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A fluorescent dye, 1-dimethylaminonaphthalene-5-sulfonyl chloride (dansyl chloride), was covalently bonded to polylysine. The dansyl tagged polylysine was complexed to DNA by gradient dialysis. The polarization and intensity of fluorescence of the dansyl and the turbidity of the complex were measured as a function of salt concentration for a variety of salts.

By assuming a two state model for the system, namely complexed and uncomplexed polylysine, the fraction, f, of the dansyl polylysine complexed to the DNA was determined from the polarization and intensity of fluorescence data.

Under the more stringent assumption that the fluorescent intensity of the dansyl polylysine molecule was dependent only on whether or not it was bound to the DNA, f was calculated from the intensity of fluorescence data alone. The results were in good agreement with the

results of the polarization measurements.

The fractional change in the turbidity of the solution of the DNA-polylysine complex was also found to be reasonably close to the value of f as determined by polarization. This indicates that the scattering unit is a well defined system.

The effectiveness of the ions in dissociating the complex is in the order SCN^-, $ClO_4^- > NO_3^- > Br^- > Cl^- > C_2H_3O_2^-$ and $Mg^{++} > Li^+ > NH_4^+$, $Na^+ > K^+$.

The dissociation curve of a complex of polylysine with A-T rich salmon sperm DNA appears to be nearly identical to that of the G-C rich Micrococcus lysodeikticus DNA-polylysine complex.

The pH titration of the complex showed that the association of polylysine with the DNA stabilized the polylysine against the loss of its protons.

NaCl is more effective in dissociating the DNA-polylysine complex in an aqueous solution than it is when the complex is in a 50 volume percent methanol solution.

The Interaction of Polylysine with DNA as Studied by Polarization of Fluorescence

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I. INTRODUCTION

A. DNA-Protein Interaction

A wide variety of proteins are associated with the DNA of eucaryotic cells. The effects of proteins upon the biological and physical properties of DNA have been the subject of many recent investigations.

Felsenfeld, Sandeen, and von Hippel (16) found that ribonuclease destabilized the helical DNA structure and they suggested that this may be an important mode of action of the nucleases and other enzymes which act upon DNA.

Histones, the basic proteins that are associated with DNA, have been known for about a century to be present in the nucleus. In 1950, Stedman and Stedman (50) suggested that the histones might be involved in the regulation of the biological activity of DNA. It was not until 1962, however, that Huang and Bonner (25) showed that when histones were experimentally complexed to DNA, they inhibited the DNA dependent synthesis of RNA. Many workers (2, 5, 7, 24, 26, 31, 34) have since confirmed and elaborated Huang and Bonner's result.

By applying hyperchromic dispersion analysis to the denaturation of nucleohistones, Ohba (40) found that A-T pairs in the DNA

were preferentially stabilized by lysine-rich histones. However, no work has shown the histones to be specific in their interaction with DNA to the extent that the histone-DNA interaction alone would account for the selective transcription necessary for the differentiation of cells. Barr and Butler (5) inferred that the histones were not specific in their inhibition of RNA synthesis from the result that each fraction of histone caused a large inhibition of RNA synthesis, even though that fraction of histone was a comparably small percentage of the native histone.

Specificity may be introduced into the histone-DNA system by chromosomal RNA. Bonner (8) reports that when salt dissociated chromatin is reconstituted by gradient dialysis and transcribed by RNA polymerase, the RNA formed is identical to the RNA produced by native chromatin as judged by hybridization competition. However, if the chromosomal RNA is removed by nuclease treatment after dissociation of the chromatin, the reconstituted chromatin is transcribed by RNA polymerase, but the RNA produced does not compete with that transcribed from native chromatin.

Frenster (17) has reviewed much of the work which suggests that chromosomal RNA is intimately associated with selective transcription. He cites his work (18) showing that nuclear RNA is effective in the de-repression of RNA synthesis.

Ptashne (45) has shown that the λ phage repressor, which is an

acidic protein, binds specifically to an operator region of the λ DNA. Gilbert and Müller-Hill (19) have shown that another repressor protein, the lac repressor, binds specifically to the the lactose operator region of the phage DNA.

Lysine-rich histones have been implicated by Littau et al. (32) in the binding of chromatin threads to form dense masses. It thus appears possible that histones have a structural function. Olins, Olins and von Hippel (43) give evidence suggesting that protamine probably serves a structural function in facilitating the dense packing of DNA in fish sperm heads.

In summary, the above discussion emphasizes the variety of effects that various proteins may have upon the function of DNA. These effects include: (a) the possible destabilizing of helical DNA structure: (b) the general inhibition of RNA synthesis; (c) the repression of a specific operator region of DNA; (d) the structural organization of DNA.

An approach to the understanding of the general characteristics of protein-nucleic acid interactions is to use well defined polypeptides as models, and the results of such studies are reported here. The literature is discussed in the next section. Chapter III presents the results of my research on the interaction of polylysine with DNA.

B. DNA-Synthetic Polypeptide Interaction

Spitnik, Lipshitz and Chargaff (49) studied the solubility properties of polylysine-DNA complexes. The complexes were more soluble in distilled water than in 0.5 molar NaCl. However, at about 1.5 molar NaCl the complex was quite soluble. This solubility at high salt concentration is presumably due to the dissociation of the complex. These workers found also that when the precipitates, formed by DNA-polylysine interaction, were redissolved in NaCl solutions, the G-C rich DNA was dissolved more readily than the A-T rich DNA.

Akinrimisi, Bonner and Ts'o (1) measured the effective binding constant of polylysine and histone fractions to DNA as a function of NaCl concentration by the method of equilibrium dialysis. They found that the affinity of the arginine-rich histone fraction is greater than that of the lysine-rich fraction. The binding of the polylysine was essentially the same as the lysine-rich histone fraction. Tsuboi, Matsuo and Ts'o (54) studied the complexing behavior of poly-L-lysine with DNA under dilute salt conditions. They followed the helix-coil transition of the DNA by ultraviolet absorbance measurements at 260 nanometers and found, in general, a two step transition. The first transition temperature corresponded to the melting of free DNA and the higher transition temperature was attributed to the melting of the

complex. Their results suggested the existence of a polylysine-DNA system with the DNA molecules that are complexed being completely complexed with a lysine to nucleotide ratio of one, whereas the other DNA molecules were completely free of polylysine. They further investigated the complex by means of sucrose gradient electrophoresis. When the components of the solution of the complex were separated by electrophoresis, the DNA migrated in two bands. The contents of each band were isolated and measurements of the melting profile on the component in each band showed that one component was free DNA and the other was a DNA-polylysine complex, showing only the higher melting transition.

The polylysine was shown to be irreversibly bound to the DNA at low salt concentration by adding P^{32}-labeled DNA to the complex before electrophoresis. Upon electrophoresis the labeled DNA was found only in the band containing the free DNA.

Leng and Felsenfeld (30) reported that precipitates formed by the simple addition of polylysine to DNA in one molar NaCl solutions had an A-T preference. When polylysine was added dropwise to a solution containing two different bacterial DNA's, the DNA with the high A-T content was almost completely precipitated before any of the other DNA was precipitated. Similar experiments with a mixture of poly dA:dT and bacterial DNA showed that all the poly dA:dT was precipitated before the other DNA precipitated.

Radioisotope labeling was also used by Leng and Felsenfeld to show that the reactions involved in forming the precipitates in one molar NaCl were reversible. Enough polylysine was added to the P^{32}-labeled DNA to precipitate 30 percent of the DNA and then an excess of unlabeled DNA was added. After centrifugation to remove the precipitate, 96 percent of the total P^{32} was found in the supernate.

Polylysine appeared to be G-C specific in the selective precipitation of DNA when tetramethylammonium chloride was used instead of NaCl. This reversal has been explained (Gary Felsenfeld, personal communication, Corvallis, Oregon, March 10, 1968) by showing that tetramethylammonium ions preferentially bind to A-T rich regions of DNA.

Ayad and Blamire (4) have developed a method of DNA fractionation by the use of polylysine kieselguhr columns. DNA was loaded to the column and the column was then eluted by a linearly increasing NaCl gradient. They found that the A-T rich DNA was eluted at a lower NaCl concentration than was the G-C rich DNA.

Olins, Olins and von Hippel (42) formed complexes of calf thymus DNA with various basic homopolypeptides. The complexes were formed by gradient dialysis over a period of about two days. The dialysate was then centrifuged at 1700 g for 15 minutes to remove the precipitate. They carried out a rather complete study of the solubility, stoichiometry, absorption spectra, and thermal denaturation

of the complexes. They observed two step thermal denaturation curves similar to those previously observed by Tsuboi, Matsuo and Ts'o (54). The lower melting temperature was characteristic of the free DNA and the higher melting temperature was characteristic of the complex. These two melting temperatures were independent of the cationic peptide to nucleotide ratios used to make up the complex. The different polypeptides stabilized DNA against melting by different amounts. The order of stabilization was polyornithine> polylysine> polyarginine> polyhomoarginine. Analysis of the dispersion of hyperchromicity showed that poly-L-lysine and poly-L-ornithine appeared to stabilize preferentially A-T rich regions of DNA, whereas poly-L-arginine and poly-L-homoarginine did not. They further found, by ultracentrifugation of the soluble complex, that the solution could be separated into a supernatant in which the molecules melted like free DNA and a pellet which contained one basic peptide residue per nucleotide. This is similar to the result Felsenfeld and Huang (15) obtained by conductometric titration of two stranded poly (A+U) with polylysine. They detected an endpoint at a concentration of polylysine which indicated that the lysine to nucleotide ratio was one.

Olins, Olins and von Hippel (43) have extended their previous work by studying the properties of DNA-protamine and several DNA-oligolysine complexes. They found that the DNA-protamine complex was characterized by a biphasic melting profile. However, this

complex differed from the long chained polypeptide-DNA systems previously studied in that the temperature of the first melting transition of the DNA-protamine complexes increased with increasing cationic peptide to nucleotide ratio. This progressive change in melting temperature was thought to be due to some specific feature of the protamine structure such as chain length or spacing of the specific residues in the protamine. The complexes were separated by ultracentrifugation into a pellet in which the DNA phosphate to arginine ratio was one, and a supernatant fraction containing only free DNA.

Olins, Olins and von Hippel also investigated the effect of chain length on the behavior of DNA-oligolysine complexes. Tetralysine complexes had monophasic melting profiles with the melting temperature increasing as the peptide cation to nucleotide ratio was increased. Octalysine complexes showed biphasic melting profiles when the ratio of cation to nucleotide was greater than 0.5. The oligolysine with a range of 14 to 18 residues per chain showed a pronounced biphasic melting temperature profile. Tetralysine did not preferentially stabilize the A-T rich regions of DNA whereas the higher oligolysines did. They found that the ability of an oligolysine to form a stable complex with a constant second transition melting temperature correlated with its ability to stabilize A-T rich regions. The stabilization of A-T rich regions of DNA was determined by taking the difference between the melting temperatures determined for optical densities

observed at 280 nanometers and 260 nanometers.

The observation (42) that polylysine stabilizes DNA against melting more than polyarginine is not in conflict with the reports (1, 41) that lysine-rich histone is removed from DNA at a lower NaCl concentration than arginine-rich histone.

RNA-polylysine complexes show some similarities to the DNA-polylysine complexes. Sober et al. (48) formed soluble RNA-polylysine complexes with a nucleotide to lysine ratio of five. These complexes were treated with nuclease. This resulted in the formation of a precipitate which contained the complex with a lysine to nucleotide ratio of one. The supernatant contained mononucleotides and short oligonucleotides. The RNA part of the precipitate was examined to determine its chain length and G-C content. They found that the complexes contained nuclease-resistant RNA segments and these segments were the same chain length as the polylysine used in forming the complexes. Native RNA yielded a polylysine complex precipitate with a higher percent G-C content than the original RNA, whereas there was no change in base content in the precipitated complexes when heat denatured RNA was used. This specificity for producing complex fragments with a high G-C content can be explained by the polylysine forming complexes with preformed double stranded portions of the RNA. Since double strands are more likely to form in high G-C regions, this would explain the G-C specificity of the complex

formation.

Latt and Sober (28) studied the binding properties of oligolysines with poly (I+C) and poly (A+U) by equilibrium dialysis. Binding of a given oligolysine to poly (I+C) was generally greater than to poly (A+U). The binding increased linearly with oligolysine chain length.

In the third paper of their series of studies, Latt and Sober (29) described the effect of various cations on the binding of oligolysines to poly (A+U) and poly (I+C). They found that the effectiveness of the salts in dissociating the oligolysine-poly (A+U) complex was $Na^+ > Li^+ > NH_4^+$, $K^+ > (CH_3)_4N^+$, whereas for oligolysine-poly (I+C) it was $Na^+ > K^+ > NH_4^+ > Li^+ > (CH_3)_4N^+$. The binding constants were greater for the poly (I+C)-oligolysine complexes in the presence of any of the salts mentioned above.

In summary, the studies discussed in this section have shown that the DNA-polylysine complex has the following properties: (a) At low salt concentrations the complex is irreversible in the sense that there is no migration of the bound polylysine from one DNA site to another (54). (b) The complex has a definite stoichiometry of one lysine per one DNA phosphate (42, 54). This implies that a definite structural basis for the complex exists. (c) The polylysine stabilizes the DNA against melting (40, 42, 54). (d) The binding shows a degree of cooperativity (42, 54). (e) In the presence of NaCl, polylysine binds preferentially to A-T rich DNA, but the specificity is reversed

in tetramethylammonium chloride solution (30). (f) Polylysine preferentially stabilizes A-T rich regions of DNA (40, 42). (g) A degree of polymerization of eight or more is required for the oligolysine to achieve biphasic melting profiles characteristic of polylysine (43). (h) Complex formation produces turbid solutions (42).

II. POLARIZATION OF FLUORESCENCE

A. General Discussion

Polarization of fluorescence is a direct and sensitive indicator of the effective molecular volume, shape, and rigidity of macromolecules.

The polarization of fluorescence, p, is defined by $p = (I_V - I_H)/(I_V + I_H)$, where I_V and I_H are the fluorescent intensities observed for the emitted light polarized in the vertical and horizontal directions respectively. The excitation and emission beams are perpendicular to one another and are in the same horizontal plane.

For rigid ellipsoidal molecules with an axial ratio less than 10, and for either spheres or molecules for which the axes of absorption and emission are randomly oriented on the ellipsoid, Perrin (44) showed that, when the exciting light is polarized normal to the plane of the exciting and emitted beams,

$$\frac{1}{p} - \frac{1}{3} = (\frac{1}{p_o} - \frac{1}{3})(1 + \frac{3\tau}{\rho_h}).$$

p_o is the limiting polarization of the molecules when the solution is at infinite viscosity and ρ_h is the harmonic mean of the rotational relaxation times corresponding to the three principal axes of the ellipsoid:

$$\frac{1}{\rho_h} = \frac{1}{3}(\frac{1}{\rho_1} + \frac{1}{\rho_2} + \frac{1}{\rho_3}).$$

τ is the average lifetime of the excited state of the fluorescent molecules. The number of excited molecules is assumed to follow a simple exponential decay. For a sphere $\rho_h = \frac{3\eta V}{RT}$ where η is the viscosity of the solution, R is the gas constant, T is the absolute temperature, and V is the molar volume. From Perrin's equation we see that if $\frac{\tau}{\rho_h}$ is of the order of unity, then p will be a sensitive measure of changes in ρ_h and hence the unfolding, association, or dissociation of the molecules will be detected. p will also be sensitive to the internal degrees of freedom that may become active in the macromolecule when the temperature of the solution is increased.

For a sphere, Perrin's equation has the form

$$\frac{1}{p} - \frac{1}{3} = (\frac{1}{p_o} - \frac{1}{3})(1 + \frac{R\tau}{V}\frac{T}{\eta})$$

and a plot of $\frac{1}{p}$ or $(\frac{1}{p} - \frac{1}{3})$ versus $\frac{T}{\eta}$, which is known as a Perrin plot, yields a straight line.

It can be shown (21, 35, 61) that the Perrin plot does not deviate much from a straight line for oblate or short prolate ellipsoidal shaped molecules. Long prolate molecules show a curvature concave to the $\frac{T}{\eta}$ axis.

If the fluorescent dye attached to a protein molecule has some degree of internal rotation, the p for the system will be low in comparison to what it would be if the dye was rigidly attached to the protein. Increasing $\frac{T}{\eta}$ will allow the dye to increase its rate of internal

rotation until its effect in reducing p is saturated. The Perrin plot for this case is initially concave toward the $\frac{T}{\eta}$ axis with the curve leveling out as the saturation point is reached. At higher values of $\frac{T}{\eta}$, the Perrin plot is characteristic of the protein molecule (21, 61).

The rotational diffusion of a rigid molecule should be equally affected by equivalent changes in the temperature or in the viscosity of the medium. The Perrin plot should be independent of how $\frac{T}{\eta}$ is varied. However, if an increase in temperature activates a previously hindered degree of freedom, then the Perrin plot will deviate from what it would be for the same $\frac{T}{\eta}$ at lower temperatures. The plot will show a curvature convex to the $\frac{T}{\eta}$ axis in the region corresponding to temperatures where some of the internal rigidity of the macromolecule is lost.

Weber revived interest in the polarization of fluorescence method (59, 61) and utilized it to study the conformational changes of bovine serum albumin and ovalbumin with changes in pH (60). The protein molecules were labeled covalently with a fluorescent dye by reacting the protein with 1-dimethylaminonaphthalene-5-sulfonyl chloride (dansyl chloride).

Many investigators have since used the polarization of fluorescence technique to study a number of properties of proteins. These include studies on the association of enzyme subunits (9), the binding of coenzymes (11, 22), and the study of the binding sites of proteins

(63). Antibody-antigen binding has been investigated (13, 23) and the effect of inorganic ions on enzymes has been studied (27, 61). Chen (10) gives a recent review of the use of the polarization of fluorescence method to study proteins. He also discusses the correlation of the intensity and spectra of the fluorescence of the bound dyes with the structure of the protein. Steiner and Edelhoch (52) review much of the work done with fluorescent protein conjugates up to 1961.

The fluorescence of the naturally occurring aromatic amino acids in proteins has not yet been utilized to study the polarization behavior of the macromolecules. Lynn and Fasman (33) have recently reported on preliminary work with copolymers of glutamic acid and tryptophan. They noted that the copolymer showed a characteristic peak in the polarization spectrum for the helical form that disappears at a pH at which the random coil is present.

There have been a few instances of the use of polarization of fluorescence to study the rotatory diffusion of polynucleotides. Churchich (12) devised a method of covalently binding acriflavine to yeast s-RNA. $NaIO_4$ was used to oxidize the ribose moiety of the terminal ribonucleoside of s-RNA. Acriflavine would then react at the oxidized site. The polarization of fluorescence data yielded a straight line Perrin plot.

Miller and Steiner (39) have used acriflavine conjugates of poly-A prepared by covalently bonding acriflavine to the poly-A by the

method of Churchich (12). They found that they could use p to follow the helix-coil transition which occurs at about a pH equal to 5.8 in 0.1 molar KCl solution. They also found that the addition of poly-U to a solution of labeled poly-A resulted in a progressive increase in p with increasing mole fraction of poly-U. The maximum polarization was attained when the mole fraction of poly-U reached 0.67, which corresponded to the formation of a three stranded complex consisting of one strand of poly-A and two strands of poly-U.

Millar and MacKenzie (38) used acriflavine covalently conjugated to s-RNA to study the helix-coil transition. The axial ratio of s-RNA in the helical form was estimated to be about six or seven, which was in good agreement with the axial ratio estimated from sedimentation, viscosity, and molecular weight data. This indicates that in the helical form the translational and rotational kinetic units appear to be of similar size and that the polarization of the dye is characteristic of the s-RNA molecule. Millar, Cukier and Nirenberg (37) have used acriflavine labeled poly-U to study the thermal dissociation of ribosomal RNA-poly-U complexes.

Millar (36) investigated the effect of mercuric chloride and methyl mercury hydroxide on E. coli s-RNA which was conjugated with acriflavin. The thermal stability of the s-RNA was determined by finding the temperature above which the system showed a deviation from the straight-line Perrin plot. S-RNA in the presence of

mercuric chloride was found to be more stable than the s-RNA free from the salt. Methyl mercury hydroxide destabilized the s-RNA.

Anufrieva, Vol'kenshtein and Sheveleva (3) studied the binding of acridine orange to DNA by following the polarization of the acridine orange. They found that the complex gave a linear Perrin plot, but that at temperatures above 75° C, the polarization of fluorescence decreased sharply indicating a cooperative change in the DNA.

There has been surprisingly little use of the polarization of fluorescence method to study the interaction of proteins or polypeptides with polynucleotides. The only paper known to me is that of Steiner (51). He labeled bovine serum albumin and egg white lysozyme by reacting the proteins with dansyl chloride. Complexes of each of these dansyl-proteins were then formed with thymus DNA. It was noted that the lysozyme or albumin gave high polarization values when complexed with DNA. Increasing the ionic strength of the solution caused the polarization to decrease until finally the polarization reached a low limit characteristic of the free protein. At a pH where the albumin was negatively charged, there was no appreciable binding to the DNA. Even though the solution viscosity was high due to the presence of the DNA, the polarization of the protein-DNA system at high salt was equal to the polarization of the free protein in water when no DNA was present. This seems to indicate that the increase in solution viscosity due to the DNA was not influencing the rate of

Brownian rotation of the protein. From the polarization data, Steiner determined the mole fraction of lysozyme bound to the DNA as a function of ionic strength.

B. Specific Application to Dissociation

Consider a system of dye-labeled molecules, each molecule of which can be in just one of two states. In one state the labeled molecule is free in solution and in the other state the labeled molecule is complexed to another species of molecule in solution. An example would be a solution of dansyl polylysine and DNA. We assume that the solution would consist of a combination of free dansyl polylysine and dansyl polylysine bound to DNA.

If the completely complexed system can be dissociated by changing the solution environment then it becomes of interest to determine the fraction of the dye-labeled molecules that are bound, f, as a function of the parameter causing the dissociation. In the dansyl polylysine-DNA case, this parameter may be the salt concentration of the solution, c. Under the above assumption of just two possible states for each molecule an analysis of the polarization of fluorescence data will yield f.

Let p_1 be the polarization of the light emitted by the bound dye-labeled molecules and p_2 be the polarization of the light emitted by the free dye-labeled molecules. If p is the polarization observed for

the over all system then Weber (59) has shown that

$$u = u_1 \phi_1 + u_2 \phi_2 \tag{1}$$

where $\frac{1}{u_i} = \frac{1}{p_i} - \frac{1}{3}$ and ϕ_i is the fraction of the light emitted by the molecules in the ith state. The subscript 1 refers to the bound state and 2 refers to the free state.

Under the assumption of essentially only two states

$$\phi_1 + \phi_2 = 1 \tag{2}$$

Equations (1) and (2) then give

$$\phi_1 = \frac{u - u_2}{u_1 - u_2} \tag{3}$$

For dilute solutions the number of molecules of a certain species that fluoresce is proportional to $\epsilon q N$, where ϵ is the molar extinction coefficient, q is the quantum yield for the molecule and N is the number of molecules of that species present. Therefore,

$$\phi_1 = \frac{f \epsilon_1 q_1}{f \epsilon_1 q_1 + (1-f) \epsilon_2 q_2} \tag{4}$$

and solving this for f gives

$$f = \frac{\phi_1}{\phi_1 + (1 - \phi_1)\frac{\epsilon_1 q_1}{\epsilon_2 q_2}} \tag{5}$$

In general the molar extinction coefficients and the quantum yields may vary with c, the salt concentration, assuming that the added salt is the only agent responsible for the dissociation. It is

possible, but unlikely, that such variations in ϵ and q might be due to a direct interaction between salt and dye. What appears to be a more likely possibility, however, is that variations would be due to indirect effects. Thus, the conformation of a dye-labeled molecule could alter as the salt concentration changed which, in turn, would place the dye in a different environment, thereby changing the spectral properties of the dye. In the bound state the dye-labeled molecules will be subject to the constraints of the complex and hence the conformation of the bound dye-labeled molecules will be insensitive to salt concentration. We can then assume that $\epsilon_1 q_1$ is independent of salt concentration, while $\epsilon_2 q_2$ might vary with salt concentration.

For the general case, the intensity of fluorescence of the system as a function of c is

$$I(c) = K \left[\epsilon_1(c) q_1(c) f + \epsilon_2(c) q_2(c)(1 - f) \right] \qquad (6)$$

where K is a constant. The assumption that all the dye-labeled molecules are bound at very low salt concentration holds for some systems. Tsuboi, Matsuo and Ts'o (54) and Olins, Olins and von Hippel (42) have shown that at low salt concentration all the polylysine is complexed to the DNA; therefore under this assumption f = 1 when c = 0. Using this in equation (6) gives

$$I(c) = \frac{I(0)}{\epsilon_1(0) q_1(0)} \left[\epsilon_1(c) q_1(c) f + \epsilon_2(c) q_2(c)(1-f) \right]. \qquad (7)$$

Eliminating $\epsilon_2 q_2$ between equations (5) and (7) yields

$$f = \frac{I(c)}{I(0)} \quad \frac{\epsilon_1(0)q_1(0)}{\epsilon_1(c)q_1(c)} \quad \phi_1 . \tag{8}$$

With the assumption that $\epsilon_1 q_1$ is independent of c,

$$\frac{\epsilon_1(0)q_1(0)}{\epsilon_1(c)q_1(c)} = 1 \tag{9}$$

and therefore

$$f = \frac{I(c)}{I(0)} \quad \phi_1 . \tag{10}$$

Using equation (3) for ϕ_1 in equation (10) yields

$$f = \frac{I(c)}{I(0)} \quad \frac{u - u_2}{u_1 - u_2} . \tag{11}$$

Equation (11) permits the calculation of f from polarization and intensity of fluorescence data. For vertically polarized excitation, it can be shown (59) that the intensity of fluorescence is determined by $I = I_V + 2I_H$.

If the more stringent assumption is made that both $\epsilon_1 q_1$ and $\epsilon_2 q_2$ are independent of the salt concentration, then equation (5) yields

$$f = \frac{\phi_1}{\phi_1 + (1 - \phi_1)\frac{I(0)}{I(\infty)}}$$

$$f = 1 \Big/ \left[1 + \frac{I(0)}{I(\infty)} \quad \frac{u_1 - u}{u - u_2} \right] . \tag{12}$$

In this case it is necessary to measure the intensity at only two salt concentrations.

If both $\epsilon_1 q_1$ and $\epsilon_2 q_2$ are independent of c, it is also possible to compute f from intensity measurements alone. Under this assumption, equation (7) yields

$$f = \frac{I(c) - I(\infty)}{I(0) - I(\infty)}. \tag{13}$$

III. POLARIZATION OF FLUORESCENCE STUDIES OF THE DNA-POLYLYSINE INTERACTION

A. Subject of Study

This chapter reports on the effect that various agents have upon the complexing of DNA and polylysine. By using reasonable assumptions, it was shown in Section B of Chapter II that the fraction, f, of polylysine bound to DNA may be determined from emission measurements. f was calculated from measurements of the polarization of fluorescence of a dye conjugated to the polylysine. This methodology permits a relatively rapid determination of a parameter sensitive to complexing.

B. Preparation of DNA-Polylysine Complex

Poly-L-lysine was synthesized by the N-carboxyamino acid anhydride method (6). Thanks are due to Robert P. Cory and Raymond L. McKenzie for carrying out the syntheses. The polylysine was analyzed by Spencer L. Baird, Jr. for its molecular weight distribution by chromatography on a carboxymethyl cellulose column using an exponential sodium chloride gradient (53).

A variation of a well known method (60) was used to conjugate the dye to polylysine. A typical preparation was as follows. Three drops of saturated NaOH solution was added to 26 ml of polylysine

(1.93×10^{-2} molar in lysine) to bring the pH to 9.4. 9.0 mg of dansyl chloride (Aldrich Chemical Co.) was dissolved in 70 ml of acetone. 35 ml of the dansyl chloride solution was added dropwise to the polylysine. After 1.5 hours the acetone was removed by flash evaporation. Then the remaining 35 ml of the dansyl chloride solution was added dropwise to the polylysine and the mixture was permitted to stand for several hours and then the acetone was removed by flash evaporation. The solution was dialyzed extensively against water, and then passed through a Sephadex G-10 column.

The dansyl polylysine was complexed to DNA by gradient dialysis (26) in the following manner. The dansyl polylysine and DNA solutions were both made 2.6 molar in NaCl and then the dansyl polylysine was slowly added to the DNA solution with vigorous stirring. In 2.6 molar NaCl the dansyl polylysine and DNA are essentially uncomplexed. The mixture was dialyzed against successively lower concentrations of salt in cacodylate buffer. Details are indicated in Tables I, IV, V, VI and VII.

C. Salt Series for the Dissociation of the Salmon Sperm DNA-Polylysine Complex

The first complex used for the salt dissociation studies is designated as complex A. The composition and characteristics of the solutions used to prepare complex A are shown in Table I.

Table I. Data on Complex A

Before Dialysis	
Lysine to dansyl ratio in polylysine	21.7
Approximate degree of polymerization of polylysine	275.
Salmon sperm DNA(P) molarity	1.92×10^{-3}
Polylysine molarity in lysine	4.42×10^{-4}
Dansyl molarity	2.04×10^{-5}
DNA(P) to lysine ratio	4.35
NaCl molarity	2.6
After Dialysis	
Days of gradient dialysis at 6° C and pH = 5.5	40.
Molarity of sodium cacodylate buffer	0.10
Molarity of NaCl	0.050
Centrifugation (g)	30,000.
Minutes of centrifugation at 5° C	90.
Supernatant after Centrifugation (Complex A)	
Salmon sperm DNA(P) molarity	1.02×10^{-3}
Polylysine molarity in lysine	8.29×10^{-5}
DNA(P) to lysine ratio	12.3
Dansyl molarity	3.82×10^{-6}
Molarity of sodium cacodylate buffer	0.10
pH	5.5
NaCl molarity	0.050
Apparent absorbance of 1 cm at 500 nm	0.034

After the NaCl concentration in the gradient dialysis reached values less than about one molar, the solution in the dialysis bags became turbid and a visible precipitate formed. After completion of the dialysis, the precipitate was removed by centrifugation. The ratio of the concentration of DNA(P) in the supernatant to the total concentration of the DNA(P) before centrifugation was 0.53, whereas

the ratio of the concentration of polylysine in the supernatant to the total polylysine concentrate was only 0.19. The supernatant, which for convenience is designated as complex A, was used for subsequent dissociation measurements.

The concentration of the DNA (salmon sperm, Calbiochem) was determined from ultraviolet absorbance measurements.

The dansyl concentration in the complex was determined from fluorescence measurements in the following manner. NaCl was added to a sample of complex A to bring it to 2.1 molar NaCl. At this salt concentration essentially all of the polylysine is dissociated. The intensity of fluorescence of this sample was measured and then a series of known volumes and concentrations of dansyl polylysine in 2.1 molar NaCl was added to the sample and the intensity of fluorescence upon each addition was measured. An extrapolation to zero intensity of a plot of fluorescent intensity versus concentration of added dansyl yielded the concentration of dansyl in the original sample.

The polarization and intensity of fluorescence were measured using an instrument built by Spencer L. Baird, Jr. and Irvin Isenberg. The instrument is a continuously recording polarization spectrometer having features in common with those described by Weber and Bablouzian (62) and Deranleau (14). Light from an Osram, 450 watt, high pressure, xenon arc lamp passes through a Jarrell-

Ash 0.25 meter Ebert monochromator and then through a Glan-Thompson prism polarizer and an American Time Products 150 hertz tuning fork light chopper. This light excites the sample. The emission is detected by two EMI 9524B photomultipliers at right angles to the exciting beam on opposite sides of the sample. One photomultiplier senses light polarized parallel to the polarization of the vertically polarized exciting light. The intensity of this beam is designated as I_V. The other photomultiplier senses light polarized in the direction of the exciting beam and perpendicular to the polarization of the exciting beam. The intensity of this horizontally polarized light is called I_H. Filters before the photomultipliers block the passage of scattered exciting radiation.

The photomultiplier currents pass to the summing points of P25AU Philbrick operational amplifiers having 10^8 ohm feedback resistors. The output voltages are applied to Princeton Applied Research lock-in amplifiers. A variable gain amplifier is in the I_V branch of the circuit. $p = (I_V - I_H)/(I_V + I_H)$ is computed by an analog computer using a Leeds and Northrup 444185 Multiplier-Divider printed-circuit card. The intensity of fluorescence, $I_V + 2I_H$, is computed with the use of a Philbrick P85AU operational amplifier and a standard adding circuit. In practice, the polarizer in the exciting beam is first set for horizontally polarized light. With this setting $I_V = I_H$, and the polarization of fluorescence should

be equal to zero. The variable gain amplifier is adjusted, therefore, so that the computer gives $p = 0$. This adjustment compensates for mismatched sensitivities of the photomultipliers and the associated circuitry. It is found that if $p = 0$ is set for one excitation wavelength, the instrument reads $p = 0$ for all exciting wavelengths down to 270 nm.

After adjustment of the variable gain amplifier, the Glan prism in the excitation beam is set for vertically polarized light. Using rhodamine B as a test substance, the instrument yields polarization values close to those reported by Weber (58) and Deranleau (14), with a difference of about $\delta p = 0.002$.

The samples were turbid and had weak emission. Consequently, the stray light from the monochromator was not negligibly small compared to the intensity of the fluorescence. Without precautions, this stray light would have raised the measured p above the actual p. The possibility of this artifact was eliminated by the use of a Corning 7-51 filter in the exciting beam. This use of complementary filters has been described by Weber (59). An alternate method of reducing the stray light was also used. In this method, a double monochromator was placed in the exciting beam. Possible artifacts due to scattering of the emitted beam were checked by comparing results using samples in cuvettes of varying length. The polarization of the samples was the same, to three significant figures,

whether measured by using 1 cm x 1 cm cuvettes or 2 mm x 2 mm cuvettes.

The salt dissociation runs were carried out by adding dry salt (reagent grade, J. T. Baker Chemical Co.) to a sample in a graduated test tube. The solution was mixed on a cyclomixer. To eliminate bubbles the solution was then centrifuged for several minutes at the highest speed of an International Equipment Co. Model CL clinical centrifuge. The mass of the added salt was measured and the volume of the solution before and after the addition of salt was noted. The apparent absorbance, A, of the turbid solution was measured, with water as a reference, in a Cary Model 14 spectrophotometer at 500 nm. The polarization and intensity of fluorescence were then measured on the instrument described above. The temperature of the cell compartment of the fluorometer was 25.0 ± 0.2^{o} C for all the measurements reported in this thesis. Corrections were made for dilution of the sample by the added salt and for possible slow drifts of the lamp intensity in the fluorometer. The dansyl in complex A was excited at 355 nm which was near the wavelength yielding maximum emission. The polarization of fluorescence was about 0.290 for the complex with no salt added. This value dropped upon the addition of salt (Figure 1) reaching a final value of about 0.200 for most salts. Dissociation by $NaClO_4$ and NaCNS resulted in final p values near 0.225.

The fraction of the dansyl polylysine that is bound to the DNA, f, was calculated by three fundamentally different methods. The first method of calculation used equation (11); the second method used equation (13). The third method calculated f from

$$f = \frac{A(c) - A(\infty)}{A(0) - A(\infty)}. \tag{14}$$

For equation (14) to be valid it must be assumed that the complexed state and the dissociated state each have a characteristic apparent absorbance or turbidity due to light scattering.

Figures 2-10 show f versus salt concentration for a number of salts with monovalent cations. f is shown as calculated by the three methods. There is close agreement among the calculated values of f.

For salts containing monovalent cations, the turbidity appeared to decrease monotonically with increasing salt concentration. As shown in Figure 12, the addition of $MgCl_2$ caused the turbidity to show an initial rise followed by a subsequent decline. Figure 11 shows that p for $MgCl_2$ monotonically decreased. Low concentrations of Mn^{++} and Cu^{++} precipitated the complex.

Figure 13 shows the variation of intensity of fluorescence for dissociation of complex A by LiCl. All of the salts showed the qualitative shape of the LiCl curve for complex A. As will be seen, a small initial rise in intensity is observed for another type of complex, termed complex F.

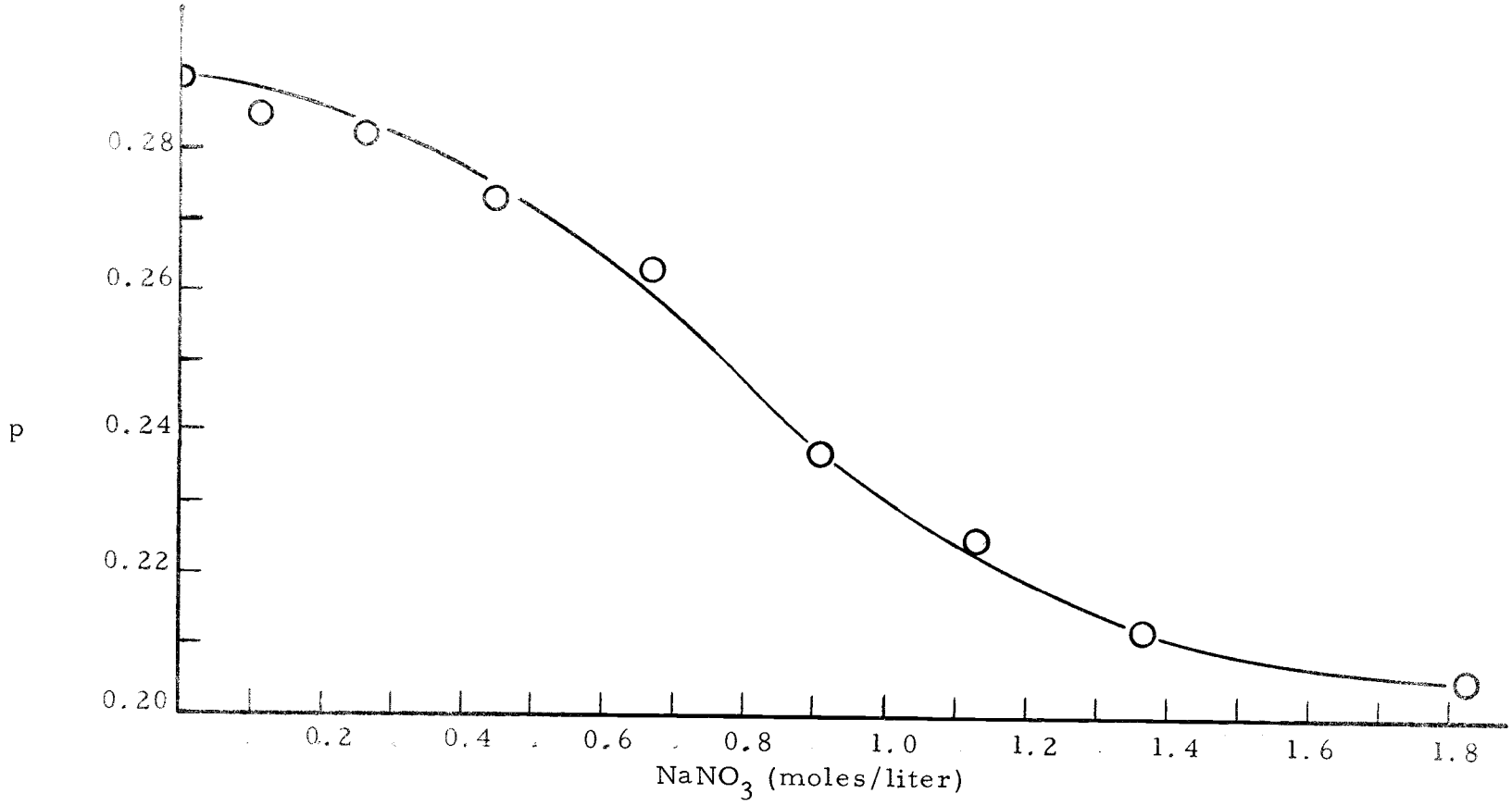

Figure 1. Effect of $NaNO_3$ concentration on the polarization of fluorescence of complex A.

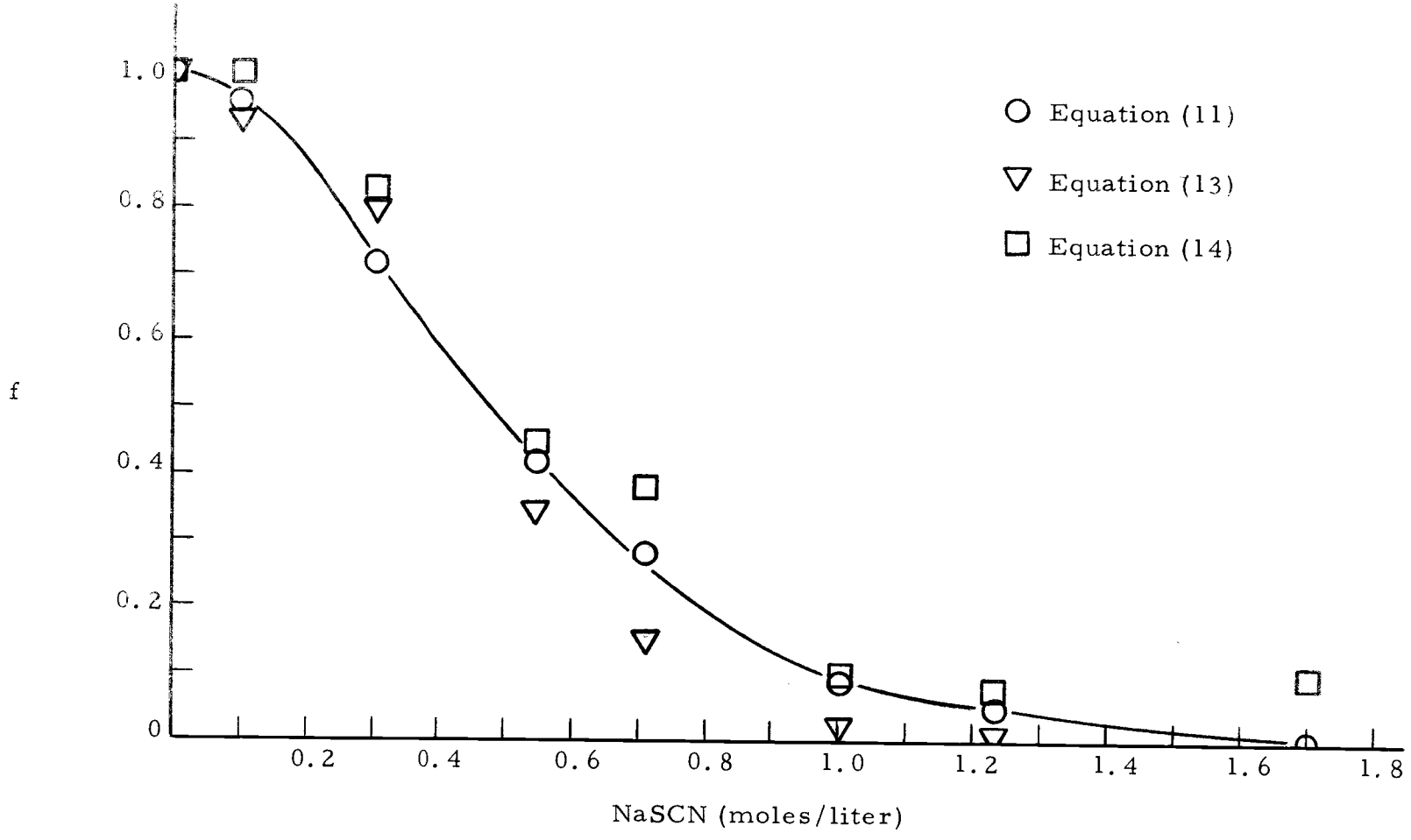

Figure 2. NaSCN dissociation of complex A.

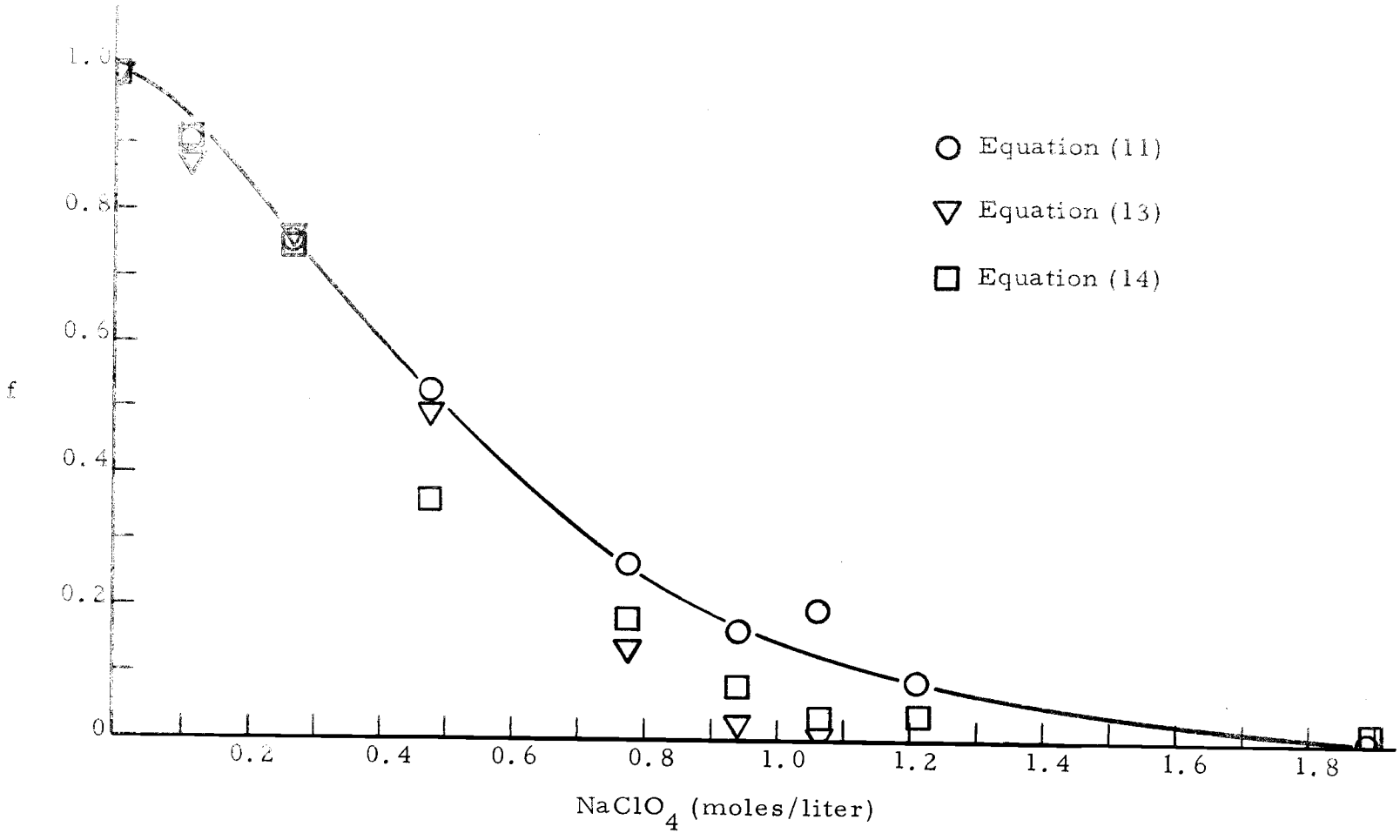

Figure 3. $NaClO_4$ dissociation of complex A.

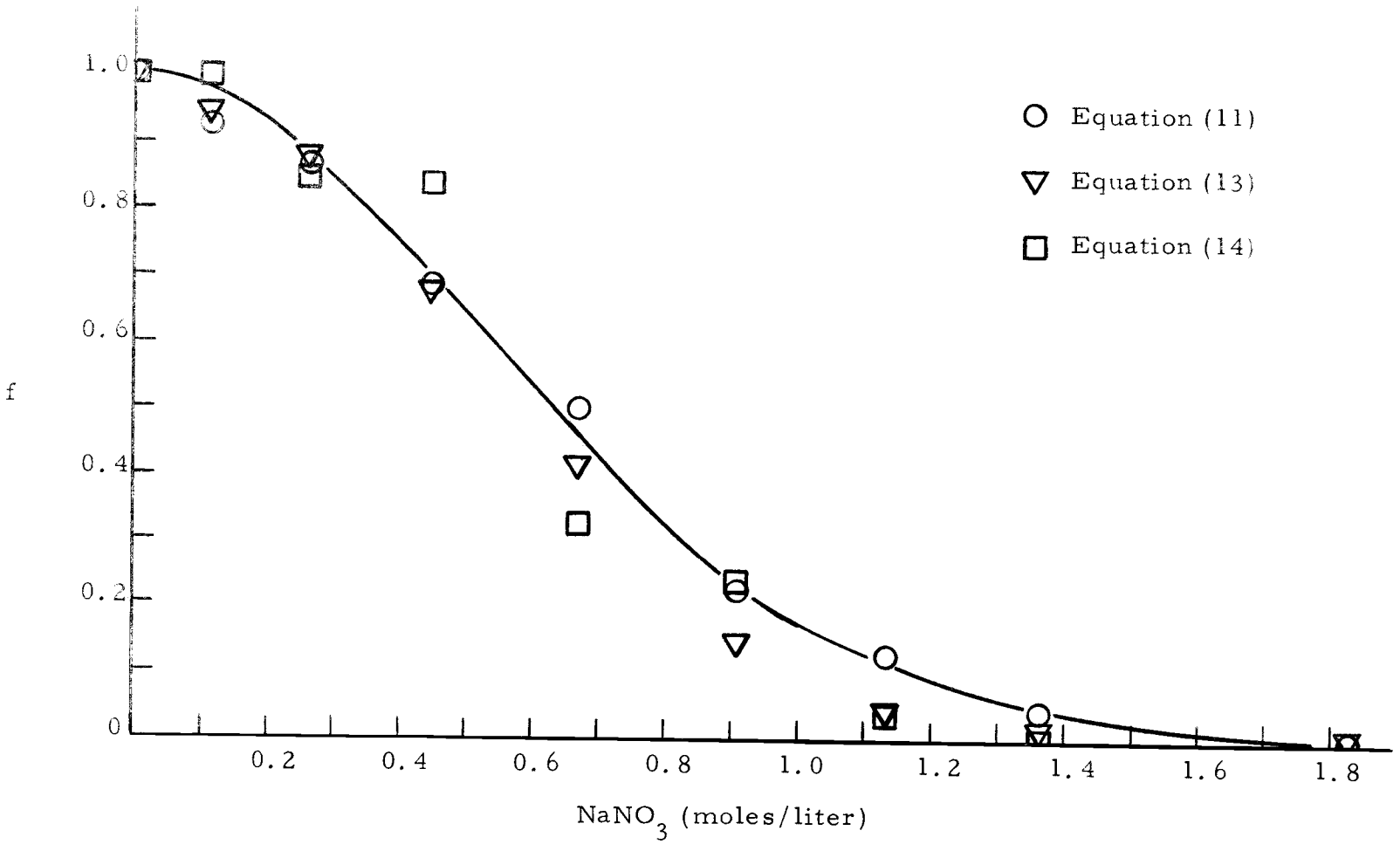

Figure 4. $NaNO_3$ dissociation of complex A.

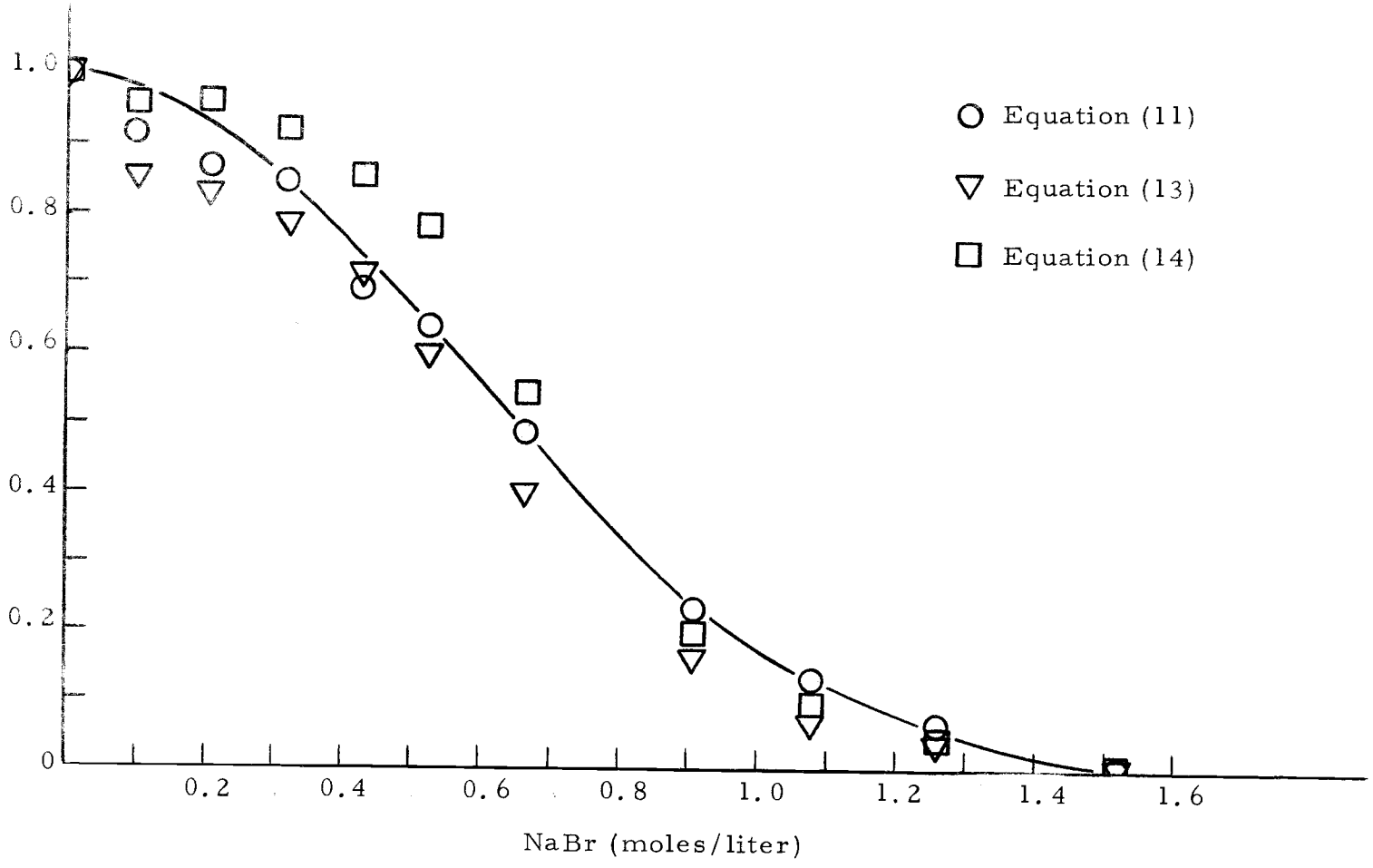

Figure 5. NaBr dissociation of complex A.

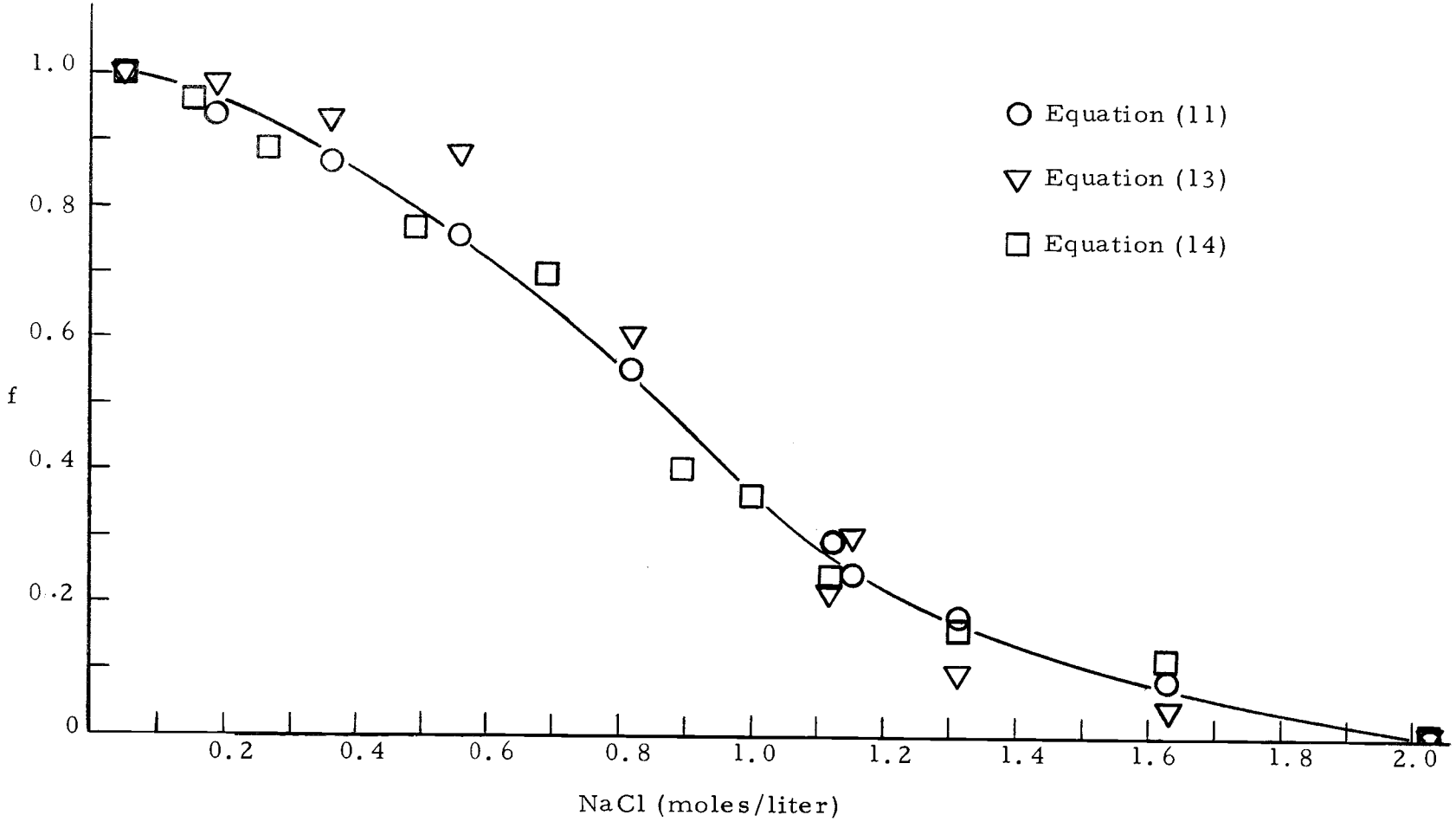

Figure 6. NaCl dissociation of complex A.

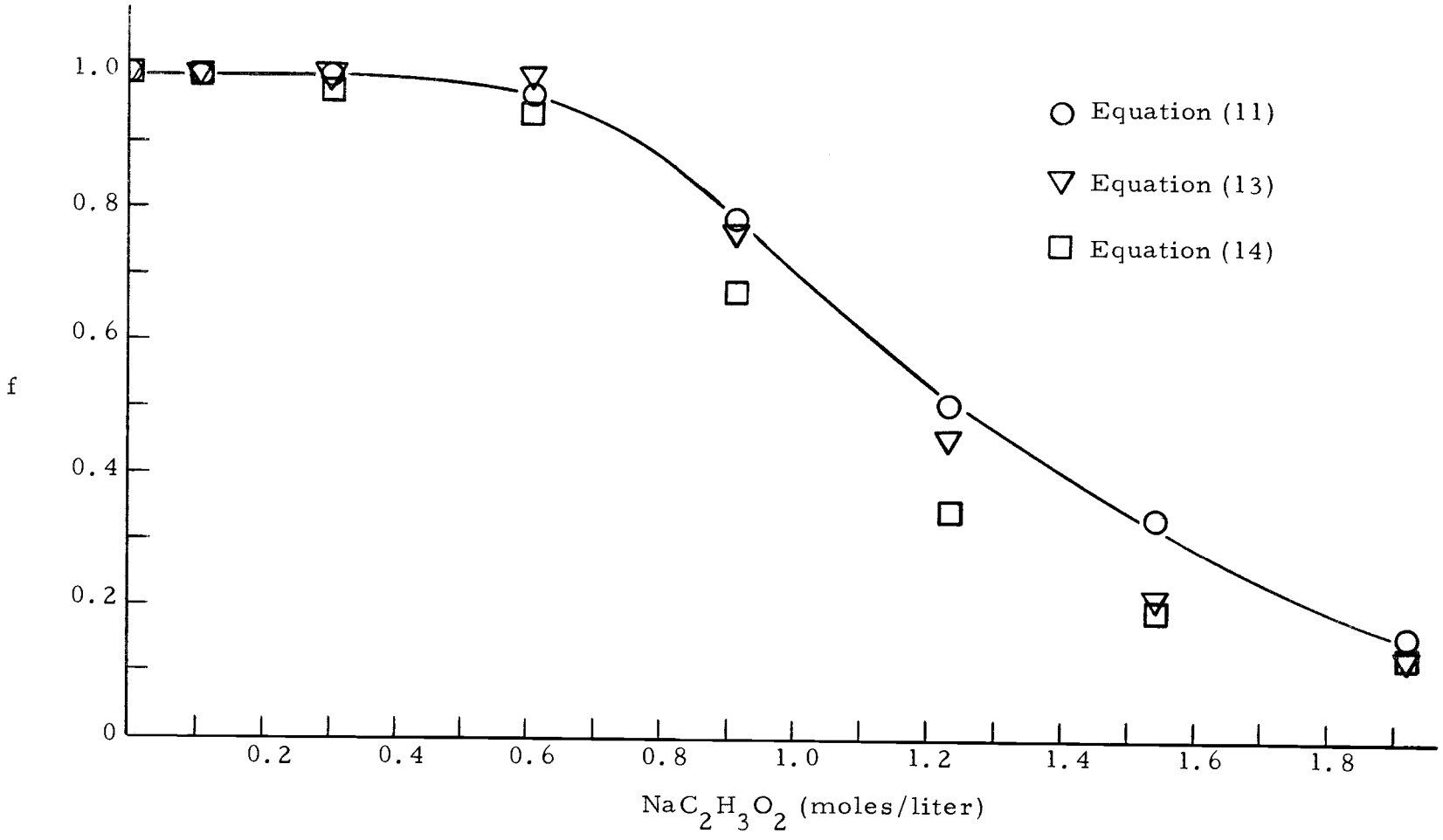

Figure 7. $NaC_2H_3O_2$ dissociation of complex A.

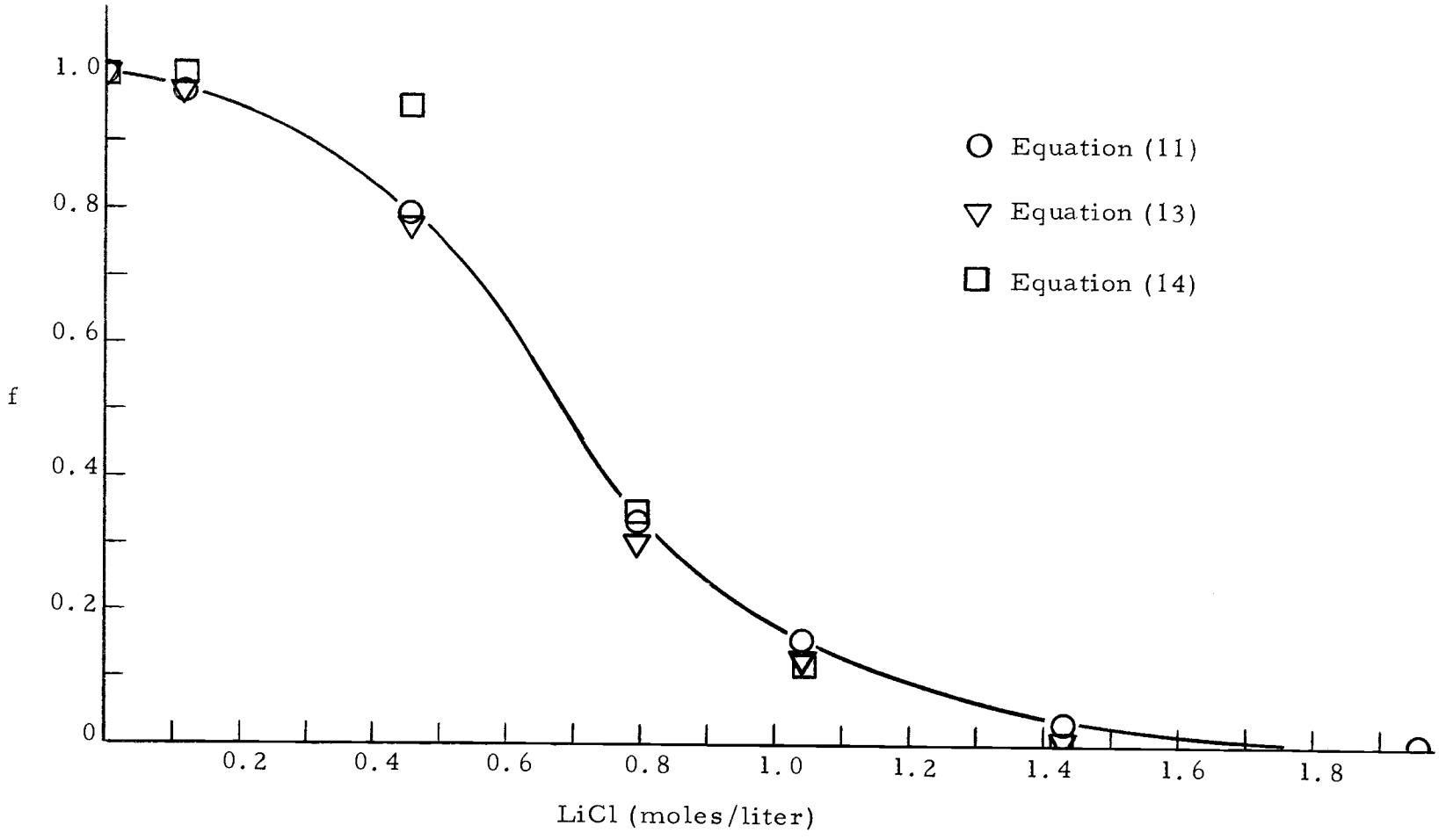

Figure 8. LiCl dissociation of complex A.

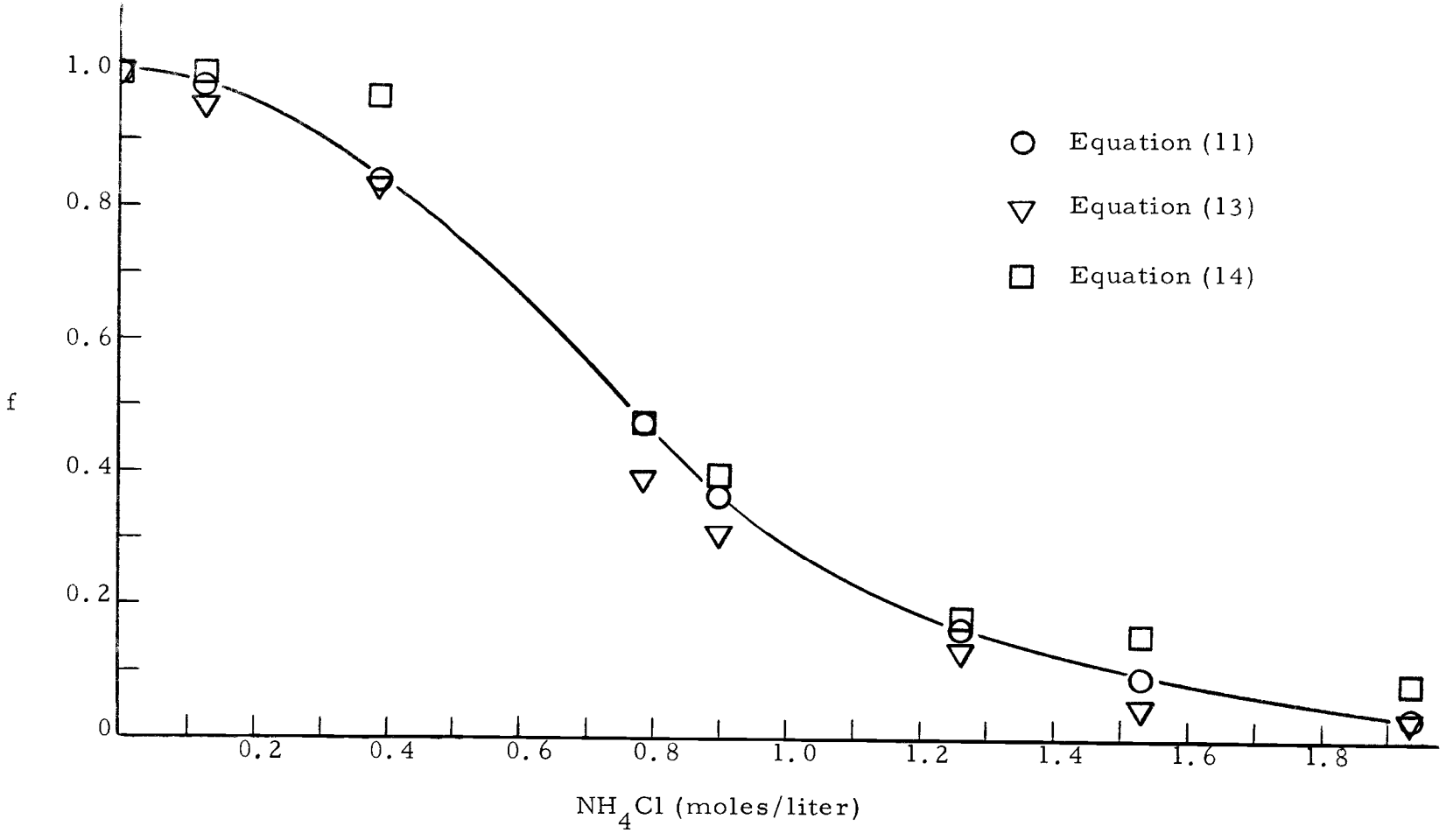

Figure 9. NH_4Cl dissociation of complex A.

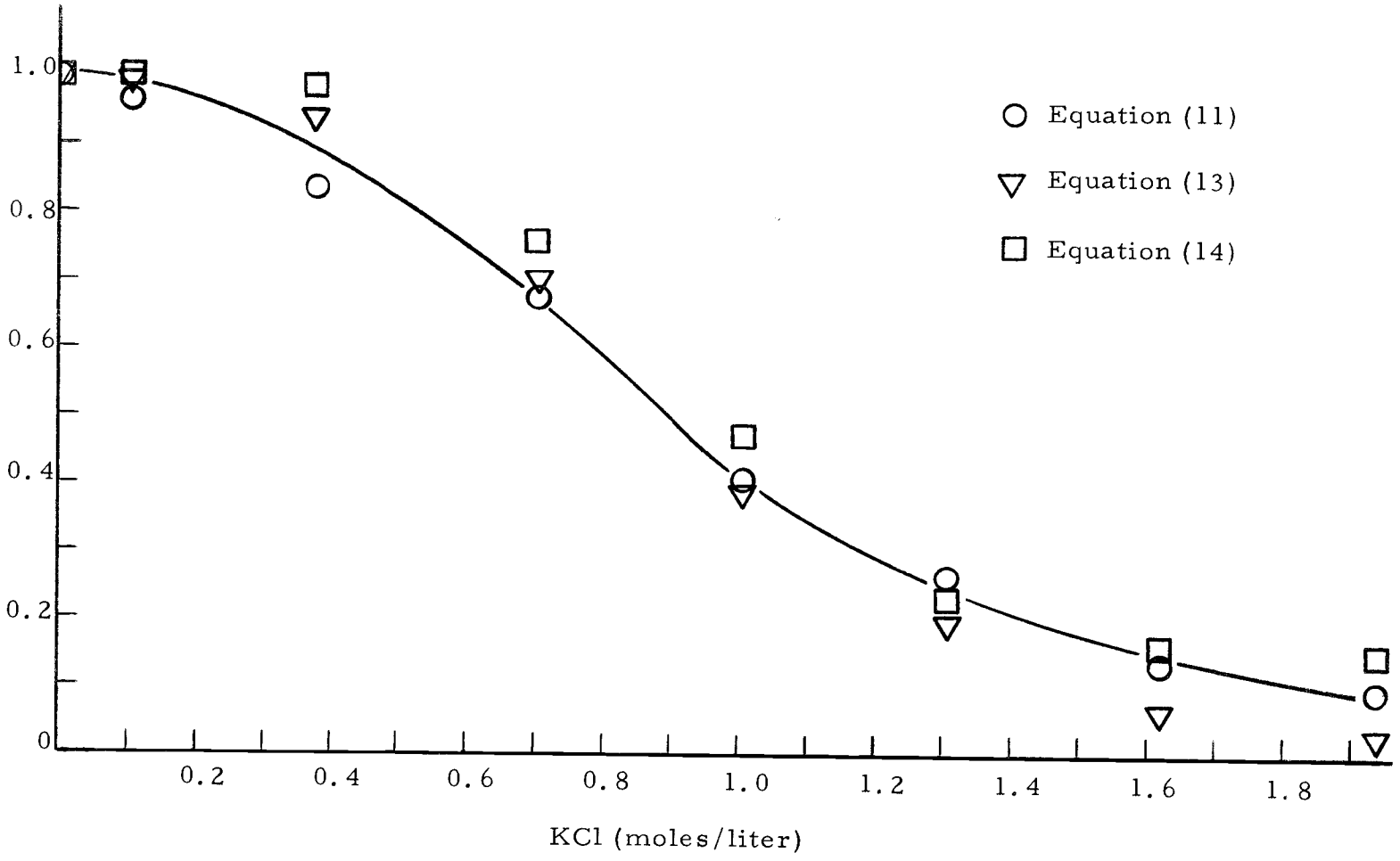

Figure 10. KCl dissociation of complex A.

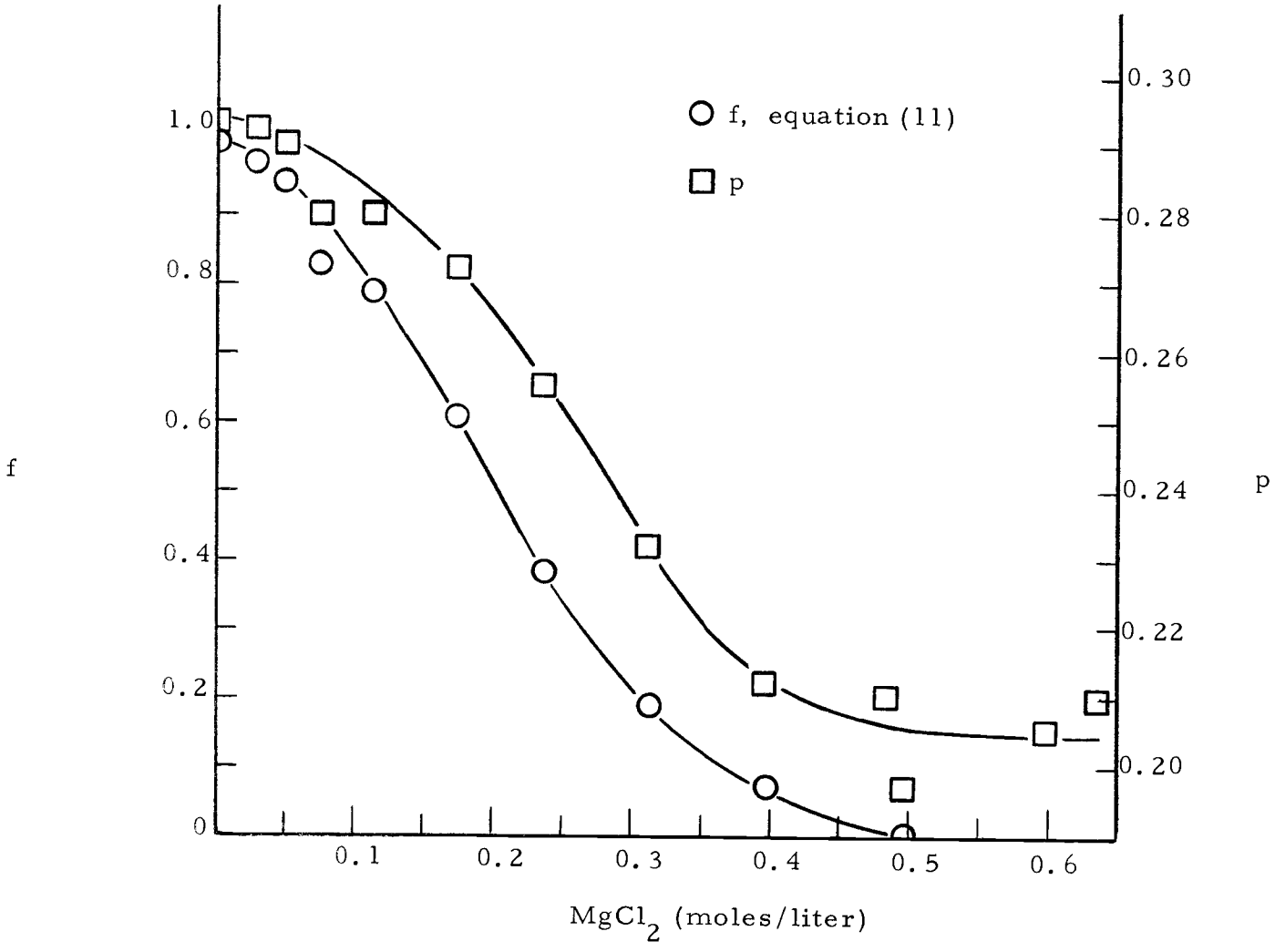

Figure 11. $MgCl_2$ dissociation of complex A.

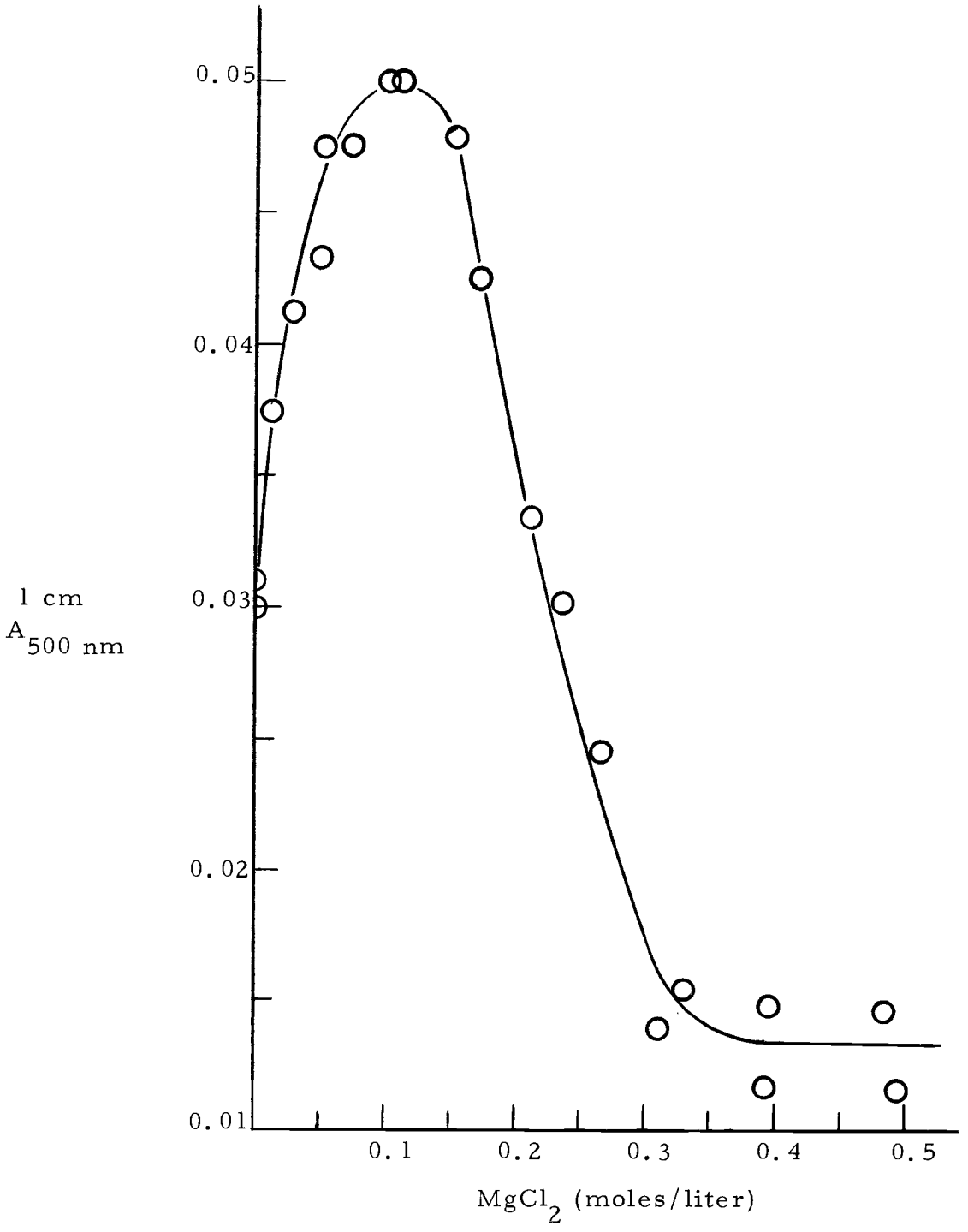

Figure 12. Effect of $MgCl_2$ concentration on the apparent absorbance of complex A.

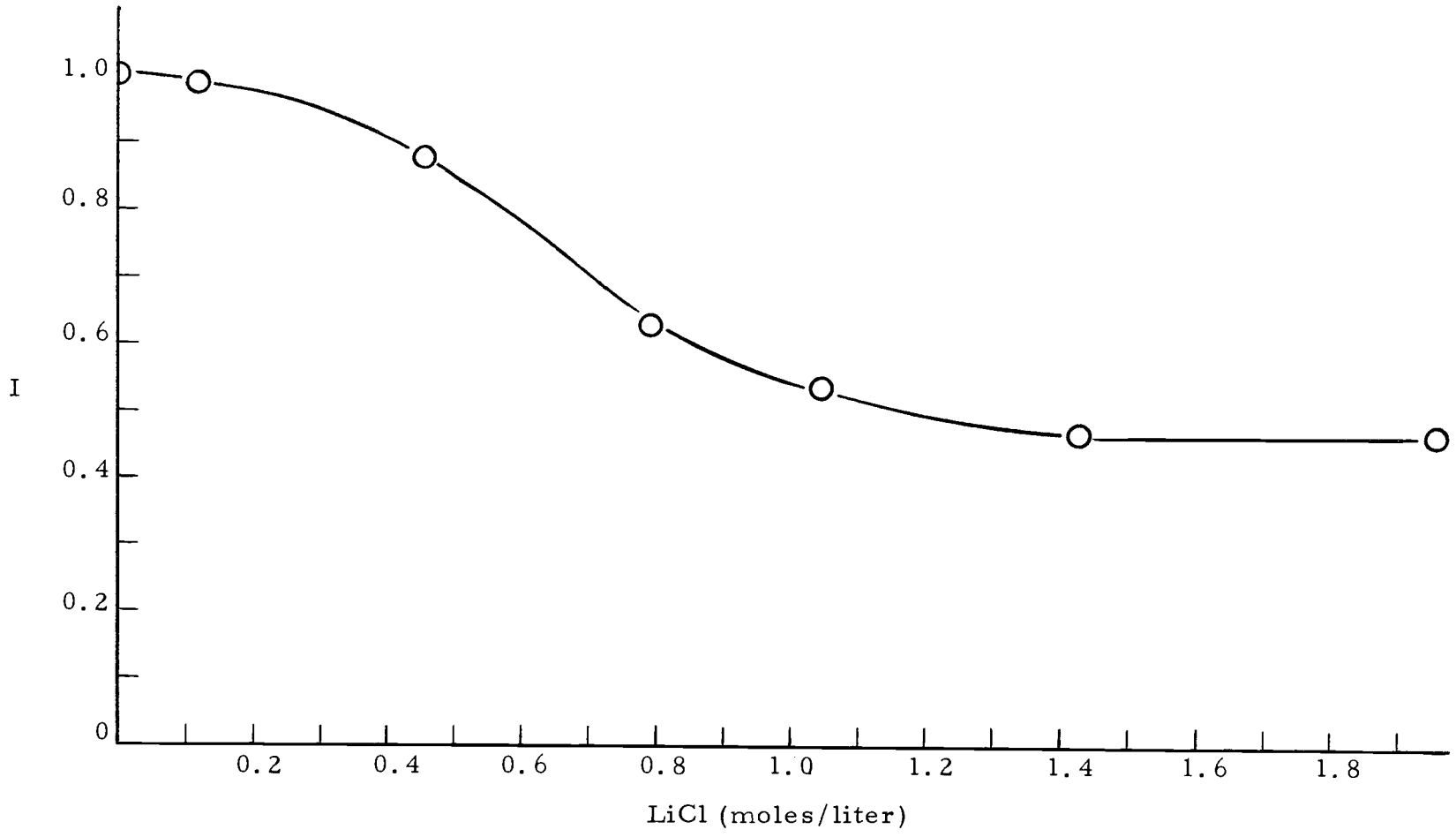

Figure 13. Effect of LiCl concentration on the intensity of fluorescence of complex A.

Table II gives a comparison of the salt concentrations for f = 0.50 as determined by the three methods for a variety of salts. The concentration of sodium acetate needed for f = 0.50 is seen to show a wide variance among the three methods. This was easily shown to be an artifact due to the presence of fluorescent impurities in the sodium acetate.

Table II. Salt Concentrations Yielding f = 0.50 for Complex A

Salt	Salt molarity at f = 0.50		
	Using polarization (equation 11)	Using turbidity (equation 13)	Using intensity (equation 14)
NaSCN	0.47	0.51	0.47
$NaClO_4$	0.50	0.42	0.46
$NaNO_3$	0.64	0.61	0.61
NaBr	0.65	0.68	0.60
NaCl	0.86	0.83	0.88
$NaC_2H_3O_2$	1.24	1.06	1.18
$MgCl_2$	0.20	--	0.19
LiCl	0.69	0.71	0.67
NH_4Cl	0.76	0.77	0.72
KCl	0.89	0.97	0.89

Table III shows the order of ion effectiveness in dissociating the complex. For comparison, the order of effectiveness for conformational changes of a number of other systems is also shown. The relative effectiveness of ions in destabilizing the native form of collagen, ribonuclease, DNA, and myosin is taken from von Hippel and Wong (56). The effect of ions on the binding of oligolysine to poly (A+U) and poly (I+C) is from Latt and Sober (28).

Table III. Relative Effectiveness of Ions

⟵ Helix	Coil ⟶
⟵ Complex	Dissociate ⟶

Collagen-Gelatin

$SO_4^{=} < CH_3COO^- < Cl^- < Br^- < NO_3^- < ClO_4^- < I^- < SCN^-$

$(CH_3)_4N^+ < NH_4^+ < Rb^+, K^+, Na^+, Cs^+ < Li^+ < Mg^{++} < Ca^{++} < Ba^{++}$

$(CH_3)_4N^+ < (C_2H_5)_4N^+ << (C_3H_7)_4N^+, (C_4H_9)_4N^+$

Ribonuclease

$SO_4^{=} < CH_3COO^- < Cl^- < Br^- < ClO_4^- < SCN^-$

$(CH_3)_4N^+, NH_4^+, K^+, Na^+ < Li^+ < Ca^{++}$

$(CH_3)_4N^+ < (C_2H_5)_4N^+ < (C_3H_7)_4N^+ < (C_4H_9)_4N^+$

DNA

$Cl^-, Br^- < CH_3COO^- < I^- < ClO_4^- < SCN^-$

$(CH_3)_4N^+ < K^+ < Na^+ < Li^+$

Myosin

$Cl^- < Br^- < I^- < SCN^-$

$K^+ < Li^+$

Oligolysine-Poly (A+U)

$(CH_3)_4N^+ < K^+, NH_4^+ < Li^+ < Na^+$

Oligolysine-Poly (I+C)

$(CH_3)_4N^+ < Li^+ < NH_4^+ < K^+ < Na^+$

Polylysine-DNA

$CH_3COO^- < Cl^- < Br^- < NO_3^- < ClO_4^-, SCN^-$

$K^+ < Na^+, NH_4^+ < Li^+ < Mg^{++}$

It is important to see if the presence of the dye tag appreciably affected the binding of polylysine to DNA. The close similarity between f values calculated from polarization data using equation (11) and from turbidity data using equation (14) permitted an evaluation of this question. To this end, complex B was prepared in a manner similar to complex A, except that untagged polylysine was used instead of dansyl polylysine. Table IV shows the data on the preparation of complex B. Note that the polylysine degree of polymerization is 140 for complex B, whereas it was 275 in complex A. So for close comparison with the dissociation of complex B another dansyl polylysine complex with DNA was prepared. Complex C was prepared from polylysine with the same degree of polymerization as the polylysine used in complex B. Table V gives data for complex C.

Figure 14 shows that the dissociation of the complex with dansyl polylysine is essentially the same as the dissociation of the complex with the untagged polylysine.

A comparison of the dissociation curve of complex A with that of complex C is shown in Figure 15. The dissociation of complex E, which has polylysine with the same degree of polymerization as the polylysine in complex C, is also shown in Figure 15. The difference between the curves may be due to the difference in the degree of polymerization of the polylysine. However, a systematic study

Table IV. Data on Complex B

Before Dialysis	
Dansyl molarity	0.00
Approximate degree of polymerization of polylysine	140.
Salmon sperm DNA(P) molarity	1.94×10^{-3}
Polylysine molarity in lysine	3.91×10^{-4}
DNA(P) to lysine ratio	4.96
NaCl molarity	2.6
After Dialysis	
Days of gradient dialysis at 6°C and pH = 5.5	29.
Molarity of sodium cacodylate buffer	0.10
Molarity of NaCl	0.050
Centrifugation (g)	30,000.
Minutes of centrifugation at 5°C	90.
Supernatant after Centrifugation (Complex B)	
Salmon sperm DNA(P) molarity	1.19×10^{-3}
Molarity of sodium cacodylate buffer	0.10
pH	5.5
NaCl molarity	0.050
Apparent absorbance of 1 cm at 500 nm	.02

has not yet been made and a rigorous demonstration of the possible effect of polylysine chain length on the dissociating properties of the complex must await further investigation.

Figure 16 shows the polarization and intensity of fluorescence as a function of NaCl concentration for dansyl polylysine. The dansyl was excited at 355 nm. The lysine to dansyl ratio was 21.7 and the dansyl concentration was 10^{-5} molar. The degree of polymerization of the polylysine was about 275. The pH ranged from 5.7 at zero salt to 5.4 at the final NaCl concentration of 3.10 molar. It is

Table V. Data on Complex C

Before Dialysis	
Lysine to dansyl ratio in polylysine	13.9
Approximate degree of polymerization of polylysine	140.
Salmon sperm DNA(P) molarity	1.87×10^{-3}
Polylysine molarity in lysine	4.31×10^{-4}
DNA(P) to lysine ratio	4.34
NaCl molarity	2.6
After Dialysis	
Days of gradient dialysis at 6°C and pH = 5.5	19.
Molarity of sodium cacodylate buffer	0.10
Molarity of NaCl	0.050
Centrifugation (g)	30,000.
Minutes of centrifugation at 5°C	90.
Supernatant after Centrifugation (Complex C)	
Salmon sperm DNA(P) molarity	7.92×10^{-4}
Polylysine molarity in lysine	3.94×10^{-5}
DNA(P) to lysine ratio	20.1
Dansyl molarity	2.84×10^{-6}
Molarity of sodium cacodylate buffer	0.10
pH	5.5
NaCl molarity	0.050
Apparent absorbance of 1 cm at 500 nm	0.006

evident that the changes in p shown in Figure 16 do not correspond to the p changes in the DNA-polylysine salt titration and so we need not consider the intrinsic polarization change of dansyl with salt concentration as an artifact in the p decrease measured on the dissociation of the complex.

A comparison of Figure 1 with Figure 16 reveals that at 2.0 molar NaCl the polarization of the dansyl in the dissociated complex is considerably higher than the polarization observed in the dansyl

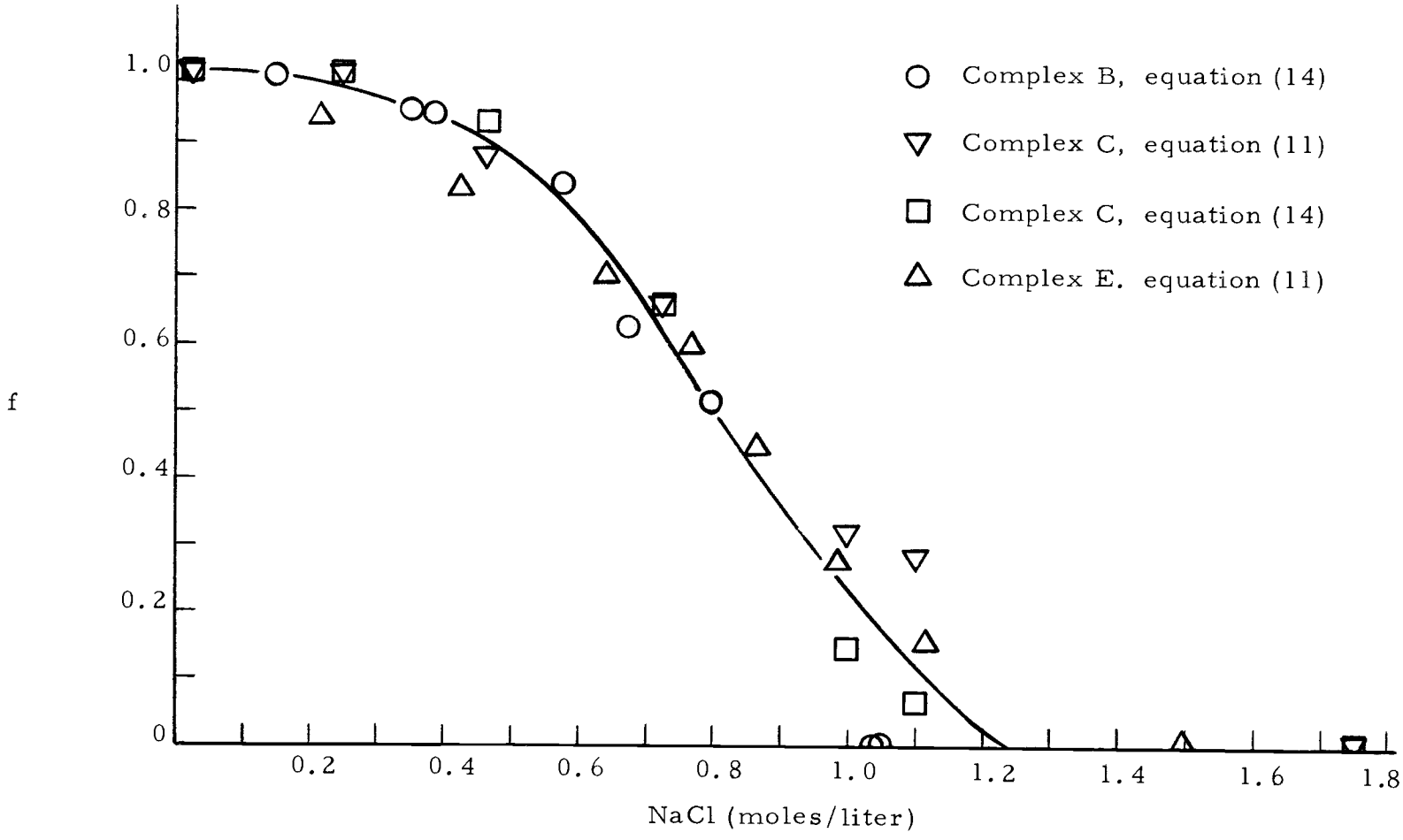

Figure 14. Comparison of the NaCl dissociation of complex B with the dissociation of complexes C and E.

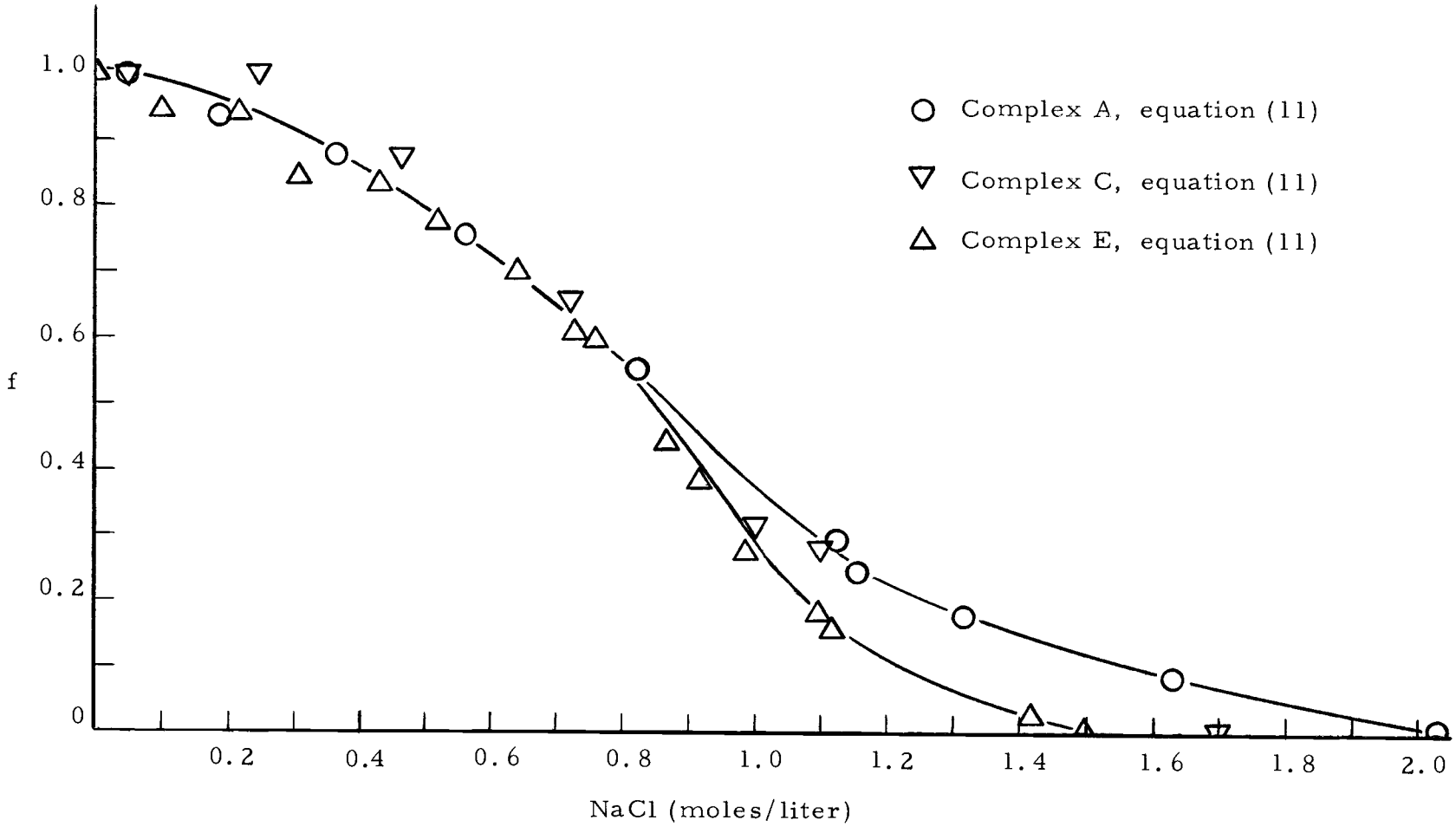

Figure 15. Comparison of the NaCl dissociation of complex A with the dissociation of complexes C and E.

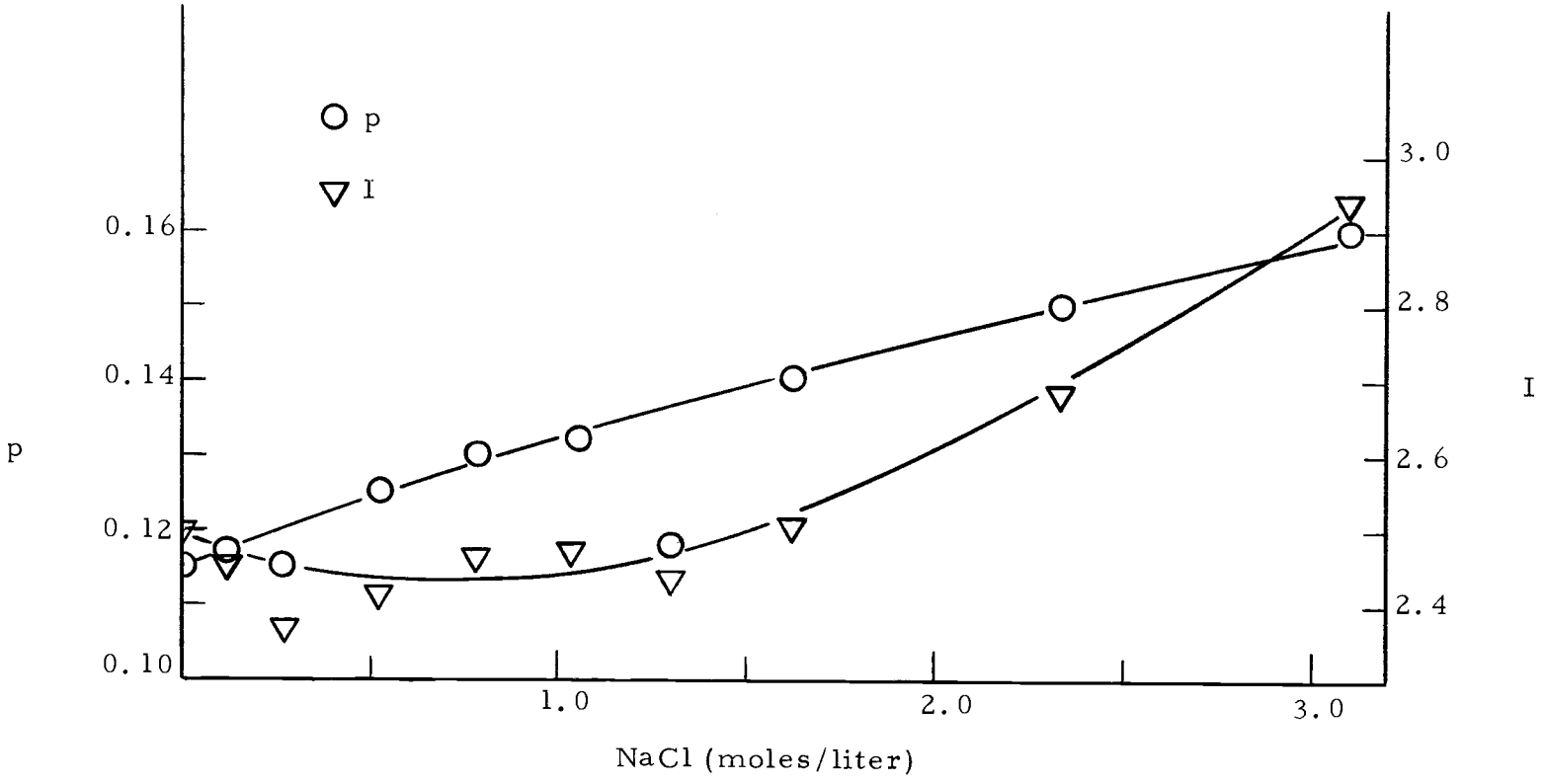

Figure 16. Effect of NaCl concentration on the polarization and intensity of fluorescence of dansyl polylysine.

polylysine with no DNA. It appears that the presence of DNA restricts the freedom of rotation of the dissociated dansyl polylysine.

D. Dissociation of the Micrococcus lysodeikticus DNA-Polylysine Complex

The specificity of polylysine for A-T rich DNA (30, 42) suggests that complexes of polylysine with Micrococcus lysodeikticus DNA, which is 28 percent A-T, might dissociate at different salt concentrations than the 58.8 percent A-T salmon sperm DNA complexes.

Table VI shows the data on the Micrococcus lysodeikticus complex, designated complex D.

Table VI. Data on Complex D

Before Dialysis	
Lysine to dansyl ratio in polylysine	13.9
Approximate degree of polymerization of polylysine	140.
M. lysodeikticus DNA(P) molarity	1.43×10^{-3}
Polylysine molarity in lysine	3.16×10^{-4}
DNA(P) to lysine ratio	4.52
NaCl molarity	2.6
After Dialysis	
Days of gradient dialysis at 6°C and pH = 6.5	15.
Molarity of sodium cacodylate buffer	0.050
Centrifugation (g)	30,000.
Minutes of centrifugation at 5°C	90.
Supernatant after Centrifugation (Complex D)	
M. lysodeikticus DNA(P) molarity	5.35×10^{-4}
Molarity of sodium cacodylate buffer	0.050
pH	6.5
NaCl molarity	0.0011
Apparent absorbance of 1 cm at 500 nm	0.005

Complex E was also prepared. It was a salmon sperm DNA-dansyl polylysine complex identical to complex C except that the gradient dialysis was carried to a NaCl concentration of 0.0011 molar and the final sodium cacodylate buffer concentration was 0.050 molar. The data of Table V applies to complex E except for the differences mentioned above.

Figures 17 and 18 show the comparison of the dissociation of complex D with the dissociation of complex E. The difference between the two is small enough to be within the experimental error.

E. Effect of pH on the DNA-Polylysine Complex

The effect of pH on the complex was determined by titrating the solutions with NaOH and measuring p, A, and I. The measurements were taken on complex E and on a complex prepared by following the procedure of Olins, Olins and von Hippel (42). Their method used lower concentrations of DNA and a faster dialysis. Very little precipitate formed in comparison to the previously discussed complexes. Table VII gives the data for this complex. As before, the complex used for measurements was the supernatant formed after centrifugation of the final dialysis mixture. This supernatant is called complex F.

Figures 19 and 20 show the results of the pH titration. Figure 19 is a composite of several different series of measurements.

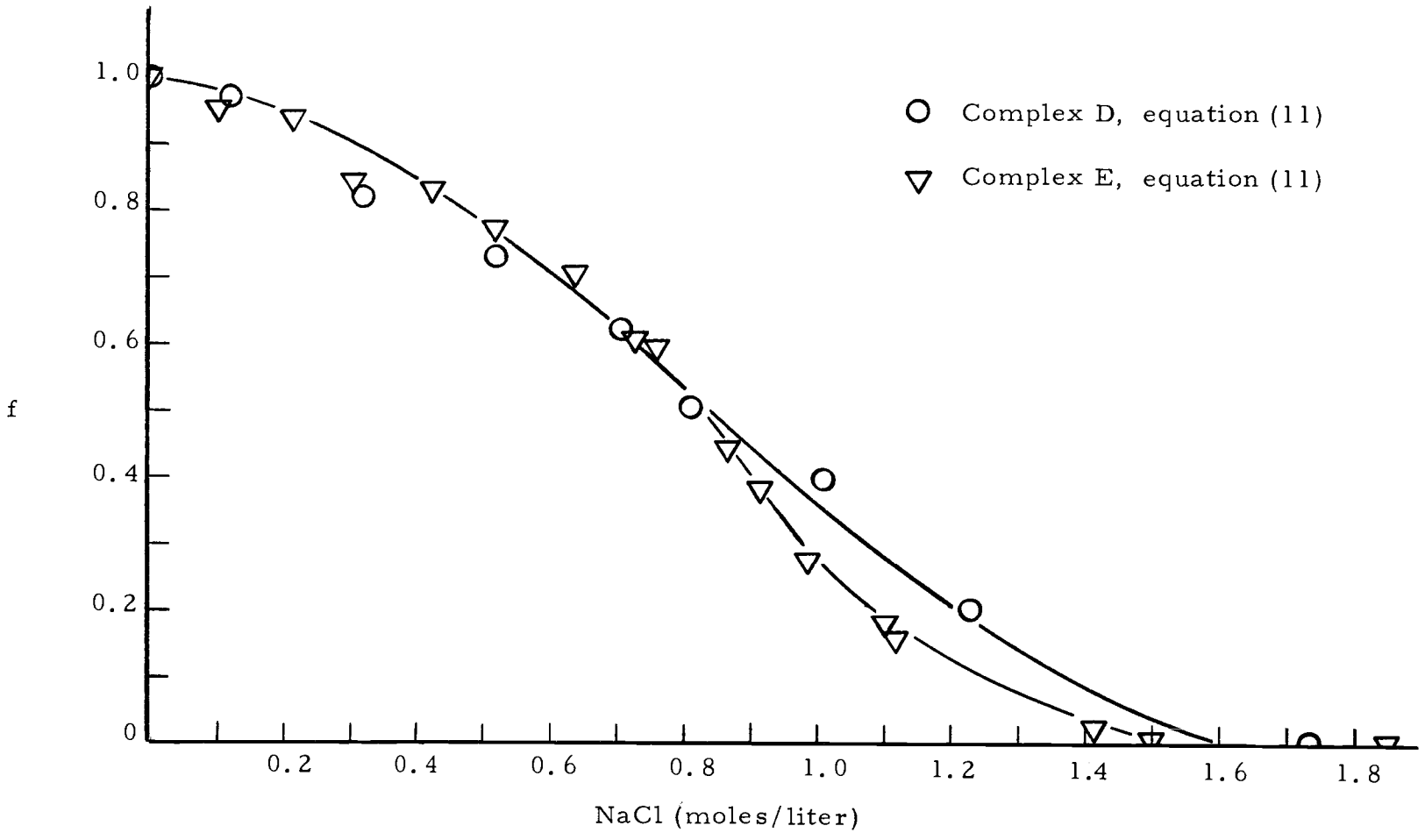

Figure 17. Comparison of the NaCl dissociation of complex D with the dissociation of complex E.

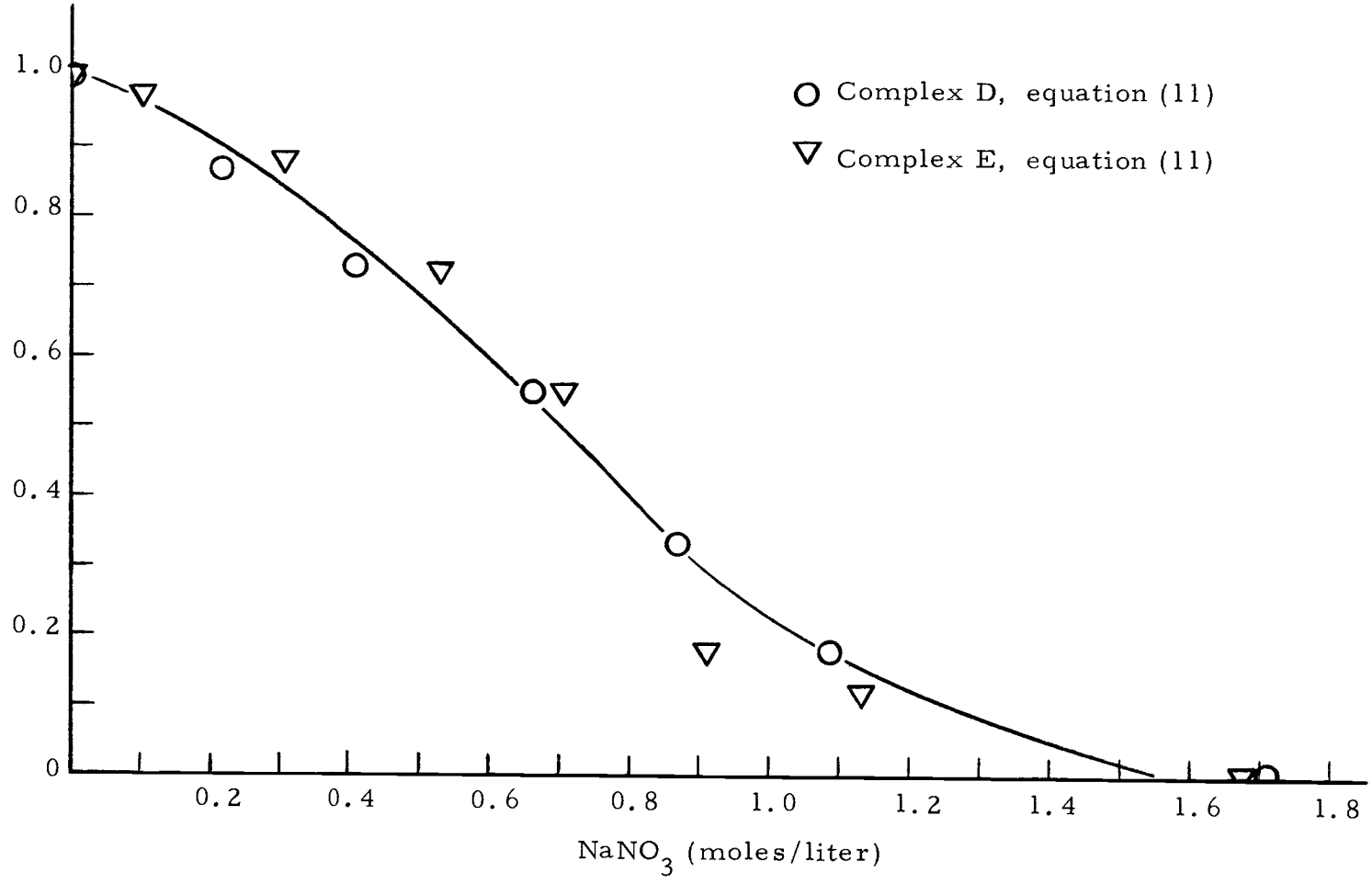

Figure 18. Comparison of the $NaNO_3$ dissociation of complex D with the dissociation of complex E.

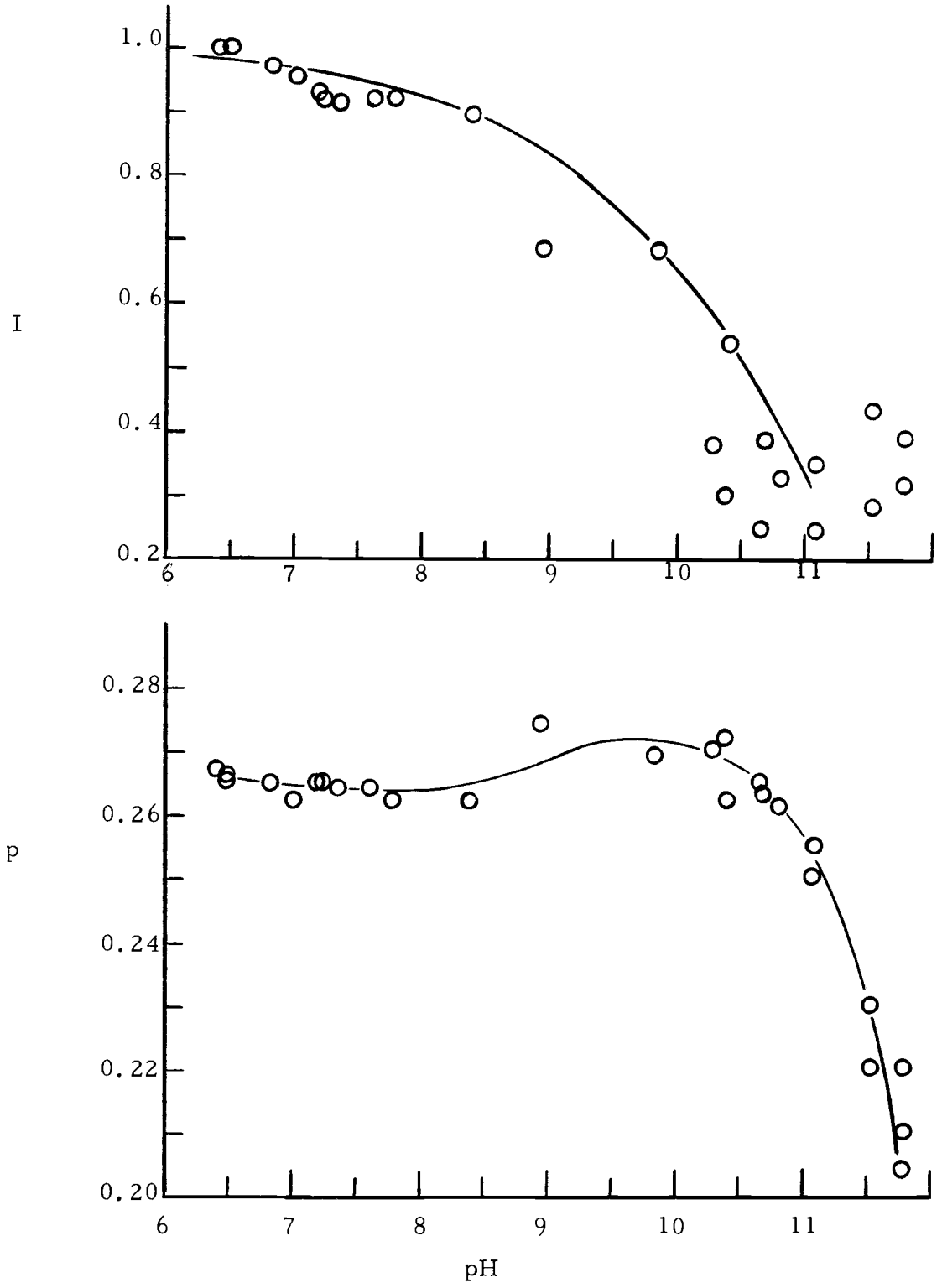

Figure 19. Effect of pH on the polarization and intensity of fluorescence of complex E. Dansyl excited at 350 nm.

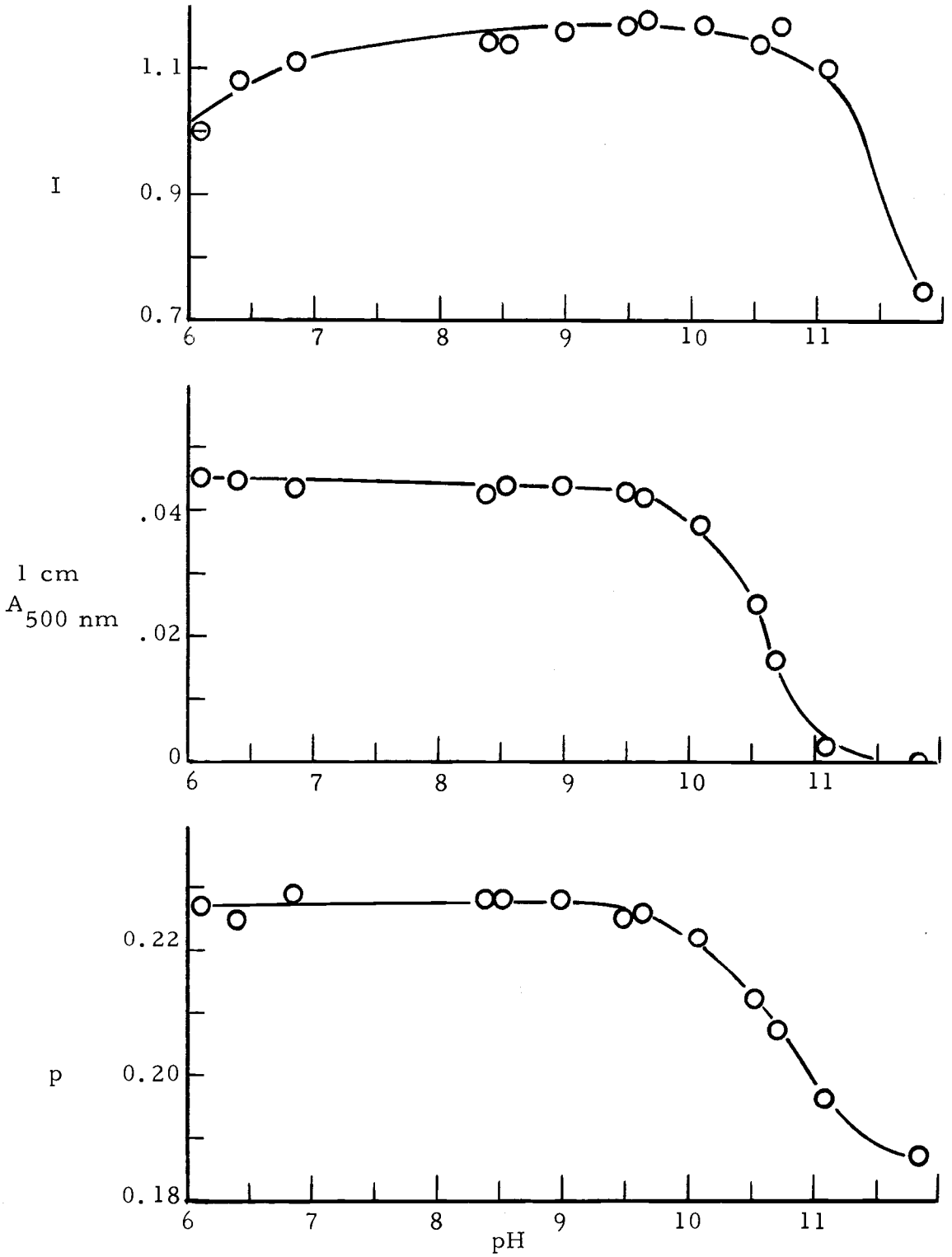

Figure 20. Effect of pH on the apparent absorbance, polarization, and intensity of fluorescence of complex F. Dansyl excited at 345 nm.

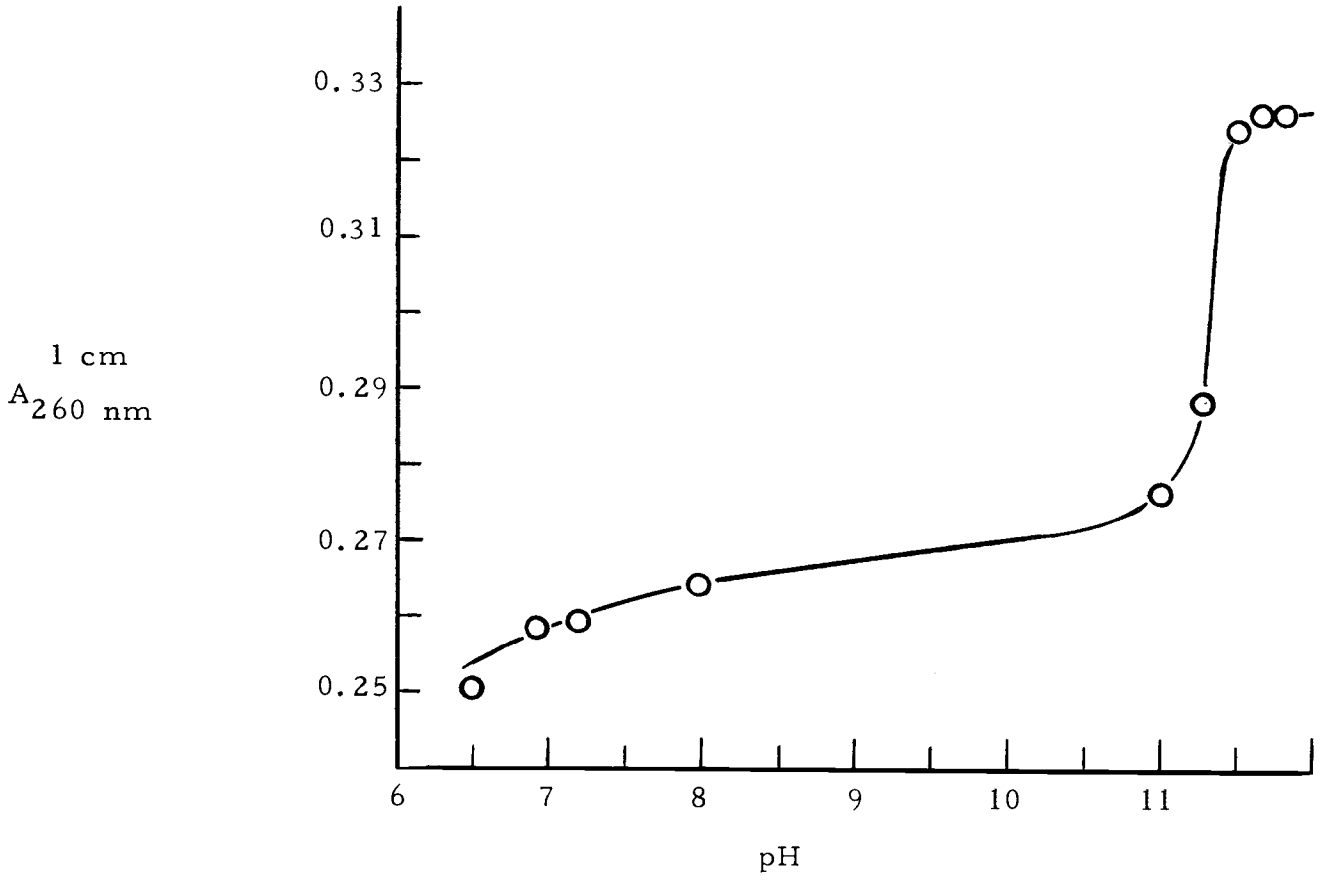

Figure 21. Effect of pH on the absorbance at 260 nm of complex E.

Table VII. Data on Complex F

Before Dialysis	
Lysine to dansyl ratio in polylysine	13.9
Approximate degree of polarization of polylysine	140.
Salmon sperm DNA(P) molarity	2.50×10^{-4}
Polylysine molarity in lysine	1.25×10^{-4}
Dansyl molarity	9.0×10^{-6}
DNA(P) to lysine ratio	2.00
NaCl molarity	2.0
After Dialysis	
Days of gradient dialysis at 6°C and pH = 6.1	2.
Molarity of sodium cacodylate buffer	0.001
Molarity of NaCl	0.000
Centrifugation (g)	1,700.
Minutes of centrifugation at 0°C	15.
Supernatant after Centrifugation (Complex F)	
Approximate salmon sperm DNA(P) molarity	2.2×10^{-4}
Approximate polylysine molarity in lysine	1.1×10^{-4}
DNA(P) to lysine ratio	2.
Molarity of sodium cacodylate buffer	0.001
pH	6.1
NaCl molarity	0.000
Apparent absorbance of 1 cm at 500 nm	0.05

Figure 21 shows the absorbance at the DNA absorption peak as a function of pH for complex E. It shows that the DNA starts denaturing at a pH of 11 and that the DNA is about half denatured at pH = 11.3. This reveals that the drop in polarization at a pH =10 is not due to the denaturation of the DNA.

Figure 22 shows the pH titration of dansyl polylysine. The dansyl concentration was 8.95×10^{-6} molar and the lysine to dansyl ratio was 13.9. The approximate degree of polymerization of the

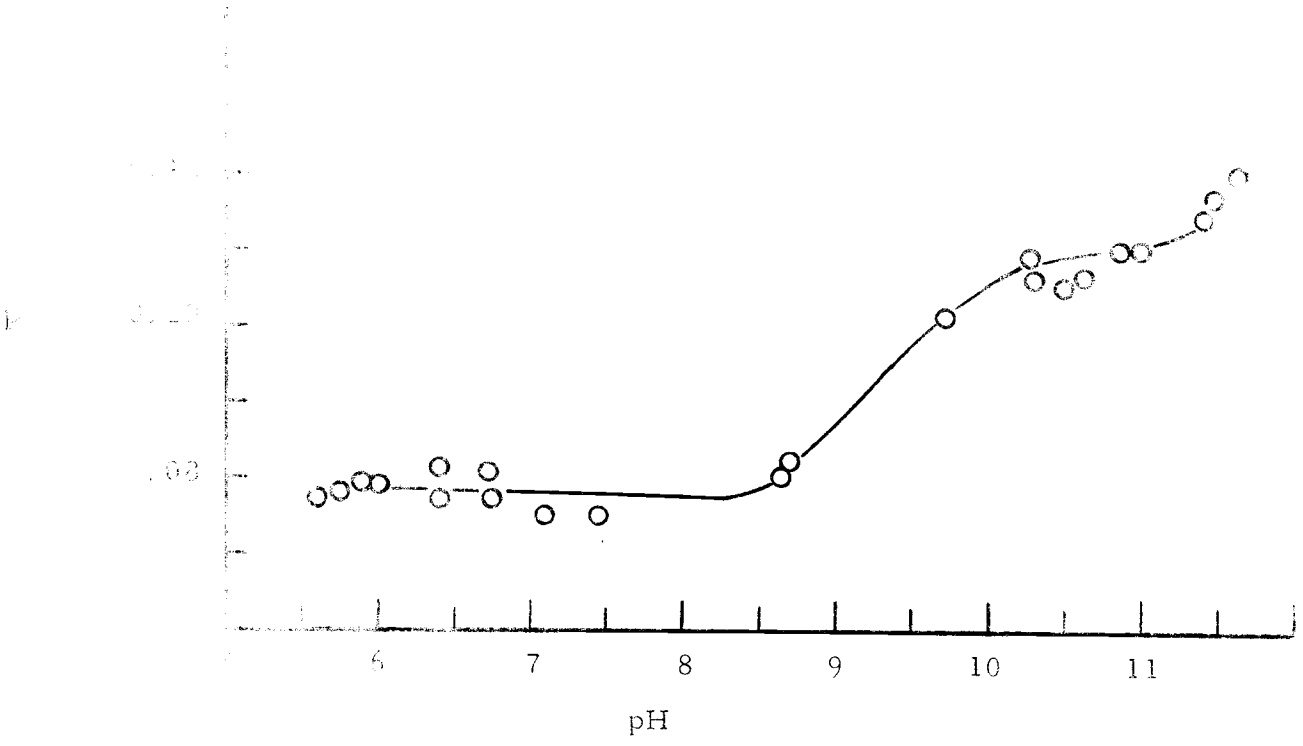

Figure 22. Effect of pH on the polarization of fluorescence of dansyl polylysine. Dansyl excited at 335 nm.

polylysine was 140. The dansyl was excited at 335 nm. The helix coil transition of the polylysine is readily detected by the polarization measurement. This has been previously demonstrated by Gill and Omenn (20). The results shown in Figure 22 are in close agreement with those reported by Gill and Omenn.

Since the preparation of complex F differed from the preparation of previous complexes, its salt dissociation characteristics were determined. Figure 23 compares the salt dissociation of complex E with that of complex F. It is evident that both methods of complex preparation yield preparations that dissociate at the same salt concentration.

The intensity of fluorescence and apparent absorbance for complex F showed an appreciable increase upon the addition of NaCl. A and I would then decrease upon the addition of more NaCl as shown in Figure 24. This behavior was not noticed in the other complexes, except for the relatively large increase in turbidity upon the initial additions of Mg^{++}. It should be noted, however, that complexes A, B, and C already had 0.05 molar NaCl and 0.10 molar cacodylate buffer before additional salt was added for a dissociation run.

F. Effect of the Presence of Methanol on the Dissociation of the DNA-Polylysine Complex by NaCl

The presence of methanol in a solution of DNA-polylysine

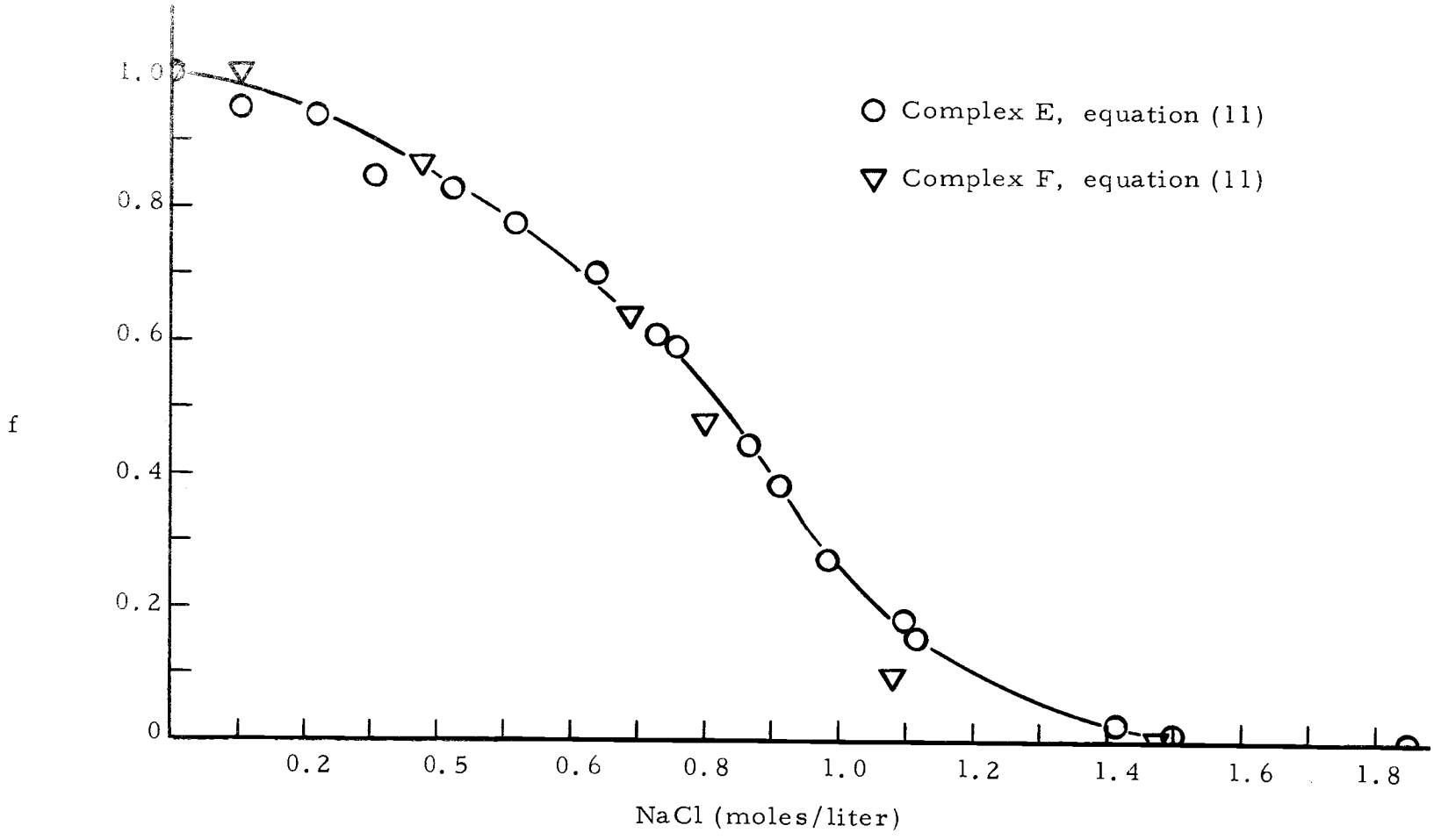

Figure 23. Comparison of NaCl dissociation of complex E with the dissociation of complex F.

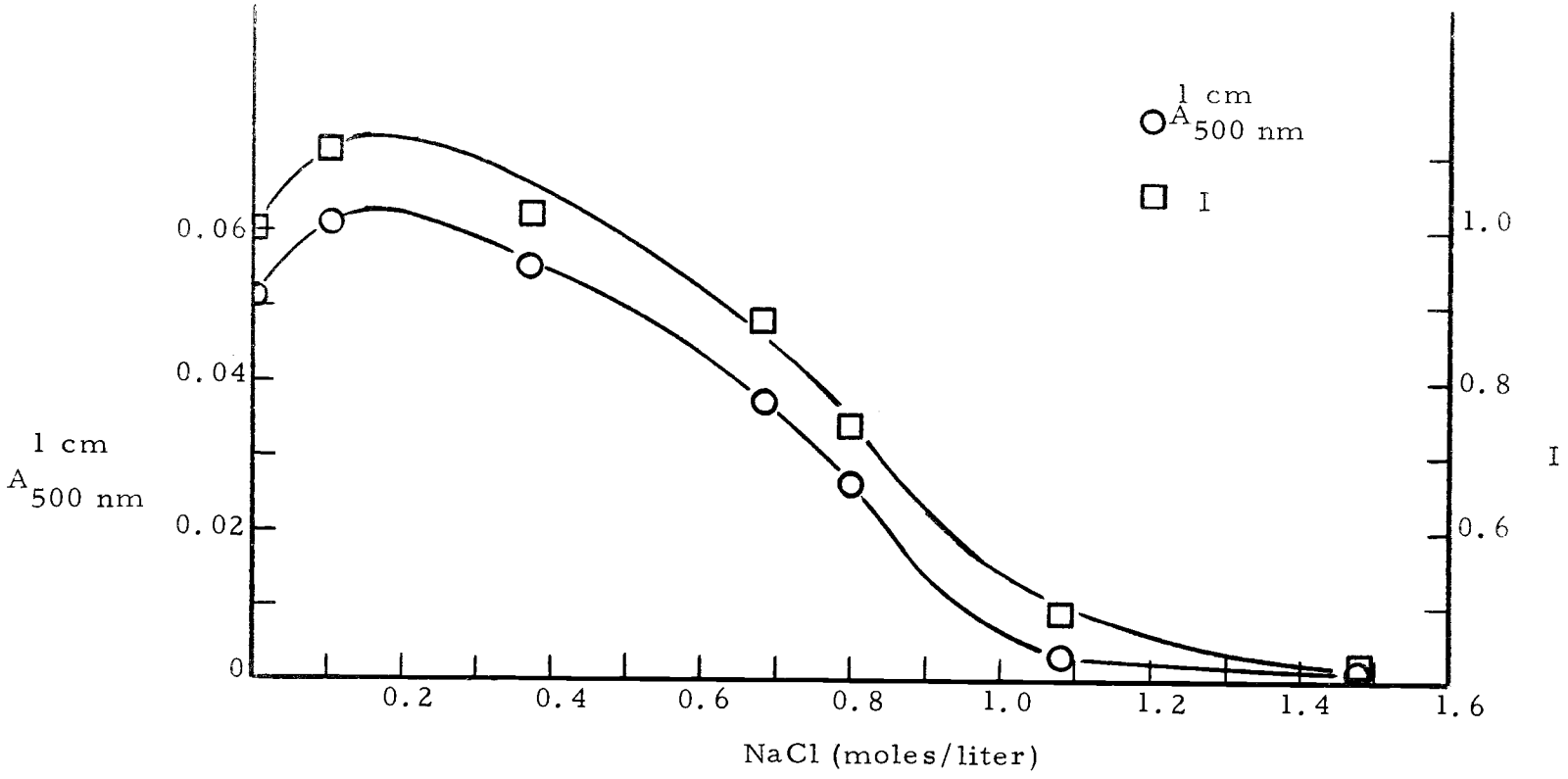

Figure 24. Effect of NaCl concentration on the intensity of fluorescence and apparent absorbance of complex F.

complex would be expected to modify the dissociation of the complex by NaCl in primarily two ways. The dielectric constant of methanol is much smaller than water and hence the electrostatic forces in the complex would be stronger in methanol than in water. This effect would make it more difficult for the NaCl to dissociate the complex. This is complicated by electrostatic interaction of the sodium and chloride ions with free polylysine or DNA which would also be larger in methanol and this would tend to shift the f vs. c curve to the left.

Another interaction of possible importance is the hydrophobic interaction in the complex, which would be weakened by the presence of methanol.

The effect on methanol was tested by dialysing complex F against enough spectroscopic grade methanol and cacodylate buffer so that the final composition was 50 volume percent methanol in 0.001 molar sodium cacodylate buffer at pH = 7.1. Upon dialysis the initial volume of 7 ml inside the bag was reduced to about 5 ml.

Figure 25 compares the salt dissociation of complex F with the dissociation of the complex in the presence of methanol. It appears that methanol stabilizes the complex against salt dissociation.

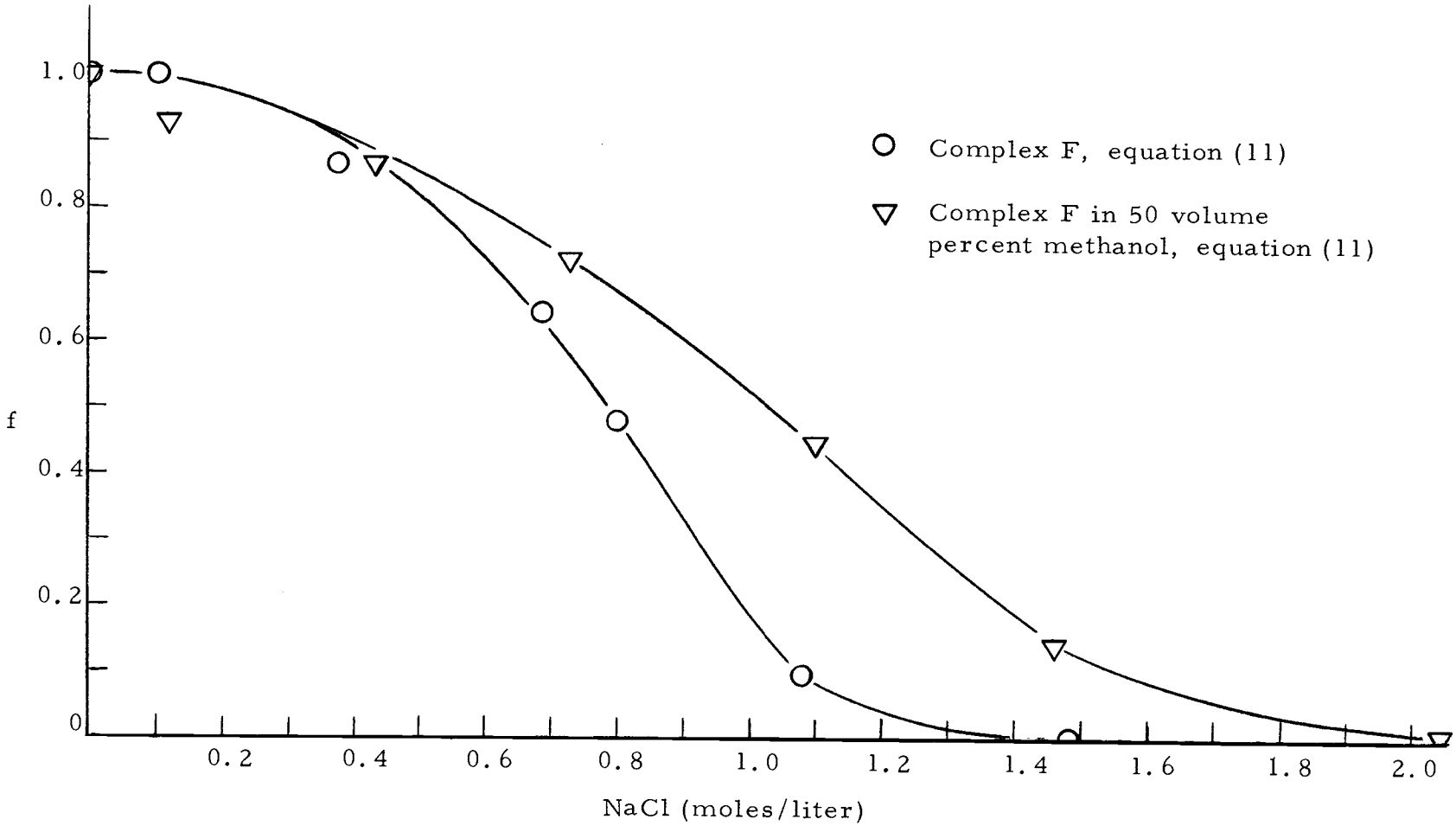

Figure 25. Comparison of the NaCl dissociation of complex F with the NaCl dissociation of the complex in 50 volume percent methanol.

IV. DISCUSSION

The quantitative determination of the dissociation properties of a complex by the polarization of fluorescence technique is based upon a two state model for the complex. Assumptions are also made regarding the absorption and emission properties of the dye. The weakest set of assumptions allows for the possibility that the product of the molar extinction coefficient and the quantum yield, ϵq, for the dissociated state may be a function of the concentration of the agent causing the dissociation. However, it is still necessary to assume that ϵq remains constant in the complexed state.

A stronger and more restricting assumption is that both states of the complex have characteristic ϵq values which remain constant throughout a dissociation of the complex. If this assumption is valid, the intensity of fluorescence alone may be used to determine the dissociation curve for the system. This appears to be the case for the dansyl polylysine-DNA system since the calculations of f under both the weak and strong assumptions were in close agreement.

As shown in Figure 24, the turbidity and intensity of fluorescence of complex F was observed to increase with the initial addition of salt. The polarization, however, did not show this initial increase. It appears that the initial addition of salt to the complex may cause it to change its state of aggregation. The polarization does not

increase upon this change. We conclude, therefore, that the frictional forces limiting the rotatory diffusion of the dye transition moment do not show a corresponding change.

The increase in aggregation at low salt concentration may be correlated to the lower solubility of the DNA-polylysine precipitates at these concentrations of salt as observed by Spitnik, Lipshitz and Chargaff (49).

The two state model does not adequately represent the DNA-polylysine system over the complete range of salt concentration. For a complete study a three state model would be needed: the state existing at essentially zero salt concentration, a second state which exists at low salt concentration, and the final dissociated state. The use of the more complicated three state model was avoided in most of the salt dissociations reported in this thesis by starting the complex in the second state. This was achieved by using an initial solution of the complex with buffer and salt concentrations in the range of 0.05 molar to 0.15 molar. When this is done, the turbidity and intensity appear to decrease monotonically upon the further addition of salt and f calculated from turbidity or intensity of fluorescence alone agrees well with the polarization determinations.

The chief results of this investigation on the properties of the complexes are as follows: (a) The calculation of f is insensitive to the assumption regarding the dependence of $\epsilon_2 q_2$ on the salt

concentration. It appears that the major change in intensity is due to the dissociation of the complex and that changes in intensity due to other causes are small in comparison.

(b) Calculation of f based on polarization and intensity data agrees well with the calculations of f based on turbidity data. If the association between polylysine and DNA were random, f from turbidity measurements would be weighted toward the larger aggregates and one would not expect a close agreement with polarization and intensity determined values of f. It is known that the polylysine-DNA complex is stoichiometric and structurally well defined and, if the scattering unit were either the complex, or a simple and well defined association of complexes, then the turbidity should be proportional to the number of scattering units. In this case the approximate agreement between f calculated from turbidity data and f determined from p and I data is consistent with the model of a rather well defined complex.

(c) The order of effectiveness of the cations for dissociating the complex is $Mg^{++} > Li^{+} > NH_4^{+}, Na^{+} > K^{+}$. The order of effectiveness of monovalent anions is $SCN^{-}, ClO_4^{-} > NO_3^{-} > Br^{-} > Cl^{-} > CH_3COO^{-}$. This is the Hofmeister series and it has been found to be the order of effectiveness in denaturing a wide variety of biopolymers. This has been cited by von Hippel and Wong (56, 57) as suggesting that the difference in the effects of these salts is due to their different effects

on water structure which in turn has corresponding effects on the hydrophobic interactions of the polymers. Robinson and Jencks (46) presented arguments that the Hofmeister series arises from the ability of the various salts to interact directly with the dipoles of the amide groups of proteins. Schrier and Schrier (47) studied the effects of salts on the solubility of the model compounds, N-methyl proprionamide and N-methyl acetamide. They were able to analyze their system by assuming the salting-out coefficient of a model compound could be expressed as the sum of the number of salting-out coefficients corresponding to the various groups in the compound. The salting-out coefficient for the methylene group followed the usual Hofmeister series whereas the corresponding coefficient for the polar amide group was independent of salt type. They interpreted this as suggesting that the Hofmeister series applies only to nonpolar parts of the molecule. The generalization of this idea would support the suggestion of von Hippel and Wong mentioned above.

Von Hippel and Schleich (55) have written a comprehensive review of the effects of neutral salts on the structure of biological macromolecules. They reach the conclusion that the interaction among nonpolar groups, salts, and the solvent is almost certainly responsible for the order of effectiveness of neutral salts almost universally following the Hofmeister series.

If the above interpretation of the Hofmeister series is correct,

it would imply that the DNA-polylysine interaction involves a significant nonpolar or hydrophobic interaction as well as the obvious ionic interactions. However, more direct evidence as to the nature of the binding energy is certainly needed.

(d) The dissociation curve of the A-T rich salmon sperm DNA-polylysine complex by NaCl is essentially the same as the dissociation curve for G-C rich Micrococcus lysodeikticus DNA-polylysine complex. This is unexpected in view of the A-T specificity noted for polylysine (30, 40, 42). The differences in the dissociation curves may be small and it would be useful to develop a sensitive differential method for comparing the dissociations of the two complexes.

(e) The pH titration of the DNA-polylysine complex shows that the complex starts its dissociation at about pH = 10, whereas the titration of the DNA-free solution of dansyl polylysine shows that there is nearly a complete loss of protons by the free polylysine at this pH. This can be interpreted as illustrating that a higher pH is required to remove the protons from the polylysine when it is complexed with DNA in comparison to the pH required to neutralize the uncomplexed polylysine. The charged form of the polylysine is stabilized by its association with DNA.

(f) The presence of methanol in a solution of the DNA-polylysine complex makes it more difficult to dissociate the complex in

comparison to the dissociation when no methanol is present. This stabilization of the complex by methanol is not unexpected in a case where ion-ion electrostatic forces are predominant.

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