). In 6-N,N-dimethyl adenine there are no amino hydrogens available; but there is a much weaker ("Third level") binding of silver to ring nitrogens without any additional proton release.

The strongest binding position for silver is obviously the 9-position, as can easily be seen by comparison of Figure 10 with other binding curves. Adenine and 6-N,N-dimethyl adenine both bind silver far stronger than the other compounds studied, and both release almost one proton per silver bound up to $r_b = 1$ (Figure 11). Precipitates were noticed in all solutions at low r_b values. After this point there are interesting distinctions.

For 6-N,N-dimethyl adenine there is a levelling off of binding at $r_b = 1$, whereat the $[Ag^+_{free}]$ increases one-hundred fold before significant excess silver is bound. At $[Ag^+_{free}] = 10^{-4}$ F, r_b is only ~ 1.1. The eventual secondary binding (beyond $r_b \sim 1$) is accompanied by negligible proton release. The "total proton release" was very close to 1.0 protons per 6-N,N-dimethyl adenine molecule present (Table I), indicating one site capable of releasing a proton.

Adenine, however, continues to bind silver and continues to release protons past $r_b = 1$, reaching $r_b \sim 1.6$ at $[Ag^+_{free}] = 10^{-4}$ F (Figures 10,11). Also, the "total proton release" for adenine

Figure 10 Binding Comparisons of Adenine, 6-N,N-dimethyl adenine

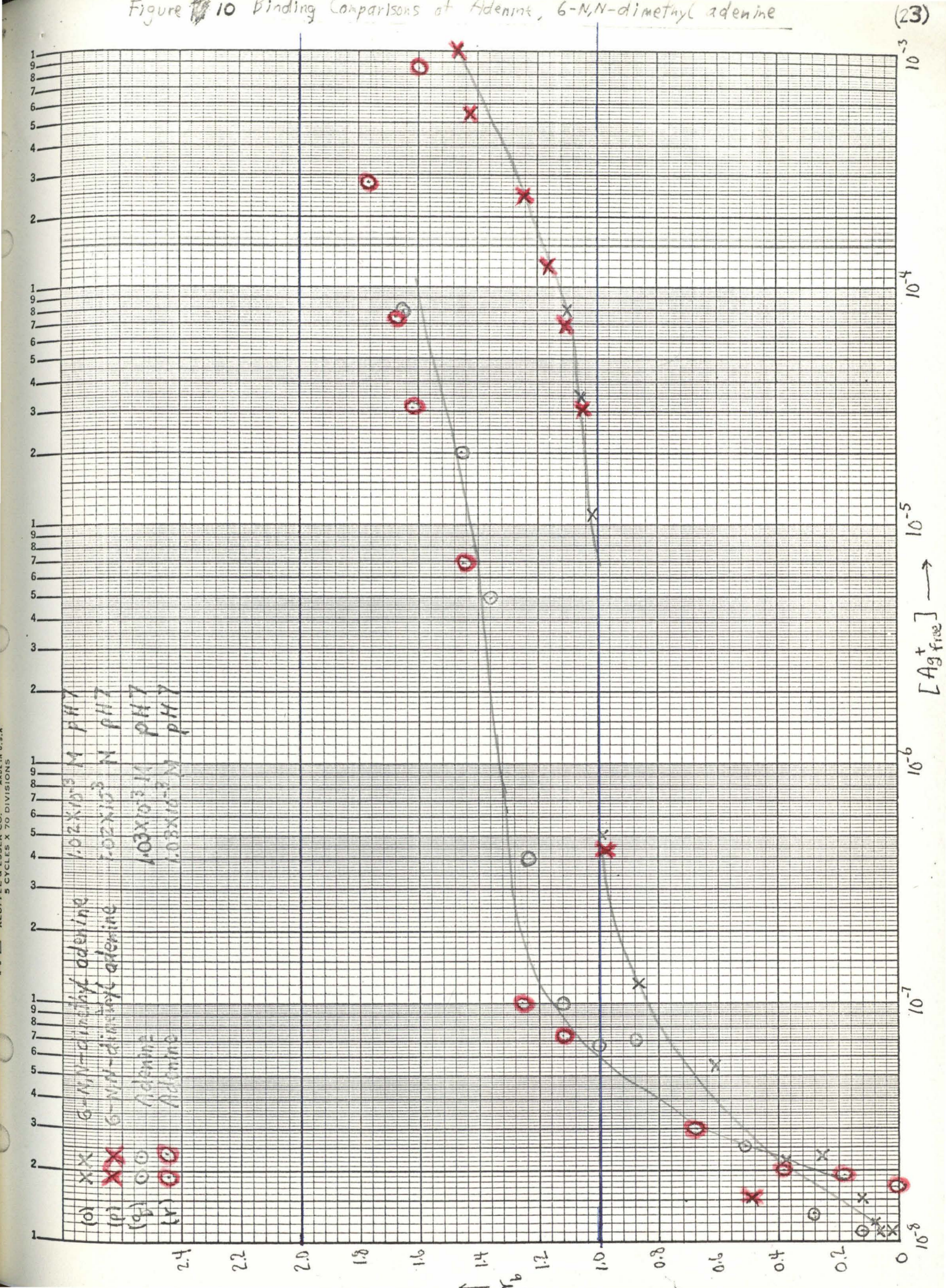


Figure 11 Proton Release Data

(at $r_b = 1.6$) was approximately 1.5 (Table I), seemingly not yet a saturation value, and clearly indicating more than one available proton.

The adenine and 6-N,N-dimethyl adenine results are very reproducible despite the fact that three of the four experiments were done prior to the electronic grounding system.

These results indicate clearly that a silver ion is very strongly bound to the 9-position of adenine and that a proton is released during the binding. The differences between adenine and 6-N,N-dimethyl adenine must be due to the 6-amino group; this would indicate that the secondary binding for adenine must be associated with the amino group. Accompanying this binding is some proton release, perhaps amino hydrogens displaced by silver. The second silver bound by 6-N,N-dimethyl adenine could be to a ring nitrogen involving no proton displacement; this somewhat weaker binding will be referred to henceforth as "third-level binding."

It is fairly easy to accept the 9-position as the strongest binding position for silver; but electronically similar mercury(II) binds to ring nitrogens more readily than to the amino groups.[5] Nevertheless, although the secondary binding need not be to the amino group of adenine, it obviously must be associated in some way with the amino group; and the simplest solution would be the displacement of an amino hydrogen. It must also be remembered that the primary binding causes precipitation and probable polymerization, making analyses of binding mechanisms more difficult.

Returning to adenosine, adenylic, acid, and 9-methyl adenine, we see that the newly proposed silver binding scheme

fits many of the binding results rather well. The "stronger binding" in each of the species could easily have involved release of an amino hydrogen; the "weaker binding" might have been to ring nitrogens.

Discussion

A secondary (associated in some way with the amino group) binding and a "third-level binding" (probably associated with ring nitrogens) have certainly been indicated by the results of a number of experiments; yet the "primary" binding to the 9-position is the only type of complexing that seems reasonably well understood. With this in mind, it might be best to formulate a reaction mechanism for the 9-position of 6-N,N-dimethyl adenine as a preliminary to other quantitative models. The results of the 9-position analysis would give an upper level to the constancy attainable for models which correctly describe other binding mechanisms. Assuming that the primary binding for 6-N,N-dimethyl adenine is associated with the 9-position only, and assuming that this reaction is complete before other positions are attacked by silver, the following mechanism can be written:

$$Ag^+ + AH \longrightarrow AgA\downarrow + H^+$$

$$K_{II} = \frac{[H^+]\,a_{AgA}}{[Ag^+][AH]} = \frac{[H^+]\,a_{AgA}}{[Ag^+][AH_0][1-r_b]}$$

For a given $[HA_0]$ and pH 7 at equilibrium

$$K_{II}' = \frac{1}{[Ag^+][1-r_b]}$$

Hence $(Ag^+)(1-r_b)$ should be a constant up to $r_b \sim 1$. Reference to Table II shows that the constants (computed from different experimental binding points) vary by as much as a factor of three from each other. Nevertheless, we believe the model is essentially correct. Now, it would be desirable to find a model with that much constancy in describing other more interesting binding sites.

Table II

Experiments (o) and (p): 6-N,N-dimethyl adenine, 1.02×10^{-3}, pH 7

Ag^+_{free}	r_b	$(Ag^+)(1-r_b) \times 10^8$
1.1×10^{-8}	0.049	1.04
1.2	0.074	1.11
1.5	0.123	1.31
2.3	0.246	1.74
2.2	0.37	1.38
5.5	0.61	2.14
1.2×10^{-7}	0.86	1.68
5.0	0.98	1.0
- - - -		
1.5×10^{-8}	0.49	0.75
4.2×10^{-7}	0.98	0.84

For adenylic acid the following model can be proposed embodying two independent binding positions for silver:

1) $HAd_{-?} + Ag^+ \rightleftarrows AgHAd_{+?}$ $\quad K_1 = \dfrac{[AgHAd_{+?}]}{[HAd_{-?}][Ag^+]}$

2) $HAd_{?-} + Ag^+ \rightleftarrows Ag\,Ad_{?+} + H^+$ Independent of reaction 1), $K_2 = \dfrac{[Ag\,Ad_{?+}][H^+]}{[HAd_{?-}][Ag^+]}$

where $HAd_{-?}$ means: 1st site empty, 2nd site empty or filled (i.e. ?)

Let $r_b = r_1 + r_2$. Suitable manipulation yields $\dfrac{[H^+]}{K_1K_2} = \dfrac{[Ag^+]^2(1-r_b)}{r_1(r_b-r_1)} + [Ag^+]^2$

First, taking $r_1 = r_2 \Rightarrow \dfrac{[H^+]}{K_1K_2} = \dfrac{[Ag^+]^2[1-r_b]}{r_b^2/4} + [Ag^+]^2 = K_{1/2}$

Table III shows $K_{\frac{1}{2}}$ to be unreasonable.

Taking $r_2 = 2r_1$ up to $r_b = 1.5$ as implied by UV studies $\Rightarrow$

$$\frac{H^+}{K_1K_2} = \frac{[Ag^+]^2(1-r_b)}{2r_b^2/9} + [Ag^+]^2 = K_{1/3}$$

$$K_{1/3} = K_{1/2} + \frac{1}{2}\frac{[Ag^+]^2(1-r_b)}{r_b^2}$$

$K_{\frac{1}{3}}$ diverges even faster than $K_{\frac{1}{2}}$; it is also easy to show that no other independent mechanism (obtained by altering the ratio of r_1 and r_2) will give results better than $K_{\frac{1}{2}}$.

Another mechanism to try is :

$$2\,Ag^+ + HAd \rightleftarrows Ag_2Ad + H^+$$

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

In Table III K_{21} is shown to be unreasonable.

The fact that all of the experiments have precipitates at high r_b values indicates that extensive polymerization is likely in the binding complex. The best assumption is that a silver attaches to two bases forming a link between the two. The fact that the binding curves have such a large slope (Figure 3) would indicate that there is a co-operative mechanism in silver binding; i.e. once one silver has com-

Table III

Experiment (a): Adenylic acid, 1.22×10^{-3} F, pH 8

Ag^+_{free}	r_b	$K_{\frac{1}{2}} \times 10^8$	$K_{21} \times 10^2$	$K_n \times 10^{-2}$
5.7×10^{-5}	0.033		5.15	
9.0	0.082		5.27	10.6
1.1×10^{-4}	0.22	78.9	10.2	7.6
1.2	0.46	16.2	20.8	7.4
1.3	0.69	6.11	31.1	7.4
1.55	0.91	3.44	34.8	6.3
1.6	1.07	1.93	44.9	6.9
1.9	1.28	1.15	49.2	6.3
2.2	1.49	0.58	60	6.7
2.8	1.76	0.157		8.7
4.5	2.02			
7×10^{-4}	2.2			
1×10^{-3}	2.4			

Experiment (d): Adenylic acid, 2.54×10^{-3} F, pH 8

Ag^+_{free}	r_b	$K_n \times 10^{-2}$
6×10^{-5}	0.13	11.7
7	0.24	9.1
7	0.35	9.8
8	0.54	8.4
9	0.73	7.7
1.0×10^{-4}	0.90	7.2
1.4	1.26	5.4
2.4	1.81	7.1

Table III (continued)

Experiment (b):

Adenylic acid, pH 7

0.98×10^{-3} F

Ag^+_{free}	r_b	$K_n \times 10^{-2}$
3×10^{-4}	0.102	11.9
3.3	0.275	10.9
3.7	0.44	9.6
4.1	0.60	8.7
4.8	0.82_2	7.6
5.6	1.04	6.8
7.0	1.30	5.1
9.1	1.59	6.0
1.26×10^{-3}	1.71	4.5
1.70	1.74	2.7
2.0	1.94	8.5
2.85	1.96	6.3

Experiment (e): Adenylic acid

2.54×10^{-3} F, pH 7

Ag^+_{free}	r_b	$K_n \times 10^{-2}$
1.5×10^{-4}	0.095	18.4
1.7	0.20	15.2
1.8	0.31	14.4
2.0	0.51	13.2
2.2	0.67	12.3
2.5	0.87	11.2
3.5	1.2	8.1
7	1.6	4.0

Experiment (f): Adenylic acid, 2.54×10^{-3} F, pH 6

Ag^+_{free}	r_b	$K_n \times 10^{-2}$
2.2×10^{-4}	0.067	84
2.0	0.30	116
2.4	0.55	94
2.5	0.71	98
3.0	0.83	75

plexed to an adenylic acid, it is easier for a second silver to attach. Without assuming a large co-operative factor, it would be very difficult to build up large polymers, especially at low r_b^{12}; yet, experimentally there is precipitation (and presumably polymerization) for adenylic acid run (f) at $r_b \sim 0.3$.

The following model might be a fairly good representation of possible polymerization for adenylic acid:

$$2n\,Ag^+ + n\,HAd \rightleftharpoons \underline{Ag_{2n}Ad_n}\downarrow + n\,H^+$$

$$K_n' = \frac{a_{ppt.}\,[H^+]^n}{[Ag^+]^{2n}\,[HAd]^n}$$

Assuming that the activity of the polymer (like a precipitate) remains fairly constant over a large range of binding, the n^{th} root of the constant can be extracted to obtain another approximate constant:

$$K_n = (K_n')^{1/n} \propto \frac{2\,[H^+]}{[HAd_o]\,[Ag^+]^2\,(2-r_b)}$$

The constant K_n not only works well for five separate adenylic acid experiments each under different pH and concentration conditions (Table III); but its value is also very reproducible from one experiment to another (with the exception of the pH 6 experiment)! For a given adenylic acid experiment the total range of K_n is less than a factor of five; the total range for all four experiments at pH 7 and 8 is less than a factor of seven. The fact that the constant at pH 6 (though reproducible from point to point) is off by a factor of ten from the other experiments might be a reflection of the effects of protonation of the

phosphate group[11].

This polymer-formation model of course also works well for adenosine titrations at pH 7 and low r_b values (Figure 7). Yet, it must also be noted that the higher proton release at the beginning of an adenosine titration (Figures 4 and 8) argues for a two-step mechanism similar to:

$$Ag^+ + Ads \rightleftharpoons AgAds + H^+$$
$$AgAds + Ag^+ \rightleftharpoons Ag_2Ads$$

with polymerization complications possible.

A two-step reaction may not fit the binding studies as well as the K_n-polymer model does; but it does a better job of explaining the spectrophotometric experiment previously mentioned. The adenosine spectrophotometric experiment fits fairly well to the following two-position reaction scheme with (a) favored two to one over (b):

(a) $n\,Ag^+ + n\,Ads \rightleftharpoons (-Ag-Ads-)_n \downarrow + n\,H^+$ POLYMER

(b) $Ag^+ + Ads \longrightarrow Ads\text{-}Ag^+\text{-}(ClO_4^-)$

Undoubtedly, a rather complicated binding scheme, involving both 1) silver binding to at least two different distinct types of sites and 2) extensive polymerization, is indicated by the data. Unfortunately, it would be very difficult to theoretically handle any more complications in the reaction mechanism.

For 9-methyl adenine the polymer-formation model gave a reasonably constant K_n (Table IVI) for $r_b < 1$. Another mechanism involving the formation of AgA and Ag_2A precipitates also gives a good fit to the data at low r_b's. Neither mechanism, however, could adequately describe the bin-

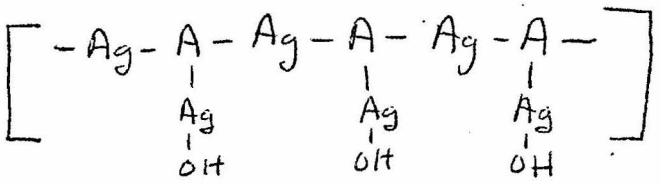
Table IV.

Experiment (l) : 9-methyl adenine, 1.00×10^{-3} F, pH 7

Ag^{+}_{free}	r_b	$K_n \times 10^{-2}$
0.97×10^{-4}	0.10	111
1.05	0.20	100
1.1	0.29	95
1.1	0.39	103
1.2	0.58	98
1.3	0.67	89
1.4	0.76	83
1.6	0.84	67
1.8	0.97	60
2.1	1.09	50
2.5	1.18	39
3.0	1.25	29.6
3.8	1.32	
5.6	1.34	
7.0	1.40	

ding past $r_b = 1$; again, the actual binding is probably altered considerably from ideality by precipitation.

Nothing has been said about the possibility of a silver binding to a ring nitrogen and carrying a co-ordinated hydroxide along with it . This could, of course, account for proton-release data. Yet, the silver that binds in this way could not be a link in a polymer chain, since a silver ion does not very easily form three co-ordination bonds[13]. Still a suitable polymer of $r_b = 2$ can be built up:

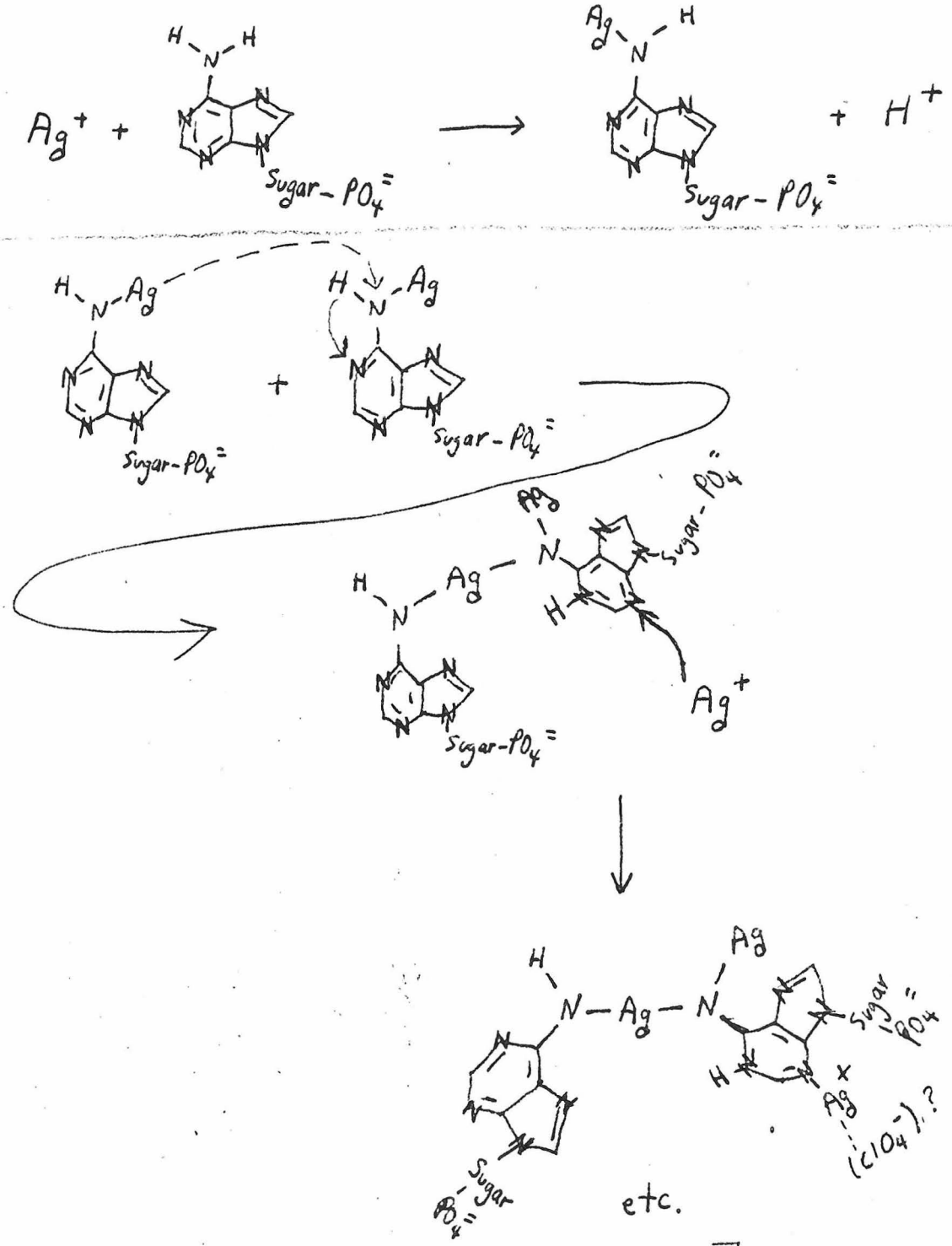


See Figure 12

In this polymer the ring nitrogens could supply all of the possible binding sites. Yet, this proposal certainly does not indicate a reason for the distinctly different binding of adenine and 6-N,N-dimethyl adenine. The AgOH theory would also seem to be non-specific as to the 1, 3, and 7 nitrogens; and no preference could be easily rationalized. If the three positions were essentially equivalent, how can the "one proton released per molecule" rule be explained? The -AgOH theory is then unlikely, although not impossible.

The binding results studied have proven to be very complicated; no correlation with the results for silver-DNA binding seems at present possible.

Figure 12 A Polymer Building Attempt



To form : $\left[-A-Ag-\underset{\underset{Ag^+}{|}}{A}-Ag-\underset{\underset{Ag^+}{|}}{A}-Ag-\right]$

$?\,(ClO_4^-)$ (ClO_4^-) (ClO_4^-)

Acknowledgements

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Prospectus

Previous work has emphasized potentiometric data in analyses of binding mechanisms; future work may accomplish much by putting equal emphasis on spectrophotometric data and its quantitative study. Hence, more spectrophotometric titrations would be useful.

Running adenosine at different pH's (to see if binding curve is still the same as for adenylic acid under comparable conditions) would be very enlightening. Such experiments might eliminate the confusion surrounding pH 6 titration of adenylic acid.

Other variations in pH and concentration for titration of the various species studied might suggest better binding mechanisms than the ones presently proposed.

Now that the equipment is seemingly so much better, it might be wise to check some of the silver-DNA binding curves.

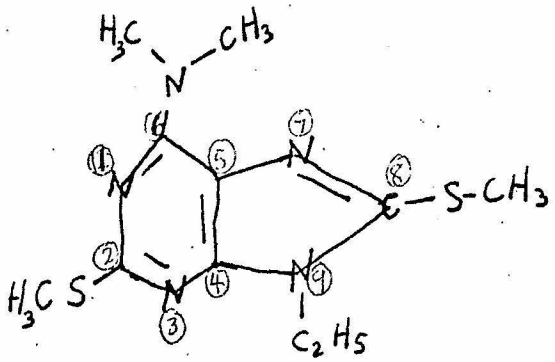
In many binding studies, analysis of precipitates, if possible, would be extremely helpful.

Different titration methods might answer doubts that have been raised as to equilibrium in the binding studies. Adenylic acid aliquots could, for example, be added to a solution of silver nitrate to see if the binding is the same as in standard titrations.

Most important, though, two new compounds have been obtained and should be titrated. The first is a few mg sample(probably just enough for one titration) of 6-N,N^4- dimethyl, 9-methyl adenine. Its 9-position and its amino group are both blocked; hence, the theory formulated in this paper would predict "third-level"

binding only, with probably little proton release. The second compound is 2,8-bis-methylmercapto-6-dimethylamino-9-ethylpurine. It could be titrated by itself (for it is completely blocked); or Raney nickel desulfurization could be used to produce 6-dimethylamino-9-ethylpurine (6-N,N-dimethyl, 9-ethyl adenine).

2,8-bis-methylmercapto-6-dimethylamino-9-ethylpurine :



Epilogue

Since the completion of the work described in this thesis, Ron Jensen has explored further into the problem of complexing silver with adenine and its derivatives. His work has lended much support to my conclusions; and he has also verified a few of the predictions in this thesis. A quick mention of Jensen's work would be a valuable addition to this thesis.

Jensen has titrated a sample of 9-methyl-6-N,N-dimethyl adenine with silver. As predicted in my paper (Prospectus), he found only a very weak "third-level binding" with no proton release. This binding is presumably to a ring nitrogen, since the 9-position and the amino group are both blocked.

For adenosine and 9-methyl adenine, Jensen has proposed the following reaction scheme:

$$3\,Ag^+ + 2\,HA + ClO_4^- \rightleftharpoons [Ag_3A_2 \cdot ClO_4]\downarrow + 2H^+$$

This scheme fits well with the proton release data, with the binding curves, and with the fact that the binding seems to reach a partial saturation at $r_b \sim 1.5$. It is interesting to note that the above reaction scheme is just a simpler way of writing the "two overlapping reactions" mechanism which I proposed for adenosine titrations (Pages 15 and 33 of this thesis)!

Jensen's conclusions for adenine, 6-N,N-dimethyl adenine, and adenylic acid are in agreement with my conclusions.

A paper has been prepared combining Jensen's results with the results of my thesis. It is: Keith Gillen, Ronald Jensen, and Norman Davidson, "Binding of Silver Ion by Adenine and Substituted Adenines." This paper has been accepted by J.A.C.S. for future publication.

Legend for Figures

Figure 1: A sketch of the titration cell.

Figures 2,3,7,9,10: Binding comparison curves

Plots of r_b vrs. log $[Ag^+_{free}]$ for various experiments

Figures 4,8,11: Proton release data

$\frac{\Delta H^+}{\Delta A_{g_{bd}}}$ plotted vrs. r_b for various experiments

Figure 5: Ultraviolet absorption spectrum of a sample of adenylic acid during a titration with silver. Spectrum is shown at various r_b values during the titration.

Adenylic acid = 1.22×10^{-3} F; pH = 8

Figure 6: Absorption data from spectrophotometric titration

Plot of absorbance vrs r_b for 1.22×10^{-3} F Adenylic acid (pH 8) at 295 mμ and 260 mμ. Non-linearity indicates more than one complex species.

Figure 12: A sample polymer buildup scheme for adenylic acid with silver. The polymer shown would have a final $r_b = 2.0$ and would have a "total proton release" of 1.0. Many other such suitable polymers are theoretically just as reasonable.

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