INTRACTION OF SODIUM , CALCIUM AND MAGNESIUM IONS WITH DEOXYRIBONUCLEIC ACID

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This thesis is dedicated to my late Father

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ABSTRACT

The interaction of sodium (i), oaloium (ii) and magnesium (II) with Deoxyribonucleic acid (DNA) of oalf thymtts has been studied by dialysis equilibrium, conductance measurements, ultraviolet light absorption and light scattering methods. The results have been summarised as follows:

(i) DNA interacts with sodium, calcium and magnesium ions and more strongly with the two divalent cations.

(ii) Magnesium and calcium ions bind quantitatively to DNA to form complexes of the following stoiohiometries: 0.125:; 0.30:1 and 0.50:1 moles of divalent cation to one mole of nuoleate phosphorus. In alkaline medium, a 1:1 mole oomplex was formed.

(iii) The stability of the complexes formed between the divalent cations and DNA depends on (a) the ooncentration of sodium chloride supporting electrolyte, and (b), the pH of the solution. The stability decreases with increasing salt concentration and with increasing acidity for eaoh DNA-M^{2+} system. The stability constants for the DNA-Mg complexes are comparable with those of DNA-Ca^{2+} oomplexes.

(iv) At room temperature, DNA retains its native structure when prepared in solution of sodium chloride of at least 10^{-4} and in both caloiura and magnesium ohloride of about one hundredth of this concentration.

The molar absorptivity e(P) of DNA becomes a function of the concentration of $NaCl_2$ $CaCl_2$ and HgClp only when the acid is denatured, but is completely independent of salt concentration when in its native form. DNA denaturation temperature (Tm) is increased in the presence of increasing concentrations of NaCl, $CaCl_2$ and MgC_2. Caloium and magnesium ions are more effective than the sodium ions in causing this increase in (Tm), showing a stronger interaction of the divalent oations than of the monovalent sodium ions with DNA.

Low oonoentrations of calcium and magnesium ions (of the order of and below) have no destructive influence on the secondary structure of DNA. However, high concentrations of these divalent oations (of the order of 0.05H and above) have been suspected to produce a denaturation followed by a precipitation and/or aggregation of a system of DNA-M on heating beyond the denaturation temperature (Tm).

(v) The method of light scattering has shown that the molecular weight of DNA depends on the concentrations of calcium and magnesium ions up to a oertain maximum concentration of these oations, when the molecular weight of the DNA is doubled. This suggests that calcium and magnesium ions are engaged in an intermolecular bonding to DNA. The molecular weight of DNA was found to be independent of the concentration of sodium ions.

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CHAPTER 1

IN Til 0 DU C T ION

Recently, efforts have been concentrated on the study of macromoleculec of the cell nucleus such as the nucleic acids and proteins. These inacroriolecules, especially nucleic acids, have been found to be present in the nucleus of each cell of all living organisms together with certain inorganic ions, among whioh are magnesium (II) and calcium (II) . One would naturally envisage that these divalent cations, magnesium and calcium, night interact with the mcromolecules and possibly influence their biochemical functions. The ions nay possibly form oomplcxes with the macro-molecules in the cell.

The interaction between deoxyribonicloic acids and magnesium, calcium and other cations such as iron, copper and silver, and some organic ions and molecules like acridines have been observed to be important biologically.

It has been recently discovered that the specificity of amino acid incorporation associated in a given condon can be changed as a function of magnesium concentration [1,2].

Acridines have been found to act eutagenically upon bacteriophage, probably by causing the insertion or deletion of a base pair in its ENA [3]> and these characteristics have been used in determining the coding ratio [4] . This system therefore, provides an opportunity of elucidating the molecular mechanism of the action of a muntagen.

It has also been reported that ions, particularly cations, are implicated in the enzymatio depolymerisation of DNA. Suggestions have also been made that the action of the ions may be on the DNA [5*6] rather than on the enzymes. Records have sham that the seoondary and tertiary structures of polynucleotides are profoundly affected by the nature and concentration of the metallic ions present. It has alao been reported that for the prolonged existence of the macromoleoular species in solution in their ordered forms, it is necessary that they must be associated with metallic cations [7*8]• However, certain metallic cations like copper (il) have been found to be exceptions because they destabilise the ordered form of the macromolecular species by breaking the hydrogen bonds which are responsible for the stabilisation of the secondary structure of the nuoleic acid.

The interest in studying the interation of metallic ions with nucleic acids and polynucleotides is partly due to the fact tiiat the speoifio hydrogen-bonded structure of the nucleic acid molecules collapses in solution in the absence of metallic salts [9 - 11]. Perhaps the divalent metallic ions, magnesium (II) and calcium (il) help to stabilise the native and ordered structure of the deoxyri-bonucleate or its protamine or histone oomplexes in the cell nucleus. Should there be a stabilisation of the nucleic acids by the divalent cations magnesium and calcium, one would expect it to depend on the concentrations of these cations. The stabilising ability will also probably depend on the type of complexes formed

with the nucleic acids, and on the configurational changes of the macromolecule that might accompany the interactions.

In an endeavour to resolve some of the problems of molecular and ionic interactions with deoxyribonucleic acids, I have conducted a number of investigations. I have two main aims in mind; first, to determine the structure and stoichiometry of the deoxyribonucleate-cation complex, and secondly, to study the degradative effects of high concentrations of magnesium (II) and calcium (II) on polydeoxyribonucleotides. It may be that the degradation of the polynucleotides by these ions, if it takes place at specific points in the chain, may help in the sequential studies of monodeoxyribonucleotides in the polynucleotide chain, and hence in a native DNA molecule.
One of the various biological implications of the outcome of these studies, if pursued further, might be the possibility of reconciling the stabilising concentrations of the cations to their physiological values.

To accomplish the aim of establishing the stoichiometry and structure of DNA-cation complex and the degradative effects of high concentrations of the divalent cations on the polydeoxynucleotides a number of experiments have been conducted. Among these are: dialysis equilibrium experiments; conductometric titrations; the ultraviolet absorption experiments; and the light scattering experiments.

All the results have been put together and certain conclusions have been drawn. These have been recorded in this thesis.

The structure of Deoxyribonucleic acid (I)

The Organochemical Structure

Various exhaustive chemical methods have shown that all nucleic acids, Deoxyribonucleic acid (DNA) and Ribonucleic acids (RNA) which are both found in the cell of all living organisms, contain a number of purine and pyrimidine bases, a number of sugar residues, and some phosphorus .

I.a The purine and pyrimidine bases

Two groups of cyclic nitrogeneous bases have been reported for all nucleic acids. Those found in most deoxyribonucleic acids are the purine Adenine (A) and Guanine (G); and the pyrimidines, Cytosine (C) and Thymine (T). Occasionally, Cytosine is found replaced or partially replaced by one of its derivatives, among which are the 5-methyl-cytosine and the 5-methoxy-cytosine.

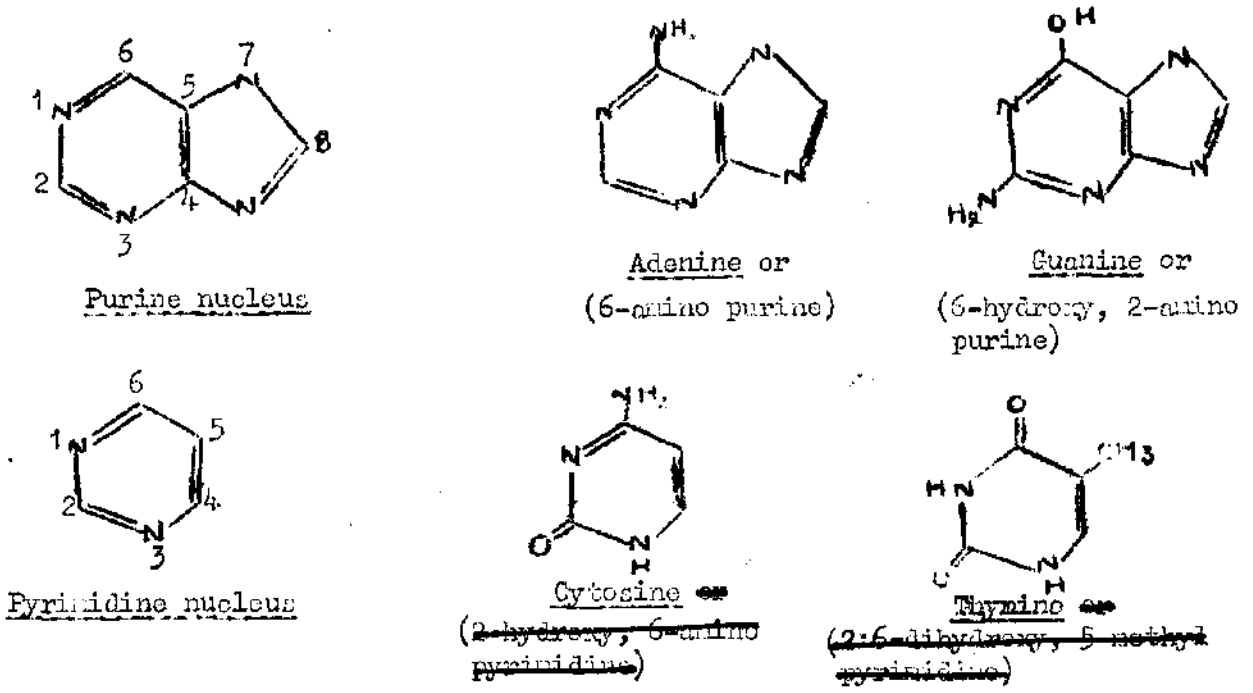


Fig.1.1 The bases commonly found in deoxyribonucleic acids.

Fischer, Traube and Roeder [12 - 15]; Wheeler and Merriam [16]; Steudel [17] and Johnson [18] have indicated the presence of these bases in deoxyribonucleic acids.

For all the bases, hypothetical possibilities of amino-imino and keto-enol tautomerisms have been assumed. The tautomers differ in the exact positions at which their hydrogen atoms are attached. Under physiological conditions, one particular form of each base is much more probable than any other. The predominating forms have been identified by the method of infrared spectroscopy [19]. Adenine and guanine have been found to exist in solution mainly in their amino and keto-amino forms respectively [20]. For cytosine and thymine, the keto-amino and diketo forms have been observed respectively [21]. The structure of deoxyribonucleic acid in solution can thus be assumed to contain bases which exist mainly in their favoured tautomerisms. The base pairing requirements for the secondary structure of the acid, which have been discussed in the later parts of this thesis will then be seen to be more restrictive.

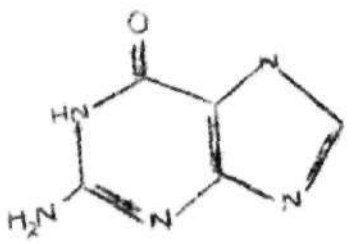


Fig. 1.2 The keto-amino form of guanine.

I b. The base composition of Deoxyribonucleic acids

As reported by Chargaff [21], deoxyribonucleic acids from different sources show some significant differences in their base contents. As determined by their base composition, DNA has been divided into three main classes. The first class, an AT-type, for which the mole ratio (A + T)/(G + C) is greater than unity; the second class, a GC-type, for which (A + T)/(G + C) is less than unity; and the third type, for which (A + T)/(G + C) is approximately unity.

Deoxyribose nucleic acids from animal sources are of the AT-type, while those from micro-organisms are of the GC-type [21].

For nearly all the unfractionated DNAs examined by various workers, the result for the mole ratios A/T ≈ G/C ≈ Purine/pyrimidine ≈ 6 - amino/6 - keto ≈ 1 have been reported for preparations from various sources.

For fractionated DNAs, the results are remarkedly different as reported by Bendich [22]. No regular patterns of variations of these ratios have been established for DNAs from various sources.

	A	G	C	T	$\frac{A+T}{G+C}$	$\frac{A}{T}$	$\frac{\text{Purines}}{\text{Pyrimidines}}$
Mammal							
Ox	29.0	21.2	21.2	28.7	1.360	1.010	1.006
Pig	29.8	20.7	20.7	29.1	1.42	1.024	1.014
Man	30.4	19.9	19.9	30.1	1.52	1.010	1.006
Micro-organisms							
Micobacterium tuberculosis	15.1	34.9	35.4	14.6	0.46	1.034	1.000
Aerobacter aerogenes	21.3	28.8	28.0	21.9	0.76	0.973	1.004

Table 1.1: Base composition of whole deoxyribonucleic acids from different sources. Data from Chargaff [21]

Fraction no.	A	G	C	T	$\frac{A+T}{G+C}$	$\frac{A}{T}$	$\frac{G}{C}$
50 - 54	1.0	0.61	0.81	0.91	1.345	1.10	0.75
59 - 64	1.0	0.71	0.82	0.94	1.268	1.06	0.81
197	1.0	0.67	0.74	0.88	1.334	1.14	0.91
244	1.0	0.86	0.90	0.94	1.103	1.06	0.96

Table 1.2: Fractionated calf thymus deoxyribonucleic acid. (data from Bendich [22] .

The macromolecular structure of Deoxyribonucleic acids (II)

II.a The Primary Structure

In their primary structures, all nucleic acids contain a number of basic small units called mononucleotides arranged linearly to form a polynucleotide chain. For DNA, a polydeoxynucleotide is formed by a number of the purnine and pyrimidine deoxyribonucleotides.

The nucleotides are compounds of a purine or pyrimidine base, a sugar moiety and a phosphate group, all joined together chemically. In all DNAs the 2-deoxy-D-ribose sugar of a furanoside structure [23 - 25] has been found.

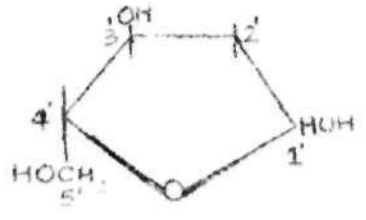


Fig. 1.3 A 2-deoxy-D-ribose sugar residue

The mononucleotides are the phosphate esters of the corresponding nucleosides, the latter being the compounds of a base and sugar, in which the base is linked by a glycosidic bond to the sugar residue at its position C_1. The β-configuration of the glycosidic bond to the sugar residue has been confirmed by Todd and Co-workers [26] ; and by Anderson et al [27], who also concluded that the sugar residue is attached respectively to the position N_9 in purine and N_3 in pyrimidine nucleus. This conclusion was previously made by Gulland and Co-workers [28-30]. By the method of X-ray diffraction, Furberg [31] has also confirmed the furanoside structure of D-ribose sugar, the β-configuration of the glycosidic linkage, and the linkage of sugar at the position N_3

of the pyrimidine ring. He was also able to show that the six atoms of the pyrimidine rings all lie in the same plane, with the base and sugar mutually perpendicular.

In a mononucleotide a phosphate group is attached to the position 3' or 5' of the sugar residue of a nucleoside.

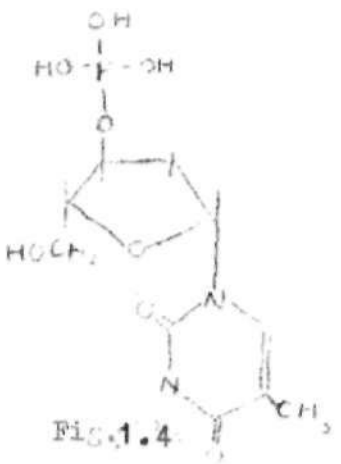


Fig.1.4

Deoxythymidine 3'-phosphate
(Deoxythymidylic acid)

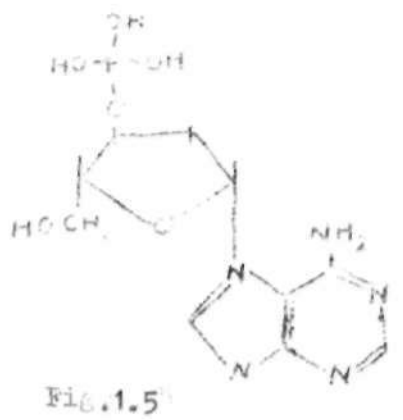
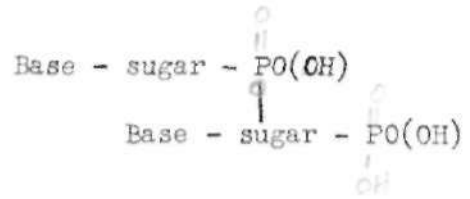


Fig.1.5

Deoxyadenine 3'-phosphate
(Deoxyadenylic acid)

The mononucleotide basic units are joined through phosphodiester linkages to form a polynucleotide chain. In a phosphodiester bond two sugar residues are linked to the phosphorus atom, each via an oxygen atom. The results of electrometric titrations have confirmed the presence of such phosphodiester bonds [3] and have also suggested that secondary and tertiary phosphate ionisations occurred to a very limited extent in the polynucleotides. Possibilities of chain branching and chain interruptions have been shown to be very small, if any. This leads to a structure

shown.



The exact sequential arrangement of the mononucleotides along a polynucleotide chain for both DNA and RNA is a problem yet to be solved. For most DNAs very little is known about the exact pattern of occurrence of the nucleotides along the polynucleotides of the native molecule. This is because it is exceedingly difficult to obtain molecularly homogeneous preparations of the acids. The high molecular weights of most nucleic acids are also a great obstacle to complete sequence determination.

For the first time the specific order of subunits in one ribonucleic acid molecule has been determined successfully by Holley [33]. Also, for a biosynthetic DNA [34], the purine and pyrimidine mononucleotides have been shown to alternate along the chain, and the 3' - 5' - mode of linkage of the phosphodiester bond has been confirmed.

The idea of alternating purine and pyrimidine mononucleotides has also been supported by Levene [35]. Shapiro and Chargaff [36]: Jones and Letham [37] and Peakocke [38] however, have produced some evidence supporting the occurrence of polypurine and polypyrimidine oligonucleotides in a synthetic polydeoxynucleotide chain.

As based on the assumption of alternating purine and pyrimidine mononucleotides, the primary structure of nucleic acids can be represented as in Fig.1.6 .

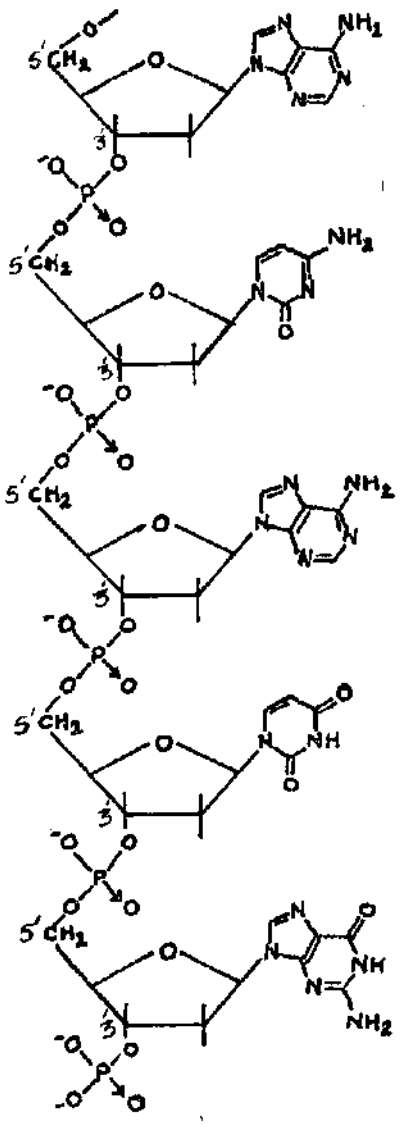


Fig. 1.6 The primary structure of deoxyribonucleic acid. Alternation of purine and pyrimidine nucleotides is assumed

II.b The secondary structure of deoxyribonucleic acids

The secondary structure of deoxyribonucleic acid refers to the stable form in which the acid exists in the cells of living organisms.

Astbury and Bell [39] ; Furberg [31] ; Pauling and Corey [40] were among the first to attempt the elucidation of the secondary structure of DNA.

Watson and Crick [41] , later proposed a structure which is now generally accepted, in broad outline, as the secondary structure of DNA.

The structure of DNA proposed by Watson and Crick consists of two coaxial polydeoxynucleotide helices of equal pitch, both following the righ-handed helices, but with the sequence of atoms running in opposite directions in the two chains. The molecular configuration is highly ordered, and the α-helical structure is generally continuous. The bases are on the inside of the helix with their planes perpendicular to the axis of the helix, while the phosphate groups are on the periphery. The sugar residue is roughly at right angle to the base.

The general structures of the double helix, especially the dimmensions, depend on the relative humidity. The A and B forms of the sodium salt of DNA have been observed [42] . The two forms differ in the dimensions of the helices. The A type exists at low relative humidity ($< 70\%$). It is highly crystalline and gives a good X-ray diffraction photograph [42] . The B form prevails at a high relative humidity ($> 94\ \%$) and is less crystalline than the A form.

The B form is the one assumed to occur in solution and in the living cells, and its structure conforms almost strictly to the one proposed by Watson and Crick.

For the B form, a deoxyribonucleotide occurs every 3.4 A° along the direction of the long axis, and adjacent deoxynucleotides are orientated at 36° to each other, with the structure repeating itself every 34 A° along the fibre axis, showing ten mononucleotides per turn of the α-helix. The arrangement of the two helices is such that a base from one helix is opposite (and in the same plane as) a base from the other, thus maintaining a good periodicity of sequence. The structure is stabilised by hydrogen bonding between the bases of two polynucleotide strands. Two hydrogen bonds are formed between adenine and thymine of opposite strands, and three bonds between guanine and cytosine or cytosine derivatives . The sites of the hydrogen bonds are shown in Fig.1.7. Fig.1.8 also represents the double helical structure of DNA of the B form. Two grooves, the small and large grooves are normally characteristic of the DNA molecule.

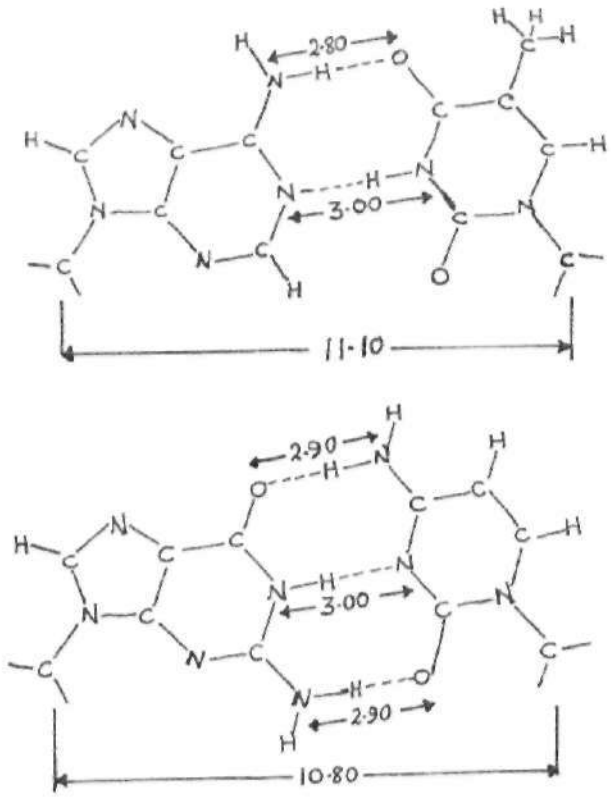


Fig. 1.7
Hydrogen bond formation between the purine and pyrimidine bases.

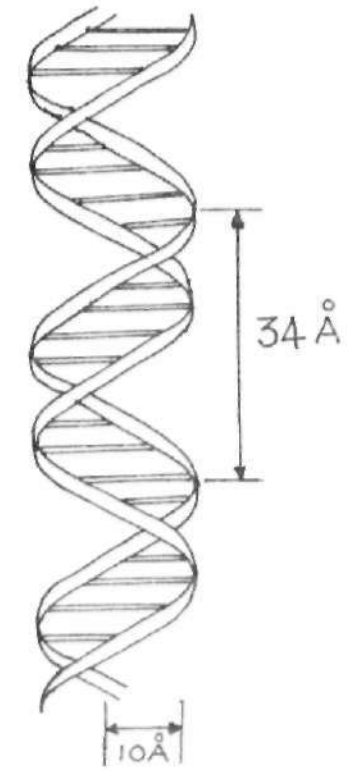


Fig. 1.8
The double α-helical structure of a native Deoxyribonucleic acid molecule, proposed by Watson and Crick [41].

The Macromolecular Structure of Deoxyribonucleic acids III

III.a The molecular weight and molecular parameters

A molecule as big as that of deoxyribonucleic acid has a very high molecular weight.

As reported previously [43 - 48] using different methods of extraction and of molecular weight determination including the methods of intrinsic viscosity, sedimentation coefficient and light scattering, the molecular weights of DNAs from various sources (mammalian) are known to vary between 2 and 16 x 10^6 . For micro-organisms, higher values of the order of up to 10^9 have been reported [48] .

Nucleate preparations from the same source have also been reported to exhibit pronounced molecular weight heterogeneity, as demonstrated by chromatographic fractionation [49, 50] . Various molecular fractions of discrete genetical activity have been isolated [50], with fractions from the same source usually varying in the proportions of guanine and cytosine bases. For example, calf thymus DNA has been found to contain seven heterogeneous fractions, varying in (A + T)/(G + C) ratio from 0.99 to 1.99; A/T from 0.99 to 1.14 and G/C from 0.75 to 1.0

The differences in these molar ratios, especially in (A + T)/(G + C), have also caused some measurable differences in some of the physical properties of the various fractions. As based on the (G + C) content of the fractions, those with a high (G + C) content melt at higher temperatures than those with high (A + T) content. A mixture of all the various fractions usually

displays a spread of melting point the breadth of which depends on the spread of the (G + C) contents of the fractions. The smaller the spread in the (G + C) contents, the narrower the breadth of the melting point. The spread in melting point (or denaturation temperature) can be measured by the method of UV absorption by the mixtures of the fractions at elevated temperatures.

For a macromolecular ion of molecular weight of about $6 - 7 \times 10^6$, containing about 9,000 mononucleotides, and maintaining the Watson-Crick structure, an overall length of about 30,000 A° has been calculated. For this molecule, a radius of gyration of about 1,500 - 2,500 A° and a mean end-to-end length of the order of 5,000 A° or more, have been measured [51] . The molecule therefore, must necessarily be a flexible coil, as supported by the results obtained from a variety of different experimental techniques, including streaming birefringence, viscosity and light scattering [43 - 48] .

III.b The stability of the DNA structures

It is already established that the secondary structure of the nucleic acids becomes unstable as a result of the cleavage of the stabilising hydrogen bonds. This leads to the denaturation of the α-helical structure of the DNA.

Denaturation of DNA, as recorded, can be achieved by an increase in temperature, treatment with acid or alkali, or dilution of the ionic (including counterion) concentration below certain critical values.

Exposure to some ultrasonic vibrations or to α and β-rays also causes the denaturation of nucleic acids. The conditions necessary for denaturation are discussed below, with the changes in the parameters of the molecule that accompany the denaturation. Acid pH for example, causes the binding of a proton by the N_1 nitrogen of either adenine or cytosine, thus eliminating the site as a hydrogen bond acceptor, and causing the breaking of one of the stabilising hydrogen bonds. On back titration another less specific structure will be formed [52 - 54].

Thomas [55, 56] studied the denaturating effects of low concentrations of electrolytes on the nucleate preparations using the method of ultraviolet radiation absorption, and found that the minimum concentrations of electrolytes, especially sodium chloride, required to maintain the secondary structure of DNA is between 10^{-4} and 10^{-3}M. For divalent electrolytes such as magnesium and calcium chloride, much lower concentrations of the order of 10^{-5}M are necessary. The mutual electrostatic repulsions between neighbouring negatively charged phosphate groups, in the absence of added electrolyte, produce some strains in the helix, and hence some denaturation of the molecule.

Cosgrove [57] was the first to demonstrate that there is an irreversible denaturation on heating sodium deoxynucleic acid in neutral aqueous solution. His results were supported by Thomas [56], who showed that the irreversible denaturation occurs only above certain critical temperature which is dependent on the ionic strength as well as on the native state and the source

of the acid. The critical temperatures recorded are between 70°C and 87°C for most native nucleate preparations. This result was also confirmed by Goldstein and Stern [58] ; Rice and Doty [59, 60] .

III.c Changes in the structure of DNA on denaturation

Simultaneous cleavage of a number of hydrogen bonds is probably required to initiate denaturation of DNA, but subsequently the process becomes cooperative, with a collapse of the weaker regions of the double helix.

For thermal denaturation the weaker regions are the sequences composed predominantly of adenine and thymine residues with only two hydrogen bonds. The stability of the adjacent regions is reduced and there is a rapidly progressive disruption of α-helical structure. There is a variation in the degree of denaturation of any given preparation which is a reflection of the compositional, and possibly sequential, heterogeneity of the DNA species. A mixture of denatured and undenatured is usually obtained [61]. Denaturation can be reversed if the critical point resulting in collapse of the total structure has not been reached. There is usually, a reformation of the secondary structure of relatively short distrupted regions on removal of the denaturating conditions.

The reduction of molecular weight to half of the original value does not always occur unless there is a complete denaturation to two single strands which finally coil up to form two compact structures.

In concentrated solutions of DNA, denaturation sometimes results in cross-linking and aggregation, and hence, gel formation.

Jordan, Mathieson and Matty [52], in their studies on the hydrogen ion titration, have confirmed the changes in the shape and flexibility of the native molecules at extremes of pH. A decrease in the radius of gyration from the original value and a decrease in the end-to-end length has been reported for the nucleic acids on denaturation [60] .

III.d Degradation of DNA

Degradation as applied to DNA usually refers to the total break down of the macromolecular structure to the subunits mononucleotides or to a multiple of mononucleotides, the oligonucleotides.

Under drastic conditions of acid and alkali, treatment with 8M Urea, during prolonged exposure to very high temperature or to α and β-radiations, complete or partial degradation of deoxyribonucleic acids has been experienced [60]. Acid and alkaline hydrolysis of the nucleate sometimes produces the nucleosides.

The molecular weight of a degraded nucleate sample usually falls considerably [62 - 64]. Similarly, the molecular size, especially the length of the nucleate is decreased.

IV. The function of Deoxyribonucleic acid and its relation to the structure

Nucleic acids, especially deoxyribonucleic acids have been reported to play key roles in cellular growth, in reproduction and in mutation.

The mechanisms of the processes of cellular growth, reproduction and mutation have been thought to have to do with either the secondary or the primary structure of the nucleic acids.

Records have shown that certain DNAs have transforming activity for streptomycin resistance in pneumococci [65], and it has been believed that only one of the two strands of the nucleic acids is required to carry information for transformation to streptomycin resistance. At least partial reformation of complementary strands into helical regions is necessary to satisfy one or more steps in the transforming process. This can occur however, only for suitably paired DNA strands. On complete denaturation the transforming activity is usually lost. Partial pairing with heterologous polydeoxynucleotide chains also causes a loss of transforming activity. Chromosomal DNAs, record shows, carry all the information necessary for cell growth and reproduction.

The genetic control of protein synthesis has also been recognised [66]. The nuclear DNA serves as a template for the synthesis of nuclear RNA, of which there are not only many different species, but at least three distinct kinds differing markedly in metabolic activity and solubility characteristics.

These are the soluble RNA (s-RNA), the messenger RNA (n-RNA) and the transfer RNA (t-RNA).

The messenger RNA carries the information from DNA in the form of specific base sequence and acts as final templates for amino acid sequence in proteins. Caldwell and Hinshelwood [67] suggested that the 'code' for each of the twenty amino acids in protein might consist of two adjacent nucleotide units, an idea that supports the formation of oligonucleotides. Gamow [68] also proposed a code according to which three nucleotide bases determined one amino acid, with the adjacent triplets overlapping and more than one triplet of bases coding for a particular amino acid.

The process of cell division is also recognised to be genetically controlled and to involve directly, the nucleic acids, (DNA). The cells divide and multiply. During the division of a cell, the hydrogen bonds holding the two strands together are broken and each strands duplicates itself in a semi-conservative way. The two separate strands serve as templates for the synthesis of new complementary chains. Watson and Crick [69] envisaged that during the replication process an unwinding of the helix is expected to enable the formation of separate strands. Levinthal and Crane [70] have found that the unwinding of α-helix is possible and found that only about 150 cal/mole of energy is required for a replicated chain link . This energy is always available with the physiological conditions of the cell.

The workers have all concluded that replication and the unwinding processes must occur simultaneously, for there to be no coiling up of the individual strands.

In view of the suggested mechanisms, in which there is chain separation, the replication procedure will in part, be determined by the stability of the helical structure, and possibly of the individual chains, though these have just a transient existence.

In the formation of some polynucleotides DNA molecules have acted as templates. For example the action of RNA polymerases is sensitive to DNAease, and requires a DNA template primer in addition to all four classical ribonucleoside 5'-triphosphates.
Polynucleotide chain formation along the deep groove of the DNA double helix has been advanced as a possible means of directly relating nucleotide sequence in DNA to that in certain ribonucleic acids.

With the RNA polymerase from E. Coli, single stranded DNA (Ø×174) has been found to produce RNA with a complementary base composition. The related double stranded material gives RNA with a composition virtually the same as that of the primer i.e. both strands can act in vitro as template primer for the production of new RNA [71] .

The secondary or primary structure of DNA comes into play for each role played by the DNA. The secondary structure of DNA also provides a means of explaning the binding between the acid and cations as recorded in this thesis.

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CHAPTER 2

SECTION I THE THEORY OF BINDING

The binding theory discussed here for the polymer and small cation system is based on the simple theory of binding between small ions and molecules. The extended theory which accounts for the interactions between the sites and ligands, or even between the bound ligands is then discussed. Where possible, methods for obtaining the quantities that appear in the various expressions have been pointed out.

I.1 Binding by a set of equivalent and independent groups.

Consider a molecule P, with n groups capable of binding to a ligand L. It is assumed that the sites of binding are structurally and electrically equivalent, and there are no electrostatic or steric interactions between them.

If P is a molecule having one (phosphate) binding site (i.e. n = 1), capable of binding to a monovalent cation e.g. Na^+, then we have at equilibrium

$$P + L \rightleftharpoons PL \tag{2-1}$$

and the association constant k_{ass} is

$$k_{ass} = \frac{(PL)}{(P)(L)} = \frac{1}{k_{diss}} \tag{2-2}$$

The association constant in equation (2-2) is usually expressed in terms of the concentrations of the ions, and for dilute solutions, the concentrations are set equal to the activities even though this may be a gross approximation.

Only one association constant is expected for this type of system provided that only one stoichiometric complex is formed.

An example of such a system is in the addition of a proton to a monobasic acid as considered in the Bronsted theory where a hydrogen ion L, interacts with a neutral or an anionic central group, P. Here corresponding association constant has been termed "Basicity constant" of the base P which is conjugated to the acid PL.

As in the special case of acid-base equilibria, the fraction α, of the binding process where (P) is the concentration of the unbound P, is given as:

$$\alpha = \frac{(P)}{(PL)+(P)} = \frac{k_{diss}}{(L) + k_{diss}} = \frac{1}{1 + k_{ass}(L)} \qquad (2\text{-}3)$$

with all the molecules of P and PL in solution represented.

The average number $\bar{v}$, of moles of ligand L, bound or the fraction of P bound to make the molecule PL, which contains a fraction α of unbound sites is given as

$$\bar{v} = 1 - \alpha = 1 - \frac{1}{1 + k_{ass}(L)}$$

$$= \frac{k_{ass}(L)}{1 + k_{ass}(L)} \qquad (2\text{-}4)$$

and $$\frac{1}{\bar{v}} = 1 + \frac{1}{k_{ass}} \cdot \frac{1}{(L)} \qquad (2\text{-}4a)$$

The quantity $\bar{v}$ has also been defined as the probability that any molecule of P, chosen at random from the solution, will be found to have a molecule or ion L attached to it.

In Bronsted's theory, the various constants have been

expressed in their logarithmic forms as:

$$\log k_{ass} \equiv pk_{diss} \equiv - \log k_{diss} \tag{2-5}$$

and from equations (2-2 to 2-5) we have

$$k_{ass} = \frac{(1 - \alpha)}{(\alpha)(L)}$$

$$\text{and } \log k_{ass} = \log \left(\frac{1 - \alpha}{\alpha} \right) + \log \frac{1}{(L)}$$

$$\text{whence } \log k_{ass} = - \log L + \log \left(\frac{\bar{v}}{1 - \bar{v}} \right) \tag{2-6}$$

1a. Cases when n > 1, and the sites are non-interacting and capable of binding the ligand, L.

Denoting by PL_i , a molecule P which contains one molecule of L attached to the i^{th} binding site, the intrinsic association and dissociation constants for the formation of PL_i from P and L are given as:

$$(k_{ass})_i = \frac{(PLi)}{(P)(L)} = \frac{1}{(k_{diss})_i} \tag{2-7}$$

For this, the corresponding average number, $\bar{v}_i$, of moles of L bound to the molecule P at site i, is

$$\bar{v}_i = 1 - \alpha_i = \frac{(k_{ass})_i (L)}{1+(k_{ass})_i (L)} \tag{2-8}$$

and since the binding sites are equivalent in all respects $\bar{v}_i$ is the same for all the sites and

$$\bar{v} = \sum_{i=1}^{n} \bar{v}_i = n \bar{v}_i \tag{2-9}$$

where the subscript i has been dropped.

In equation (2-9), $\bar{v}$ is the total average number of occupied sites per molecule of P. For n equivalent sites therefore,

equation (2-4) becomes

$$1 - \alpha = \frac{\bar{v}}{n} = \frac{k_{ass}(L)}{1 + k_{ass}(L)} \qquad (2\text{-}10)$$

and $$\frac{\bar{v}}{n - \bar{v}} = k_{ass}(L) \qquad (2\text{-}10a)$$

Equation (2-10a) is the usual Langmuir Isotherm, and has been written in the form which permits the direct determination of the term n from a measurement of the mole of ligand bound per mole of P at equilibrium concetration (L) of the ligand. A plot of $1/\bar{v}$ versus $1/L$ should be linear with a slope of $1/nk_{ass}$ and an intercept of $1/n$, if equation (2-10a) is obeyed. This type of plot, experience has shown, does not always give an accurate value of n, regardless of whether or not the Langmuir equation is obeyed.

Putting $\frac{\bar{v}}{n} = \theta$, equation (2-10a) has been expressed in terms of the intrinsic association constant k_o for one molecule of the adsorbate, and n, the molar concentration of the free adsorbate as

$$k_o n = \frac{\theta}{1-\theta} = \frac{1 - \alpha}{\alpha} \qquad (2\text{-}10b)$$

which is further expressed as

$$\frac{r}{n} = K_o B - K_o r \qquad (2\text{-}11)$$

in which $\theta = r/B$; and r, B are the number of occupied sites and the total number of sites per molecule respectively.

For one type of binding site, and if the Langmuir isotherm is valid, r/n in equation (2-11) is expected to vary linearly with r, and from the limiting values of the slope and the intercept at $r = 0$, k_o and B can be evaluated.

I.1b. Two non-interacting binding sites and one ligand.

Where two equivalent and non-interacting binding sites on a central molecule are involved in binding quantitatively to one ligand L, the association constant for the stoichiometric complex formed has been derived from the equation of the reaction as follows:

(a) Two-step Reaction

$$P + L \rightleftharpoons PL \qquad k_1 \tag{2-12}$$

$$P + PL \rightleftharpoons P_2L \qquad k_2$$

$$k_1k_2 = k = \frac{(P_2L)}{(P)^2(L)}$$

or (b) One-step Reaction:

$$2P + L \rightleftharpoons P_2L \tag{2-12a}$$

$$k = \frac{(P_2L)}{(P)^2(L)}$$

In either case, the fraction of sites unbound (α) is given as

$$\alpha = \frac{(P)^2}{(P_2L)+(P)^2}$$

$$\text{and } \bar{v} = 1 - \alpha = \frac{k_{ass}(L)}{k_{ass}(L)+1} \tag{2-13}$$

$$\text{and } \frac{\bar{v}}{1-\bar{v}} = k_{ass}(L) \tag{2-14}$$

$$\text{where } \log(\bar{v}/1-\bar{v}) = \log k_{ass} + \log(L) \tag{2-15}$$

Equations (2-6) and (2-15) are identical showing that the association constant of the binding is independent of the valence of the ligand.

As an extension to this, suppose there are y ligands, L, and y equivalent and non-interacting central groups, each of two equivalent and non-interacting sites, e.g. y molecules of adenosine diphosphete as the central molecules then the association constant will be y multiple of that in equation (2-15).

In practice if a macromolecule could be constructed with y such central small molecules, covalently joined together with no interactions between the separate parts such that the mode of binding of such a system with y ligands would obey the Langmuir isotherm, the value of the association constant to be expected from a plot of $\log\left(\frac{\bar{v}}{1-v}\right)$ against log (L) would be that for only one of the complexes formed. I have assumed here that each P_2L set could be taken as a separate complex. The overall association constant k_{ass}, which could be taken as the sum of all the various $(k_{ass})_i$, $(k_{ass})_j$ ..... $(k_{ass})_y$ then becomes $(k_{ass})_i \times y$ or $(k_{ass})_{ob} \times y$. Hence for a macromolecule, equation (2-15) can be written as

$$\log\left(\frac{\bar{v}}{1-\bar{v}}\right) = \log(k_{ass})_{ob} + \log(L) \qquad (2\text{-}15a)$$

where $(k_{ass})_{ob}$ = association constant observed

and $$(k_{ass})_{ob} = \frac{k_{ass}}{y} \qquad (2\text{-}15b)$$

For a nucleate ion with say 18,000 phosphate negatively charged sites, binding quantitatively with 9,000 moles of magnesium or calcium, y is 9,000 and the overall association constant will be 9,000 $(k_{ass})_{ob}$.

Rather than define the association constant $(k_{ass})_{ob}$ as that for the i^{th}, j^{th} ..... y^{th} complex, it is more meaningful to define it as the mean association constant, allowing for the interactions (non-electrostatic) between the bound and unbound ligands during the binding process.

I.2 Binding by a set of equivalent and interacting groups.

The latter part of the last section has considered the problem of binding between a number of ligand, L, with twice its number of sites on central molecules, P, with each central molecule possessing two identical and non-interacting sites.

In the problem of binding of small molecules or ions with a polyelectrolyte e.g. a polynucleate ion, the situation is slightly different. Here the binding sites are no longer non-interacting (at least electrostatically). Two types of electrostatic interactions have always been considered in this problem. The first one, the vertical nearest neighbour interaction and the second, the horizontal interactions.

The vertical nearest neighbour interactions is the more important and is the one discussed. As discussed in chapter four, the electrostatic interactions especially the vertical nearest neighbour interactions between the identical charged sites will often lead to an extension of the polymer chain, and will modify its binding with the ligands.

The presence of some metallic ions has been reported to cause a diminution of the expansive electrostatic interaction in a polymeric ion. In other words, the presence of the electrostatic repulsive interactions would encourage the binding of metallic cations to the macro-ion. As a result of this charge neutralisation through binding, there will be a shortening of the statistical and contour length of the polymer.

The mutual interactions of the bound ligand molecules or ions will alter the effective free energy of association of unbound ligands with the macro-ions, and for a given set of external conditions of e.g. temperature and pressure, the free-energy of association is a complicated function of the fraction of the available sites which are occupied by the adsorbates [l],[m] . Hence it is to be expected that the binding of cations by a macro-ion would be strongly ionic-strength dependent and the free energy of association, ω, would decrease with increasing ionic strength.

The state of affairs as observed for nucleic acids is slightly different from the general case in which all the binding sites are directly above each other. In this case, the doubly stranded polynucleotides undergo a helix-coil transition and as a result may possess a varying helix content. Hence the vertical interactions between neighbouring sites are affected by the formation of the helix, since the sites are no longer directly above the immediate neighbour.

An additional set of horizontal interactions between the molecules bound to the two strands of a helical region is therefore set in. This is negligibly small as compared to the vertical interactions, however. The two interactions have often made the complete theoretical description of the binding system very difficult, and generally, where there are two or more different types of binding sites, the situation usually becomes complex.

I.2a. Binding of small ions or molecules to a macro-ion with n identical, but interacting sites.

For a macromolecular ion of n identical sites which interact with one another, at least electrostatically, such that binding at any site affects the binding affinities at other sites, the interaction effect is accounted for by allowing the association constant k_{ass} and the standard free energy change ΔF° to vary with $\bar{v}$, where $\bar{v}$ still carries its usual meaning.

We define the intrinsic association constant, k_{int}, as the limiting value of the association constant k_{ass}, when $\bar{v} = 0$, i.e. before any interaction due to binding has occured. Corresponding to an intrinsic association constant is an intrinsic standard free energy change ΔF°_{int} . Using an arbitrary function $\phi(\bar{v})$ to account for the interaction effect, for $\bar{v} > 0$, we have

$$\Delta F^{\circ} = \Delta F^{\circ}_{int} + RT\,\phi(\bar{v}) \qquad (2\text{-}16)$$

$$\text{or} \quad k_{ass} = k_{int} \cdot e^{-\phi(\bar{v})} \qquad (2\text{-}17)$$

From equations (2-16) and (2-17) it can be seen that if

$\phi(\bar{v})$ increases with $(\bar{v})$ the occupation of sites makes binding at other sites more difficult, and if it decreases with $(\bar{v})$, there are cooperative interaction between sites. When $\bar{v}$ is zero however, the function $\phi(\bar{v})$ also becomes zero.

Apart from the interactions between the sites, which have been accounted for in equations (2-16) and (2-17), the sites are no longer strictly identical since they are interacting during the binding process. This is because the sites located nearest an occupied site will usually be subjected to a stronger interaction than those further away. The quantities ΔF and $\phi(\bar{v})$ as here defined, are therefore, average properties of all sites and configurations at any value of $\bar{v}$.

Hence equation (2-14) for the prevailing conditions of interaction can be written as $\frac{\bar{v}}{n-\bar{v}} = k_{ass} L = k_{int} \cdot e^{-\phi(\bar{v})} \cdot L$ (2-18)

and

$$\log\left(\frac{\bar{v}}{n-\bar{v}}\right) = \log k_{int} - 0.434\, \phi(\bar{v}) + \log L \qquad (2\text{-}19)$$

Equation (2-18) can be utilized in one particular way. If the combining sites can be shown to be identical and their number, n, is known then equation (2-19) can be applied directly to determine the qualitative nature of the interactions between sites. This equation has been applied to different binding problems, especially to those of binding of oxygen molecules with haemoglobin in which the function $\phi(\bar{v})$ decreases with $\bar{v}$.

Cases where $\phi(\bar{v})$ increases with $\bar{v}$ are however more common especially where there are electrostatic types of interactions between the sites or between the molecules of the adsorbates bound.

I.2.b. The electrostatic term $\phi(\bar{v})$ for the polyelectroytes

The predominant form of interaction for the case of binding between small molecules or ions and a polyelectrolyte is electrostatic, i.e. $\phi(\bar{v})$ is directly related to the effect of charged sites on the chemical potential of the reacting species. The electrostatic forces may be non-specific, hence account must be made of all charges, bound or unbound, present in the solution in computing $\phi(\bar{v})$. The intrinsic properties of the molecule must therefore be redefined as those properties which it would have in the absence of any charges rather than when $\bar{v} = 0$.

For polyampholytes which possess no form completely devoid of charge, it is necessary in the definition of the intrinsic properties of the molecules to refer to a hypothetical discharged state rather than the state when the net charge is zero. It can be assumed that all electrostatic interactions become a minimum when the average net charge per molecule $\bar{z}$, is zero. Hence the intrinsic properties of a site of a polyampholyte are those it possesses when $\bar{z} = 0$.

For many polyelectrolytes, the state $\bar{z} = 0$ is actually a discharged state, but with polyampholytes with the two types of charges positive and negative the interactions between these charges are in general, not zero when $\bar{z} = 0$, and the intrinsic properties there defined will then be different from those for a completely discharged state.

The standard free energy of the molecule therefore becomes at any stage of the binding,

$$\Delta F^{\circ} = \Delta F_{int} + \bar{\nu} RT \, \phi(\bar{z}) \qquad (2\text{-}20)$$

The function $\phi(\bar{z})$ in equation (2-20) is a positive function of $\bar{z}$ for combination with a positive ion and a negative function for combination with a negative ion i.e. a net (positive) charge will always tend to repel a positive ion and attract a negative one. Analogous to equation (2-18).

$$\frac{\bar{\nu}}{n-\bar{\nu}} = k_{ass} \, L = k_{int} \cdot e^{-\phi(\bar{z})} \cdot L \qquad (2\text{-}21)$$

The function $\phi(\bar{z})$ has been related to the electrostatic free energy, Wel, of the macromolecule in a simple approximation that the average free energy per macromoleculr in a solution can be replaced by the free energy of the average molecule (characterised by the average charge, $\bar{z}$). Considering the process of adding, on the average, $d\bar{\nu}$ ions of charge z_i to a macromolecule which already has on the average $\bar{z}$. On the average, $d\bar{\nu}/n$ ions will be added to each one of the n identical sites for binding. The resulting free energy change dF per mole of sites will be just $d\bar{\nu}/n$ times the value of ΔF given by equation

$$\Delta F = \Delta F^{\circ} + RT \ln \frac{\alpha}{(1-\alpha)L} \qquad (2\text{-}22)$$

$$\text{i.e.} \quad dF = \left[\Delta F^{\circ} + RT \ln \frac{\alpha}{(1-\alpha)L} \right] \frac{d\bar{\nu}}{n} \qquad (2\text{-}23)$$

By considering the energies involved in the process of completely discharging the macromolecule from $\bar{z}$, and then charging it to a value of $(\bar{z} + z_i\, d\bar{v})$, it has been shown that the free energy change dF in equation (2-23) can also be expressed as

$$dF = \left[\Delta F - NW_i + Z_i N \left(\frac{dW_{el}}{d\bar{z}} \right) + RT \ln \frac{\alpha}{(1-\alpha)\, L} \right] \frac{d\bar{v}}{n} \qquad (2\text{-}24)$$

where ΔF has been used for the standard free energy change for reaction of the discharged species, which may be different from that of the reaction of the charged species ΔF°, W_{el} is the electrostatic free energy of a single macro-ion, N, the Avogadro's number and W_i the work of charging a single ion of charge Z_i .

From equations (2-23) and (2-24) we have

$$\Delta F^\circ = \Delta F - NW_i + Z_i\, N\, dW_{el}/d\bar{z} \qquad (2\text{-}25)$$

The electrostatic free energy W_{el} has been shown to be equal to $A(\bar{Z})^2$ where A is a constant for the particular molecule, and includes terms in the radius of the molecule, the ionic strength and dielectric constant of the medium, and the temperature of the system.

Differentiation of the expression, $W_{el} = A(\bar{Z})$ ... with respect to $(\bar{Z})$ and the substitution of the result into equation (2-25) gives

$$\Delta F^\circ = \Delta F - NW_i + 2AN_{zi}\, \bar{Z} \qquad (2\text{-}26)$$

and $\Delta F^\circ_{int} = \Delta F - NW_i$

Since by definition, $\Delta F^\circ = \Delta F^\circ_{int}$ where $\bar{z} = 0$

Hence

$$\Delta F^\circ = \Delta F^\circ_{int} + 2AZ_i\, \bar{Z} \qquad (2\text{-}27)$$

and from equations (2 - 20) and (2 - 27)

$$RT\ \phi(\bar{Z}) = 2AN z_i \bar{Z}$$

$$\text{and} \qquad \phi(\bar{Z}) = \frac{2ANz_i\ \bar{Z}}{RT} \qquad (2\text{-}28)$$

and making the substitution $\omega = AN/RT$ we have

$$\phi\ (\bar{Z}) = 2\ \omega\ z_i\ \bar{Z} \qquad (2\text{-}29)$$

The term, ω, which appears in equation (2-29) is a constant which is independent on z_i and $\bar{Z}$, but depends on the model chosen to represent the macromolecule, i.e. on which particular expression is used for A in equation $W_{el} = A(\bar{Z})^2$, and on the parameters occuring in the equation. A few examples have been quoted.

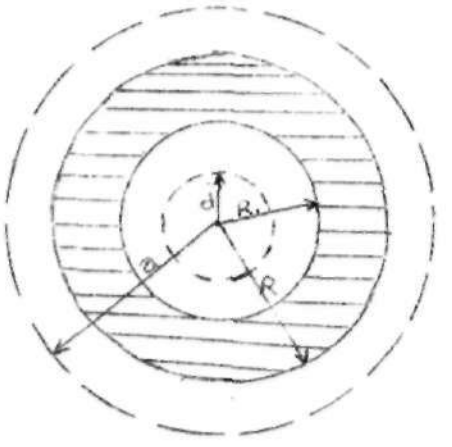
(i) For flexible linear polyelectrolytes,

$$W_{el} = \frac{3z^2\ e^2}{2DR_e} \left\{ \frac{1}{K^2R_e^2} - \frac{3}{2K^5R_e^5} \left[K^2R_e^2 - 1 + (1+KR_e)^2 . e^{-zKR_e}\right] \right\} \qquad (2\text{-}30)$$

where R_e is the radius of a sphere equivalent to the polyelectrolyte ion, and it is a quantity of the same order of magnitude as the radius of gyration, R_G, D is the dielectric constant of the medium and K is the reciprocal Debye radius depending on the ionic strength. Equation (2-30) was derived by Hermans and Overbeck [n] and has been employed in calculations on the properties of polyelectrolytes.

(ii) In particular, the approximate electrostatic energy of long rod-shaped ions has been computed by Hill [0] for a number of polyelectrolytes. The expressions for W_{el} obtained by Hill have been assumed to hold for nucleic acids for the structure proposed by Crick and Watson. One of the two expressions developed by Hill is included here.

Hill in his treatment has considered a cylindrical molecule of a cavity radius, R, down its centre, which contains solvent. For this molecule, there is a layer from which ions are excluded both on the inside and on the outside, i.e. there are mobile ions only where $r < d$ and $r > a$ in the model shown



Hill's model with z charges spread uniformly over the outside of the cylinder ($r = R$)

For this model Hill has shown that

$$W_{el} = \frac{z^2\epsilon^2}{DL} \frac{\left[\frac{K_o(Ka)}{K_a K_1(K_a)} + \ln\frac{a}{R}\right]\left[\frac{D_i\, I_o(Kd)}{DKd\, I_1(Kd)} + \frac{D_i}{D}\ln\frac{R_1}{d} + \ln\frac{R}{R_1}\right]}{\left[\frac{D_i\, I_o(Kd)}{DKd\, I_1(Kd)} + \frac{D_i K_o(Ka)}{DK_a K_1(Ka)} + \frac{D_i}{D}\ln\frac{aR_1}{dR} + \ln\frac{R}{R_1}\right]} \qquad (2\text{-}31)$$

In equation (2-31), D_i is the dielectric constant between $r = R_1$ and $r = R$ i.e. the dielectric constant of the macro-ion material. The K functions are modified Bessel functions of the second kind, and the I functions are modified Bessel functions of the first kind [P] .

It is difficult to assign a value to D_i .

In conclusion, until one is able to determine all the separate quantities that appear in equations (2-30) and (2-31) accurately, the theoretical evaluation of W_{gl} may not be possible, and the use of equations (17, 18, 19 and 21) in this chapter to determine the association constant k_{ass} in the binding process may be impracticable.

SECTION II: Account of Previous Work on Binding of Cations, Particularly Magnesium (II) and Calcium (II) with DNA

Almost at the beginning of the study of the interactions of nucleic acid with metallic ions, Shack, Jenkins and Thompsett [1] discovered that the addition of a simple electrolyte reduced the molar absorptivity $\epsilon(P)$, of a well prepared nucleate solution. From their UV absorption studies of nucleic acid solutions at 259 mμ they found that the effect of added electrolyte depended on the charge of the cation, divalent cations like magnesium (II) and calcium (II) being more effective than the sodium ions or univalent ions in lowering the absorption of the nucleate solution. The authors also reported that the presence of anions such as Cl^- in sodium chloride, or in calcium chloride, exerted no influence on the absorption.

They also showed that the quantity of divalent cation needed to bring about the transformation was about the same as that of the phosphorus in the nucleic acid. They concluded that the lowering of $\epsilon(P)$ arose from the binding of the cations by the phosphate groups of the nucleate. In their study of different concentrations of the nucleate, it was found that the extent of the spectral change was primarily a function of the ratio of the divalent cation concentration to nucleate phosphorus concentration, and was not directly a function of the salt concentration itself.

By the method of conductance measurements, they showed that divalent cations are more effective in binding at far lower concentrations than the univalent cations.

The results of the experiment and the conclusions of Thomas [2], Steiner and Beer [3] have supported the idea that the metallic ions are associated with the ordered structure of the nucleic acid. This association has been shown to be a necessary requirement for the prolonged existence of the macromolecular species in solution.

It has also been recognised that the denaturation temperature (Tm) of a nucleate preparation is a function of the (G + C) ratio (i.e. the percentage of guanine and cytosine) of the preparation, in an expression given as Tm = 69.3 + 0.41 (G + C), in 0.2M sodium chloride, but it is also influenced by the concentration of metallic cations [4].

Most investigators have assumed that binding of metallic cations to nucleic acids and polynucleotides is predominantly a function of the charged phosphoryl residues [5], as supported by the comparison of estimates of binding obtained from potential measurements and analytical data [6].

II.1 Binding of magnesium (II) and calcium (II) with nucleic acids and polynucleotides

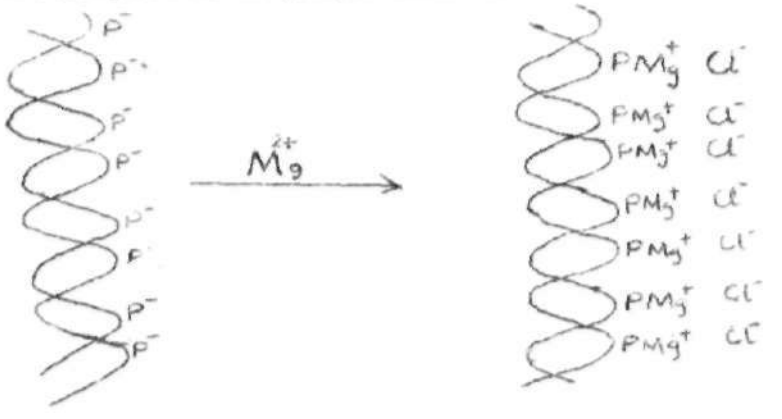
Various investigations on the binding of magnesium with nucleic acids had been in progress for about twenty years now.

Cavalieri [7], Shack, Jenkins and Thompsett [6]; Zubay and Doty [8]; Zubay [9]; Shack and Bynum [10]; Pullman and Pullman [11] have shown that, in the presence of low concentrations of sodium chloride, magnesium is strongly bound to both denatured and

undenatured DNA, binding by the undenatured DNA being the stronger. In the presence of higher concentrations of sodium ions, however, binding of magnesium is reported to be considerably reduced.

The authors have suggested that the primary sites involved in the binding are the charged phosphate groups, and envisaged that under suitable conditions, chelation with the purine rings in single stranded or denatured polynucleotides could presumably occur.

In experiments in which the activity coefficient of magnesium ions was measured in MgDNA - $MgCl_2$ mixtures [12], it was found that magnesium ions was bound to an extend greater than one mole of the ion to two moles of DNA phosphorus. This indicated that some magnesium ions were rendered inactive either by binding to other portions of the DNA than the phosphate sites, or by ion pairs at the phosphate sites, with a 1:1 mole ratio. The concept of ion pairing has been considered as the more probable, and would lead to charge reversal on the segment of DNA polyion, producing areas with the properties of polycations with univalent counterions (Cl^-).



Charge reversal in the binding of magnesium to DNA

The authors have further concluded that charge reversal could lead to a destabilisation of the double helix of the nucleic acid because of the vertical electrostatic repulsive interaction between the magnesium ions carrying one charge each.

Shack and co-workers [1], by the method of conductance measurements have found that in distilled water there appeared to be a sharp end point to the titration of DNA solution with magnesium or calcium ions at about 0.8 equivalent of magnesium or calcium per mole of polymer phosphate. On increasing the number of experiemental points, another end-point at 1.0 equivalent of divalent cation was obtained, and on lowering the pH from 7.6 to 5.8 before starting the titration, most of the combining groups on the polymer appeared to have been rendered inactive.

Similar observation has been made by Fresco and Doty [13] in their study of poly A. They assumed a transition of poly A to a new form below pH 6.5 in distilled water. Beers and Steiner [14] have supported this view and have suggested that the phosphate groups in the new structure are involved in internal "salt linkages" with positively charged adenine groups in such a manner as to reduce the affinity of the phosphate groups for divalent cations.

Shack and co-workers [1] have concluded therefore, that there is binding of cations with polynucleotides up to the point where the conductance curve with DNA becomes parallel with that without DNA,

and none beyond this point. They further suggested that the first end point might be the point at which sodium ions, about half of which are probably held tightly in the vicinity of untitrated polynucleotides, begin to be replaced with higher efficiency by divalent cation, or it might represent a major change in the structure of the polynucleotide [6, 8, 15]. Similar results have been obtained for calcium chloride and barium chloride.

Felsenfeld and Huang [16], have however disagreed with the conclusions of Zubay and Doty, that the purine bases are involved in the interaction with the cations.

The most direct experiments on magnesium binding to DNA have been reported by Shack and Bynum [17]. These experiments have indicated that magnesium is bound tightly by DNA at ionic strength 10^{-3}M (sodium chloride), that magnesium is replaceable by sodium ion in high concentration of sodium ion, thus supporting Thomas [18]. Shack and Bynum also concluded that magnesium is more strongly bound to the native than the denatured DNA in conflict with the results of conductance studies reported in references [8, 9, and 19] In these references, magnesium has been reported to bind more strongly to denatured than undenatured DNA.

The stabilising effect of magnesium binding with DNA has been reported by a number of workers. Eichhorn [20] has reported that the presence of magnesium ions raised the thermal denaturation temperature of a particular DNA preparation from 63° to 80°C.

The work of Dove and co-workers [21] has shown that magnesium and cobalt are bound almost stoichiometrically by DNA at a concentration of about 3×10^{-4} M, and that the melting temperature (T_m) increases generally by 35° to 45°C. Their study has shown that the observed breadth of transition is greater for half equivalent of divalent cation per mole of DNA phosphate than for zero or one equivalent. This finding has been attributed to stronger ion binding by native than by denatured DNA.

Lyons and Kotin [22], by means of spectrophotometric and magnetic resonance techniques have studied the effect of magnesium ion on the thermal and phase stability of DNA. They concluded that (i) magnesium interacts with DNA at the phosphate site only; (ii) whereas aqueous solutions of pure magnesium salt of DNA are relatively resistant to thermal denaturation, their thermal stability is reduced in the presence of added magnesium chloride. (iii) there is a precipitation of DNA with an excess of magnesium.

They proposed a mechanism where site-bound magnesium forms ionic links between separated DNA strands through -P-O-Mg-O-P-complexes, leading to the exposure of the bases to solvent. This is followed by hydrophobic base-base interaction, leading to large aggregates, and finally, phase separation.

Earlier, Lyons and Kotin [23] had shown that, in the absence of added simple salt e.g. sodium chloride, MgDNA is more resistant to thermal denaturation than is NaDNA, i.e. the complex of MgDNA is more thermally stable than NaDNA, indicating a stronger binding by magnesium

than sodium ion to DNA. For a heat denatured MgDNA, Lyons and Kotin have found that, after quenching and then treating with magnesium chloride, a precipitate was formed. This type of precipitate was not obtained when the native MgDNA was treated with magnesium chloride. The authors have concluded that the role of magnesium(II) or magnesium chloride is in the ionic linkage between strands of Mg-DNA.

Eichhorn and Butzow [24] have discovered that in the presence of magnesium ions, denaturation temperature of a nucleate preparation is not only increased by about 35°C, but there is a corresponding and greater decrease in the UV dbsorption on cooling. This greater decrease in absorption on cooling has also been observed and explained by Lyons and Kotin [12]. Their explanation is that magnesium ions hold single chains of DNA in close proximity after the hydrogen bonds are split during heating, so that more of the hydrogen bonds can reconnect during the cooling process. These authors have assumed that calcium, barium, nickel and cobalt would all behave like magnesium.

Eisenger and co-workers [25] have shown that, unlike the MgDNA system, thermally denatured NaDNA aggregates in the presence of sodium chloride, but with no precipitate formed.

Other experiments which have been reported on the interaction of magnesium with DNA include those of Blout and Asadourin [26].

Blout and Asadourin have found that the addition of sodium chloride to an aqueous solution of DNA lowers the absorbance (between pH 6.5 and 7.0) by an amount varying linearly with the logarithm of the salt concentration. They have visualised the action of salt as effectively separating the absorbing groups from each other, and thus lowering the absorption.

Thomas [18] has studied the addition of sodium ions in the form of sodium perchlorate to a system of DNA-Mg with one equivalent of magnesium bound per mole of phosphorus. He found that the melting point (Tm) decreased significantly with increasing sodium ion concentration, then increased as the linear dependence of (Tm) on log $[Na^+]$ was achieved.

The sodium ion can replace magnesium ion at rather low concentrations of the latter, but do not stabilise the native structure as well, once bound.

An almost complete breaking down of the primary structure of nucleic acid, i.e. of the polynucleotides, has also been reported in the presence of magnesium ions. In 1952, Tamm and co-workers [5] reported that magnesium (II) ions degrade denatured DNA to soluble nucleotides to the extent of 20% in 160 hours at 37°C.
Berg [27] in his personal communication [28] also reported a certain optimum pH of degradation for certain divalent cations such as zinc and magnesium.

For magnesium he has reported no degradative reaction at neutral pH and initially at pH 9. However, he has observed a reaction at pH 10 with no reason for this given. He indicated that at high concentration of magnesium there is degradation even below pH 10. Butzow and Eichhorn [29] have reported the degradation of poly A (polyadenylic acid) by magnesium, and by manganese, cobalt, nickel and copper.

II.2 Copper - DNA System

As reported by Eichhorn [20], copper ions can effect the lowering of the denaturation temperature (Tm) of a nucleate preparation from about 63° to 42°C. Eichhorn observed no decrease in the UV absorption of the nucleic acid-copper system on cooling.

The effect of copper on (Tm) of nucleic acid has been explained by Michelson [30], as due to the coordination of the copper ions with the electron donor groups in the nucleate bases, thus destroying the hydrogen bonds and preventing their reformation, as with denaturation in the presence of formaldehyde. This idea has been supported by Eichhorn [20] and by Hiai [31].

Copper, in a 1:1 ratio to polynucleotide prevents the formation of poly (A + U) i.e. a polymer of adenylic acid and uridylic acid, and brings about the dissociation of the poly (A + U) complex produced in the absence of the metal [32]. Poly (I + C) is similarly dissociated by copper (II) ions. The addition of sufficient electolyte as sodium ions has been found to reverse the copper (II) induced dissociation of poly (I + C). The effect has been reported to be the same for RNA.

Generally, a temperature of about 60°C is required for the degradation by copper ions to go to completion in one day. This type of degradation has been reported only for RNA and not DNA where there is no 2'-hydroxy group. The sugar-phosphate backbone of DNA therefore remains unaffected by copper ions [24], [33].

Eichhorn has also observed a reversible effect of copper ion on (Tm) of a DNA preparation. The reversibility is achieved in high ionic strength of sodium chloride as reported by Eichhorn and Bamann [32]. Eichhorn has drawn the conclusion that the reaction of copper with DNA is a technique for reversibly breaking and remaking hydrogen bonds. For denaturation of RNA, milder conditions of temperature have been found necessary.

In part of the experiments conducted by Eichhorn and Tarien, it was discovered that copper did not bind to the uracil or thymine base in a nucleoside linkage. Copper has also been found to react with poly-A, but not with poly-U, and poly-A reacts with poly-U at low ionic strength of copper. In high concentrations of copper ion, no poly (A + U) was formed.

The formation of poly (A + U) is faster in the presence of a small quantity of copper ion than in its absence. This has been assumed to be because, copper initially bound to DNA, binds to the phosphate groups, and stabilises the DNA double helical structure. Additional quantities of the metallic ions are then bound to the bases and cause the denaturation of the DNA [34].

Copper ion in its high concentrations destroys poly (I + C) at high temperature [32] .

The formation constants of DNA - Cu^{2+} complexes have been determined polarographically by Miller and Bach [35] . It was found to decrease with the degree of binding. This has made the authors conclude that binding causes a reduction in the electrostatic potential ψ on the DNA molecule. The binding constants computed have been found to conform to the relation, $K = K_o \exp [- ze/kT]$, where (- ze) is the effective charge on the nucleate, k the Boltzmann's constant and T, the absolute temperature. K_o is the binding constant at zero potential. Its value has been found to depend on the nature of the competing counterions to the phosphate group of the DNA. It has been found to be apparently small when sodium serves as the competing counterions. When copper ion is bound to DNA, the electrostatic potential of the nucleate is reduced, and the binding constant for further binding is lowered [36] . This suggests that the lowering of the binding constant by the ions must occur via their effect on the electrostatic potential.

The binding constant to a site on a macromolecule has been found to be affected by the previously bound molecules . In the case of copper, the effect is non-cooperative [37] - [39] .

II.3 Zinc - DNA System

Poly-A is degraded in a 2-hour period by zinc (II) at the neutral pH. Again, at the neutral pH, zinc ions have also been found to degrade poly-C, poly-U, and RNA at approximately the same rate, with poly-I degraded very much more slowly [29] . It has been shown that the zinc-poly-I complex is unordered, at least to the extent that hypochromicity could be an adjunct of order. The mid-point of the transition from the triple-helical structure of poly-I to the unordered structure has been found to occur in 1.0M sodium chloride at 42°C.

The zinc-poly-I complex (with only 1×10^{-3}M added sodium nitrate), on the other hand, has been found to exhibit the absorbance characteristic of the unordered structure only at 25°C, and no transition was observed when the temperature of the system as raised or lowered.

The fast degradation of poly A by zinc has been attributed to the fact that the completely filled 3d shell of zinc results in zero change in crystal field stabilisation energy on substitution [28].

Eichhorn and co-workers [29] have also assumed that the slowness of the reaction of zinc with poly I is due to the weakening of the zinc-phosphate bond through simultaneous binding of zinc to the inosine base, and perhaps because there are many more hydrogen bonds in the poly-I complex.

The results on zinc degradation of poly-A and poly-I have indicated that only the 2' and 3' monophosphates are produced in large quantities, and that zinc breaks the phosphodiester linkage at the 5'-phosphate position.

No inorganic phosphates were reported to be produced in the degradation of poly-A and poly-I with zinc during a 50-hour incubation period [24], [40].

II.4 Lanthanum - Polynucleotide System

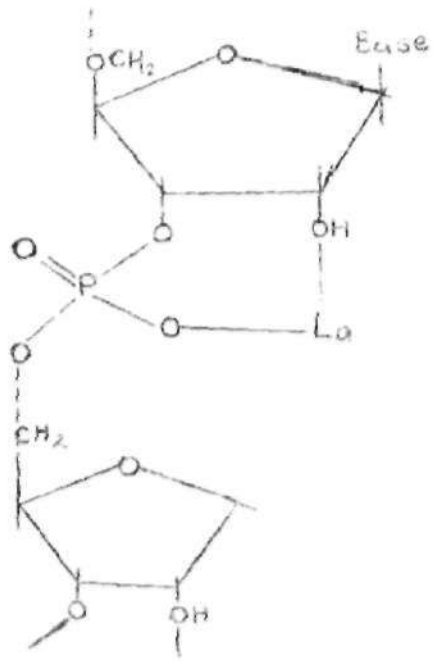
The interaction of lanthanum (III) with nucleic acids and polynucleotides has been widely studied. Hamann, et al [33] have used lanthanum (III) and cerium (III) in dephosphorylating mononucleotides. They have shown that both ions are also capable of degrading RNA at 37°C and over a period of many days.

Eichhorn and Butzow [24] used the findings of Bamann as a basis and studied the heating of poly-A, poly-U, poly-C and poly-I in the presence of lanthanum at 64°C. The amount of "soluble nucleotide" was determined with time of reaction. They observed that there was no increase in the amount of soluble nucleotide in the absence of lanthanum, even when heating was done for 40 hours. They have also observed that the degradation of poly-I proceeded much more slowly than that of the other polynucleotides, with the kinetics of degradation not following a simple order.

A mechanism of degradation by lanthanum was proposed which proceeds through a process in which the large polynucleotides are attacked randomly wherever phosphate happens to be at any position along the chain.

These conclusions and proposals of Eichhorn and Butzow have been supported by the results of the study of sedimentation coefficient of the homopolynucleotides in the presence of lanthanum, that the metallic ion attacks the polynucleotide chain throughout its length.

Baumann et al [33] have proposed a structure for the intermediate during the cleavage of the phosphate bond of lanthanum-polynucleotide complex. Evidence for the structure proposed has been obtained in their studies.



The structure of an intermediate in the cleavage of a polynucleotide by lanthanum proposed by Baumann et al.

Bamann and co-workers have suggested that probably the lanthanum ion functions to weaken the electron density of the hydrogen-susceptible bond, as other metal ions generally do [41, 42].

For poly-A, poly-C, and poly-U only the 3'-monophosphates have been formed in significant quantities as reported. For poly-I, however, both 3'- and 2'-monophosphates have been obtained in large quantities. In either case, lanthanum breaks the phosphodiester linkage at the 5'-phosphate position.

Dimroth et al [43] have also succeeded in degrading RNA with lanthanum and other trivalent cations like Co^{3+} and Bi^{3+} to its nucleosides. In all the experiment conducted, one mole of La^{3+} was found to bind three nucleotides.

No degradation of DNA by lanthanum has been reported. This is because of the absence of the 2'-hydroxyl group in DNA [33].

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CHAPTER 3

The preparation of Deoxyribonucleic acid (Sodium salt)

The preparation of deoxyribonucleic acids can be discussed under two headings.

i) The preparation of the polyacid-polybase (deoxynucleoprotein) in which deoxynucleic acid is chemically anchored to a basic protein, histone or protamine.

This preparation requires mainly, the removal of ribonucleoprotein from the deoxynucleoprotein. Excluding the methods which involve the use of acid or alkali, three main methods have been used for removing ribonucleoprotein. The first uses water or an aqueous solution of low ionic strength in sodium chloride [1-9]. The second method however, uses solutions of high ionic strength in sodium chloride [10-12].

The first method is preferred to the second since there is dissociation of the deoxynucleic acid from protein at high ionic strength of sodium chloride [13-16]. On precipitation, a protein-nucleate is obtained which is unlikely to resemble in specific detail, the original nucleoprotein.

The third method was used by Kirby [17] in the preparation of ribonucleic acid. It consists of homogenising the tissue in a mincer and treating the homogenate with an equal volume of phenol solution (90 per cent. w/v) with stirring, after filtration through a Buchner funnel to remove fibrous tissue.

Two layers are obtained after centrifugation. The lower phenol layer contains the deoxynucleoprotein and protein. The top aqueous layer contains the ribonucleoprotein and polysaccharides and is removed by suction. The lower phenol layer is washed with water to obtain a mixture of dexynucleoprotein and protein only.

ii) After preparing the deoxynucleoprotein in solution of low ionic strength, or one of high ionic strength, the protein is removed using one of, or a combination of a number of agents such as chloroform [10]; carbontetrachloride/amyl alcohol mixture [10], high concentrations of sodium chloride [1]; a detergent such as sodium dodecyl sulphate [19]; Duponol detergents, Xylene sulphonate, or by digestion with chymotrypsin [18]. In most cases, the protein-nucleic acid bond is severed after the treatment with one of the above agents, with the protein precipitated simultaneously, and removed by centrifugation. Treatment of the supernatant nucleate solution with 95% ethanol or methanol leaves a fibrous, white material, the sodium salt of deoxynucleic acid.

In practice, all the methods of nucleic acid extraction lead to the formation of nucleate of various degrees of purity. On the account of this, the method giving a nucleate of highest purity is often preferred.

The method of deprotenising with chloroform gel used by Gulland, Jordan and Threlfall [10] gives a preparation having low molecular weight, following a marked fractionation during isolation and extraction.

Similarly, the methods using saturated sodium chloride [1] and Chymotrysin [18] give poor yield and fractionated material.

The method using anionic detergent to deprotenise the nucleoprotein [19] however, has been found to be most commonly used, because it has the advantage of a good yield of a material of good qualities.

The method of anionic detergent normally produces a nucleate with a high molecular weight, of a phosphorus content of about 9.2%, of a maximum absorption in the UV region between 257 and 261 mu and of molar absorptivity ε(P) of 6600. It is usually fibrous and of a high viscosity in its aqueous solution. It is usually free of protein and ribonucleic acid or its protein complex. This method resolves the problem of enzyme depolymerisation which normally confronts the workers by using a sodium citrate solution and working at $0^{o}C$. Both of these conditions suppress the enzyme action of depolymerisation of the nucleate.

The method of Kay, Simmons and Dounce [18], as employed to the extraction of the nucleic acid sample used for the investigations reported in this thesis

About 150 grammes of fresh calf thymus were chopped into small fragments and homogenised in a high speed mincer for about three minutes with 600 mls of ice-cold 0.9% sodium chloride solution, containing 0.01M sodium citrate. (The pentosenucleoproteins are generally removed into solution at this stage, since they are quite soluble in this salt concentration of 0.9% or 0.15M. The deoxynucleoproteins are insoluble in the salt concentration, and are separated from the ribonucleoproteins by centrifugation at 2500 rev/min). The supernatant solution of ribonucleoprotein was discarded and the sediment washed by homogenisation with the sodium chloride-citrate solution, followed by centrifugation thrice.

The sediment was homogenised for one minute in a high speed mincer with three litres of ice-cold 0.9% sodium chloride, and the material treated with 270 mls of detergent solution (5gm of sodium dodecyl sulphate in 100 mls of 45% ethanol), added with stirring. At this stage the deoxynucleoprotein complex was dissociated to deoxynucleic acid and protein.

The resulting gel was stirred vigorously at room temperature for three hours, after which a viscous, syrupy solution was obtained. Taking the sodium chloride concentration to one molar by adding about 160 grammes of sodium chloride, a solution with a marked drop in viscosity was formed after stirring for about 10 minutes to homogenise solution. The solution was centrifuged at room temperature at 3000 rev/min to remove the protein sediment. Ninety five percent ethanol was added with stirring to obtain a gelatineous, fibrous, white precipitate, which was wound up on the end of a stirring rod. The entrapped supernatant which was a crude product, was pressed out washed three times with 95% ethanol and then with acetone, until the washings were no longer cloudy.

The Crude product (about 5 grammes) was suspended in about 1750 mls of distilled water at room temperature, and dissolved by rapid stirring. On complete dissolution, about 160 mls of the detergent solution were added followed by a further stirring for another one hour. The sodium chloride concentration was brought to one molar by adding 112 grammes of solid sodium chloride, with stirring (at this stage the nucleate which is highly soluble in this concentration of salt remained in solution, while the residual protein and ribonucleoprotein, if any, precipitated out). The mixture was centrifuged at 13,000 rev/min for $1\frac{1}{2}$ hours.

The deoxynucleic acid was precipitated from the supernatant by the addition of one volume of 95% ethanol and washed as before. A fairly pure product was obtained as determined by the phosphorus percentage.

The whole process follwed after the formation of the crude product was repeated using 1750 mls of distilled water for solution and 0.9% sodium chloride, at which concentration any protein remaining was precipitated with a little amount of nucleate. The solution was centrifuged at room temperature at 13,000 rev/min for $1\frac{1}{2}$ hours, and the sodium chloride concentration adjusted to one molar. A careful addition with stirring, of 95% ethanol produced a fibrous product which was washed as before with ethanol, and then with acetone, and finally dried in air. The yield of the final product was about 3 grammes.

The nucleic acid extracted, in the form of its sodium salt, and used for the measurements, contained about 9.15% phosphorus. It has been labelled (CTA). Its molecular weight as determined by the method of light scattering is 12.44×10^6. The nucleate exhibits a maximum of absorption at 259.5 mμ and of $\grave{\epsilon}(P)$ value of 6594 at 259.5 mμ, in neutral aqueous solution. The percentage of water in the fibrous material, as determined by heating a sample of the material to a constant weight in an oven at 110°C is 2%. From the percentages of the water and phosphorus contents, the nucleate contained a negligibly small amount of protein or other contaminants.

As obtained here and as from other methods, the sodium salt of dexynucleic acid is the final product. It has been shown to be a good representation of the native molecule both molecularly and structurally.

The method of phosphorus determination

The method of estimation of phosphorus in the nucleate sample [20] was modified in this laboratory, and it consists in the hydrolytic degradation of the pure material and conversion of the product to inorganic phosphates which are capable of showing absorption at 680 mu on reacting with an ammonium molybdate solution.

Each dexyribonucleic acid sample was first dried in the oven at 110°C for one and a half hours, and was kept in a dessicator.

For each sample, a solution containing about 0.005 gm of DNA in 10 mls was prepared. 1 ml portions of each solution were measured into three kjeldahl flasks. The material in each flask was treated with 0.30 ml of 18M H_2SO_4, 0.50 ml of $CuSO_4$-K_2SO_4 solutions, and a few grains of selenium, and digested for about five minutes until water had finished distilling off. A fourth flask was prepared for the blank, containing all other things, except nucleate. It was also digested.

The four flasks were heated in a hot water bath at the boiling point of water (99°C) for one hour. Each of the cold solutions was treated with 0.60 ml of 5M H_2SO_4, 1.0 ml of the secondary phospate reagent*, 1.0 ml of ammonium molybdate solution [containing 26.323 gm/litre of $(NH_4)_6 \cdot Mo_7O_{24} \cdot 4H_2O$], and with 5.0 mls of water. The flasks were heated in the water bath for fifteen minutes, during which time a blue colour developed in the solutions. The solutions were cooled, and made up to 10 mls in 10 ml standard flasks with distilled water. The absorption of

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* The secondary phosphate reagent contained 12.50 ml of the primary phosphate reagent, and 0.0500 gm of 1-amino-2-naphthol-4-sulphonic acid, made up to 50 mls, and prepared fresh every week. The primary phosphate reagent contained 55.50 gm of $Na_2S_2O_5$ plus 2.0 gm of Na_2SO_3 in 250 mls of solution.

each solution at 680 mu was measured using an EEL colorimeter.

The phosphorus concentration of each DNA sample was evaluated from a calibration graph previously obtained using an inorganic phosphate standard, containing 0.10 mg phosphorus in 5 mls of solution.

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CHAPTER 4

SECTION I

CONDUCTANCE STUDIES

THEORETICAL

I.1 Solution Properties of DNA

As generally observed for many polyelectrolytes, the configuration of the polydeoxynucleate ion may be expanded by the electrostatic repulsion between the charged phosphate groups. Such an expansion is often manifested in a large intrinsic viscosity. [1,2]. The associated counterions, i.e. the small ions which are always found in close proximity with the polymer, e.g. sodium ions in the sodium salt of DNA may diffuse through a hypothetical membrane of the macro-ion into the outer solution. This has often been found to cause an increase in the concentration of the mobile ions in the solution. In effect, there is a residual net charge within the domain of the macro-ions. This results in an increase in the potential within the molecule relative to the surroundings and further loss of cations is consequently discouraged. This implies that some of the counterions are more strongly associated with the macro-ions than the others, and these will always be found in the domain of the molecule.

The polymer ion, as a result of the increase in potential within it, will expand, especially if a statistical portion of its unit is ionised. The addition of a simple salt such as potassium bromide or sodium chloride to the solution of the polyelectrolyte tends to equalise the concentrations of the ions inside and outside the macro-ion,

and thus decreases the osmotic expansive force, and perhaps causes an increase in the mobility of the macro-ion.

The average negative charge on the nucleate ion in the absence of added salt decreases with decreasing nucleate concentrations, especially at acid pH. In the neutral pH region, this has been found to be almost constant for different nucleate concentrations [3]. With increasing concentration of nucleate ion, the polyions have been observed to change continuously from an extended to a more coiled form [4].

I.1.a Conductance behaviour of DNA

The conductance behaviour of polyelectrolytes in general has been examined [5a and b]. It has been found that polyelectrolyte solutions behave differently from the simple binary electrolytes like sodium chloride when contained between conductance electrodes. Essentially, the relatively large size of the macro-ion, coupled with their changing configuration and orientation imposes some restrictions on the direct application of any relatively simple theory of conductance to polyelectrolyte solutions.

Generally, solutions of polyelectrolytes, especially of DNA, show large conductances as observed in the series of experiments reported in this thesis. The large conductances arise from the excess charge (or net charge) which resides on the surface of the polyion.

The large conductances have not been shown to vary in any specific manner e.g. linearly, with the nucleate concentration. Most likely, they will not vary linearly with nucleate concentration because of the interaction between the polyions which is expected to become appreciable in high nucleate concentration.

The interaction between the various phosphate charged sites on all the polyions cannot be reduced to any desired extent by dilution, unlike those of associated colloidal micelles. This is because the charge carrying groups occupy fixed positions on the polymer chains, so that local regions of high charge density persist even at extreme dilutions.

Thus, apart from knowing the density of the ionised groups along the polymer chains which affects the extension of the polymeric nucleate ion and its physical properties that may depend on the chain extension, the electrochemistry of the polydeoxynucleate solution as regards the effect of the polyion on the ionic activity coefficients, ion-pair formation, and electrophoretic phenomena must also be known. However, it is not always easy to estimate the effects mentioned. In particular, it is difficult to evaluate the effect of the polyion on the ionic mobility in a polydeoxynucleate simple electrolyte system because of the interionic interactions.

In this study, no attempt has been made to make the above evaluation since it may not be necessary to furnish the information required about the structure and stoichiometry of the macro-ion divalent ion complexes formed in the solution. Rather, some simple behaviour

which is generally expected in the study of conductance of an electrolyte has been considered.

Practically all mobile ions in solution are expected to contribute to the conductance measured. The magnitude of the contribution by each ion is dependent on its mobility and size, apart from other factors, such as the external field and pressure. Interference between the ionic atmosphere of various ions are also expected to produce some changes in the overall conductance of the electrolyte. The binding between the divalent cations such as calcium and the macro-ion is also expected to modify the contributions to the conductance from the counterions and possibly that from the macro-ion itself.

For a simple electrolyte like calcium chloride, in its aqueous solution, the calcium and chloride ions both contribute independently to the overall conductance in a sufficiently low concentration of the electrolyte. For such a system, the specific conductance K is given as

$$\Lambda = \frac{1000\ K}{C} \qquad (4\text{-}1)$$

where Λ is the equivalent conductance and C, is the concentration of the electrolyte (mobile ions).

In the presence of macro-ion, the effective concentration of the mobile ions is not known, and is different from that calculated by summing up the concentrations of the macro-ions, counterions, and all other mobile ions put together. The contributions to the overall specific conductance of the electrolyte by added simple ions such as calcium (II) or magnesium (II) would also be difficult to estimate.

This is because of the complexity introduced into the system of interacting buffer, macro-ions and counterions. It would be expected however, that for a calculated value of the concentration of simple electrolyte, the specific conductance K, should be higher than that observed in practice.

During conductometric titration, the pattern of variation of the conductance will manifest any major changes in the parameters of the macro-ions, and any changes in the stoichiometry and possibly of the structure of the complex or complexes formed. On complete occupation of the binding sites on the macro-ions by the small ions, there should be an increase in conductance thereafter, showing that the ions are free to move, and independently too, in their low concentrations. Any interaction between bound and unbound simple cations or anions will also affect the conductance in a way not easily predictable.

As in the simple case of acid-base titrations, or in precipitation titration, an end point is usually denoted by a point of specific change in the slope of the specific conductance/concentration curve. These end points correspond to stages of the study when the mobile ions can be supposed to behave independently or almost so. Ocassionally, a minor change in the slope of the graph may be revealed by a short discontinuity. This may be due to interferences between the bound and the unbound counterions.

As will be found in section two of this chapter, the number of net negative charges on the polydeoxynucleate ion depends both on the pH and ionic strength (of simple electrolyte like NaCl, $CaCl_2$ etc) of the solution.

During conductometric titration therefore, there is expected, a variation in the degree of dissociation of the macro-ion as divalent cation is added. This variation in the degree of dissociation is expected to be a smooth one over the concentration of the added simple ions, and is expected to affect the degree of binding of small cations with the polyanions.

I.2 Experimental

I.2(a) Apparatus and material

The conductance studies of binding between DNA and magnesium (II) or calcium (II) were conducted with a highly sensitive Wayne-Kerr Universal conductivity bridge (B 221 model), constructed from resistance and capacitance components.

A voltage of 230 was stabilised by an 'advance adjustable stabiliser' across the conductivity bridge. A dipping type of conductivity cell with platinum electrodes, coated with platinum black was employed. Its constant was 1.07 cm^{-1}.

Experimental solutions were placed in a cell container of about 50 ml capacity, made of polyethylene. The cell, dipping in the solution, was always kept in a water bath maintained at 25 ± 0.1°C during the experiment.

Conductivity water (1.10×10^{-7} ohm^{-1} om^{-1}) was used throughout for rinsing cell holder and for preparing solutions. The nucleic acid used was that extracted in this laboratory and labelled (CTA). This has been described in chapter three.

I.2.b Solution preparation

Three sets of DNA solutions of 0.05 per cent (wet weight) and of ionic strength of 10^{-3}M in sodium chloride were prepared in buffer solutions of ionic strength 5×10^{-3} and pH 9.1, 7.20 (both in Tris/Hcl buffer) and pH 3.63 (in acetate buffer).
Nucleate solutions were also prepared of 0.05 per cent (wet weight), of pH 7.1 and ionic strengths of 1×10^{-2} and 5×10^{-2}M in sodium chloride.
The methods of preparing the buffer solutions are found in reference [6].

In preparing the nucleate solution, the fibrous material was suspended in the appropriate solvent for about a day at 2°C, with a gentle shaking to homogenise the solutions. Vigorous and prolonged stirring was avoided since this might course some shearing in the solution, and consequently some denaturation of the DNA.
The concentrations of the nucleate solutions used in moles of phosphorus per litre, were obtained from the percentage of phosphorus and the percentage of the dry weight in the wet material taken. This was 8.227×10^{-4} mole phosphorus per litre of solutions (see chapter 3).

Stock titrant solutions of sodium chloride (0.10 gm NaCl per 100 mls of solution); calcium chloride (0.15 gm dry $CaCl_2$ per 100 ml of solution) were prepared in each buffer system.
The concentration of calcium (II) and magnesium (II) in the stock solution was determined titrimetrically as described in Section II 1.c .

I.2.c Method

A short experiment was first conducted to test the reproducibility of measurement with the conductivity bridge. In this, the specific conductance of increasing concentrations of calcium chloride or magnesium chloride in aqueous solution were measured.
Starting with 25 mls of conductivity water, 0.10 ml portions of the stock calcium (II) or magnesium (II) solution were added in steps, and the corresponding conductance of the solution determined after each addition, on the attainment of thermal equilibrium at 25 ± 0.1°C. About ten minutes were allowed for this. Only low concentrations of solutions of calcium or magnesium ions, for which the simple expression (1) in the theory holds were investigated. A straight line was obtained in the plot of specific conductance against concentration of the electrolyte. This suggested that the mobile ions in the electrolyte solution contributed to the overall conductance independently.

At each pH value, for each ionic strength in sodium chloride, and for each divalent cation two sets of experiments were conducted. The conductances of solutions containing divalent cations, with and without the macro-ions were measured, each time starting with 25 mls of nucleate solution, and solvent, respectively.
A total of 4.00 mls of the solution of calcium chloride or magnesium chloride was added in steps of 0.10 ml, using a pipette of 0.10 ml capacity. Correction for the dilutions of the nucleate solution was made after each addition of 0.10 ml of the stock titrant solution, and a graph of specific conductance against the concentration of divalent cation was plotted in each case.

I.3 Results and discussions

The interpretation of the results of the conductance studies of binding between divalent cations and DNA ions in salt solution is quite difficult. This is because all the ions in solution contribute to the total conductance measured, though not independently. However, since the only species whose concentrations were effectively changing are the divalent ions and the corresponding anions, one can attribute the variations in the conductance of the system to variations in the concentrations of these small ions. In the particular case of high salt concentration, the pattern of variation of the conductance of the solution of the nucleate would depend mainly on the contributions by the macro-ion as is explained later.

The assumption is made that any major changes in the pattern of variation of conductance can be attributed to changes in the concentrations of the added divalent cations, (for the case of low ionic strength of sodium chloride $\leq 1 \times 10^{-2}$), since these are the only ions that bind appreciably to the polyanions. Binding by the anions such as ($C\bar{l}$) is assumed not to be significant in the system of polyions interacting with added electrolytes.

All the mobile ions except the polyanions may be assumed to contribute linearly to the conductance of the solution as the concentration of the added salt was increased.

As anticipated for a native DNA preparation, the conductance of the water-cation system was always lower than that for the water-DNA-cation-system at any given concentration of added salt of divalent cation except for the case of high ionic strength in sodium chloride ($\geq 5 \times 10^{-2}$).

Figs (1 - 9) represent the plot of the conductance derived from the added salt against the concentration of the added electrolyte. Each graph was obtained by subtracting the initial conductance contributions by water or water-DNA from any of the subsequent values for the different concentrations of the divalent electrolyte.

In these figures, the graph for water-cation is above that of water-DNA-cation owing to the subtraction of the initial values which was by far greater for the water-DNA-cation system.

Sodium chloride of concentration 10^{-3}, 1×10^{-2} and 5×10^{-2} was

employed, both to stabilise the secondary double-helical structure of the nucleate ion, and to provide the necessary concentration of sodium ions in the study of the effect of sodium ion on the binding of the divalent cations by DNA.

The presence of these concentrations of added sodium chloride, especially the 5×10^{-2}M, has been shown to affect the binding of the divalent cations to DNA and to modify greatly the pattern of variation of the conductance of the nucleate solution as solution of divalent electrolyte was added, as shown by fig. 6 for DNA-magnesium.

Lower concentrations of sodium chloride ($< 10^{-3}$M) have been reported to cause some denaturation in the native molecule.

Experiences in this studies have also indicated that the best and most detailed results which can be most easily interpreted can be obtained only at ionic strengths of sodium chloride down to 10^{-3}M, but not less.

I.3.a The end points of the titration

Three end points have been observed in each of the titrations conducted. These correspond to 0.125, 0.30 and 0.50 mole of divalent cation to one mole of nucleate phosphorus, or 0·25, 0·60 and 1·0 gm equ. of M^{2+}/P (see figs 4 and 10, and tables 4·1 and 4·2).

The results are in partial agreement with those of Shack and Thompsett [7]; and those of Zubay and Doty [8], who have reported less than half a mole of divalent cation per mole of phosphorus.

The results are however, in full harmony with those of Felsenfeld and co-workers [9] . For the DNA-magnesium system, the two end points reported by Felsenfeld for undenatured DNA i.e. 0.30 and 0.50 mole of divalent cation per mole of phosphorus have also been observed here for the DNA-calcium system.

A third end point of 0.125 mole divalent cation per mole of nucleate phosphorus has been reported here for the first time for both the DNA-Mg^{2+} and DNA-Ca^{2+} systems.

The end points have been found to be ionic strength and pH independent. Even for high ionic strengths of sodium chloride they still have the same values.

Felsenfeld, Zubay and Doty [3] have attributed the occurrence of the end points at 0.30 to denaturation of the nucleate ions, but this can be challenged. Even in high concentrations of supporting electrylyte (NaCl of the order of 5×10^{-2}M) these end points, including that at 0.30, have appeared. ~~in one form or another.~~ With this condition of high ionic strength denaturation of the nucleate is least expected, especially at the temperature of the experiment and with careful preparations of nucleate solution. Also the precise value of the end-point would be expected to depend on the degree of denaturation i.e. on the concentration of supporting simple electrolyte like sodium chloride. Such a dependence of the end-points (even that at 0.30) on ionic strength has not been noted in these series of investigations, hence none of the end-points can be attributable to denaturation of the nucleate.

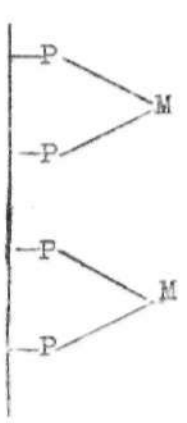
Rather I would suggest that the end-points, particularly 0.125 and 0.30 mole divalent cations per mole of phosphorus would determine the various configurations and structures (including flexibility) of the nucleate ions, as is further explained in I.3.b. That at 0.50 mole cation to one of phosphorus can be taken to represent a quantitative binding between the cations and the phosphate groups on the nucleate ions.

I. 3.b. The effects of ionic strength on the end-points of titrations.

The results presented in this section for the experiments on conductometric titrations of DNA solutions with divalent cations with three end-points indicate clearly that these end-points are ionic strength independent. The first conclusion that can be drawn here is that the sodium ions did not bind strongly or appreciably, to DNA in its aqueous solution in the presence of magnesium or calcium, but modified the mode of binding of the latter to the macro-ion and the pattern of variation of the conductance of the solution of polynucleate ions.

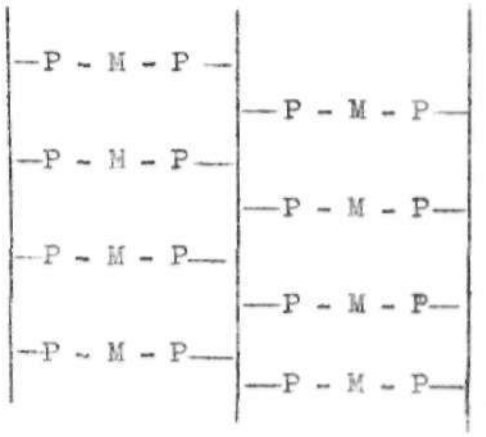
The effect of high concentration of sodium chloride which is seen in a complete change in the shape of the conductance/concentration curve is quite difficult to explain. There is an increase in conductance to a certain point and then a decrease past its zero value to values much lower than the initial. The decrease in conductance below the initial value can be assumed to have been caused by either (a) the precipitation of the nucleate ions by the added divalent cations, or (b) aggregation of the polyions by an interchain linkage via the divalent cations, thus effectively becoming less mobile.
The latter suggestion of aggregate formation seems more acceptable here as precipitation of the nucleate would be expected to lead to a much greater decrease in the conductance than was observed. Precipitation would also probably cause a large decrease in the UV absorption of the nucleate solution.

No such decrease in absorption at 260 mμ was observed for the nucleate solution after the titration. This again suggests the concept of aggregation of the polyions. Aggregation as applied here may be the coiling up of the separate DNA strands to form a compact structure, or the bridging of two or more nucleate ions by divalent cations or both. The monovalent electrolyte present in a high concentration (of the order of 5×10^{-2}M) possibly served in maintaining the secondary structure of the nucleate ion, and possibly in causing the individual α-helices to be orientated parallel, so that the in-coming divalent cations could bind the polynucleate ions intermolecularly and intramolecularly as explained by the diagram.



Low ionic strength

Intramolecular interaction of divalent cations



High ionic strength

Intermolecular interaction and aggregation through cross-linking by divalent cations.

Intermolecular mode of binding is expected to produce an appreciable decrease in the mobility of the macro-ion, and in the overall conductance measured. This suggestion of aggregate formation in explaining the mode of variation of conductance for nucleate solution of high ionic strength in sodium chloride would be acceptable only, ~~and only~~ if it could be shown that the rate of decrease of the conductance contribution by the nucleate is by far greater than the rate of decrease of overall conductance measured.
Assuming this condition is satisfied, then segments of conductance/ concentration curves would be obtained as for low inonic strengths of sodium chloride in which there was a continuous increase in conductance as the divalent cations were added stepwise.

I.3.c The effect of pH on the end-point of titration

The results showing that the stoichiometry of the binding of calcium (II) and magnesium (II) to DNA is pH independent, as studied by conductance titration, seem difficult to understand on the account of the variations in the degrees of ionisation of the polyion with variations of pH. It would be anticipated that the degree, or the extent of binding, should depend on the degree of ionisation of the polyion, and so should decrease as the acid pH range was ascended. The third end-point in particular, should vary in such a way as to be in harmony with the general rule. The following explanation has however been considered for the departure of the actual results from the expected.

Deoxyribonucleic acid is isolated as its sodium salt. In its aqueous solutions, it carries a number of negative charges on the ionised phosphate groups which depends on the pH of the solution. In acid pH, it can be assumed that only electrostatic interactions (the types found between sodium ions and the phosphate negatively charged sites) exist between the protons (H^+) and the phosphate groups. In varying degrees of acidity therefore, the situation would be similar to that of varying amounts of sodium counterions in close proximity with the polyions i.e. many more hydrogen ions are found near the negatively charged phosphate sites.

With the introduction of the divalent cations, (which presumably bind covalently with the phosphate groups), they would compete with the hydrogen ions for the phosphate sites. The divalent cations would over-power the hydrogen ions and make them available for contributing to the conductance of the solution.

Therefore, the conductance of the nucleate solution, rather than the degree of binding would be expected to be pH dependent and any pH effect which might be expected would be on the slope of the various parts of the conductance/concentration curves. No regular changes in the slopes of the curves have been noted for the various pH values investigated however.

I.3.d Conductance Curves and the Structure of DNA.

As it might be expected, there may be some structural changes in the macro-ions/divalent ion complexes as a region of the curve is left for the one immediately next to it.

The binding of the divalent cations to the polyions would be expected to produce a shortening of the length of the polyion, or at least to reduce the electrostatic expansive force between the charged phosphate groups, The polymer ion becomes less mobile as the phosphate sites are neutralised or bound to the cations. This is one of the main reasons why the conductance contribution by the nucleate-cation complex should decrease with increasing concentration of added divalent electrolyte.

The increase in conductance from one end point to another can be considered as due to the combined effect of added divalent electrolyte particularly the anions, and the displaced sodium counterions. After the third end point (ie. 0.50 mole M^{2+}/P) all the phosphate sites can be assumed to be bound and possibly the conductance of the nucleate-cation complex might become zero or negligible.

The possibility of a complex of more than half a mole of divalent cation to one mole of phosphorus has also been considered and reported in Section II of this chapter. The method of conductance measurement does not seem too reliable in studying this, possibly because the divalent cation concentration was getting too high.

After the formation of the complex of 0.50 mole M^{2+}/one mole phosphorus, the additional divalent cations, as they are introduced, could interact with the complex already formed to produce a complex of higher proportion of cation to phosphorus. This could be regarded as a hypothetical polycation, whose stability would be profoundly dependent on the pH of the solution and the electrostatic interactions between the positively charged sites.

pH	$\frac{\text{mole } M^{2+}}{\text{mole P}}$	Slope of Curve	Relative Slope
9.10	0.125	0.260	0.718
	0.30	0.304	0.840
	0.50	0.335	0.924
	after 0.50	0.362	0.999
	water - Ca^{2+}	0.362	1.000
7.05	0.125	0.194	0.538
	0.30	0.267	0.743
	0.50	0.350	0.973
	after 0.50	0.360	1.001
	water - Ca^{2+}	0.359	1.000
3.63	0.125	0.197	0.609
	0.30	0.284	0.878
	0.50	0.323	0.999
	after 0.50	0.324	1.000
	water - Ca^{2+}	0.324	1.000

Table 4.1 The end points of conductance titration in the binding studies calcium (II) with Calf thymus DNA.

$\frac{\text{Mole } M^{2+}}{\text{mole P}}$	pH	Slope of Curve	Relative Slope
0.125	9.10	0.360	0.697
0.30		0.468	0.907
0.50		0.495	0.958
after 0.50		0.520	1.007
water-Mg^{2+}		0.5163	1.00
0.129	7.05	0.349	0.808
0.30		0.385	0.891
0.50		0.418	0.967
after 0.50		0.430	0.996
Water-Mg^{2+}		0.432	1.000
0.120	3.63	0.290	0.620
0.30		0.422	0.905
0.50		0.447	0.957
after 0.50		0.466	0.999
Water-Mg^{2+}		0.467	1.000

Table 4.2 The end points of conductance titration in the binding studies of magnesuin (II) with Calf thymus DNA.

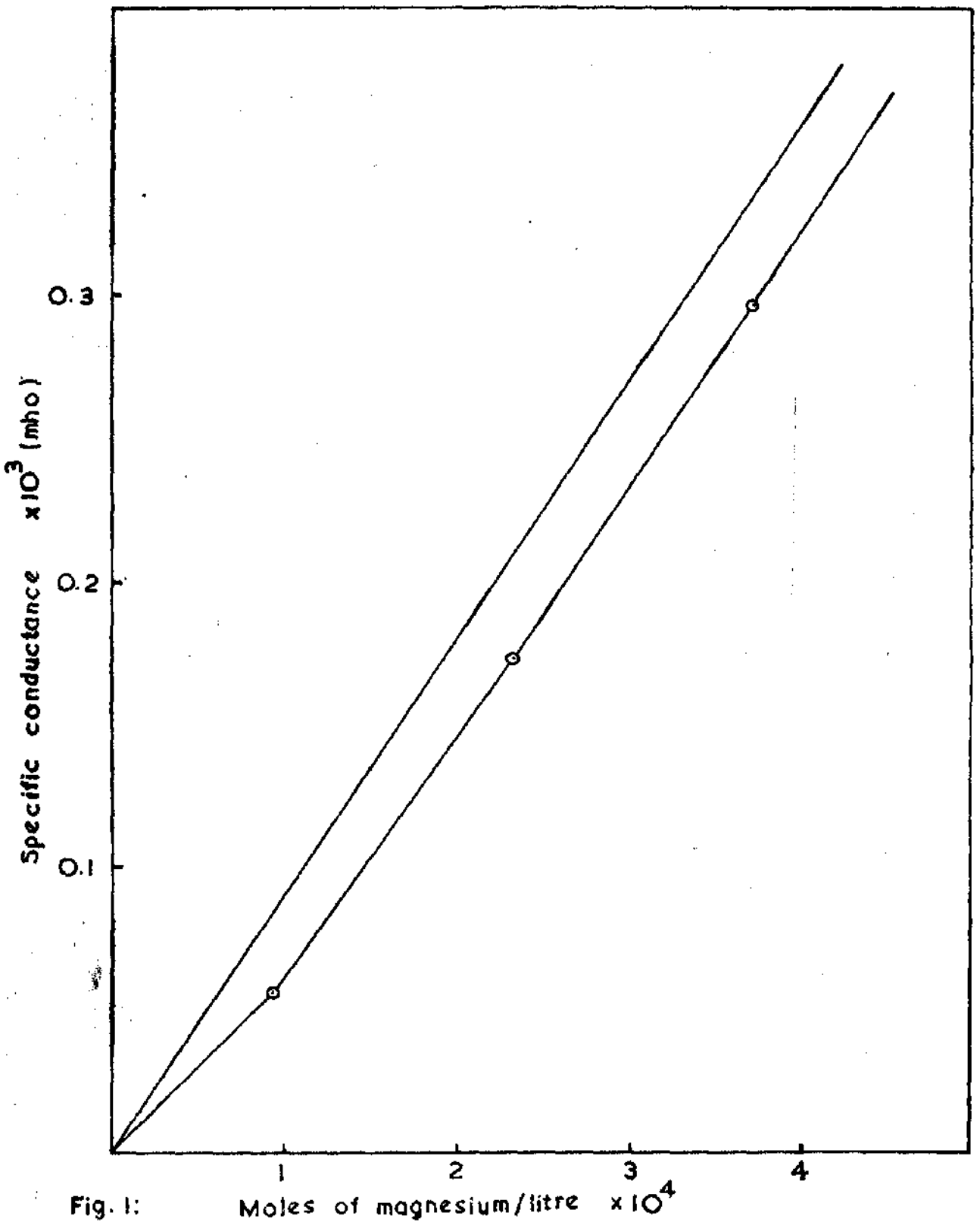

Specific conductance x10³ (mho)
0.3
0.2
0.1
1
2
3
4
Moles of magnesium/litre x10⁴

Fig. 1:

pH 3.63

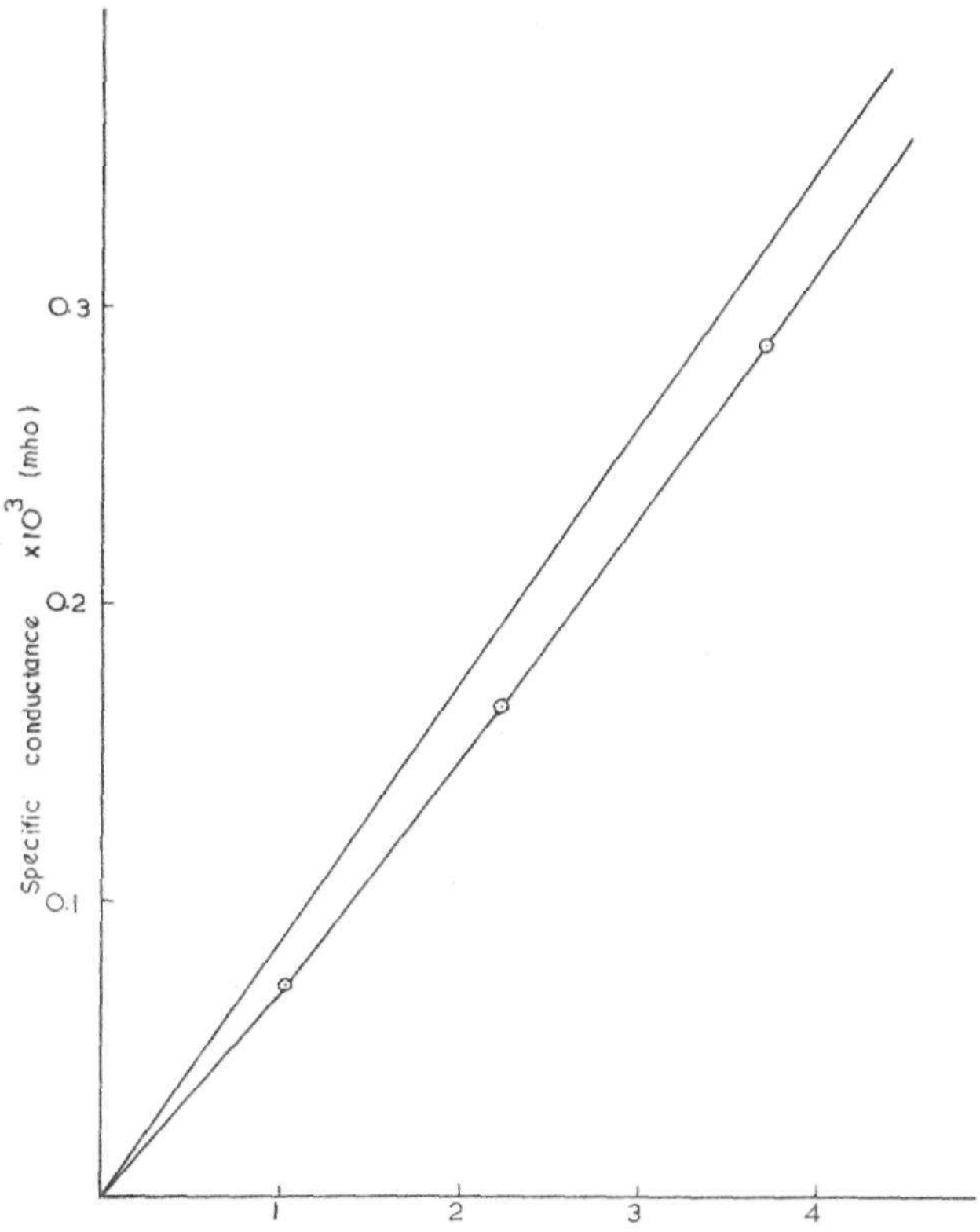

Specific conductance x10^3 (mho)
0.3
0.2
0.1
1
2
3
4
Moles of magnesium/litre x10^4
pH 7.05

Fig. 2:

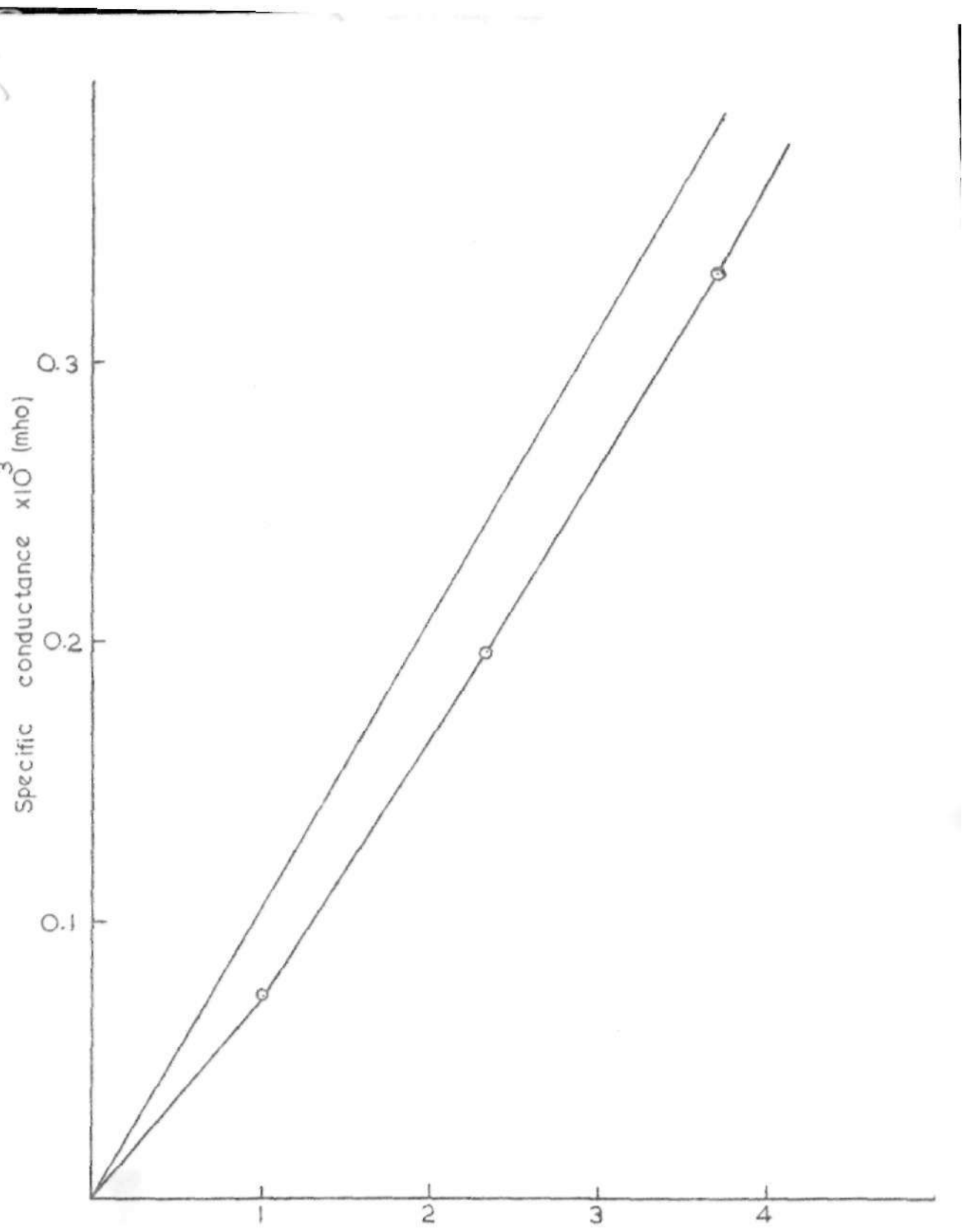


Fig. 3: Moles of magnesium/litre x10^4

pH 9.10

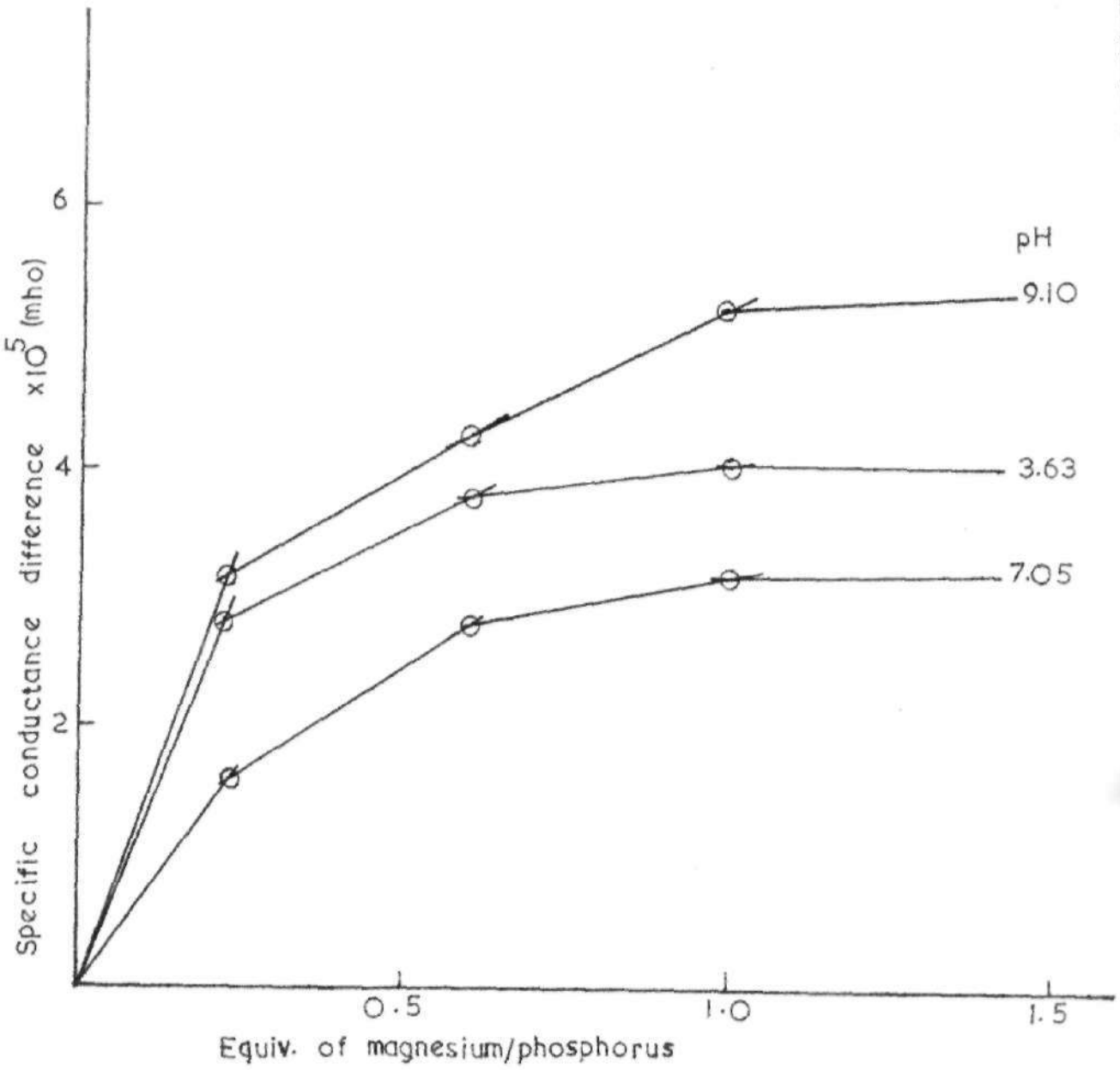

Specific conductance difference x10^5 (mho)
6
4
2
pH
9.10
3.63
7.05
0·5
1·0
1·5
Equiv. of magnesium/phosphorus

Fig. 4:

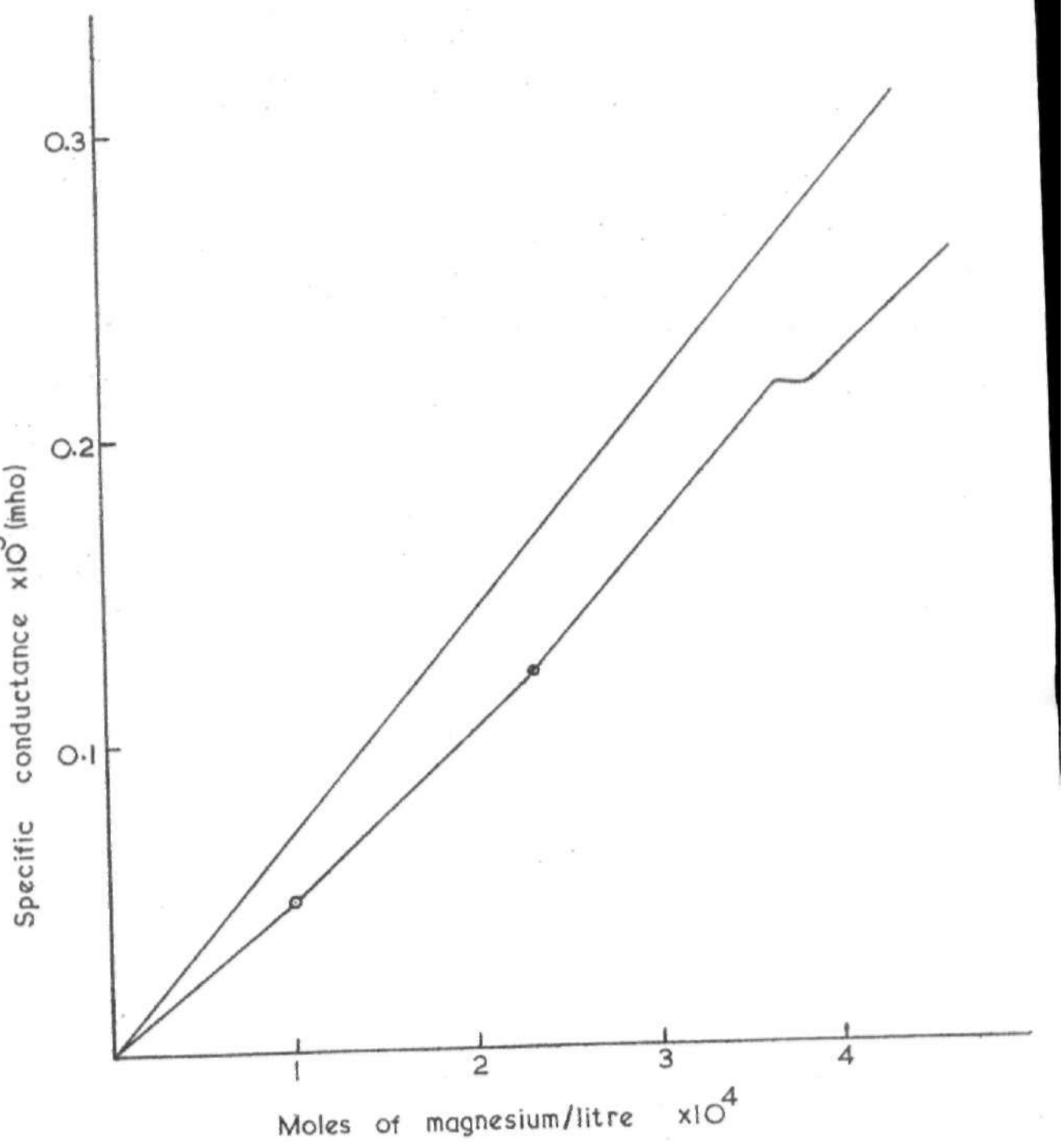
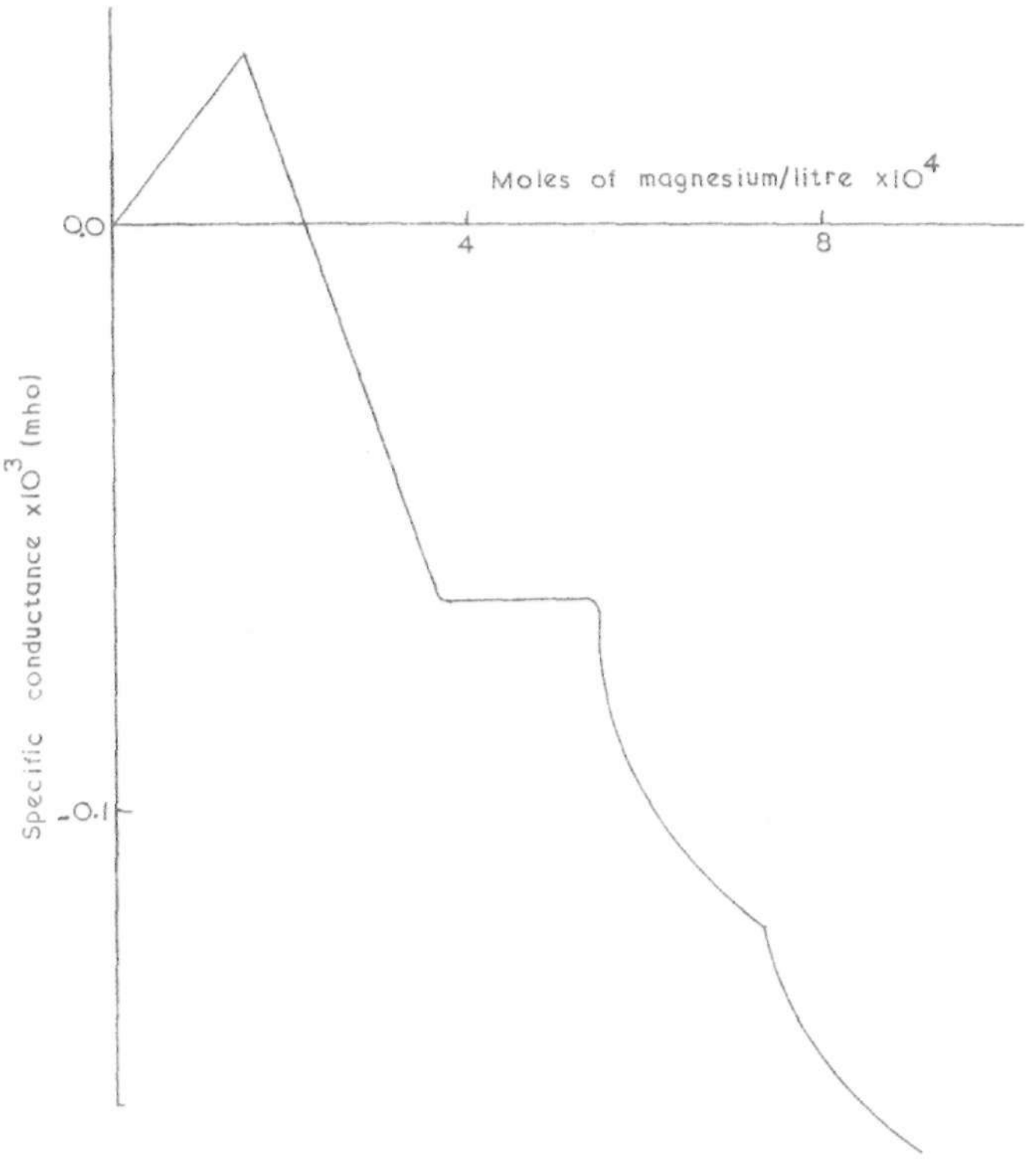


Fig. 5: (pH 7.05 and [I] = 0.01 in NaCl)



(pH 7.05 and [I] = 0.05 in NaCl)

Fig. 6:

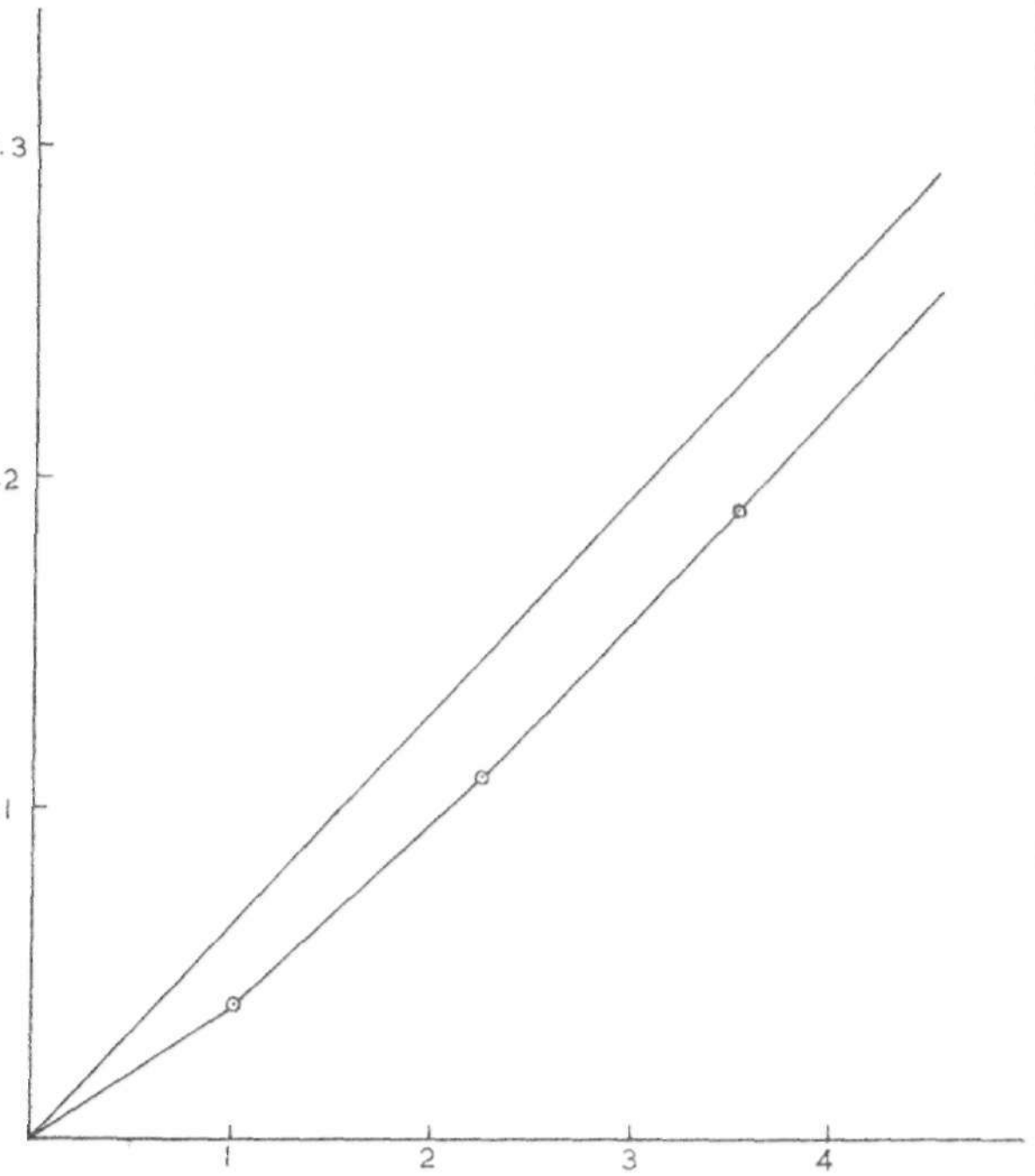


Fig. 7: Moles of calcium/litre $\times 10^{4}$

pH 3.63

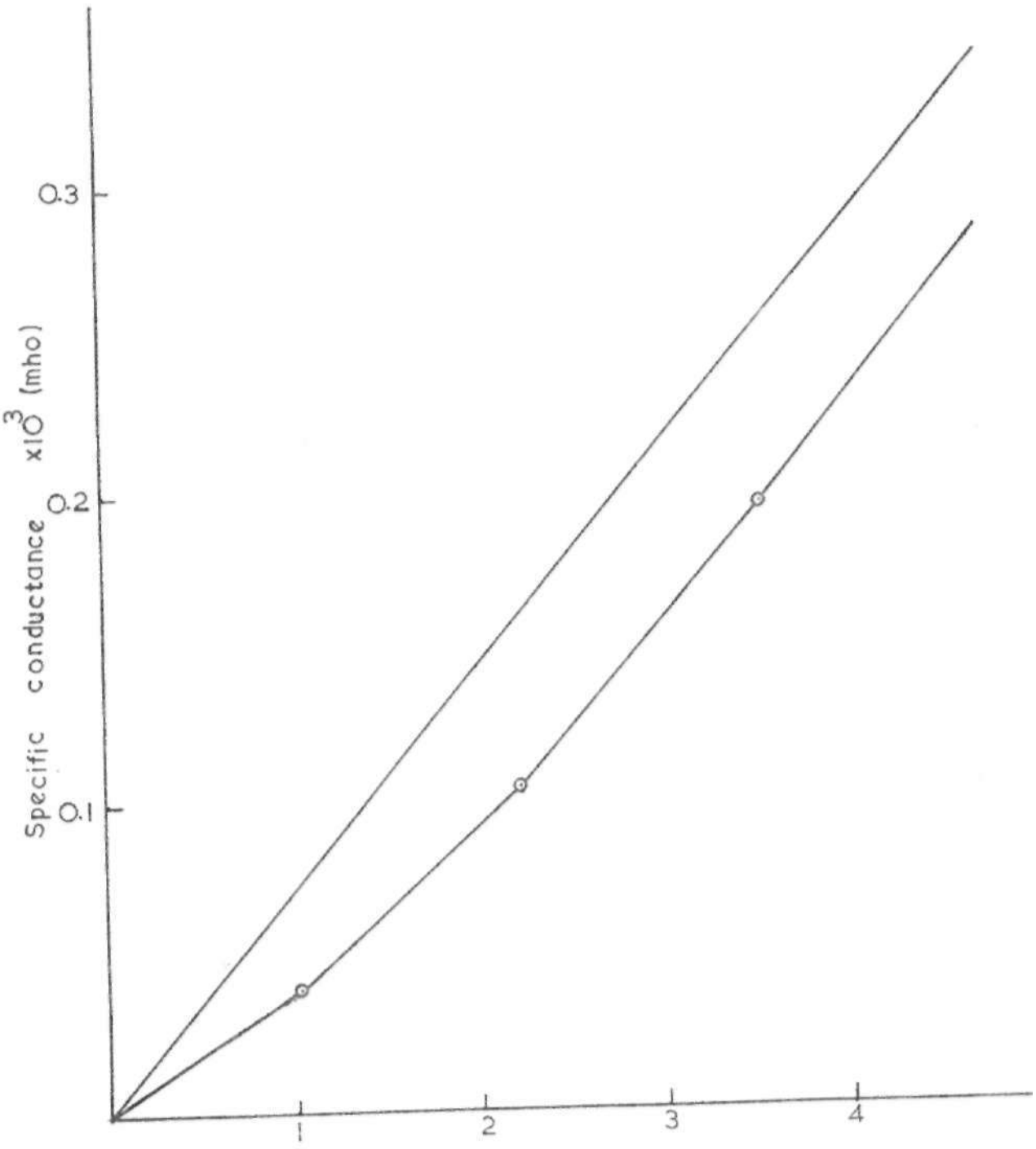

Specific conductance x10^3 (mho)
0.3
0.2
0.1
1
2
3
4
Moles of calcium/litre x10^4

Fig. 8: pH 7.05

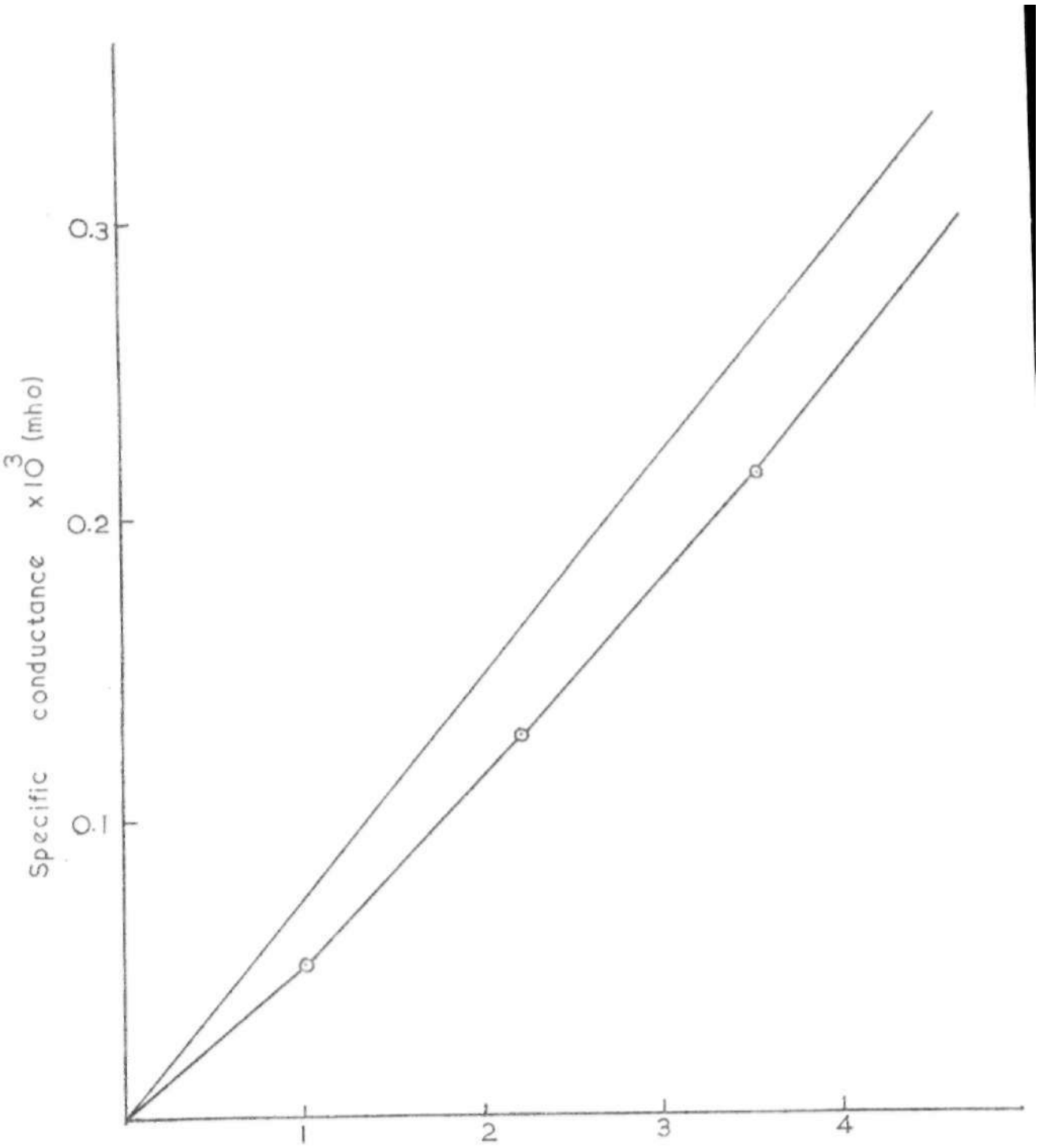


Fig. 9: Moles of calcium/litre x10^4

pH 9.10

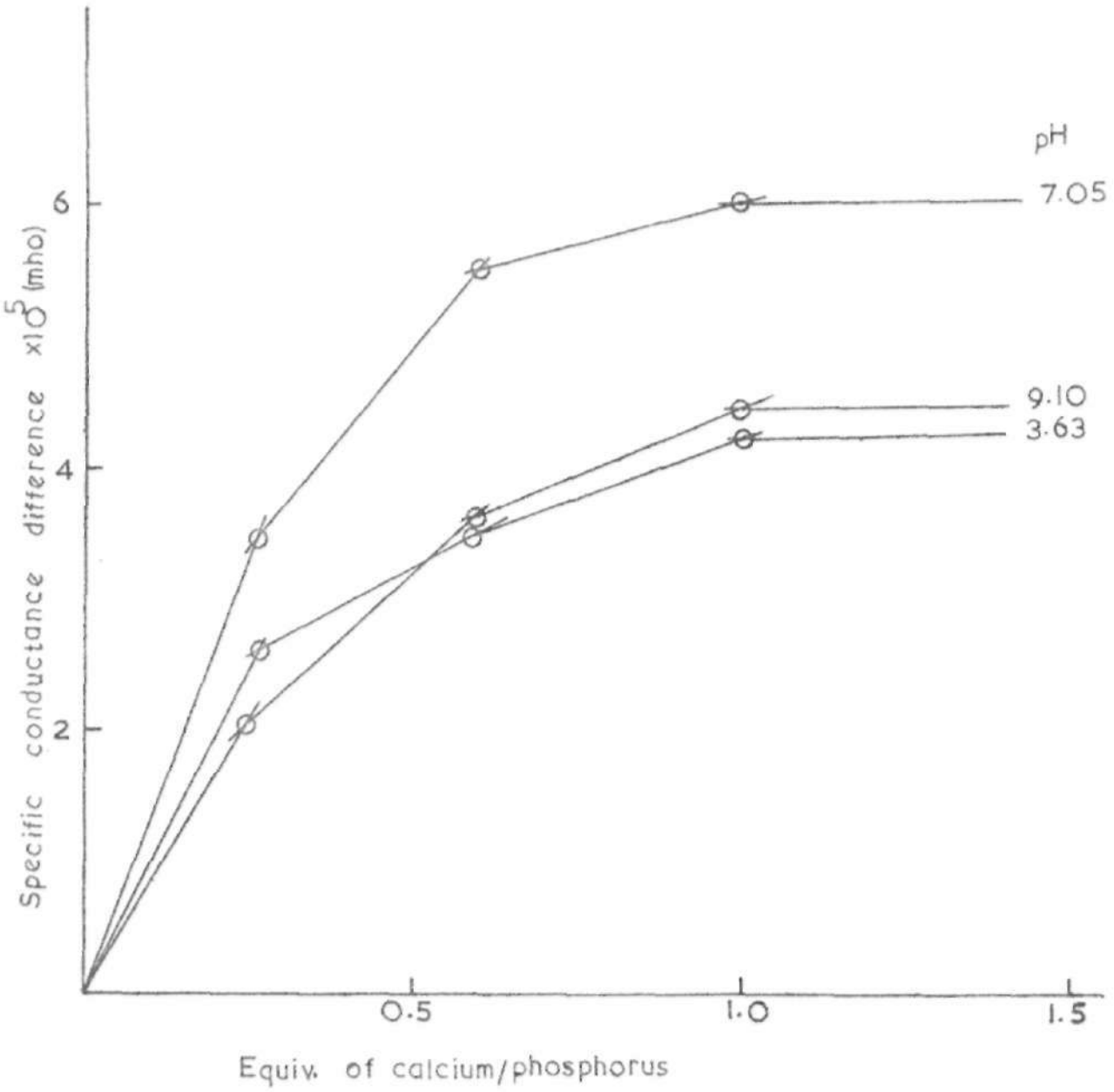
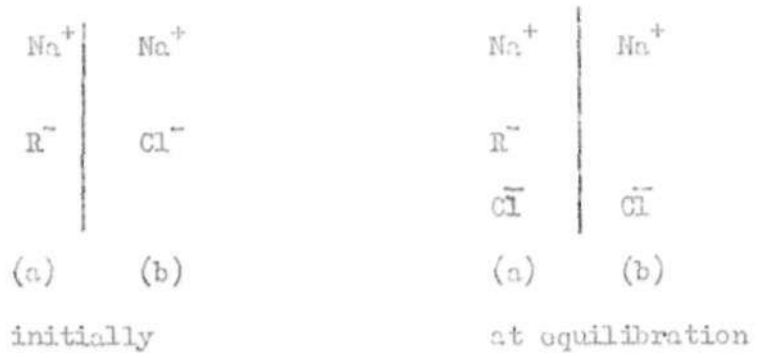

pH
7.05
9.10
3.63
6
4
2
Specific conductance difference x10^{5} (mho)
0.5
1.0
1.5
Equiv. of calcium/phosphorus

Fig. 10:

II.1. The Theory of Equilibrium Dialysis

In a system where two phases areseparated by a permeable membrane there is a flow of ions or molecules across the membrane in either direction. If the membrane is permeable only to some of the molecules and not to others, (i.e. semipermeable), there is a movement of ions or molecule from one phase into the other. Systems of this kind are readily set up. They include proteins, nucleic acids and polymeric molecules in general with a membrane partition between their solutions and those of small ions or molecules.

Consider the sodium salt of deoxyribonucleic acid (DNA) inside a cellophane bag, then the large molecule to which the membrane is permeable is the DNA.



A system of two phases (a) and (b) separated by a semipermeable membrane shown in a vertical line.

The solution of the macro-ion inside the membrane is dialysed against a solution of sodium chloride.

Thermodynamic considerations have shown that to a first approximation, at equilibrium the product of the concentrations of a pair of diffusible ions (in this case Na^+ and $C\bar{l}$ ions) on one side will be the same as the product of the concentrations of the same ions on the other side of the membrane.

$$\text{i.e. } [Na^+]_a\,[C\bar{l}]_a = [Na^+]_b\,[C\bar{l}]_b \qquad (4\text{-}2)$$

At equilibrium there must be electrical neutrality, and

$$[Na^+]_a = [R^-] + [C\bar{l}]_a \qquad (4\text{-}3)$$

Similarly, $[Na^+]_b = [C\bar{l}]_b$

$$\text{Hence, } [Na^+]_a > [Na^+]_b, \text{ and } [C\bar{l}] < [C\bar{l}]_b \qquad (4\text{-}3a)$$

Differences in the concentration of diffusible ions will always lead to differences in potential on the two sides of the membrane. These differences in potential are believed to account for certain phenomena in living matter.

II.1.a Detailed thermodynamic considerations of equilibrium dialysis

By the method of thermodynamics, equations have been formulated which account for the Donnan equilibrium (the distribution of mobile ions in the system), and Donnan corrections.

We considered two phases separated by a semipermeable membrane. One phase (β), contains a macromolecular ionic species which cannot diffuse through the membrane to the other phase (α).

Small ions i, j, k, ..., of cations and anions which can freely pass through the membrane are considered to be present. For the solvent (designated by the subscript 1),

$$\mu_1(P) = \mu_1^o(P) - RT \phi \Sigma r_i \qquad (4\text{-}4)$$

where $\mu_1^o(P)$ is the chemical potential (a function of pressure) and ϕ is the osmotic coefficient defined by

$$\ln \frac{P_1}{P_1^o} = - \phi \Sigma r_i \qquad (4\text{-}5)$$

The term r_i is the mole ratio of the ionic species i to solvent, while P_1 and P_1^o are the vapour pressures of the solution and pure solvent, where,

$$\mu_1^o(P) = \mu_1^o + PV_1 \qquad (4\text{-}6)$$

Equation (4-6) then becomes,

$$\mu_1(P) = \mu_1^o + PV_1 - RT \phi \Sigma r_i \qquad (4\text{-}7)$$

In equation (6), μ_1^o is the solvent standard chemical potential at zero pressure and V_1, its partial molar volume. For the ionic species i, equation (4-7) has been written as

$$\mu_i = \mu_i^o + RT \ln a_i + Z_i F \psi + PV_i \qquad (4\text{-}8)$$

Where Z_i is the valence; F, the Faraday; ψ, the electrostatic potential, and V_i the partial molar volume. This species in the two phases at equilibrium should have the same chemical potential, whence

$$\mu_i^\alpha = \mu_i^\beta \qquad (4\text{-}9)$$

and since the same solvent is found on both sides of the membrane,

$$(\mu_i^o)^\alpha = (\mu_i^o)^\beta \qquad (4\text{-}10)$$

Hence,

$$Z_i F(\psi^\alpha - \psi^\beta) + V_i\,(P^\alpha - P^\beta) + RT \ln \frac{a_i^\alpha}{a_i^\beta} = 0 \qquad (4\text{-}11)$$

Similarly, expressions can be written for species i and k .... at membrane equilibrium.

For all the ions present, for electrical neutrality, we have

$$Z_i\, F(\psi^\alpha - \psi^\beta) = 0 \qquad (4\text{-}12)$$

$$\text{and} \quad RT\ \Sigma \ln \frac{a_i^\alpha}{a_i^\beta} + (P^\alpha - P^\beta)\ \Sigma V_i = 0 \qquad (4\text{-}13)$$

and from equations ~~(4-6) and (8)~~ to (4-8),

$$(P - P) = \frac{RT}{V_1}\ (\phi^\alpha\ \Sigma r_i^\alpha - \phi\ \Sigma\ r_i^\beta) \qquad (4\text{-}14)$$

An alternative equation for expression (4-13) can therefore be

$$\Sigma \ln \frac{a_i^\alpha}{a_i^\beta} = -\ \frac{\Sigma V_i}{V_i}\ (\phi^\alpha\ \Sigma\ r_i^\alpha - \phi^\beta\ \Sigma\ r_i^\beta) \qquad (4\text{-}15)$$

and in terms of the activities of the ionic species, where $a_i = m_i\ \gamma_i$, and M_i is the molality, and γ, the activity coefficient,

$$\sum \ln \frac{m_i^\alpha\ \gamma_i^\alpha}{m_i^\beta\ \gamma_i^\beta} = -\frac{\Sigma V_i}{V_i}\ (\phi^\alpha\ \Sigma\ r_i^\alpha - \phi^\beta\ \Sigma\ r_i^\beta) \qquad (4\text{-}16)$$

For dilute solutions, the right hand side of equation (4-16) is zero and, $\Sigma\, n_i^{\alpha}\, \gamma_i^{\alpha} = \Sigma\, n_i^{\beta}\, \gamma^{\beta}$.

The quantities that appear in equation (4-16) are obtainable only for the electrolyte and not for the individual ions. For an electrolyte, where ν_i, ν_j are the number of ions per molecule of the electrolyte containing ions of the type i and j, then

$$\ln \frac{(n_i^{\beta})^{\nu_i} (n_j^{\beta})^{\nu_j} (\gamma_{ij}^{\beta})^{(\nu_i+\nu_j)}}{(n_i^{\alpha})^{\nu_i} (n_i^{\alpha})^{\nu_j} (\gamma_{ij}^{\alpha})^{(\nu_i+\nu_j)}} = \frac{V_i+V_j}{V_i} \left[\phi^{\alpha}(r_i^{\alpha}+r_j^{\alpha}) - \phi^{\beta}(r_i^{\beta}+r_j^{\beta}) \right] \quad (4\text{-}17)$$

as in the case of sodium chloride, with the ions Na^+ and $C\bar{l}$ denoted by n_i, n_j

For a binding study between a macro-ion and a divalent electrolyte such as calcium chloride, in the presence of sodium chloride as supporting electrolyte, similar, but more extended equations have been obtained. The i, j and k ions found here are Ca^{2+}, $C\bar{l}$ and Na^+, and expression (4-17) becomes

$$\ln \frac{(n_i^{\beta})^{\nu_i} (n_j^{\beta})^{\nu_j} (n_k^{\beta})^{\nu_k} (\gamma_{ij}^{\beta})^{(\nu_i+\nu_j)} (\gamma_{jk}^{\beta})^{(\nu_i+\nu_k)}}{(n_i^{\alpha})^{\nu_i} (n_j^{\alpha})^{\nu_j} (n_k^{\alpha})^{\nu_k} (\gamma_{ij}^{\alpha})^{(\nu_i+\nu_j)} (\gamma_{ik}^{\alpha})^{(\nu_j+\nu_k)}} =$$

$$\frac{V_i + V_j + V_k}{V_i} \left[\phi^{\alpha} (r_i^{\alpha} + r_j^{\alpha} + r_k^{\alpha}) - \phi^{\beta}(r_i^{\beta} + r_j^{\beta} + r_k^{\beta}) \right] \quad (4\text{-}18)$$

II.1.b The osmotic coefficients, mole ratios, and partial molar volume

In very dilute solutions, the right hand side of equation (4-18) is negligible. In many systems, especially in the one studied here, Σr_i is assumed to be small, and $(\phi^\alpha - \phi^\beta)$ also small. The macro-ion itself will have very little effect on ϕ^β directly but will influence it by its interaction with the small ions. It seems likely that ϕ^β will be less than ϕ^α for this reason. The term V_i for the macro-ion will be relatively large. It is also possible to make approximations to V_i(DNA) since the partial specific volume is known approximately, and estimates have been made of the molecular size and shape of the molecule.

Experiments conducted on the activities of electrolytes in the presence of macro-ions can be used to obtain information about the magnitude of ϕ^β and hence the value of ϕ^α from further calculations, in theory. The approximate nature of the estimate likely to be made of V_i(DNA) and ϕ^β, the probability that the whole of the right hand side of equation (4-18) is small are very important. This, together with the fact that the whole behaviour is a second-order effect in the presence of significant ion binding to DNA, do however, make the calculations of the various quantities inaccurate.

Equation (4-18) may therefore be regarded to have only a theoretical significance meanwhile.

II.1.c Donnan Corrections

Either of two methods can be used in accounting for the Donnan effect in a system of two phases separated from each other by a semipermeable membrane, with one of them containing some macro-ions. The method of correcting for the Donnan effect (Appendix) is not very easy and has here been dropped. This might be because the expression in n_{3B} (the divalent cation bound to the nucleate) is too sensitive to any error that might be committed in experimentally determining the various quantities that appear in the equation. Also, it might be that the assumption that sodium ions did not bind to the nucleate ions is wrong. Values of n_{3B} calculated from the expression developed for the Donnan effect reported in the appendix have been found conflicting with those obtained by the simple method described below, presumably because of the reasons given above.

The alternative way, of suppressing the Donnan effect, is by the addition of enough of a simple electrolyte such as sodium chloride. In such a system where sodium chloride (MA) is used to suppress the Donnan effect, at equilibrium, the distribution of the diffusible ions across the membrane is given as

$$\frac{C_{M_1}}{C_{M_2}} = \frac{C_{A_2}}{C_{A_1}} = r \qquad (4\text{-}19)$$

where C_{M_1} and C_{M_2} are the concentrations of the diffusible cations in the two phases (1) and (2), while C_{A_1} and C_{A_2} are those for the diffusible anions.

For an electrical neutrality in both compartments,

$$C_{M_2} = C_{A_2} \tag{4-20}$$

and $$C_{M_1} = C_{A_1} + Z_P C_P \tag{4-20a}$$

where C_p is the molal concentration of the macromolecular ion, and Z_P is its valency.

Equations (19, 20 and 20a) have led to a final expression in r, the molar ratio, given as

$$r = \frac{Z_P C_P}{2 C_{M_2}} + \left(\frac{1 + Z_P^2 C_P^2}{4 C_{M_2}^2} \right)^2 \tag{4-21}$$

Equation (4-21) holds particularly for protein systems [10], and its simpler form of equation (4-19) has been tried and found to hold for the nucleic acid systems studied.

It shows that the Donnan equilibrium depends very much on the net charge on the macro-ion and that the limit of r varies with the number of net charge or with pH. (see figs 13, 14, 17 and 18) The ratio r tends to unity as the Donnan effect is suppressed, and even more so, when the polymer carries no net charge.

II.2 Experimental

II.2.a Apparatus and materials

All experiments were conducted at 25 ± 0.1 °C in a low temperature bath. Visking cellophane tubing was used for the dialysis. The tubes were prepared by boiling in the appropriate buffer solution for one hour to remove any small molecules or ions that might have been bound to the membrane, or those that could be bound to the nucleate ions during dialysis. The tubes were rinsed throughly with conductivity water.

Large glass test-tubes (6" × 1"), with rubber bungs were used to hold the experimental solution outside the cellophane bag. The test-tubes were previously washed with sulphuric acid/dichromate washing solution, and finally rinsed with conductivity water.

Deoxyribonucleic acid (CTA) of 9.15 per cent phosphorus content was used for all experiments.

II.2.b Solution preparation

The purpose of the dialysis experiments was to study the binding of magnesium (II) and calcium (II) with the DNA at various ionic strengths and pH values. Under these conditions of varying ionic strength and pH values, some of the parameters of the nucleate ion, namely, the length, diameter, and particularly, the number of net negative charges on the polyion would vary.

Five buffer solutions of different pH values but of the same ionic strength of 0.005 were prepared using standard methods [1].

The solutions were: Tris/HCl buffer solutions of pH 9.25, 8.20 and 7.05; and acetate buffer solutions of pH 5.0 and 3.63. The pH of each solution was determined with a 'direct reading' pH meter (Pye model 23A) before and after dialysis.

One half of each buffer solution was made of the appropriate ionic strength of 0.02M, 0.10M or 0.16M in sodium chloride, while the other was made twice the required ionic strength.

Nucleic acid stock solution of 0.10 per cent (wet weight) was prepared by suspending the weighed amount of material in conductivity water at 2°C, with a little shaking. This was kept in a refrigerator at about 2°C.

II.2.c Dialysis method

Solutions of 0.05 per cent nucleate (wet weight) were enclosed in collophane bags and dialysed against solutions of calcium or magnesium ions contained in the large test-tubes.
A total of 10 mls. of the solutions was used inside and outside the dialysis bag, with the different solutions mixed in the following volumes:

Solution inside the bag

Five millilitres of nucleate solution (0.10 per cent wet weight) plus 5 mls. of buffer solution of twice the required ionic strength in sodium chloride were used. No ions of magnesium or calcuim were added.

Solution outside the bag

Ten millilitres of buffer solution of the appropriate strength in sodium chloride and varying concentrations of magnesium or calcium ions were used. No nucleate solution was added.

Time of dialysis equilibration

The time of dialysis equilibration was first determined prior to the actual measurements. In this determination, four dialysis tubes containing varying amounts of added calcium chloride or magnesium chloride were set up. The initial concentrations of the divalent cations outside the bag were determined by the method of ultramicrotitration discussed later. The concentrations of the cations in the solutions outside the bag were found to be constant after about six days.

The series of experiments reported here were conducted for the various pH values and ionic strengths, allowing at least seven days for dialysis equilibration.

The concentrations of the divalent cations, initially and at equilibration, were determined in each case. By the method of difference, and after the correction for the cations adsorbed by the cellophane bag, the amount of divalent cation bound to the nucleate (in terms of mole cation per mole of nucleate phosphorus) was estimated.

II.2.d Corrections for the ion adsorbed by the cellophane bag

It was assumed that the presence of the nucleate ions in the dialysis bag did not affect the adsorption of the divalent cations by the membrane. It was therefore decided to conduct dialysis experiments without the nucleic acid.

Dialysis tubes were prepared containing 10 mls of the same solution inside and outside the bag. The solution outside the bag initially, contained known concentrations of added divalent cations. Dialysis was allowed to proceed for seven days, after which the solutions inside and outside the bag were analysed separately for the concentrations of the divalent cations. By difference of the sum of the equilibrium concentrations outside and inside the bag, from the initial value, it was possible to estimate the amount of cation adsorbed per gramme of the cellophane bag.

Using different initial concentrations of divalent cations, a Langmuir adsorption isotherm was drawn to cover the range of concentrations of the cations generally investigated by the method of equilibrium dialysis. Corrections were made for the cations adsorbed by the cellophane at any given equilibrium concentration of the divalent cations using the isotherm.

II. 2. e Analytical methods

The ultramicro-analytical method is a modified form of the general volumetric analytical method. It has been used and recommended for the analysis of solutions especially those obtainable only in small quantities. This method has been preferred to several other methods in estimating calcium and magnesium, at least for its simplicity and accuracy.

The method generally estimates the amount of free cations in an aqueous solution by the complexing of the cations with disodium dihydrogen-ethylenediamine tetraacetate (EDTA). The presence of such a cation - EDTA complex is detectable with an indicator. The complexometric reaction is normally allowed to proceed in an alkaline medium, where there are colour changes characteristic of the system in investigation.

The method of Ultramicrotitration [11] .

Only small quantities of solution (of the order of 1-200 µl) are required in this method. The required volume of each solution except the burette solution, is measured with a standard micro-pipette (made of polyethylene) into a small plastic titration cup specially made for this purpose.

The burette is a small glass tubing with the end drawn such that a polyethylene delivery needle can be connected to it, with the delivery needle pointing downward into the titration cup. Near this needle is an electric stirrer made of plastic for the purpose of stirring the solution during the titration.

The table below reveals the general quantity of each solution usually involved.

	Blank	Standard	Unknown
Distilled water	20 µl		
Standard		20 µl	
Unknown			20 µl
KOH	100 µl	100 µ l	100 µl
Indicator	10 µl	10 µl	10 µl

Table 4.3

The same pipette is used for blank, standard and unknown.
The solutions are mixed in the order shown and titrated with EDTA, usually 0.02N solution.
The burette contains a piston attached to a screw micrometer connected internally to a pointer which moves round a circular graduation from which the number of microlitre of the solution delivered can be read off directly. As the screw is engaged, the volume of burette solution delivered into the titration cup is registered on the scale (Beckmann model 150).

An estimate of about 0.005 µl has been made for the accuracy of measurement with this instrument.

Calcium (II)

In estimating calcium [11, 12], each of 20 µl samples of aqueous solution of calcium (II) was treated with 100 µl of potassium hydroxide/potassium cyanide solution (1.25N KOH with 0.050 gm KCN added), and titrated with EDTA in the presence of 10 µl of calcein indicator (fluorescein complexene solution, containing 0.025 gm of the complexene in 100 ml of 0.25 N sodium hydroxide).
The indicator was usually stored in brown polyethylene bottles in a refrigerator to prevent any colour change of the indicator.
The colour of the indicator was changed from yelow-green to orange-red at the end point of the titration.
This titration was carried out only in the day light and never under a fluorescent light or any artificial light since the colour change at the end point was very difficult to detect under artificial light.

Magnesium (II)

Various analytical methods commonly used in the estimation of magnesium in its aqueous solution were tried. Some of these were the colorimetric method, which involves measuring at 520 mμ, the absorption of the complex formed between magnesium and eriochrome black-T [13], and the titrimetric method [12].

Of all these, the titrimetric method was found to be most accurate and reproducible, and it was the one used for the work reported here.

As in the estimation of calcium, 20 μl of the magnesium (II) solution was titrated with (EDTA) in the presence of 100 μl of a buffer solution of pH 10 (67.5 gm of NH_4Cl in 200 ml. of water, plus 570 ml of NH_4OH, all diluted to one litre).

Five microlitres of eriochrome black-T solution (0.20 gm of indicator in 50 mls of methanol) was used as the indicator, with the colour changing from a distinct blood-red to bright-blue at the end point.

For both calcuim (II) and magnesium (II), standard solutions of the cations were first prepared and analysed titrimetrically to test the reproducibility and accuracy of measurements [2, 3].

pH	Equilibrium conc. of $Mg^{2+} \times 10^3$	Mole Mg^{2+} bound per 2 Phosphorus	Mole ratio of Mg^{2+}, r
9.25	1.540	0.551	1.169
	2.970	0.917	1.145
	5.034	1.004	1.123
	7.252	1.651	1.108
	11.810	2.149	1.078
	16.770	2.170	1.067
8.20	1.784	0.585	1.154
	3.414	0.926	1.127
	5.065	1.000	1.106
	7.377	1.374	1.087
	12.570	1.724	1.064
	17.260	1.808	1.049
7.05	1.844	0.541	1.141
	3.583	0.923	1.110
	5.488	0.945	1.081
	7.551	1.017	1.064
	12.960	1.191	1.042
	17.580	1.255	1.034
5.00	1.447	0.374	1.121
	2.920	0.447	1.072
	4.915	0.511	1.048
	7.626	0.568	1.035
	12.240	0.638	1.025
	17.440	0.809	1.022
3.63	1.267	0.251	1.093
	2.931	0.400	1.064
	5.043	0.455	1.035
	7.575	0.483	1.030
	11.550	0.554	1.023
	17.270	0.724	1.020

Table 4.4 Dialysis of nucleate solution (9.399×10^{-4}) mole P/litre against magnesium chloride at various pH values and ionic strength of 2×10^{-2}M in Na

pH	Equilibrium concn. of $Ca^{2+} \times 10^3$	Mole Ca^{2+} bound per 2 Phosphorus	Mole ratio of calcium,r
9.25	1.050	0.394	1.176
	3.102	0.930	1.141
	5.401	1.021	1.113
	7.656	1.776	1.110
	11.121	2.074	1.080
	16.251	2.087	1.060
8.20	1.210	0.425	1.165
	3.250	0.904	1.131
	5.327	0.968	1.100
	7.765	1.426	1.086
	10.891	1.670	1.073
	16.350	1.745	1.050
7.05	1.700	0.511	1.141
	3.165	0.857	1.116
	5.456	0.926	1.080
	7.505	1.031	1.064
	12.351	1.196	1.045
	17.33	1.278	1.035
5.00	1.421	0.364	1.123
	3.495	0.500	1.069
	5.111	0.532	1.049
	8.324	0.598	1.034
	13.050	0.685	1.025
	16.750	0.787	1.022
3.60	0.953	0.226	1.111
	3.133	0.432	1.065
	4.957	0.472	1.046
	8.154	0.485	1.029
	12.040	0.575	1.022
	16.720	0.713	1.020

Table 4.5 Dialysis of nucleate solution (9.399×10^{-4} mole P/litre) against calcium chloride at various pH values, and ionic strength of 2×10^{-2}M in NaCl.

Ionic Strength I	Equil. concn. of Mg^{2+} (mole/per litre) $\times 10^3$	Mole Mg^{2+} bound per 2 Phosphorus	Mole ratio of Magnesium r
0.02	1.844	0.541	1.141
	3.583	0.923	1.110
	5.488	0.945	1.081
	7.551	1.017	1.064
	12.960	1.191	1.042
	17.580	1.255	1.034
0.10	0.8024	0.198	1.111
	2.771	0.570	1.098
	3.897	0.760	1.093
	7.178	0.870	1.057
	11.480	1.060	1.043
	16.900	1.150	1.032
0.16	2.527	0.392	1.085
	3.987	0.660	1.072
	4.815	0.670	1.062
	7.152	0.795	1.052
	9.945	0.850	1.040
	16.570	0.940	1.027

Table 4.6 Dialysis of nucleate solution (9.399×10^{-4} mole P/litre) against magnesium chloride at various ionic strengths in sodium chloride and pH 7.05

Ionic Strength I	Equil. concn. Mole Ca^{2+} per litre x 10^3	Mole Ca^{2+} bound per 2 Phosphorus	Mole ratio of calcium r
0.02	1.700	0.511	1.141
	3.165	0.857	1.116
	5.456	0.926	1.080
	7.505	1.031	1.064
	12.351	1.196	1.045
	17.330	1.278	1.035
0.10	1.942	0.400	1.098
	3.127	0.540	1.079
	6.396	0.730	1.053
	8.726	0.820	1.043
	14.700	0.956	1.030
	17.55	0.980	1.026
0.16	1.891	0.333	1.086
	3.924	0.480	1.058
	5.637	0.551	1.046
	8.222	0.590	1.034
	12.310	0.661	1.025
	18.030	0.700	1.018

Table 4.7 Dialysis of solution (9.399×10^{-4} mole P/litre) against calcium chloride at various ionic strengths in sodium chloride and pH 7.05

Ionic Strength (NaCl)	pH	$i_{\log k}$		$m_{\log k}$
0.02	9.25	2.435		2.490
0.02	8.20	2.366		2.430
0.02	7.05	2.333		2.395
0.02	5.00	2.334	*1.623	1.650
0.02	3.60	2.334	*1.522	1.610
0.02	7.05	2.333		2.395
0.10	7.05	2.239		2.225
0.16	7.05	2.234		2.116

Table 4.8 Stability constant(k) for DNA-magnesium complexes

Ionic Strength (NaCl)	pH	$i_{\log k}$		$m_{\log k}$
0.02	9.25	2.424		2.440
0.02	8.20	2.409		2.405
0.02	7.05	2.344		2.303
0.02	5.00	2.424	*1.589	1.600
0.02	3.60		*1.520	1.529
0.02	7.05	2.344		2.303
0.10	7.05	2.204		2.078
0.16	7.05	2.195		1.900

Table 4.9 Stability constant(k) for DNA-calcium complexes

$i_{\log k}$ and $m_{\log k}$ are values computed from plots of equations (2-4a) and (2-15a) respectively.

* values of log k for second equilibrium from lines passing through unity at acid pHs.

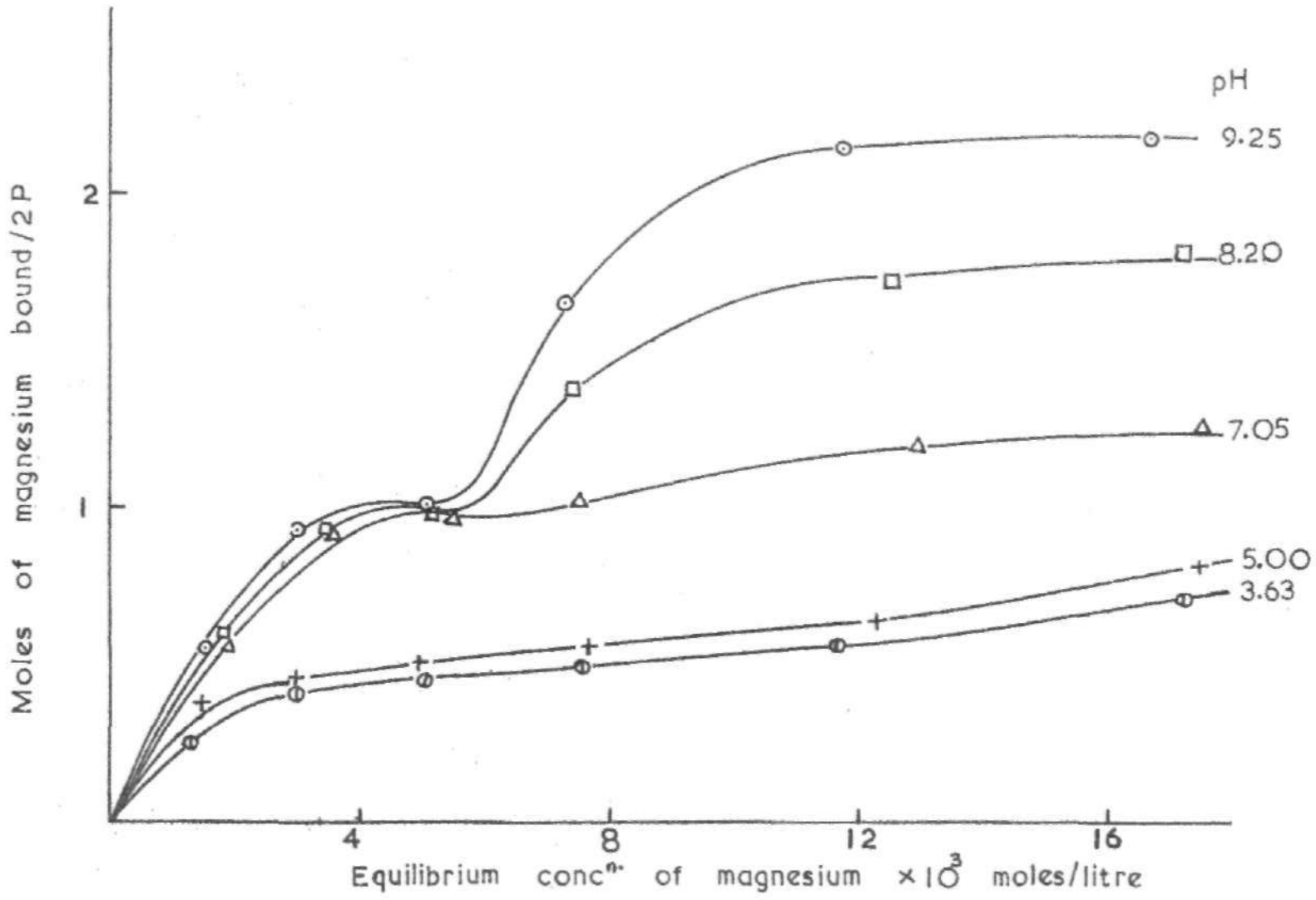


Fig. 11: Dialysis of DNA against magnesium chloride for an ionic strength of 0.02 in NaCl

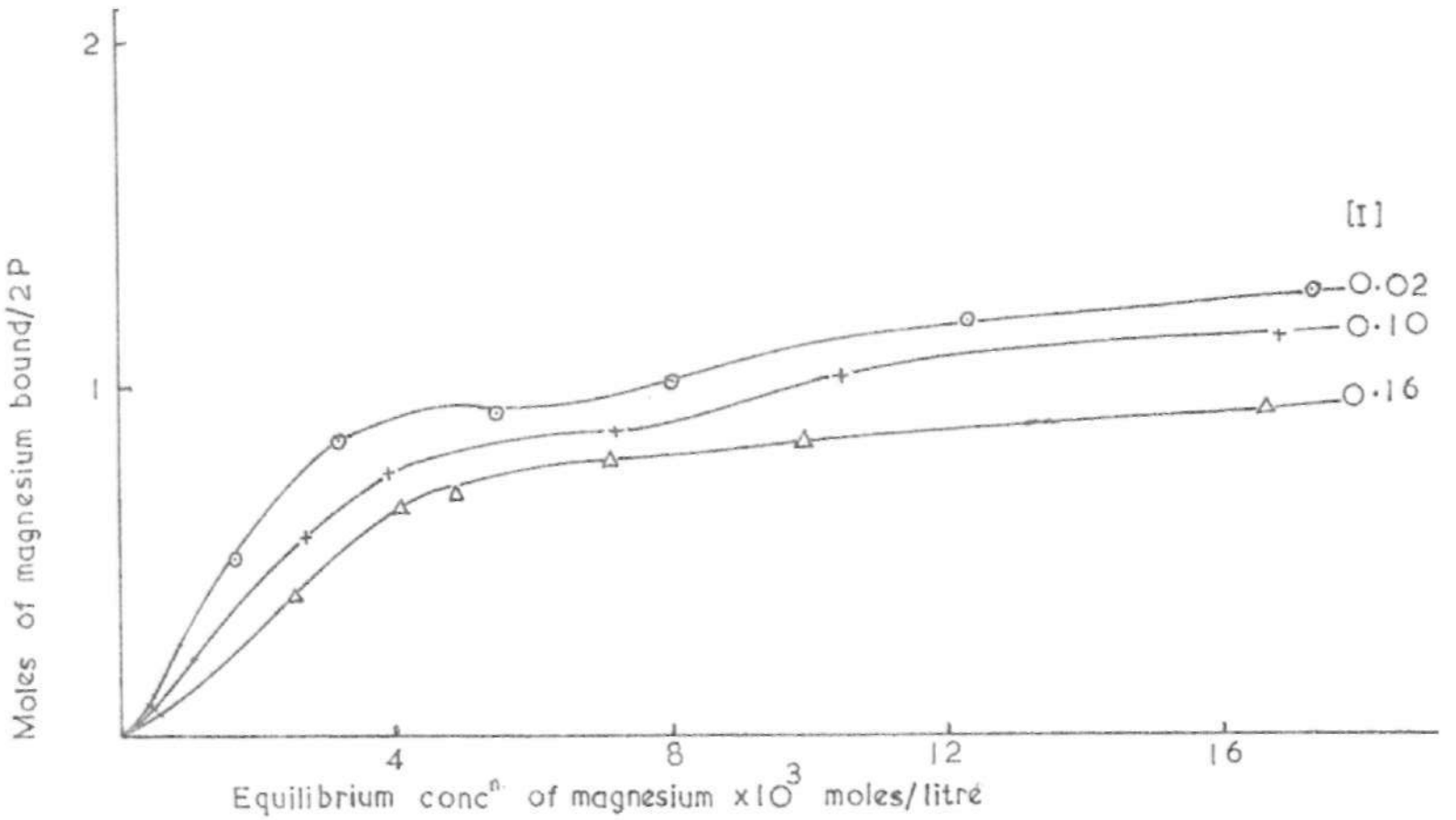


Fig 12: Dialysis of DNA against magnesium chloride at pH 7.05

Fig. 13: DONNAN EFFECT

Mole ratio vs equilibrium conc^n of magnesium $\times 10^{3}$ mole/litre for [I] = 0.02 in NaCl

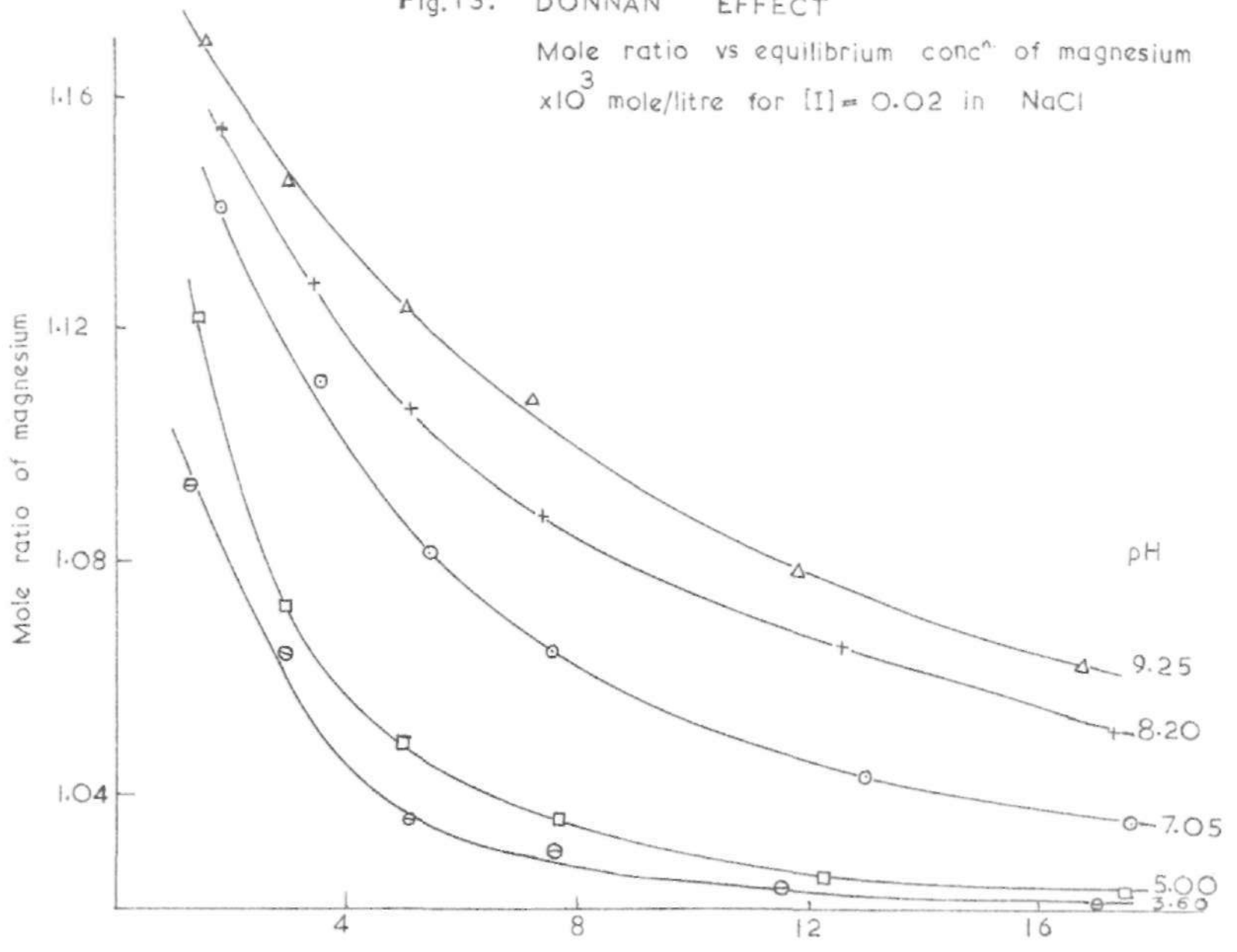
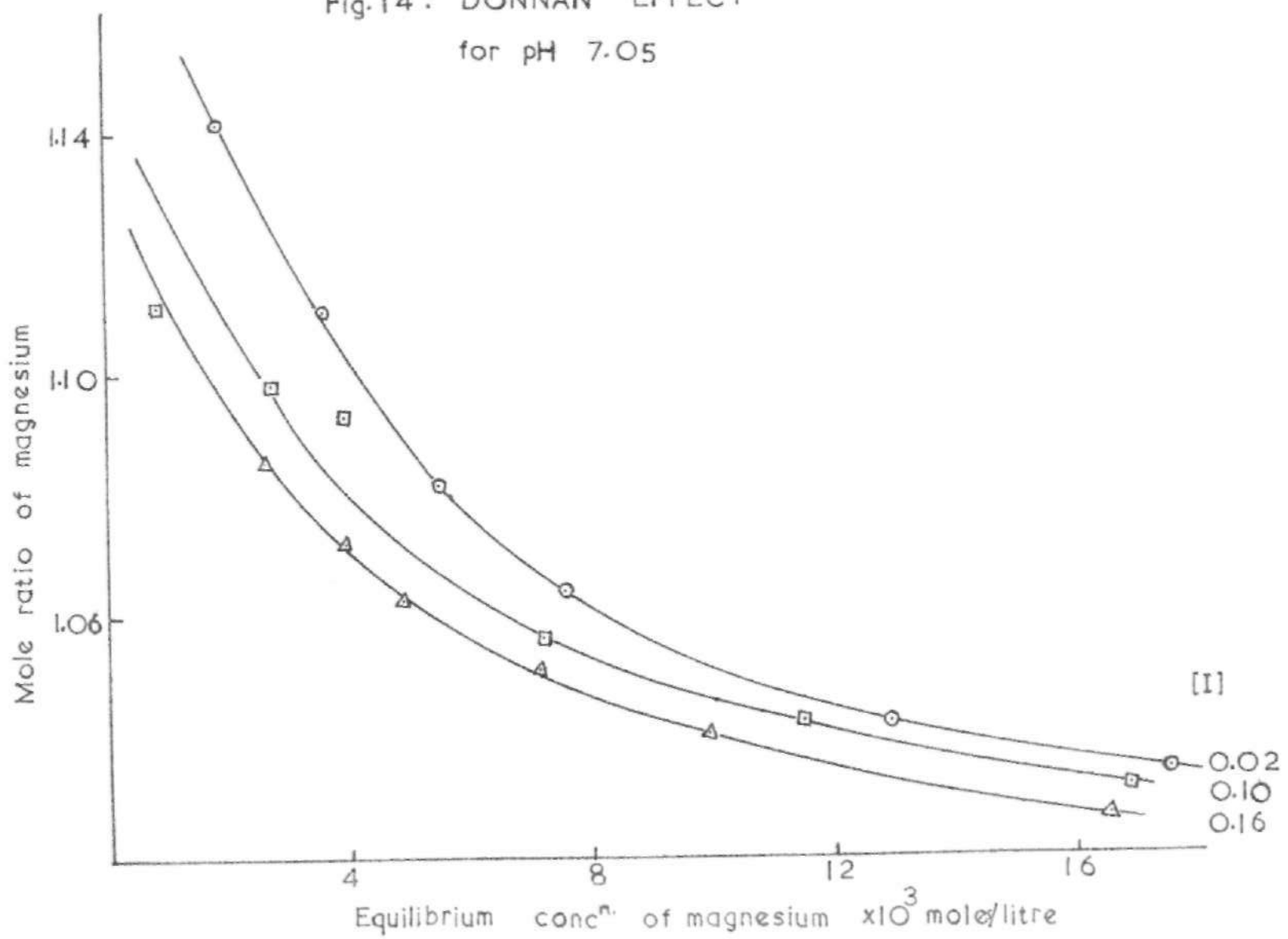


Fig. 14 : DONNAN EFFECT
for pH 7.05



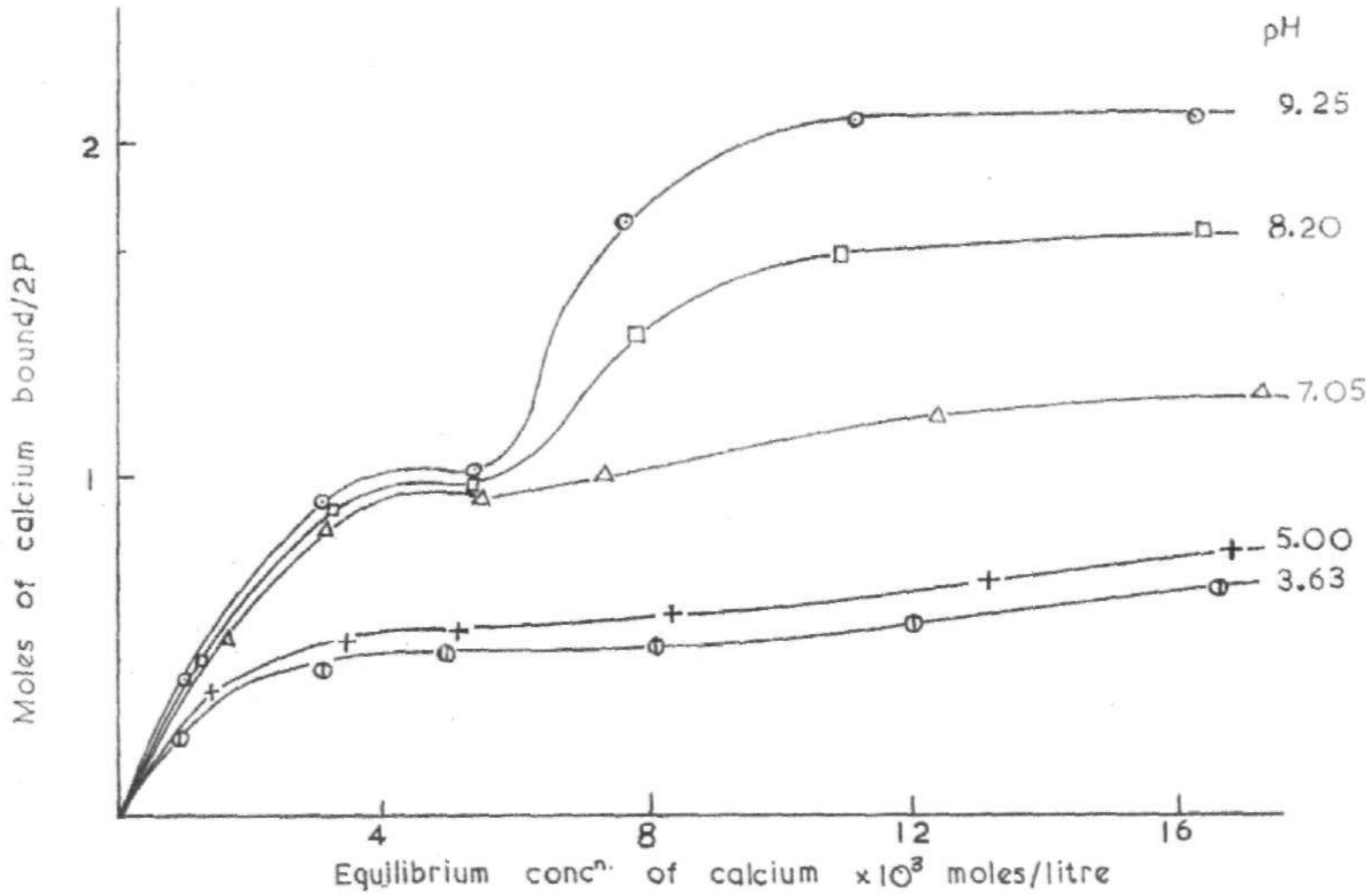


Fig. 15: Dialysis of DNA against calcium chloride for an ionic strength of 0.02 in NaCl.

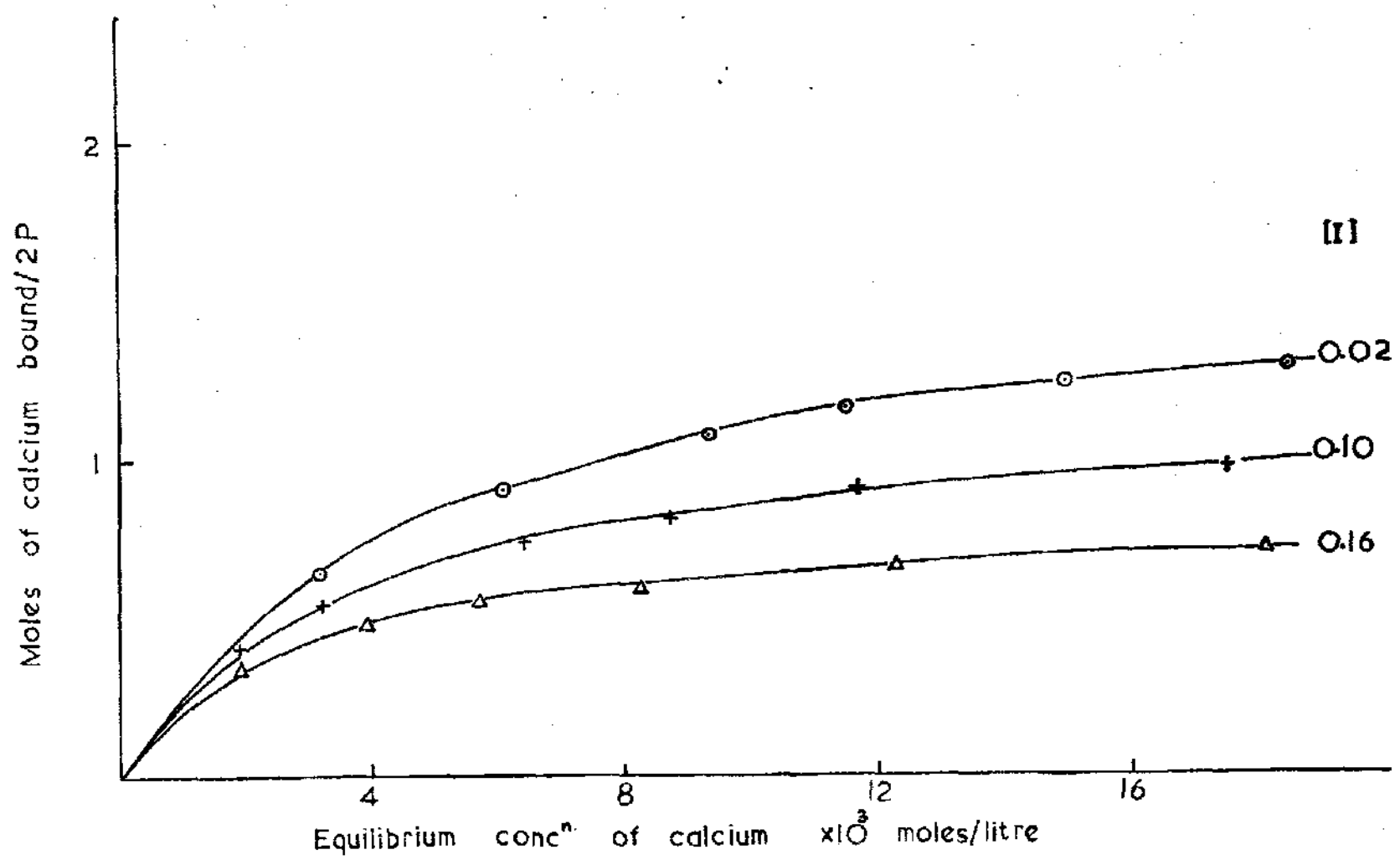


Fig. 16: Dialysis of DNA against calcium chloride at pH 7.05

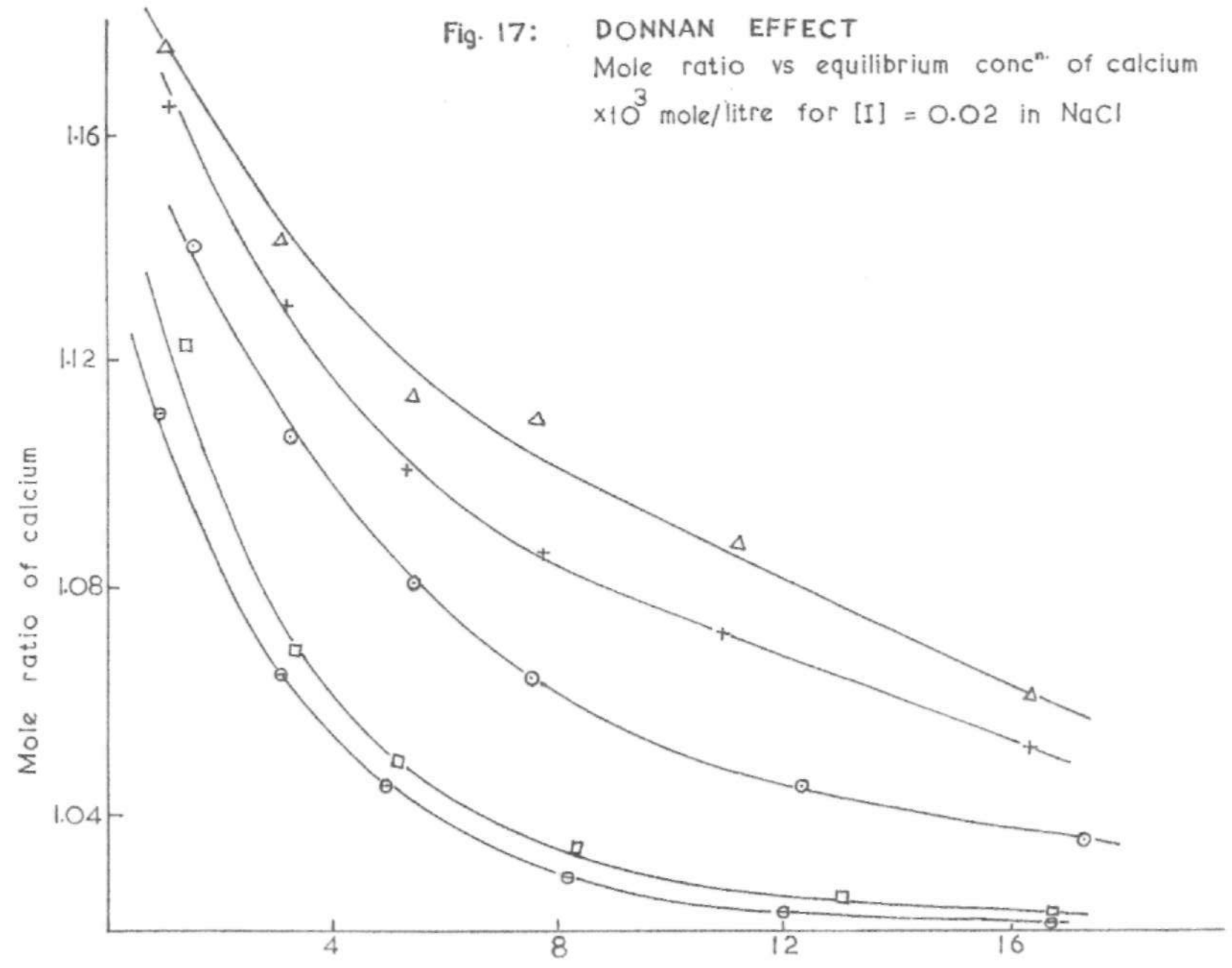


Fig. 17: DONNAN EFFECT

Mole ratio vs equilibrium conc$^{n.}$ of calcium $\times 10^{3}$ mole/litre for [I] = 0.02 in NaCl

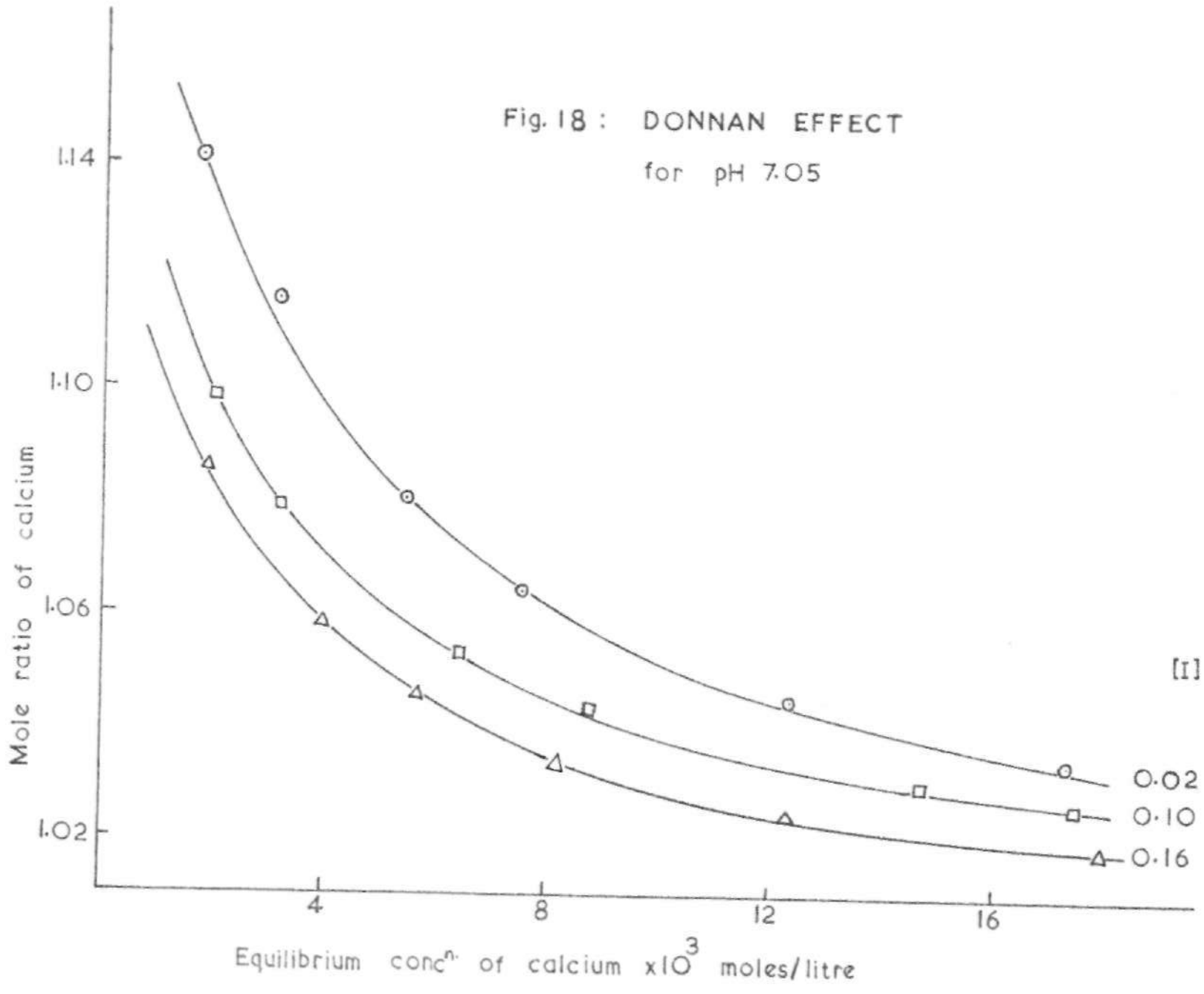
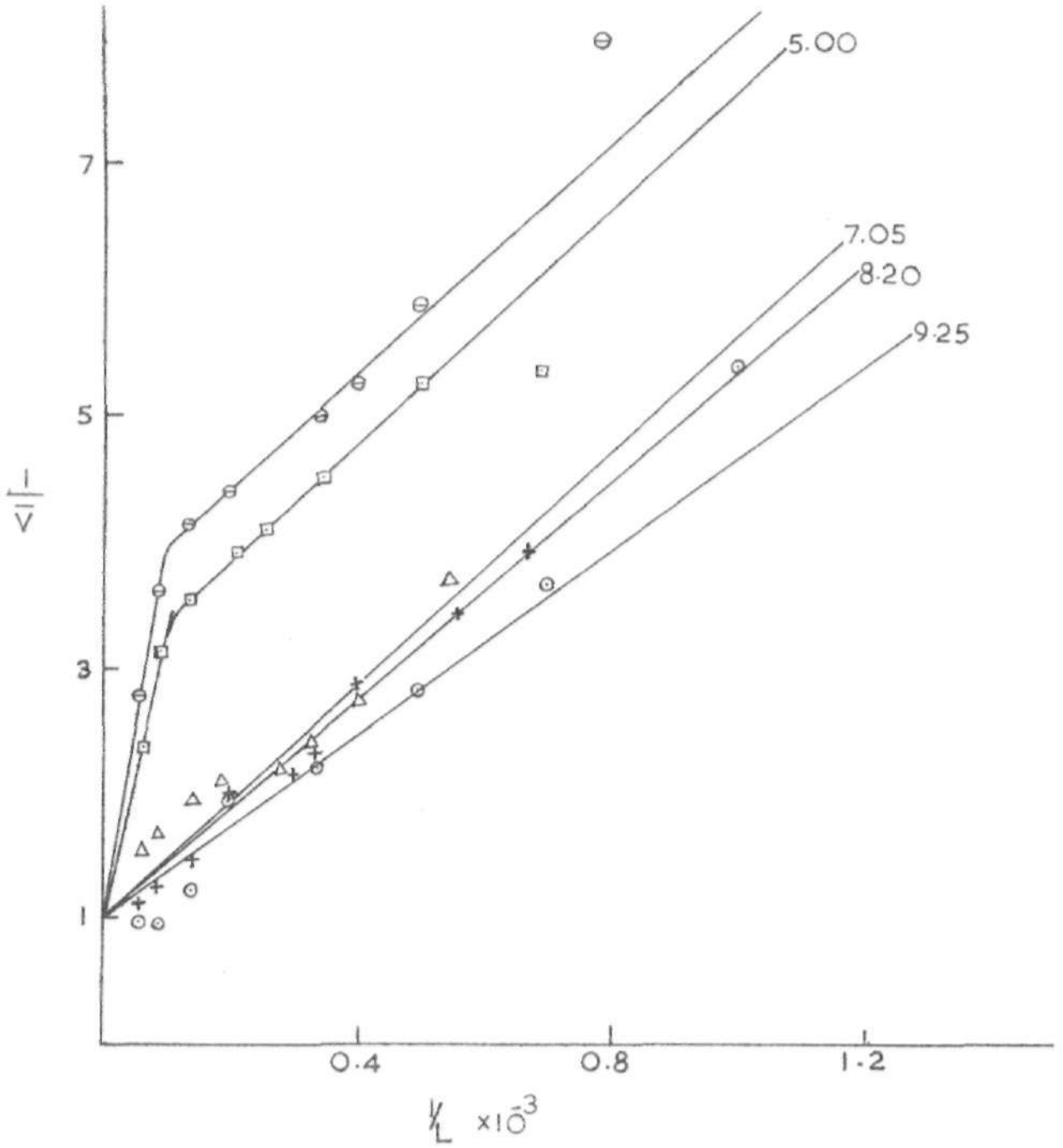


Fig. 18 : DONNAN EFFECT
for pH 7.05



Figs. 19 to 22: Computation of stability constants of DNA–Mg^{2+} complexes

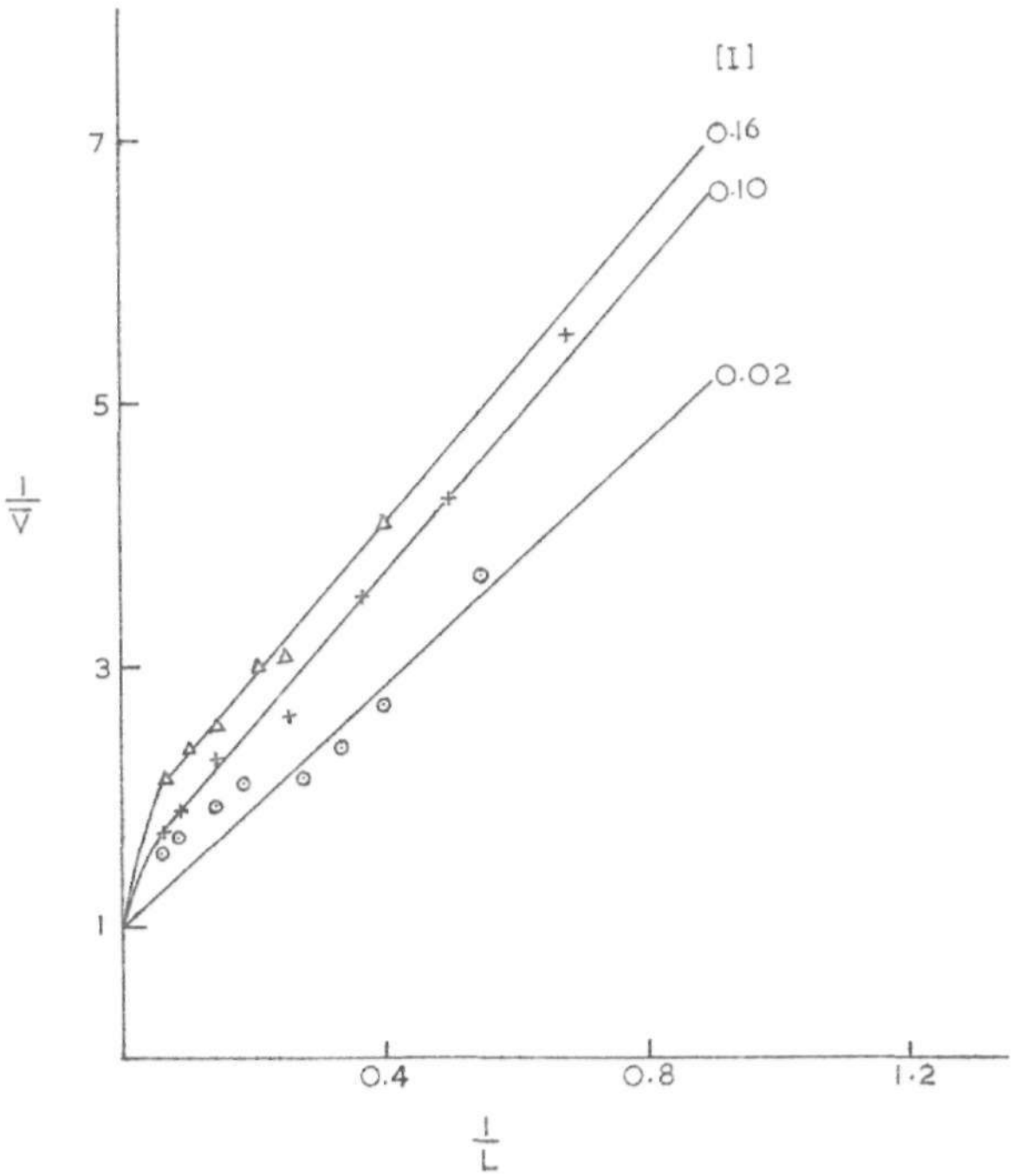


Fig. 20: Plots for three ionic strengths of sodium chloride [I] and pH 7.05

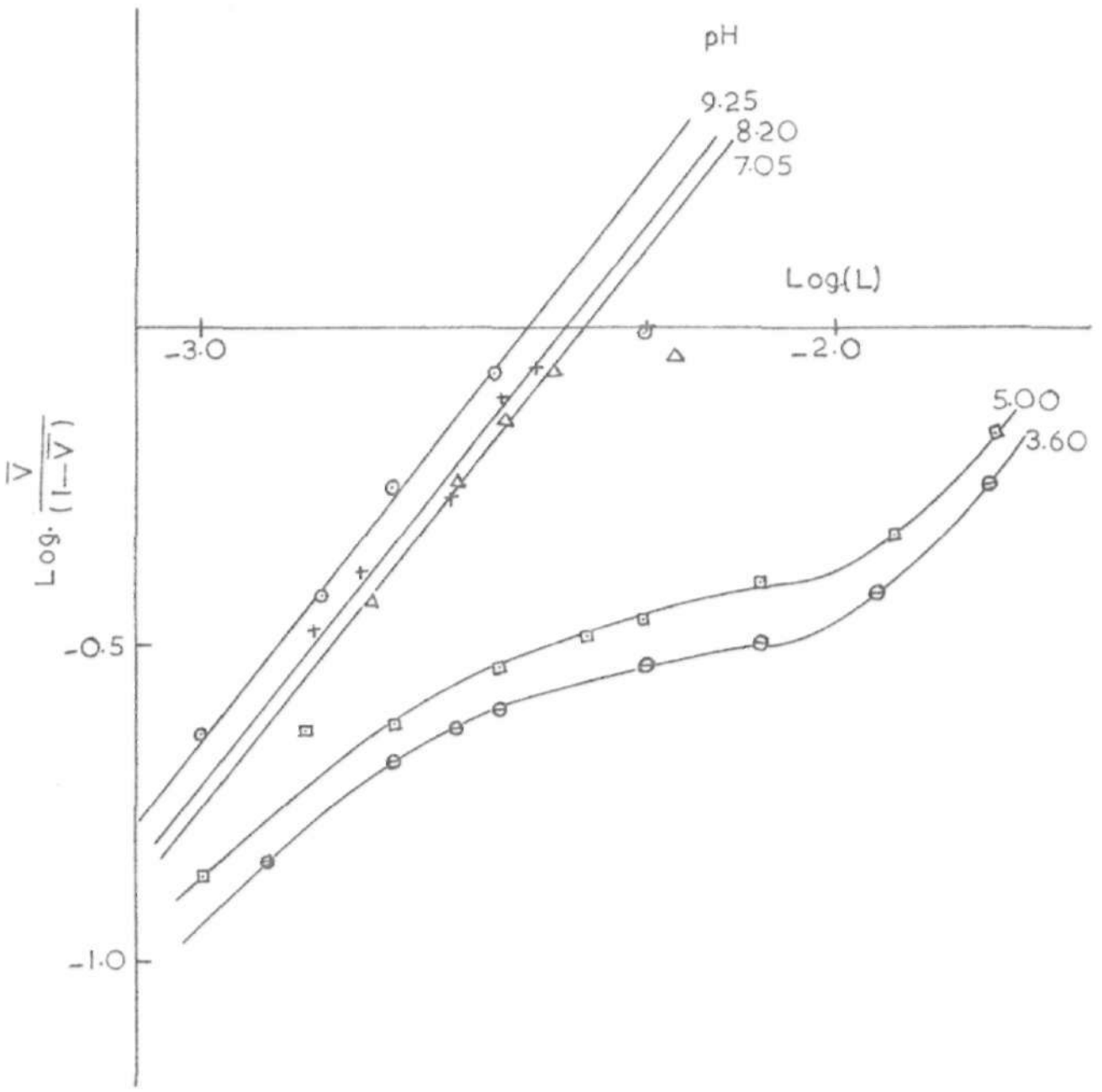


Fig. 21: Plots for different pH values and ionic strength of 0.02

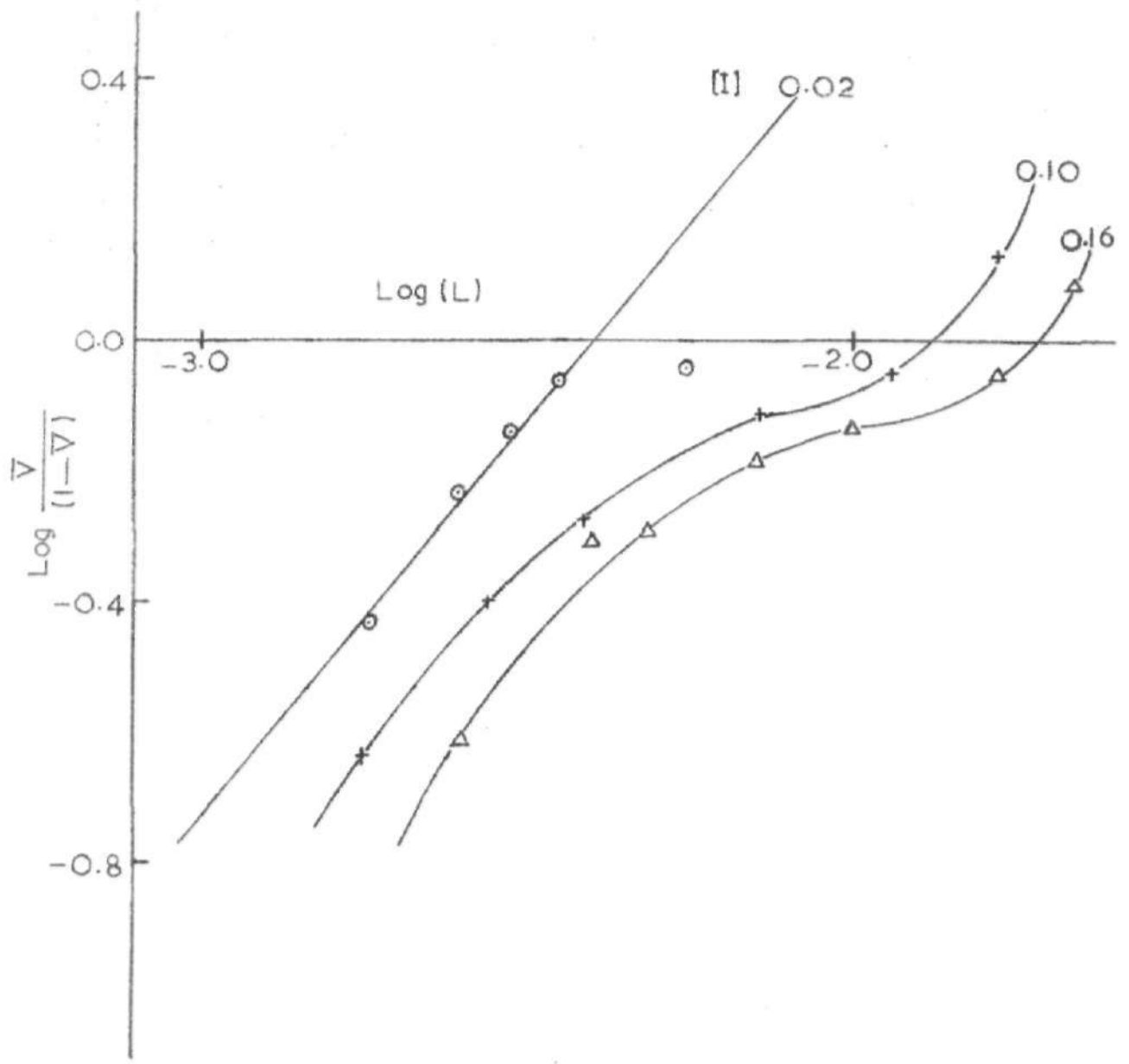
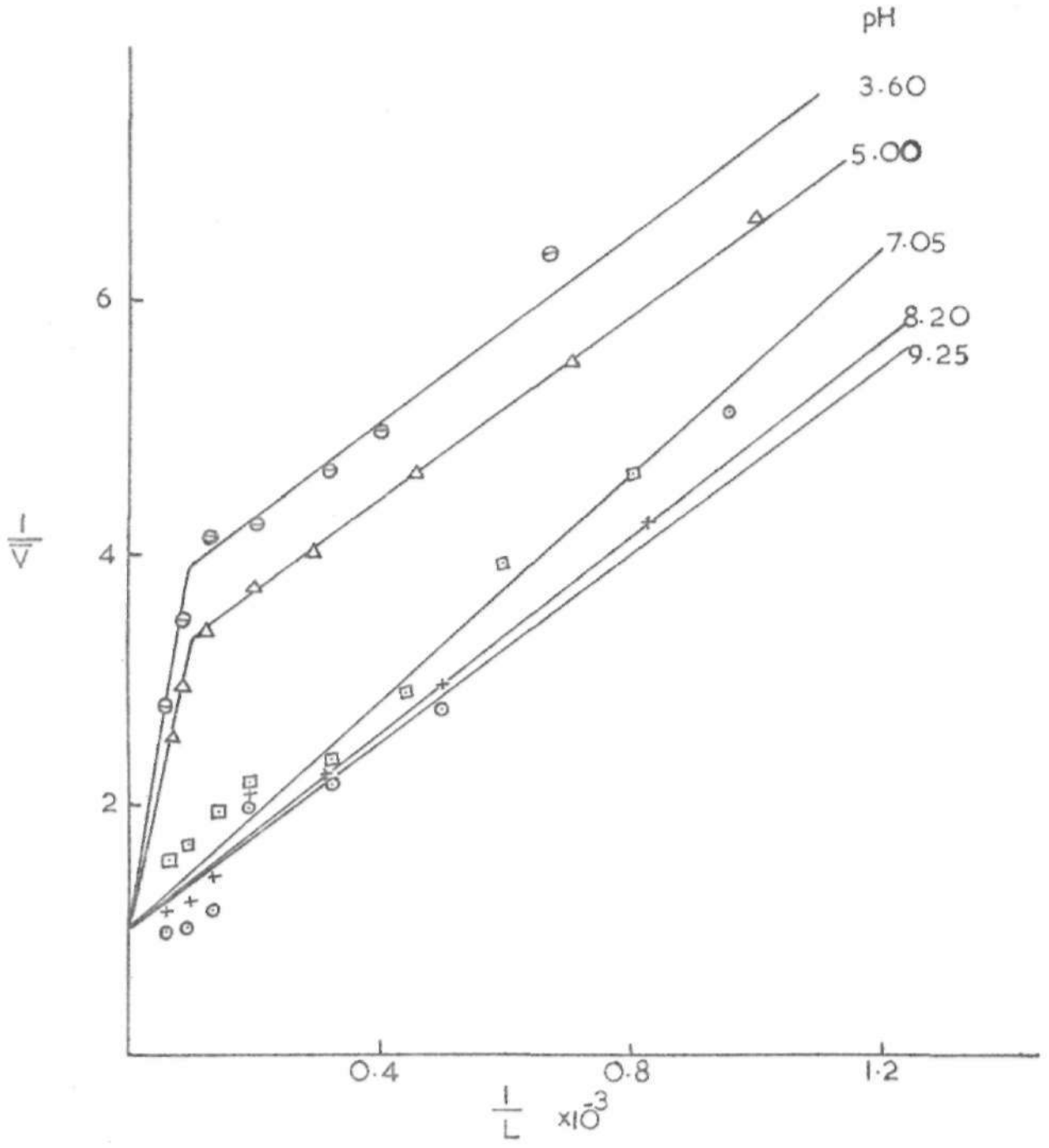


Fig. 22: Plots for three ionic strengths at pH 7.05



Figs 23–26: Computation of stability constants of DNA—Ca^{2+} complexes

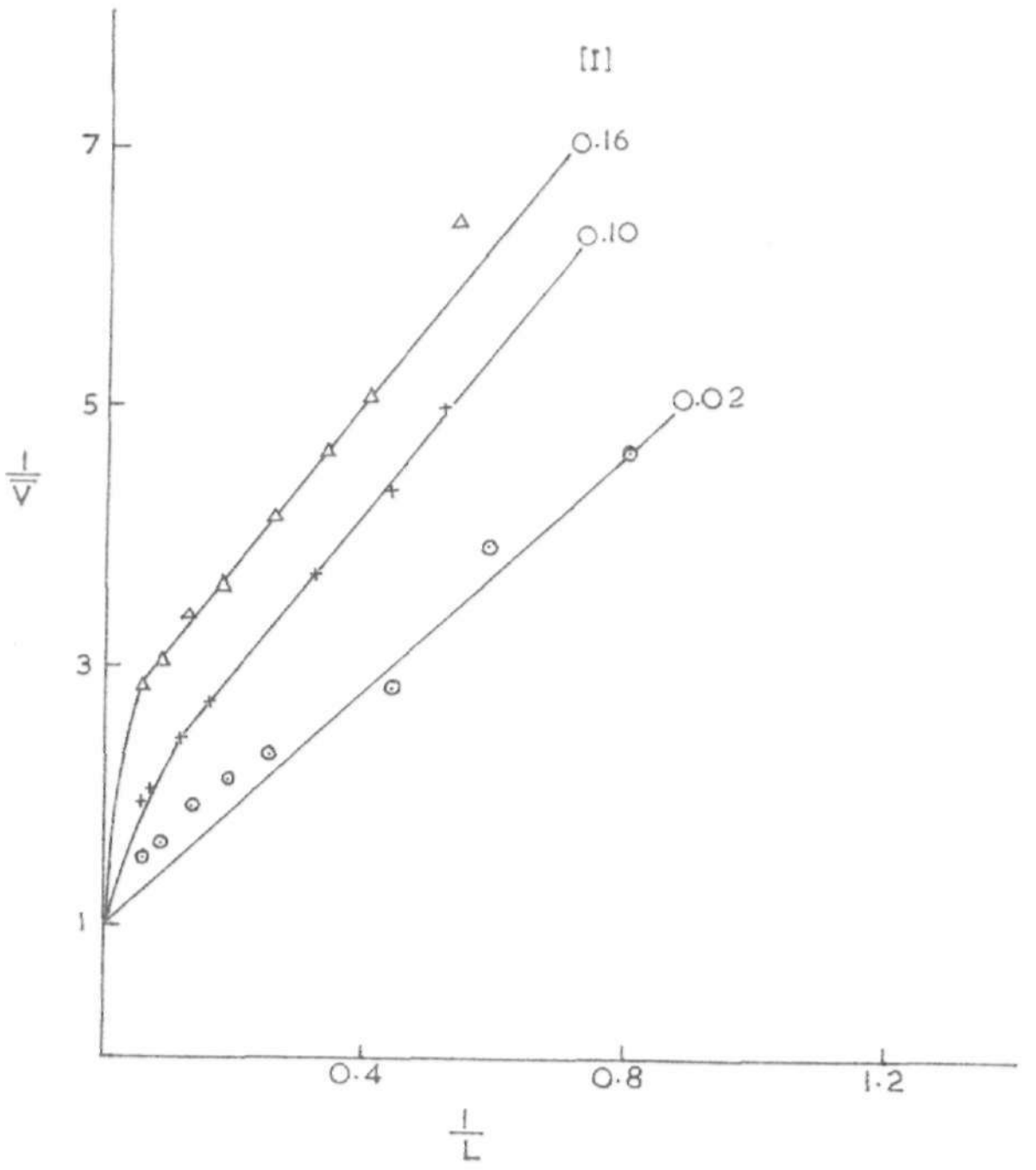


Fig 24 : Plots for three ionic strengths of sodium chloride and pH 7.05

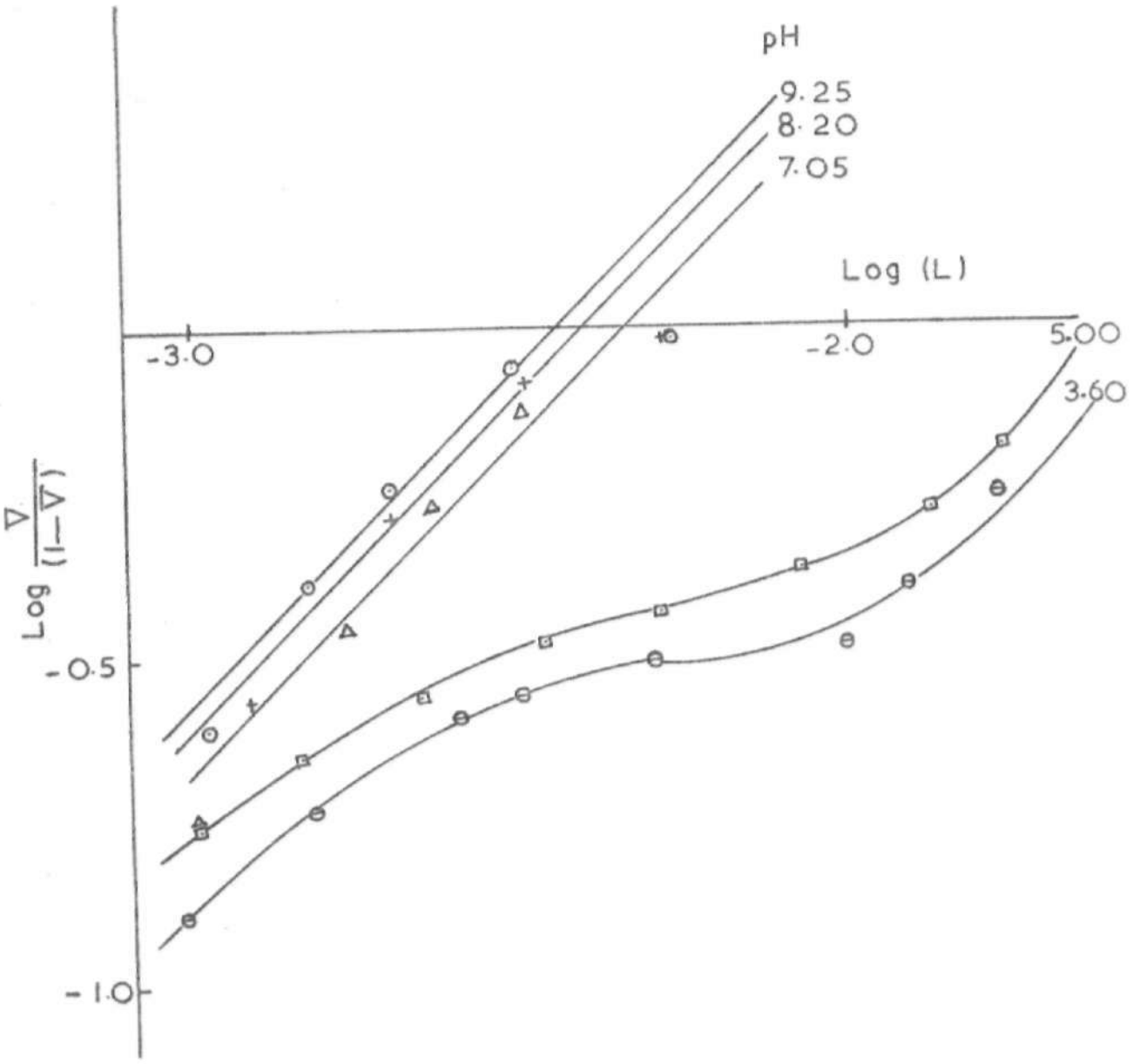


Fig. 25: Plots for different pH values and ionic strength of 0.02

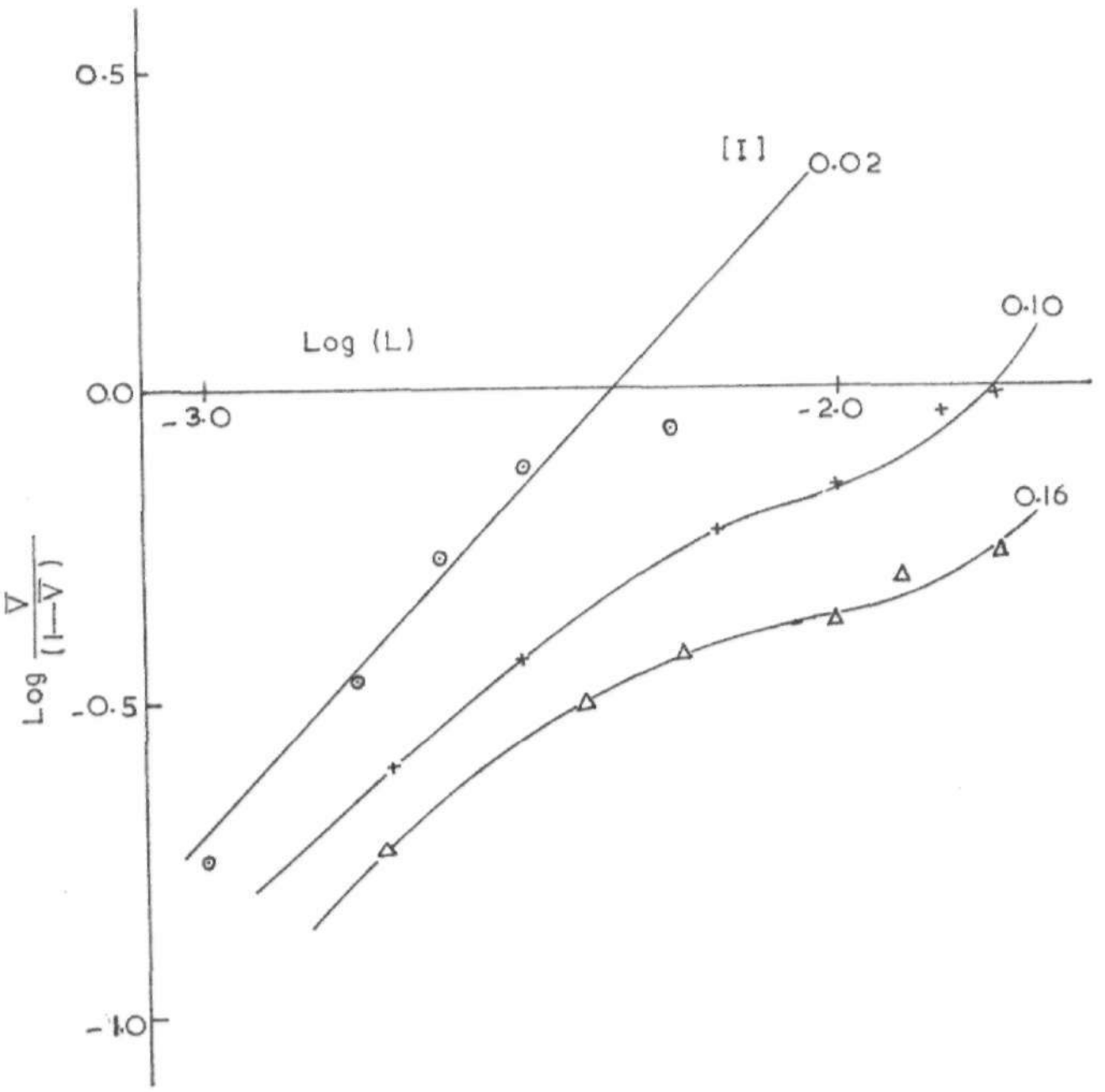


Fig. 26: Plots for three ionic strengths at pH 7.05

II.3 Results and discussion

A double equilibria has been established with two types of complexes formed (figs 11, 12, 15, 16). The first corresponds to a quantitative binding of divalent cations to the nucleate phosphorus, with half a mole of cation binding one mole of phosphorus.

The second which occurred only for neutral and alkaline pHs and for low ionic strengths, represents the formation of a complex the stoichiometry of which depends very much on the pH and ionic strength of the solution. This equilibrium might also occur for the acid pHs and high ionic strengths at higher concentrations of divalent cations.

II.3.a The effects of pH and ionic strength on binding (figs 11, 12, 15, 16)

The results in tables (44 and 47) indicate that binding decreased as the pH was moved towards the acid range (and at the ionic strength in NaCl was increased, tables 46 & 47). The formation of a complex of half a mole of cation to one mole of nucleate phosphorus is well pronounced in the region of neutral and weakly alkaline pH (i.e. pH 7 - 9).

The formation of a complex of this stoichiometry occurred for the acid pH range only at high concentrations of divalent cations.

A complex with more than half a mole of cation with one of phosphorus is also formed, but only for the neutral and alkaline pHs.

Binding at the second equilibrium (i.e. that with more than half a mole of cation to one of phosphorus) appeared to be more pH dependent than that for the first equilibrium.

This equilibrium might have been as a result of (i) binding of cation to other dissociable sites at these alkaline pH range, or (ii) the formation of a complex of more than half a mole of cation to one of phosphorus, or (iii) the interaction of cations with the buffer system. The first suggestion is unlikely to be correct here because dissociable sites other than the phosphates and the ($-NH_3$) are not expected to be dissociated at pH 8, but above pH 9.5 and below pH 5.0.
Also, since the same buffer solution is found inside and outside the dialysis bag, any difference in the concentrations of divalent cations outside from inside must be due to the amount bound to the polyanion which is found only in the bag. The equilibrium could therefore have arisen from the interaction between bound and unbound cations on the phosphates.

It is expected that this equilibrium might be obtained for acid pHs at high concentrations of divalent cations.

The drop in the binding, particularly for the first equilibrium, as a result of changes in pH towards the acid range is a revelation of changes in the number of net negative charges on the macro-ion, as reported by Jordan, Mathieson and Matty [4].

The plots of the mole ratio (i.e. the ratio of $[M^{2+}]$ inside to that outside the dialysis bag at equilibrium) against equilibrium concentration of divalent cations outside the bag have shown that the Donnan effect, discussed in the theory in this chapter, decreased with decreasing pH towards the acid range, Figs. (13, 14, 17 and 18).

This implies that possible corrections for the Donnan effect would need be made for binding at these high pH values, especially for low equilibrium concentrations of added divalent cations.

Variations in the ionic strength of the sodium chloride supporting electrolyte seems to have a similar influence on the binding as pH variations.

Binding decreased with increasing ionic strength of sodium chloride, in keeping with the idea of decreasing net negative charge on the nucleate with increasing ionic strength [4].

The effect of high ionic strength on binding is quite appreciable. Another possible reason for this might be that the sodium ions were engaged in what could be called 'a non-cooperative interaction' with the divalent cations in binding to the macro-ions. As reported [14], sodium ions in high concentrations reversibly bind to the nucleate, and displace magnesium or calcium ions in low concentrations of the latter. In high concentrations of the divalent cations, the situation would be reversed, as in the case of high equilibrium concentrations of divalent cations, when both equilibria would be obtained.

II.3.b Nature of the binding sites

As is already known, the complete hydrolysis of DNA yields the sugar residues, the purine and pyrimidine bases and phosphoric acid.

Phosphoric acid is tribasic and ionises in three widely separated pK or pH. Those are:

primary ionisation (pK < 2)

secondary ionisation (pK ≏ 6)

tertiary ionisation (pK ≏ 9)

The mononucleotides are formed by the establishment of a phospho-monoester linkage between the deoxypentose and phosphoric acid. As a consequence, the tertiary phosphate ionisation is lost, leaving only the primary and secondary ionisations.
The primary ionisation occurs at so low a pH (≤ 2) as to correspond for practical purposes to that of a strong acid.
Thus the phosphate group of a nucleotide carries a single negative charge at acid pH's and a double charge at alkaline pH's (pH > 6).

Combination of nucleotides to form a polynucleotide results in the elimination of the secondary phosphate ionisations except for that arising from the terminal phosphate, which is not involved in a phosphodiester linkage.
A diagram of a completely ionised phosphate groups on a segment of a polynucleotide is shown in chapter one.
Other ionisable groups in a polynucleotide (or DNA) are shown in table 4.10, with their corresponding pKa' values.

	Amino $\overset{+}{-}NH_3$	$-N_1H-C_6O-$	$-N_3H-C_2O-$	Imidazole - NH -
Thymine	-	9.85	-	-
Cytosine	4.5	-	12.26	-
5-methyl cytosine	4.7	-	12.4	-
Adenine	4.15	-	-	9.8
Guanine	3.2	9.3	-	12.3

Table 4.9 pKa values of some ionisable groups on a polynucleotide. Figures obtained from Nucleic acid by D. O. Jordan and contained in references [17 - 22].

Table shows that over the pH range of 3.6 to 9.25, only the amino ($-\overset{+}{N}H_3$) type of ionisation would be expected, and only for cytosine, 5-methyl cytosine adenine and guanine.

Eliminating all the other ionisable groups therefore, we are left with the primary phosphate ionisation and the amino ($-\overset{+}{N}H_3$) type of ionisation taking place in the pH range (3.6 to 9.25) investigated. Of these two types of ionisations, only the primary phosphate type would be expected to permit the binding of a cation such as magnesium or calcium.

Hence the ionised phosphate groups must be the only binding sites on the nucleate for the system of DNA interacting with divalent cations.

The amino-type of ionisation is expected to cause a reduction of the net negative charges on the polyion, and therefore produce a slight decrease in the extent of binding.

As based on the secondary structure of DNA proposed by Crick and Watson, chapter one, these phosphate groups are on the periphery of the α-helix and are accessible and susceptible to attack by the divalent cations. A possibility exists too, that the anions from the divalent salt might be bound to the positively charged bases (i.e. the - $\overset{+}{N}H_3$ group). This possibility is nevertheless very small, since the bases are within the core of the α-helix and are not quite accessible to the small anions. There could also be some interaction between the anions and uncharged amino-groups.

II.3.c Configuration of ~~binding~~ bound nucleate

Consider a simple case of a polynucleotide chain interacting with divalent cations. Initially the chain would assume a full extension owing to repulsion between the negatively charged phosphate groups. The chain would also tend to coil up.

In the presence of divalent cations the phosphate sites could be bound in several ways, one of which is shown below.
For a native DNA molecule the two polynucleotides are stabilised by hydrogen bonds formed between the bases (chapter one).
Binding with the cations can be assumed to take place round the helix. As illustrated, a sort of ring formation through the divalent cation has been assumed. This would mean that, for the structure to be stable, the cations would be in a different plane from those in which other atoms in the ring are found.

The exact arrangement of the cations around the ~~A~~-helix is not known, and the following proposals have been put forward; (i) interchain interactions (ii) intrachain interactions and (iii) intermolecular interactions. Each of these would lead to a stabilisation of the polymeric ion.

(i) interchain interactions: binding of a divalent cation to two phosphate groups, one from each chain of a DNA molecule, is a general feature of interchain mode of interaction. It can occur only in places where phosphate groups on the two chains are close together, particularly at the large and small grooves, within the acute angles. Far away from the grooves, interchain binding is improbable and the intra chain type of binding might predominate.

(ii) Intrachain interactions: this is the binding of a cation to two phosphate groups on the same polynucleotide chain. Usually, and as in the interchain binding, two immediate neighbour phosphates groups are involved.

Both the interchain and intrachain interactions are expected to be equally stable, except for the fact that, where a tendency exists for strand separation, the interchain type of binding could easily be destabilised due to strains in the molecules.

The third type of interaction could occur as explained in section I.3.b of this chapter.

II.3.d Comparison of binding for the DNA-Ca^{2+} and DNA-Mg^{2+} complexes.

Approximate stability constants for the complexes formed at all the various pH values and ionic strengths have been computed for the two types of complexes i.e. DNA-Ca^{2+} and DNA-Mg^{2+}. These are shown in table (4.8 and 4.9), and are the thermodynamic stabilities which measure the extent to which the complex DNA-M^{2+} was formed under the conditions of experiment. In other words, these values represent the formation constant for DNA-M^{2+}.

Both the graph of $\frac{1}{\bar{\nu}}$ against $\frac{1}{L}$ (where $\bar{\nu}$ and L, are respectively the average mole of cation bound per mole of phosphorus and the equilibrium concentration of divalent cation outside the bag) and log $(\bar{\nu}/1-\bar{\nu})$ against log L (fig 19-24) are not linear, indicative of (i) some possible changes in the macromolecular structure of the DNA molecule, and (ii) interactions between the sites of binding or between bound and unbound ligands.

As expected from equations (2-4a) and (2-15), the intercept along the $1/\bar{\nu}$ axis is unity and the slope of the log $(\bar{\nu}/1-\bar{\nu})$ versus log L curves is also approximately unity.

Points for low concentrations of the divalent cations up to the first equilibrium fall almost on a straight line from which the stability constant was computed. No such straight lines were easily obtainable for acid pH's and high ionic strengths.

Two values of stability constants have been obtained for acid pH values corresponding to the initial slope at the intercept of unity on $1/\bar{\nu}$ axis, and the other corresponding to that for the first equilibrium. From these two figures it could be concluded that the complex for the first equilibrium is much more stable than that for the second equilibrium.

The stability constants decreased from the alkaline pH range towards the acid pH range, and from low ionic strength in sodium chloride (supporting electrolyte) towards high ionic strengths. These figures are approximate since the electrostatic effect on the stability constant was not accounted for. If accounted for, the electrostatic effect would likely cause an increase in the values of the stability constants, as based on equation (2-19) of chapter two, section II. In this equation, the term $\phi(\bar{\nu})$ is expected to decrease with increasing $(\bar{\nu})$ i.e. as the degree of binding is increased [15]. In actual fact, with the assumption made in chapter two, section I.b, for 2y mononucleotides in a DNA molecule, the stability constant for complex formation with divalent cations would be y times the values reported in table 10.

For the purpose of comparison, it can be concluded that the two types of system formed complexes of comparable stabilities. For the same pH value or ionic strength, the stability constants are of the same order, but slightly higher for the DNA-Mg^{2+} system.

This is not unexpected on the account of the smaller ionic radius of the magnesium ion (0.65Å) as compared to (0.95A°) for calcium [16].

For each system, the stability constant first dropped sharply from the alkaline pH to a much lower value in the acid pH, and tended to remain constant as the system became more highly acidic. The lower limit for each system approached 2×10^2 in high acid pH. The upper limit cannot easily be estimated but the increase is not expected to continue indefinately, but could exhibit an inflexion in the alkaline pH, on a graph of stability constant versus pH. It may rise thereafter however, in the pH range higher than 9,5, where ionisations of other sites like the imino-and imidazole is anticipated.

In conclusion, it has been found that calcium and magnesium ions interact with DNA to form complexes of varying stability. The DNA-M^{2+} complex is most stable in the neutral and weakly alkaline pH's and in low ionic strength of sodium chloride supporting electrolyte.

II.3.e Comparison of results of conductance and dialysis studies

The results obtained from the conductance studies and the equilibrium dialysis studies have shown some agreement. At the same time, it appears as if each method has its own merits. The method of conductance studies has furnished information about the stoichiometry of binding of 0.5 mole M^{2+} to one mole of phosphorus, and it has indicated points which could be associated with structural changes in the nucleate ion. It has however failed to show any dependence of binding on the ionic strength in simple electrolyte, and pH variations.

On the other hand, the dialysis technique has been found to be capable of detecting the binding of M^{2+} with DNA to form a complex of 0.5 mole of M^{2+} to one mole of nucleate phosphorus. It has also confirmed the dependence of binding on variations of ionic strength in simple electrolyte (e.g. NaCl), and variations of pH. However, this method suffers from inability to reveal points of structural changes in the nucleate ion, such as correspond to 0.125 and 0.30 mole of M^{2+} to one mole of phosphorus, obtained from conductance measurements.

The two methods combined have however succeeded in showing that

(i) DNA interact with magnesium and calcium to form a complex of 0.50 mole of M^{2+} to one of phosphorus.

(ii) there are other points corresponding to 0.125 and 0.30 mole M^{2+} to one of phosphorus, which have been identified with either the formation of complexes of these stoichiometries, or points of changes in the structures of the nucleate ions.

(iii) binding of DNA to magnesium and calcium is pH and ionic strength (in sodium chloride) dependent, and decreases with increasing ionic strength, and as acid pH range is ascended.

(iv) the complex formed (1: 1 mole M^{2+}/P) by DNA with magnesium and clacium are stable with the DNA-Mg^{2+} complex being more stable than the DNA-Ca^{2+} complex under the same conditions of temperature, pH and ionic strength.

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CHAPTER 5

SECTION I. THEORIES OF HELIX-TO-COIL TRANSITION IN DNA

I. INTRODUCTION

Exposure of solutions of DNA to elevated temperatures results in a number of changes in the physical properties of the molecules e.g. UV light absorption, optical rotatory dispersion, infrared spectrum circular dichroism, and bouyant density [1]. For native DNA, these changes are not continuous with rise in temperature, but occur rather abruptly at a relatively high temperature. The region 75 - 95° has been found to be specific for nucleic acids [1]. Thus, heating solutions of DNA in buffer of pH 7 for one hour has little effect on the viscosity of the solution (measured after cooling) until a temperature of about 80° is reached [2]. Above this temperature a rapid decrease in viscosity occurs. For calf thymus DNA in 0.2M sodium chloride, this increase has been found to have a mid-point at 86°C.

The change in viscosity behaviour occurs rapidly at a specific 'melting' temperature, and is accompanied by a large decrease in the mean molecular end-to-end length, but with no change in the molecular weight [4]. The instability of DNA to heating as indicated by the drop in viscosity is markedly dependent on the ionic strength of the medium, and in salt free solution, the change is continuous from room temperature rather than abrupt. Failure to observe complete dissociation of the complementary strands as evidenced

by the molecular weight which remained unchanged, may be attributed to random formation of hydrogen bonds on cooling the solution, particularly when the nucleate concentration is fairly high.

The most convenient method of studying the thermal transition from native to denatured DNA employs the change in ultraviolet absorption on proceeding from a helical to a random coil structure [5].

The thermal transition is broad, suggesting that there is a distribution of specific denaturation temperatures for the DNA molecules in the preparation. This has been explained as due to the unequal contribution by the two types of base pairs to the stability of the helix.

The 'melting' temperatures of different DNA preparations from different sources usually have different values, as determined by the (G + C) content of the acid [2]. Preparations which are rich in (G + C) normally have higher melting temperatures than those with low (G + C) content on the account of higher total number of hydrogen bonds between guanine and cytosine than between adonine and thymine.

I.1 Characteristic features of a helix-to-coil thermal transition.

(i) The transitions observed can be represented by simple sigmoids defined by two parameters: the position of their mid-point, on a relative absorption versus temperature graph.

The steepness and the shapes of the transitions are generally, analogous to those observed in highly cooperative order-disorder phase transitions of crystals or other polymers.

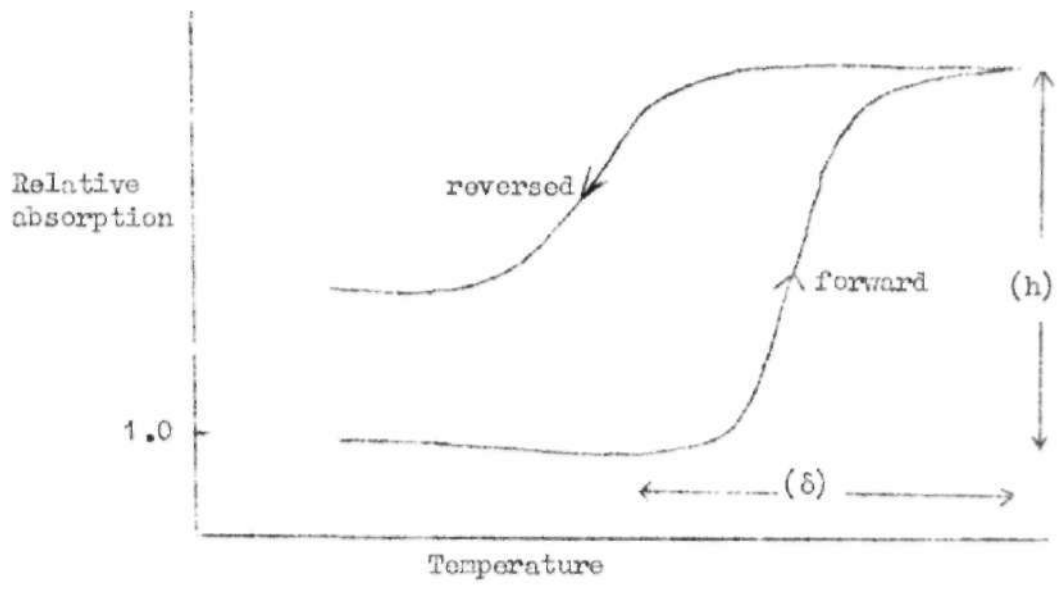
(ii) If the system under study involves two components, (i.e. if intermolecular heterogeneity is absent and the only two species involved are the native and denatured form(s) in equilibrium which are to be measured by the changes observed), and if the transition is reversible over a considerable region beyond the mid-point at which statistically 50 per cent of the molecules have been converted from the native to the denatured forms (i.e. some combination between the two extremes of all the molecules having been denatured to the extent of 50% or half of the molecules denatured to 100%), then for the transition DNA(native) $\rightleftharpoons$ DNA (denatured), the equilibrium constant is given as:

$$K_{tr} = \frac{[\text{denatured}]}{[\text{undenatured}]} = \frac{\text{fraction of base pairs in coiled regions}}{\text{fraction in helical region}}$$

The temperature at the transition mid-point $t_{\frac{1}{2}}$ °C (or $T_{\frac{1}{2}}$ on the absolute scale) is equivalent to a melting point t_m (or T_m).

(iii) The position of T_m depends directly and linearly on the composition of the DNA [2], increasing with increasing (G + C) content. No such dependence of the transition width on the composition of the DNA has been established. The value of T_m is relatively insensitive to the molecular weight of the polymer [6].

In the 0.2M sodium chloride, the relation $t_m = 69.3 + 0.41\ (G + C)$ has been established, where 69.3 is the $t_{1/2}$ of a hypothetical polymer containing only (A + T). The slope of the graph of t_m versus (G + C) is 0.41°/mole per cent (G + C) and has been found to be relatively independent of ionic strength. As observed, t_m increases by about 18°/log $[Na^+]$, for any given preparation [6]. Both of these parameters appear to be equally valid for natural DNA, and for enzymatically synthesized homopolymer absorption/temperature curve for the nucleic acid an entirely different curve, similar in shape to the first sigmoid, is expected and obtained.



Heating and cooling curves for a DNA solution.
This shows the forward and reversed sigmoid-shaped curves.

The absorption at room temperature for the forward and reverse curves differs. The values of T_m for the reversed curve is always smaller than that of the heating curve. In other words, a pre-heated DNA solution (heat denatured DNA) which has been allowed to cool to room temperature, if heated again, would follow the reversed curve for a native DNA. The T_m value of the reversed curve can be made to approach that of the heating curve by introducing some simple electrolytes like magnesium chloride to the heat denatured nucleate.

(iv) The transition breadth (δ), depends principally on three parameters [7], namely: the conformation of the molecule, the intramolecular heterogeneity and the intermolecular heterogeneity.

I.1.a Conformation of the DNA.

Single-stranded DNA shows much greater breadth of transition than does double-stranded DNA, and in general the greater the helicity of a polymer, the smaller the breadth. Hence a partially denatured sample will exhibit a large transition breadth [8]. A complete or a partial re-naturation of the denatured DNA would lead to a decrease in the transition breadth, as is achieved by the addition of simple cations like sodium and magnesium ions [8]. The presence of divalent cations capable of non-specific or specific and stoichiometric interactions can affect the transition breadth.

In general, these effects are more pronounced when the polymer is more flexible, and the most compact and rigid conformations always exhibit the most cooperativeness in the transition, and hence the smallest transition breadth.

I.1.b Intermolecular Heterogeneity:

This refers to an inequality in the molecular weight, or (G + C) composition or other physical or chemical properties of the molecules of a particular DNA preparation, or different preparations. It is clear from (ii) that, if a mixture of two different homopolymers or even two DNAs varying sufficiently in base composition are present in a mixture, two separate transitions are observable. As the compositions of the two (or more) components become more and more similar, the two sigmoids overlap more and more until their resolution into two separate curves is no longer possible. This resolution may be improved by measuring the transition at more than one wavelength. Wavelength values of 260, 230, or 280 mμ have been used. Such dispersion measurements of transitions are useful in the analysis of thermal and pH transitions of polymeric molecules.

I.1.c Intramolecular Heterogeneity:

Even for duplex DNAs of the same molecular weight but isolated from different species or cell types, the transition breadth has been found to be different.

The following general order has been found. DNA virus < small micro-organisms < large micro-organisms < higher organisms [7a]. Part of the reasons for this difference in the breadth of transition is undoubtedly due to the coexistence of DNA fragments of varying overall base composition in the preparation. These different fragments may have pre-existed in vivo or have been introduced during the isolation procedure by fragmenting the extremely long and fragile native DNA molecule. Part however, may be instrinsic. The extent of the cooperativeness of the transition may vary even in different homogenous DNA molecules and certain intrinsically less stable regions may be distrupted first without immediately affecting the remainder of the molecule.

I.2 Thermodynamics of Helix-to-Coil Transition in DNA

Contributions to the problem of the various thermodynamic quantities involved in the helix-to-coil transition in DNA have appeared in a number of publications [8 - 12]. However, no completely satisfactory treatment has yet been proposed that can be used to analyse the contributions made by various factors, e.g. electrostatic interaction and configuration, in controlling the helix-to-coil transition.

Two approaches to the thermodynamics of helix-to-coil transitions will be discussed here.

I.2.a The Approach of Kotin [11, 12]

This approach considers the electrostatic repulsion of the charged phosphate groups on opposite strands, and how they can be overcome during the interaction of the nucleate ion with simple metallic ions. The added electrolytes are assumed to screen the electrostatic repulsive forces between charged groups on opposite strands of the molecule.

Though it is not quite clear which electrostatic repulsions are being screened (vertical or horizontal interactions), a simple assumption has been made that at pH 7, and generally in the neutral pH range, the charged phosphate groups on one strand interact electrostatically with those on the other strand to reduce the stability of the molecule.

The difference in the Gibb's free energy between the helix and coil ($\Delta G_{tr} = G_h - G_c$) has been considered to be made up of two terms:

i.e. $\Delta G_{tr} = \Delta G_o + \Delta G_e$ (5-1)

In this expression, ΔG_o is a measure of intrinsic stability, practically independent of ionic strength, or is, the free energy at very high ionic strength where the electrostatic repulsions become a minimum. The other term is an electrostatic component ΔG_e arising from repulsions at low ionic strengths. It consists of reversible work against electrostatic forces required to bring two fully charged coils from infinite separation into the completely organised helical arrangement.

It is a quantity which is difficult to compute accurately on the basis of any assumed molecular model.

Dove and Davidson [6]; Kotin and Nagasawa [11] have solved the Poisson-Boltzmann equation for an assumed model for DNA, and have shown that

$$\Delta G_o/RT = C_1 - C_2 \log [\text{salt}];$$

where C_1 and C_2 are positive constants, virtually independent of temperature, and characteristic of the radius and linear charge density of the charged polyelectrolyte and [salt] is the concentration of the univalent electrolyte added.

Making use of the equation

$$\Delta G_o = \Delta H_o - T\Delta S_o \qquad (5\text{-}2)$$

where ΔH_o and ΔS_o are the enthalpy and entropy of transition at high ionic strengths and T is the absolute temperature, when ΔG_o tends to zero. The melting temperature, T_m^o, is obtained by the requirement that ΔG_{tr} in equation (5-1) should vanish.

Hence,

$$T_m^o = \Delta H_o/\Delta S_o \qquad (5\text{-}3)$$

In order to determine the effect of ionic strength on T_m, the dependence of ΔG_o on electrolyte concentration must be determined first. The equations obtained by Dove and Davidson [6], Kotin and Nagasawa [11] have led to an expression in ΔG_{tr} given as

$$\Delta G_{tr} = \Delta H_o - T\Delta S_o + RT\,(C_1 - C_2 \log [\text{salt}]) \qquad (5\text{-}4)$$

and is equal to zero at a high ionic strength, with a corresponding limiting value of melting temperature T_m°. Whence

$$T_m = \frac{T_m^\circ}{1 - (R/\Delta S_o)(C_1 - C_2 \log [salt])} \qquad (5\text{-}5)$$

which by expansion of the denominator in a Taylors series and dropping all higher-order terms gives

$$T_m = T_m^\circ \left[1 + R/\Delta S_o \, (C_1 - C_2 \log [salt])\right] \qquad (5\text{-}6)$$

and for a univalent electrolyte,

$$t_m = t_m^\circ + \frac{R\, t_m^\circ}{\Delta S_o} C_1 + \frac{R\, t_m^\circ}{\Delta S_o} C_2 \quad (\text{p cation}) \qquad (5\text{-}7)$$

In equation (5-7) t_m is the temperature in Celsius scale and (p cation) is the negative logarithm of the electrolyte concentration. A plot of t_m against pNa^+ for example is expected to be linear and of a slope equal to about - 18.5°. Hence, from equation (5-7)

$$R\, t_m^\circ \frac{C_2}{\Delta S_o} = -18.5^\circ \qquad (5\text{-}8)$$

and

$$t_m = t_m^\circ - 18.5 \left(pNa^+ + \frac{C_1}{C_2}\right) \qquad (5\text{-}9)$$

Generally, in estimating C_2, T_m° and ΔS_o must be known. Available data [9] have suggested for a DNA molecule a value of $T_m^\circ \approx 375^\circ K$ or $t_m^\circ = 102^\circ C$ and $\Delta S_o \approx 23$ cal/deg/mole base-pair for an ionic strength of 0.2 in sodium chloride and pH 7.0, for which C_2 has been found to be approximately 0.6 and C_1 which is approximately $0.5C_2 \approx 0.3$.

Equations (5-7) and (5-9) have been employed in estimating the limiting concentration of sodium ions, which just permits DNA to retain its helical conformation at 20°C and a t_m of 25°C. Inman and Jordan [13] have obtained a limiting concentration of 10^{-4}M for sodium ions for maintaining its helical structure in this way, and for those values of temperature (25°C) and salt concentration (10^{-4}M Na^+) a value of 1.1 kcal/mole base pair has been obtained for ΔG_o. This means that the electrostatic repulsion leads to a destabilisation of the helix to this extent, which is opposed by the intrinsic term ΔG_o which stabilises the helix to the same extent.

I.2.b The Approach of Walker

Changes occurring in the solvent being disregarded, the helix-to-coil transition has been considered as a first order phase change in which the helix and coil forms of the polymer are two phases of the same substance [10].

In a single component system at a constant pressure G_h and G_c, the free energies of the helix and coil respectively, are assumed to be each functions of only the temperature and ionic strength. At the melting temperature, T_m, G_h and G_c are equal, hence

$$d G_h = d G_c \qquad (5\text{-}10)$$

and

$$\left(\frac{\partial G_h}{\partial T_m}\right)_I dT_m + \left(\frac{\partial G_h}{\partial I}\right)_{T_m} dI = \left(\frac{\partial G_c}{\partial T_m}\right)_I d T_m + \left(\frac{\partial G_c}{\partial I}\right)_{T_m} dI \qquad (5\text{-}11)$$

Equation (5-11) expresses the relationship between a change in temperature dT_m and a simultaneous change in ionic strength dI,

such that the phase equilibrium is maintained.

Hence,

$$\frac{d\,T_m}{d\,I} = \frac{1}{\Delta S}\left(\frac{\partial \Delta G}{\partial I}\right)_{T_m} \qquad (5\text{-}12)$$

where $\Delta G = G_c - G_h$ and ΔS is the entropy for the transition helix-to-coil at constant ionic strength. Again, the free energy change ΔG has been considered to be made up of two terms, one electrostatic, ΔG_e and the other ΔG_h due to the change in hydrophobic interactions between the bases, and the changes caused by the breakdown of the hydrogen bonds between the base pairs and their reformation with solvent molecules. The electrostatic interaction producing ΔG_e tends to destabilise the helix while the hydrophobic interactions tend to stabilise it. It is expected that changes in the ionic strength will primarily affect ΔG_e and will have little or no influence on ΔG_h, so that ΔG_e may replace ΔG in equation (5-12). At the transition temperature, T_m,

$$\Delta G = 0 \text{ and } \Delta H = T_m \Delta S \qquad (5\text{-}13)$$

and

$$\frac{1}{T_m} \cdot \frac{dT_m}{dI} = \frac{1}{\Delta H}\left(\frac{\partial \Delta G_e}{\partial I}\right)_{T_m} \qquad (5\text{-}14)$$

In order to examine equations (5-12) and (5-14) values of ΔG_e at various ionic strengths must be determined, and models proposed for the native and denatured forms of the nucleic acid. The exact configuration of the helix and coil at the transition is seldom known exactly, however.

Walker [10] however, has assumed that long-range interactions between different parts of the same molecule native or denatured, are negligible, and has considered only a short length of the helix. Thus over a length of about ten base pairs (one turn) the helix of both DNA and RNA has been regarded as a rigid rod, so has denatured region formed by the denaturation of ten base pairs.

For DNA, a model of a charged cylinder in which the charge is spread uniformly over the surface has been considered. The electrostatic free energy, according to Hill [14] is given by

$$G_e = \frac{z^2 \epsilon^2}{Dl} \left[\frac{k_o(ka)}{k_a k_1(ka)} + \ln \frac{a}{b} \right] \qquad (5\text{-}15)$$

where z is the charge on a length l, of the cylinder; ϵ is the electronic charge; D is the dielectric constant of the surrounding medium; k is the reciprocal Debye-Hiikel radius dependent on the ionic strength; b is the radius of the cylinder; a is the distance of closest approach of counter-ions; $k_{o(ka)}$ and $k_{1(ka)}$ are modified Bessel functions of the second kind. Ge is generally difficult to determine for a denatured DNA, since the model is constantly changing and because the denaturation transition is always accompanied by decrease in the charge density of the molecule [15] .

The decrease in charge density reflects an increase in contour length and a decrease in the radius of the molecule [16]. Thus over a small length (one turn) the denatured form may be

represented by a charged cylinder of greater length, l', and smaller radius b', than that representing the helix, but with the same volume.

The values of ΔG_o have been shown by Walker to decrease with ionic strength, and to be only a slowly changing function of temperature, and to a first approximation, may be regarded as independent of temperature. Assuming that ΔS and ΔH are also independent of ionic strength, then the partial differentials on the right hand side of equations (11,12 and 14) may be replaced by complete differentials and the equations integrated to give the following results:

$$T_n = \frac{\Delta G_o}{\Delta S} + T_o \qquad (5\text{-}16)$$

$$\ln T_n = \frac{\Delta G_o}{\Delta H} + \ln T_o \qquad (5\text{-}16a)$$

in which T_o and $\ln T_o$ are integration constants and have been identified with the transition temperatures in the absence of electrostatic effects, presumably reached at very high ionic strength when ΔG_o tends to zero. From equations (5-16) and (5-16a), ΔS and ΔH can be obtained from the linear plot of T_n versus ΔG_o and $\ln T_n$ versus ΔG_o respectively.

Values of ΔG_o of the order of 13 k cal mole^{-1} base have been reported for a DNA molecule [17]. A value of 8 k cal mole^{-1} base has also been reported for the change in enthalpy from the plot of $\log T_n$ against ΔG_o from equation (5-16a) after making the necessary corrections for the interactions between the DNA and water molecules [10].

The approximately linear nature of equations (5-16) and (5-16a) as obtained by Walker, suggests that there is no marked dependence of either ΔH or ΔS on temperature or ionic strength. With the counterions strongly bound to the DNA, as in the case of interaction with magnesium or calcium, the total net charge on the molecule would be reduced, and both the electrostatic free energy and the estimated values of ΔS and ΔH would be affected.

A simple empirical equation has been found to apply to the variation of T_m with the ionic strength for low ionic strength values [10].

i.e.

$$T_m = A + B \log I \qquad (5\text{-}17)$$

where A and B are constants. The quantity A is identified with the denaturation temperature when the ionic strength is unity.

I.3 Conformations of native and denatured forms of DNA.

A number of different conformations of DNA can be expected in denaturation studies. On the one extreme, there can be the completely ordered double helical and complementary conformation. At the other extreme there can be an essentially completely flexible random coils with no extensive structure or order remaining. This corresponds to the completely and irreversibly denatured form obtained at high temperatures and extremes of pH. On removal of the denaturing agent and on returning to the standard condition of temperature (e.g. room temperature) a certain amount of order is restored.

Intermediate forms containing both extensively ordered as well as disordered regions capable of instantaneous reversion to the completely ordered starting conformation, have been identified in heat and acid denaturation. Under appropriate conditions, the complete separation of the two complementary strands can be brought about. This process can be quite slow, and requires relatively extreme conditions of temperature and pH, well beyond the mid-point of the reversible transition.

The reversible process of strand separation can also be brought about under the conditions listed below:

(1) The reaction must be thermodynamically favoured; i.e. it must be allowed to take place in a region of temperature well below t_m and at a relatively high ionic strength.

(2) It must be favoured kinetically as well. Strand recombination requires the consideration of the concentrations of the strands (or their number). It could be assumed to be a second order process (involving two strands coming together) and it will be favoured by high concentration of the strands (or the nucleic acid). The strands must be able to approach closely; this again is favoured by high ionic strength and/or the presence of specific counterions such as Mg^{2+}, as well as the absence of charges on the base i.e. at neutral pH.

(3) For complete restoration of order, all base pairs must be perfectly matched, as can be accomplished in homopolymers such as hypothetical poly (G + C), poly (A + T) and in synthetic poly (A + U). There is extensive mismatching in natural polynucleotides however. This makes reformation of the double helix difficult.

For extensive re-ordering in natural polynucleotides, the imperfect regions are normally ruptured and are allowed to reform at temperatures below t_m. This process is called 'annealing'. Re-naturation is most easily accomplished with DNA from small viruses, somewhat less easily with DNA from large viruses, still less easily with that from bacterial, and least easily, if at all, with that from higher organisms [18, 19].

SECTION II: THE UV STUDIES OF INTERACTION OF SODIUM, MAGNESIUM AND CALCIUM WITH DNA.

II.1 Photometric Accuracy

Two main types of error can be considered in the phtometric technique. The error introduced into the final result by the chemical preparative treatment, which can be considered to be negligible as compared to the instrumental error [20].

The conditions for maximum photometric accuracy has been deduced by equating the second derivative of Beer's law [20], equation (5-18)

$$I = I_o \, 10^{-abc} \qquad (5\text{-}18)$$

to zero. If this law is obeyed, then the relative error in concentration $\Delta c/c$ per unit photometric error ΔP is given by

$$\frac{\Delta C}{c} / \Delta P = 1/2.3A(10^{-A}) \qquad (5\text{-}19)$$

The left hand side of equation (5-19) has been shown to have a minimum value when A = 0.434 (or 36.8% transmittance) i.e.

$$\left[\frac{\Delta C}{c} / \Delta P \right]_{min} = -2.72 \qquad (5\text{-}20)$$

It is desirable in a photometric technique therefore to use concentrations of the absorbing molecules for which the ratio $\left(\frac{\Delta c/c}{\Delta P} \right)$ is as close to the minimum as possible.

In the plot of $\left[\frac{\Delta c/c}{\Delta P} \right]$ against transmittance, it has been found [20] that, between 20% and 60% transmittances, the ratio between the relative error in concentration and the photometric error has a value very much near 2.72. The corresponding absorption range is about 0.20 to 0.70.

Hence by using concentrations which have absorption between 0.20 and 0.70 a maximum photometric accuracy can be attained.

II.2 EXPERIMENTAL

II.2.a Instrumental

For all the UV measurements, a Unicam SP 500 spectrophotometer was used with a hydrogen discharge lamp, supplied with a stabilized current of 300 mA from a separate power pack for the range of wavelength 210 - 300 mμ.

High tension and bias voltages were derived from batteries housed within the instrument, while the lamp and the valve heaters were run from two high capacity 6V accumulators. Silica cells of 10 mm path length were used. These had ground and tight-fitting lids. The cell holder was such that hot liquid could be circulated through it from a bath of liquid connected externally.

By the use of a suction rotatory pump, hot liquid was pumped from a thermostated liquid bath into the cell compartment and out into the bath. The efficiency of circulation of liquid was such that there was a difference of only about four degrees of temperature

between the experimental cell solution and the liquid in the thermostated bath.

As it was necessary to raise the temperature of the experimental solution above 98°C, water could not be used as the liquid bath. Instead a mixture of ethylene glycol and water (boiling point 110°C) was used. This mixture allowed a temperature of up to 99°C to be measured in the nucleate solution inside the silica cell

Various devices were tried for measuring the solution temperature. The method using a thermocouple was found inaccurate because a proper contact could not be made between the cell containing the solution and the thermocouple hot end.
An alternative method of allowing the thermometer to dip into the experimental solution was adopted.

A tight-fitting wooden lid carrying a small hole for the thermometer which could be read to 0.25°C was used for the cell compartment. The themometer was allowed to dip into the blank solution at each stage of the measurement until thermal equilibrium was attained.

It is worth mentioning that during heating, air bubbles were formed in the solutions contained in the cells. These were removed by a prior heating of the solutions to about 55°C, when most of the dissolved air escaped without the nucleate ion being denatured.

With this method, only a very small and negligible air-space was left on top of the solution in the cell after each complete set of measurements.

II.2.b Experimental Methods.

(i) Dilute Solutions (not preservable)

Freshly prepared dilute solutions of different but accurately known weights of wet DNA sample (whose weights of phosphorus were known) were prepared in buffered 0.2M sodium chloride (pH 7.05 in tris/HCl buffer).

The dissolution of the fibrous acid was allowed to take place at 2°C with a minimum of shaking, to give a homogeneous solution.

The UV absorption spectrum for the wavelength range of 210 - 290 mμ and the absorption at the wavelength of absorption maximum (λ = 260 mμ) were determined for each nucleate solution from the graph of absorption against wavelength.

It was also possible to examine the range of nucleate concentration for which the Lambert-Beer Law was obeyed from the plot of absorption at 260 mμ against nucleate concentration.

(ii) Stock Solutions (preservable)

Two stock nucleate solutions (each of about 3×10^{-3} mole phosphorus per litre) were prepared at 2°C for the sets of experiments reported here.

One of the stock solutions was dialyzed in cellophane membrane tubing against four changes of conductivity water at 2°C and for twenty four hours to remove all dialysable molecules or ions such as the sodium counterions. The other stock solution was undialysed. Both of them were stored in a refrigerator at 2°C.

Dilutions to a selected nucleate concentration of about 9×10^{-5} mole phosphorus per litre of solution were made from each of the stock solutions for the denaturation studies. Fifty millilitres of the dilute nucleate solution were made up to the appropriate strength with added simple electrolyte (NaCl, $CaCl_2$ or $MgCl_2$).

Nucleate solutions of varying concentrations of added electrolytes were prepared. The absorption spectrum (at 210 mμ to 290 mμ), and the absorption at 260 mμ were determined at room temperature (26°C) for each solution, for the three electrolytes.

From these it was possible to study the dependence of absorption at 260 mμ of a nucleate solution of a constant concentration on the concentration of a simple electrolyte from the plot of the relative absorption Ai/Ao against the logarithm of the concentration of the simple electrolyte.

Here, A_i is the absorption of the nucleate solution of a given concentration of simple electrolyte, and A_o is the absorption of the nucleate of same concentration as all others, but with no added electrolyte.

In a similar way, investigations were conducted on the thermal transitions of nucleic acid in the presence of different concentrations of added simple electrolyte for each of the solutions above.

Measurements of the UV absorption were made at elevated temperatures of up to 98°C.

At each fixed temperature, an average value of the absorption at 260 mμ was determined. In step of about six degrees, away from the area of hyperchromic rise, and two degrees at the steep rise, the absorption of the nucleate in the temperature range between 26°C and 98°C was probed.

From the plot of the relative absorption A_t/A_o against temperature, it was possible to determine the denaturation temperature (T_n) for each nucleate-cation system. A_t here represents the absorption at any given temperature, t, and A_o its absorption at room temperature 26°C.

The cooling process was also studied. Measurements of the absorption at 260 mμ were made as the nucleate solution cooled.

Measurements of absorption at elevated temperatures were also made at 230 mμ and 280 mμ to examine any dispersion of absorption which could be associated with intramolecular heterogeneity in the nucleate preparation and to see what information could be obtained about the primary structure of the nucleic acid, and possibly that of the acid complex with magnesium and calcium.

Cation	Concentration mole/litre x 10^5	Relative absorption at 26°C and 260mμ
Mg^{2+}	1.968	0.991
	2.446	0.991
	3.050	0.991
	3.748	1.005
	4.920	1.004
	6.392	1.002
	10.050	0.997
	12.340	0.993
Ca^{2+}	1.279	0.996
	2.311	0.993
	2.907	0.994
	3.716	0.993
	4.835	0.994
	5.766	1.002
	7.377	0.991
	9.152	1.002

Table 5.1 Variation of the relative absorption (A_i/A_o) at 26°C and 260 mμ with concentration of magnesium and calcium ions in the nucleate solution.

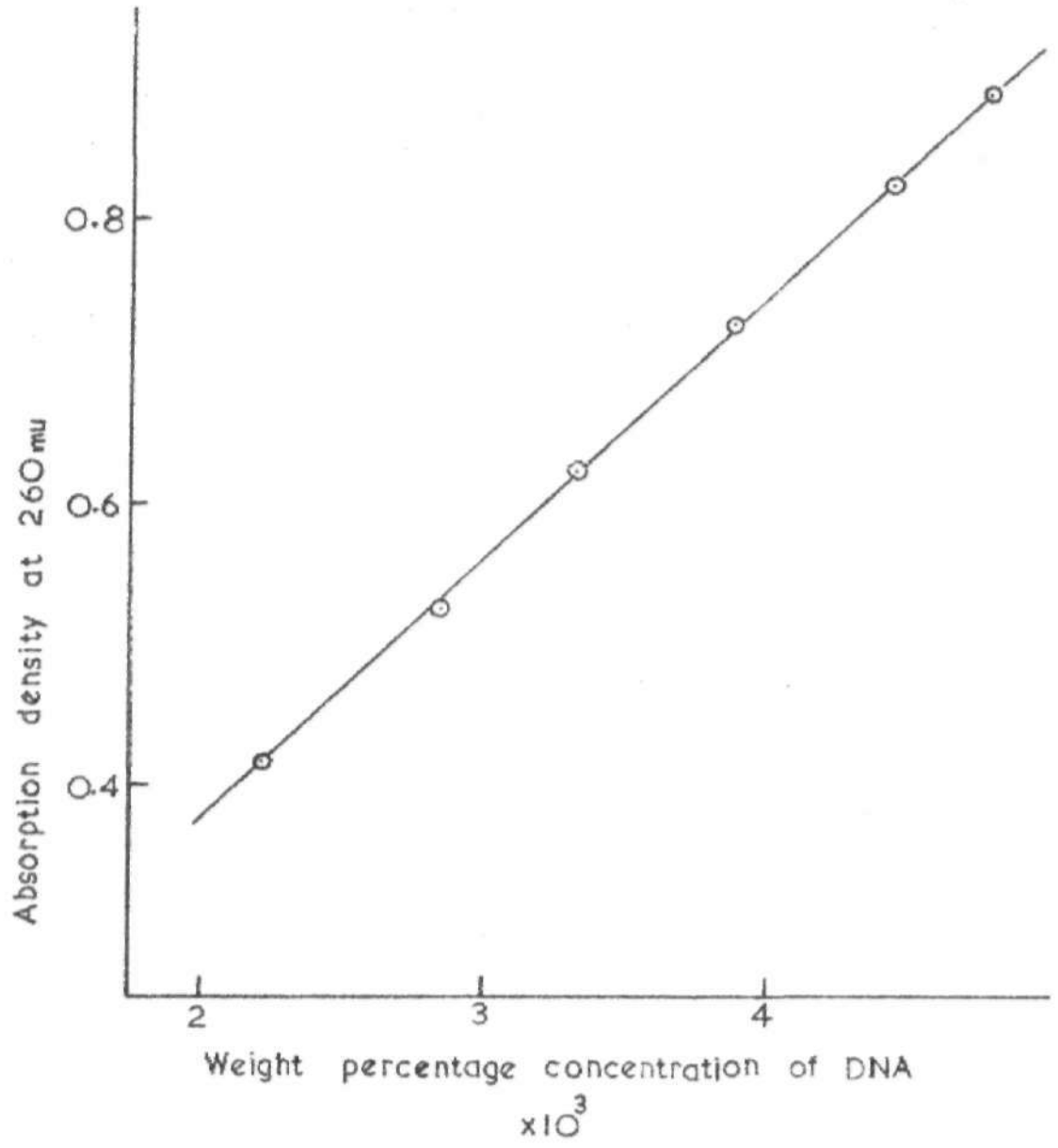
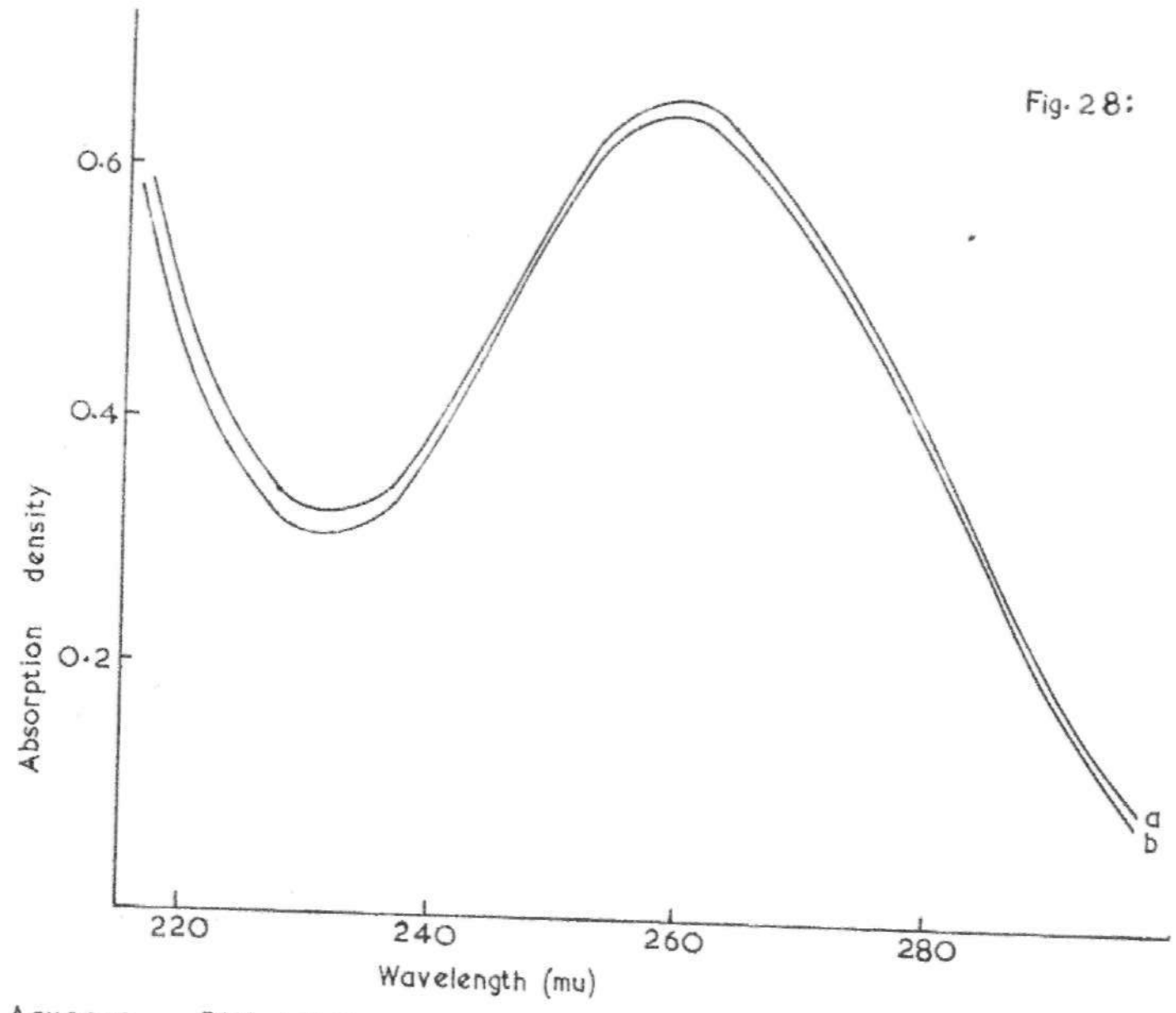

Absorption density at 260 mu
0.8
0.6
0.4
2
3
4
Weight percentage concentration of DNA
x10^3

Fig. 27:

Fig. 28:



Aqueous DNA solutions of two concentrations of NaCl
(a), 0.00 ; (b), 1.52×10^{-4} mole/litre

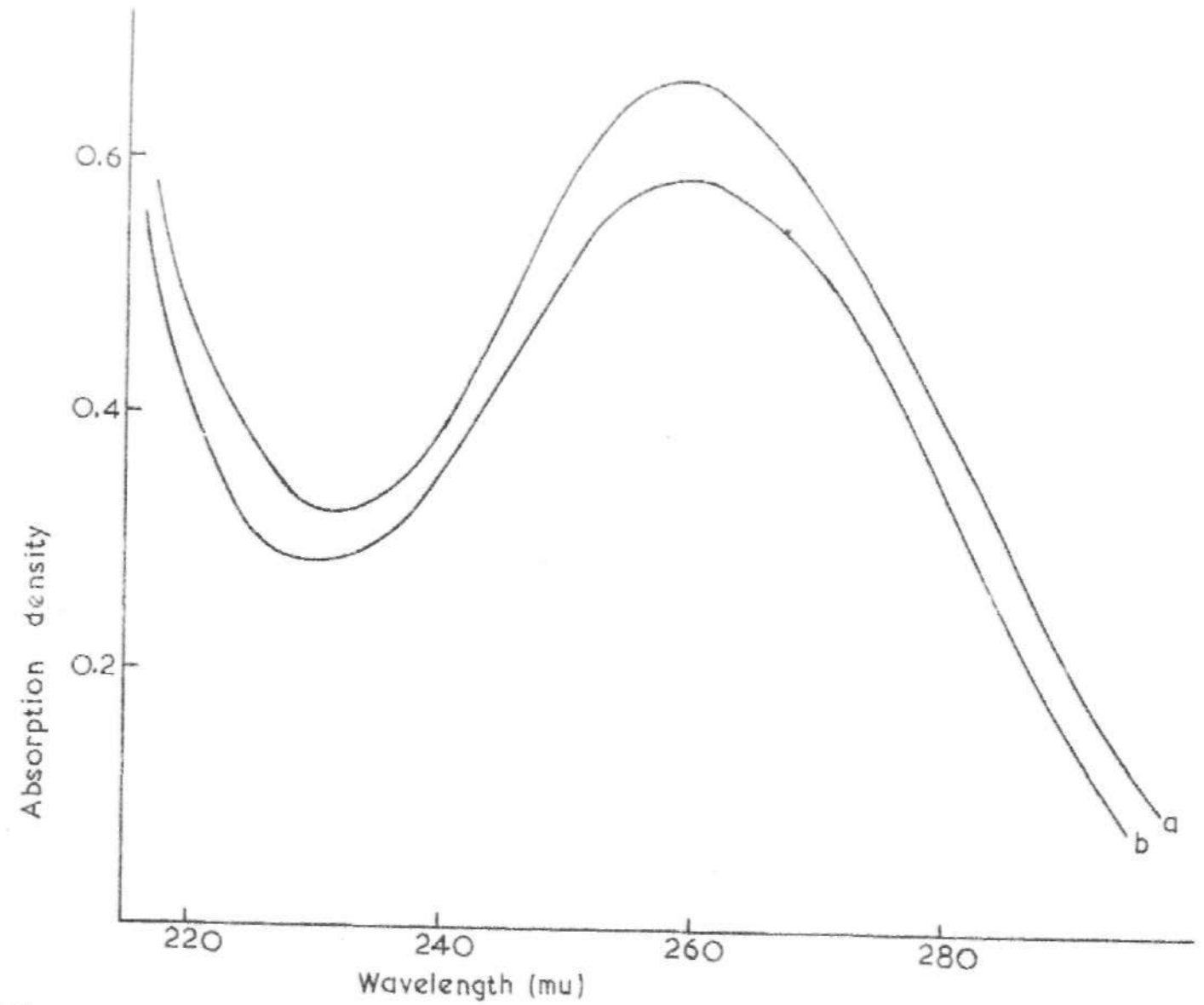


Fig. 29: Aqueous DNA solutions of two concentrations of $MgCl_2$ (a), 0.00 ; (b), 1.234×10^{-4} mole/litre.

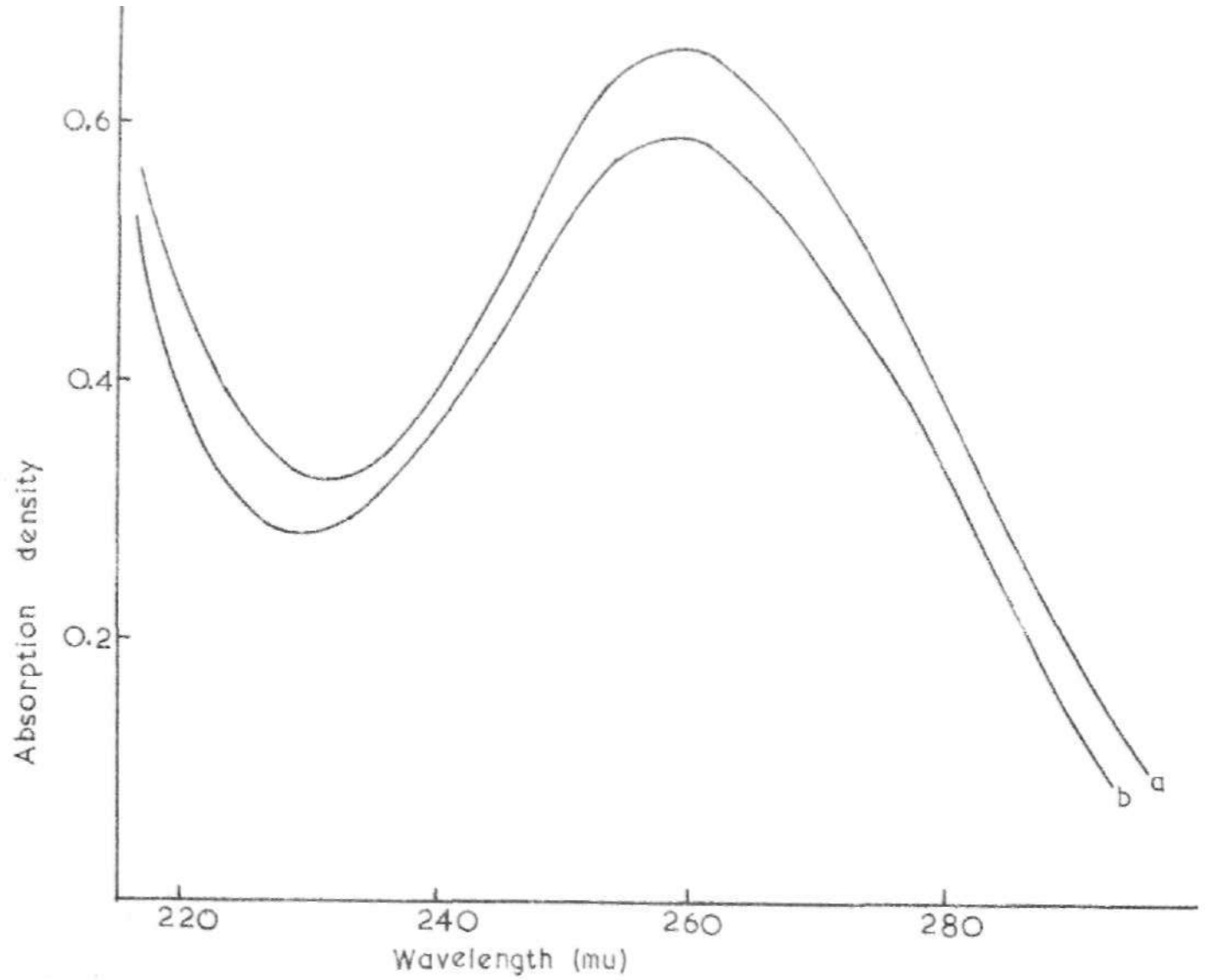


Fig. 30: Aqueous DNA solutions of two concentrations of $CaCl_2$ (a) 0·00 ; (b), 9.15×10^{-5} mole/litre.

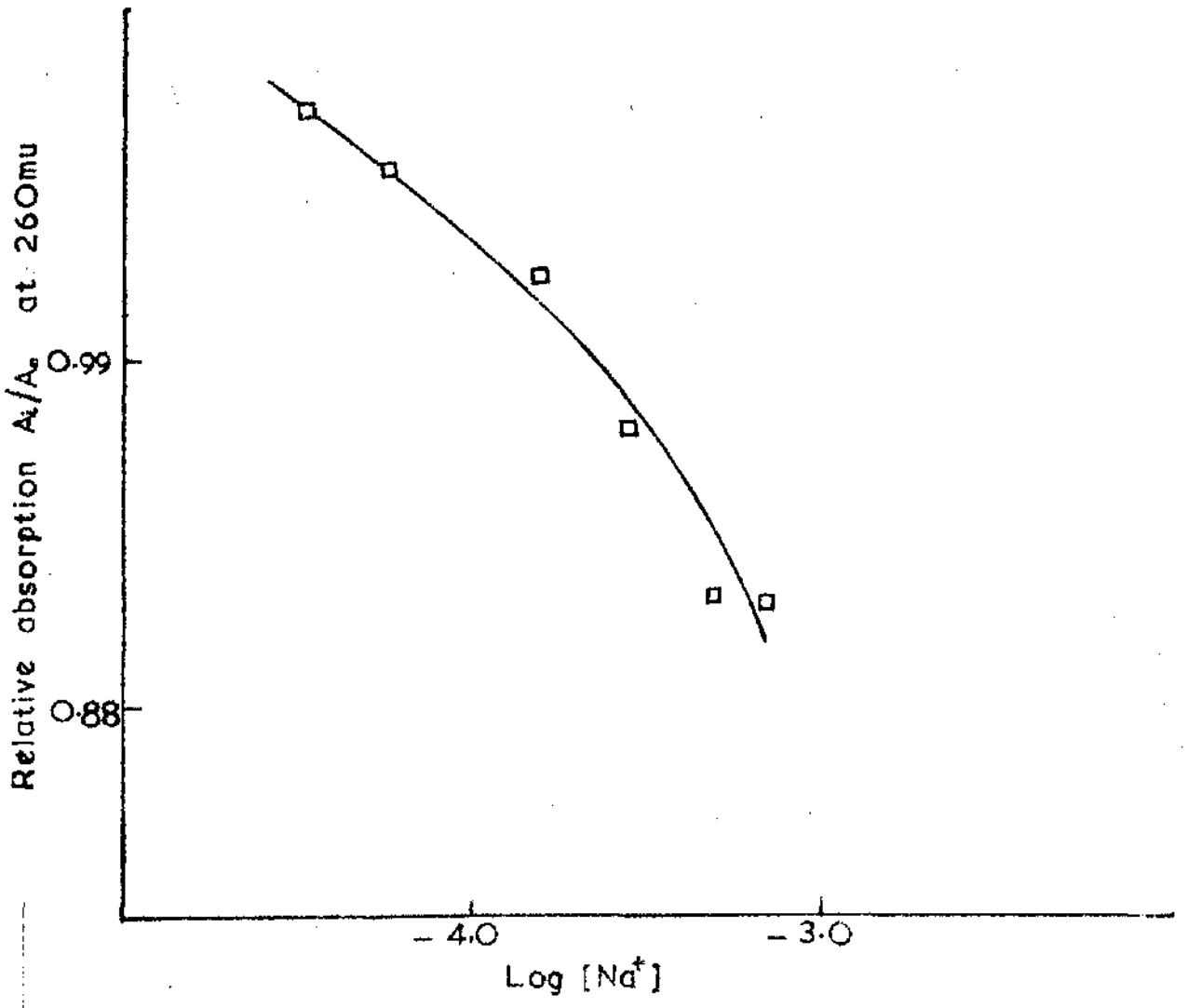


Fig. 31 : Absorption of dialysed DNA solution with various concentrations of NaCl.

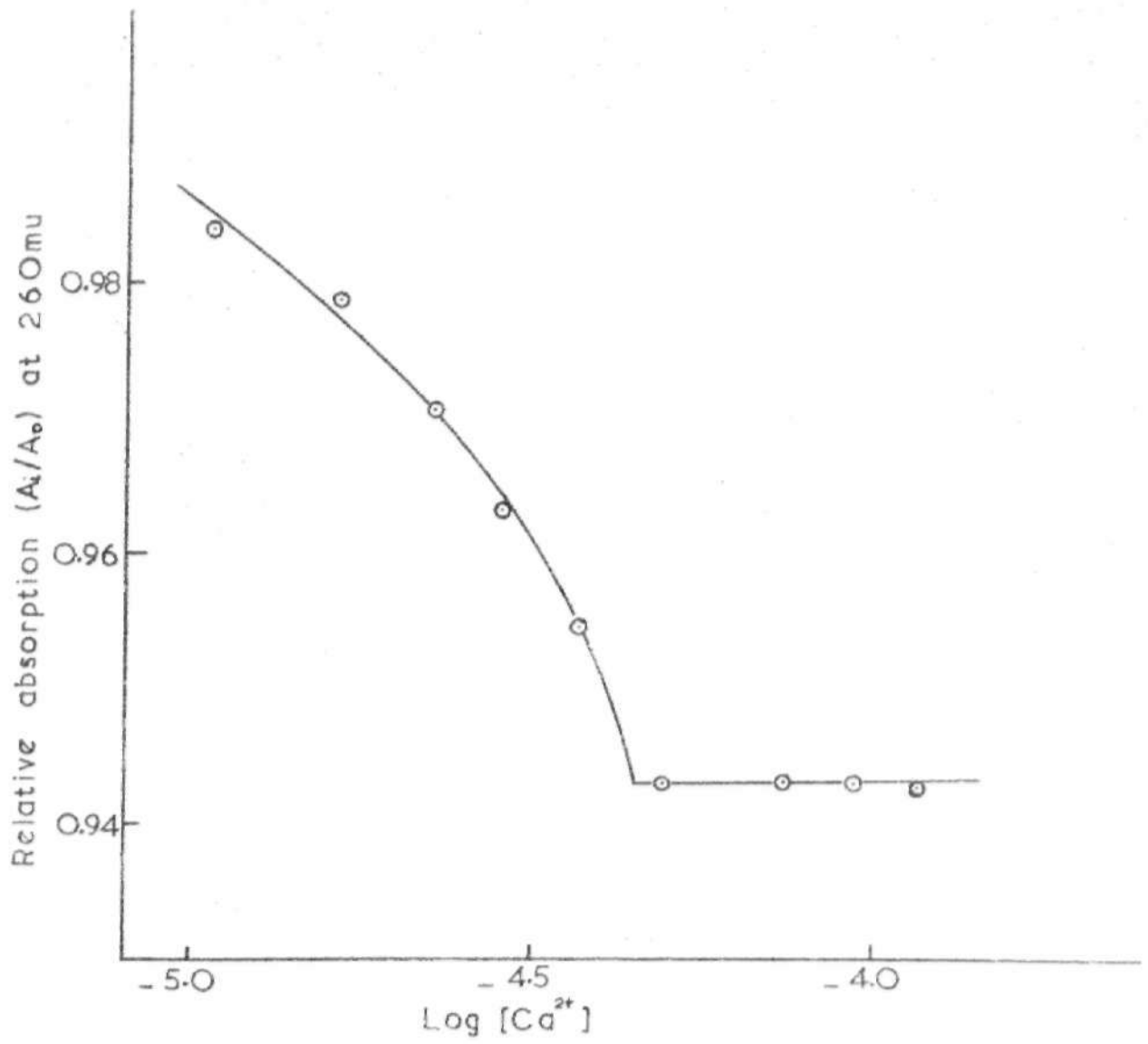


Fig. 32: Absorption of dialysed DNA solution with various concentrations of $CaCl_2$.

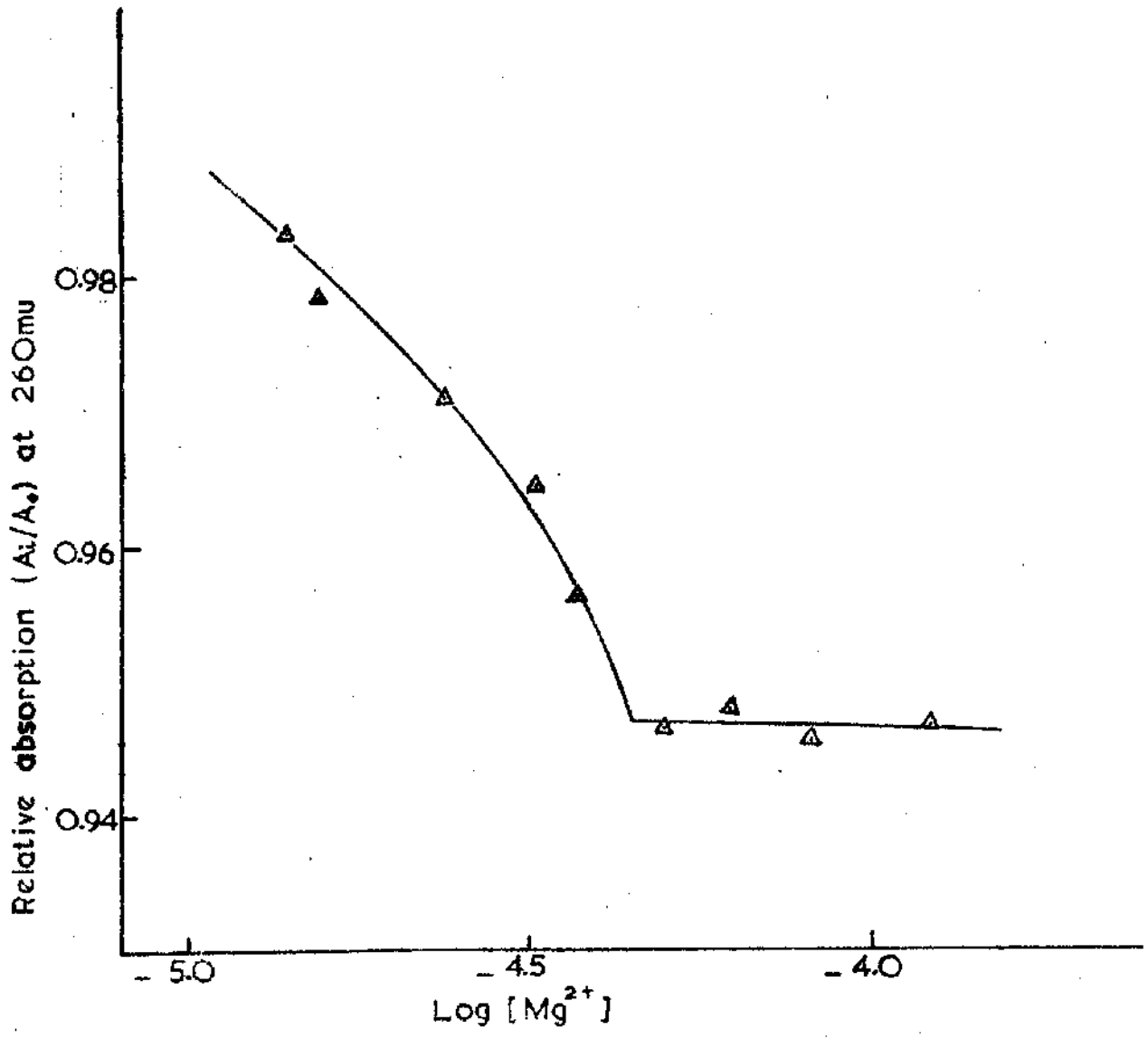


Fig. 33: Absorption of dialysed DNA solution with various concentrations of $MgCl_2$.

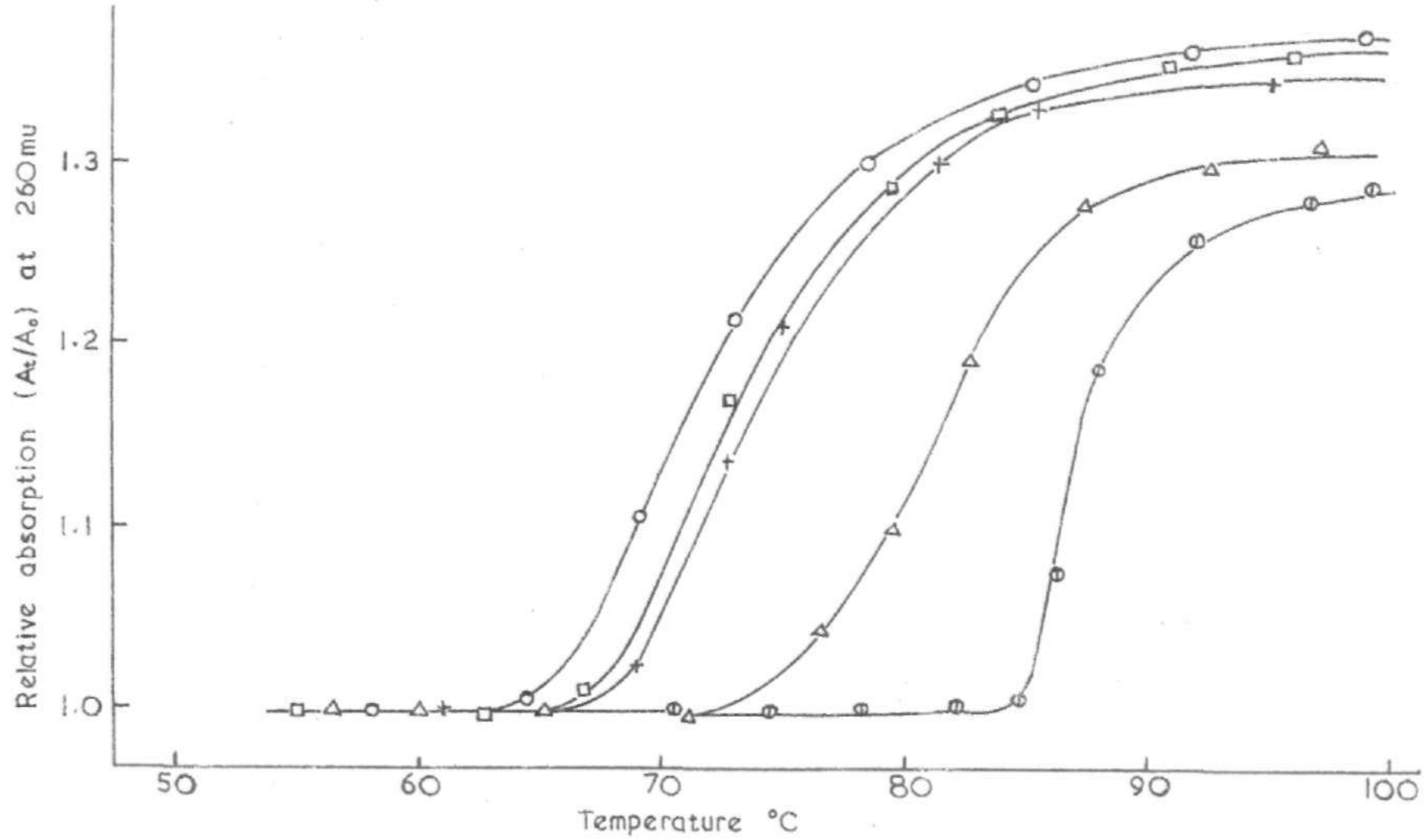


Fig. 34: Absorption–temperature profiles of DNA–Na^{+} systems of (o), 0.86 x 10^{-4} ; (□), 1.52 x 10^{-4} ; (+), 2.00 x 10^{-2} ; (Δ), 1.00 x 10^{-1} ; (Φ), 2.00 x 10^{-1} mole of sodium chloride per litre.

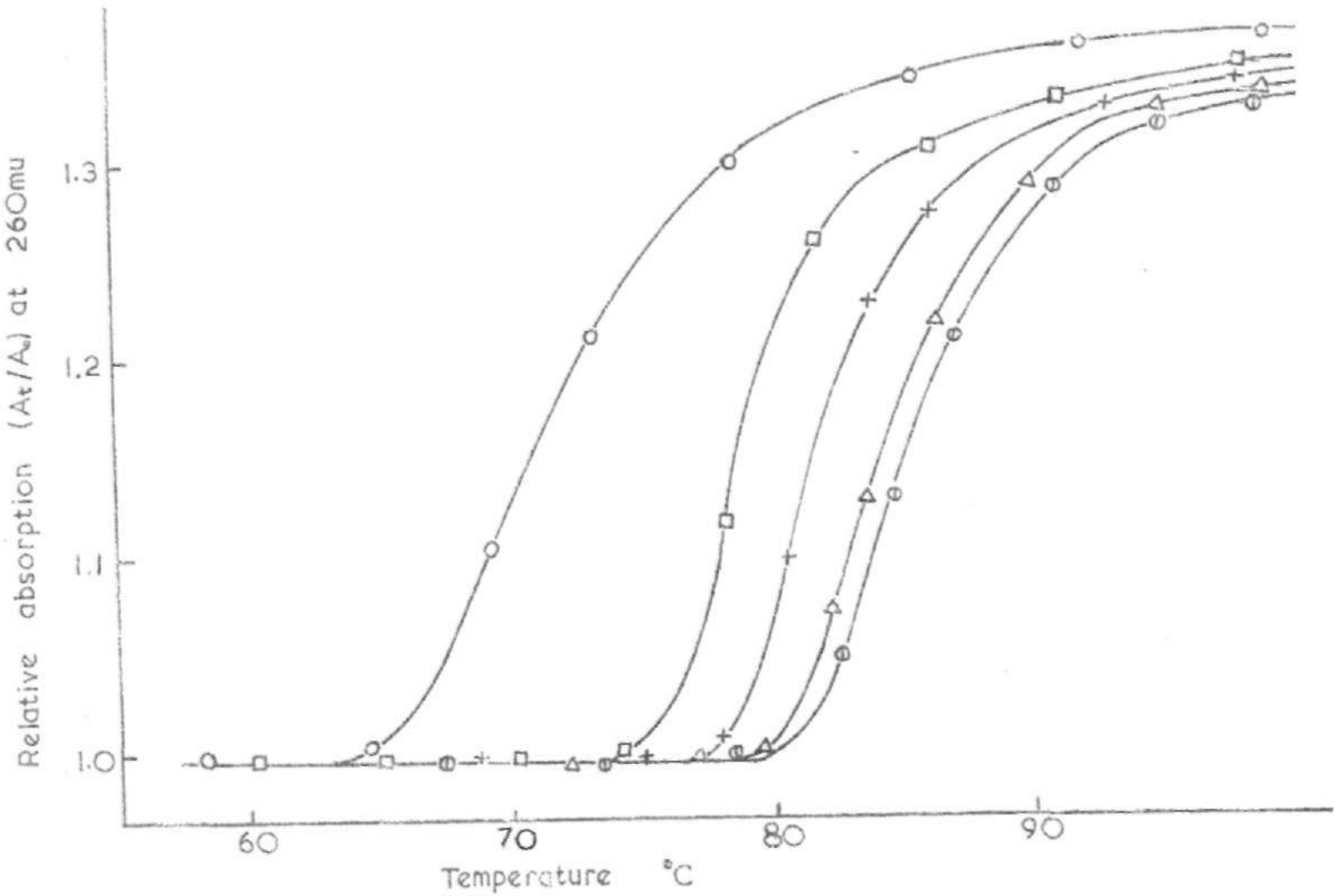


Fig. 35 : Absorption—temperature profiles of DNA—Mg^{2+} systems of (o), zero ; (□), 2.38×10^{-5} ; (+), 6.31×10^{-5} ; (Δ), 8.10×10^{-5} ; (Φ), 12.26×10^{-5} mole of magnesium chloride per litre.

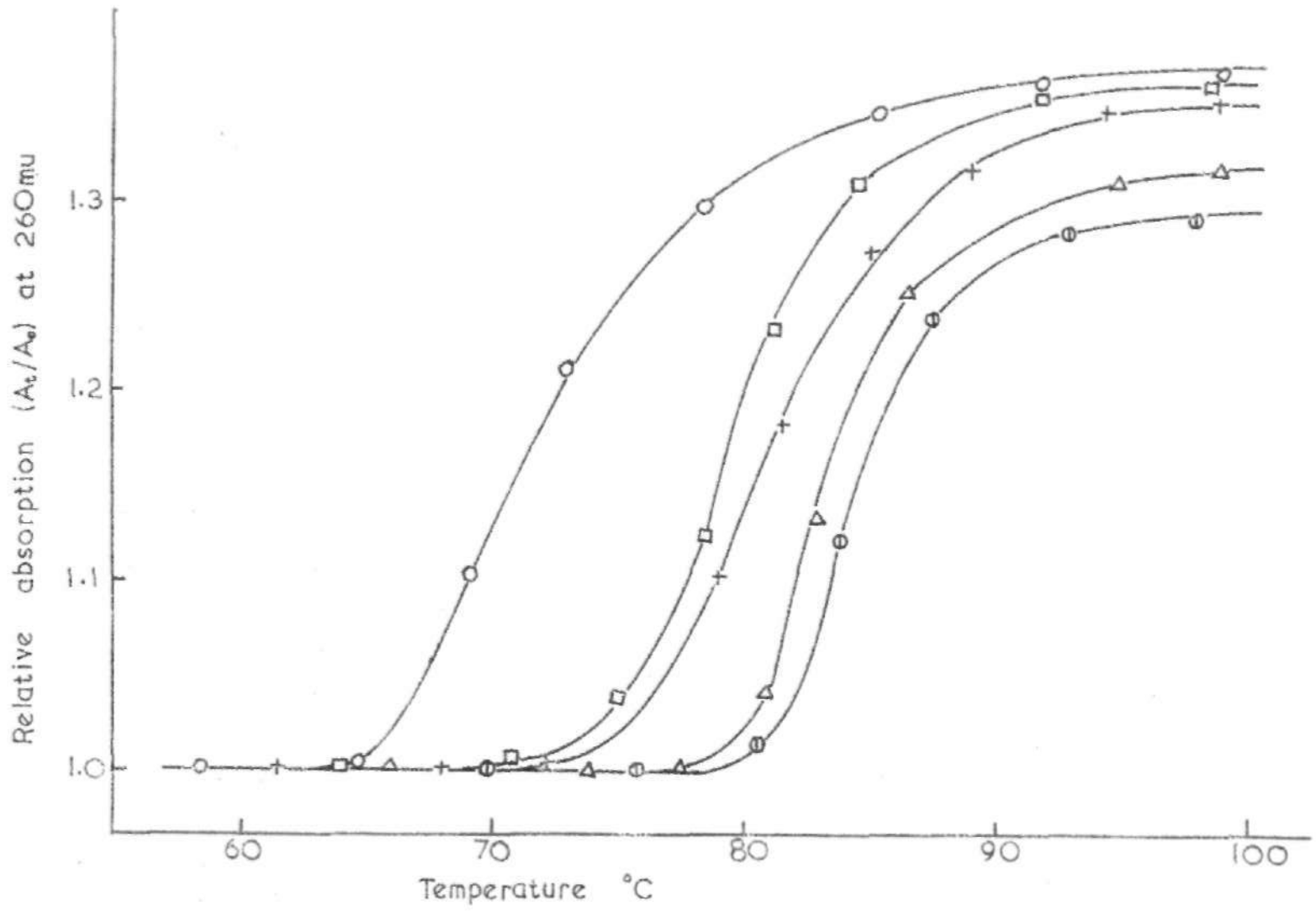


Fig. 36 : Absorption—temperature profiles of DNA–Ca^{2+} systems of (o), zero ; (□), 2.26×10^{-5}; (+), 4.89×10^{-5}; (Δ), 9.27×10^{-5}; (Φ), 11.63×10^{-5} mole of calcium chloride per litre.

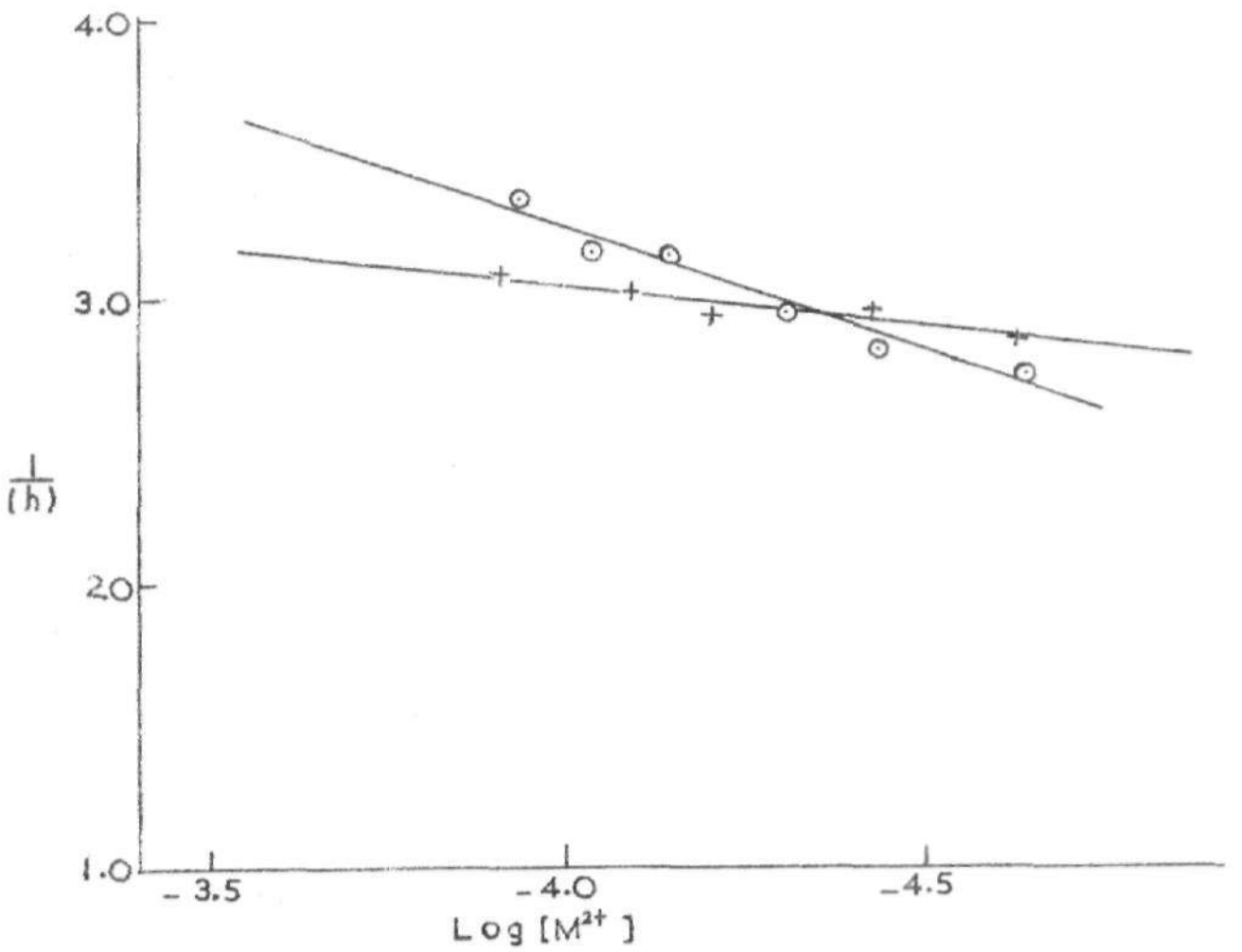


Fig. 37: The variation of the hyperchromic rise (h) at 260mu with the concentration of Ca^{2+} and Mg^{2+} ions in denatured (dialysed) DNA. (⊙), DNA–Ca^{2+} and (×), DNA–Mg^{2+}.

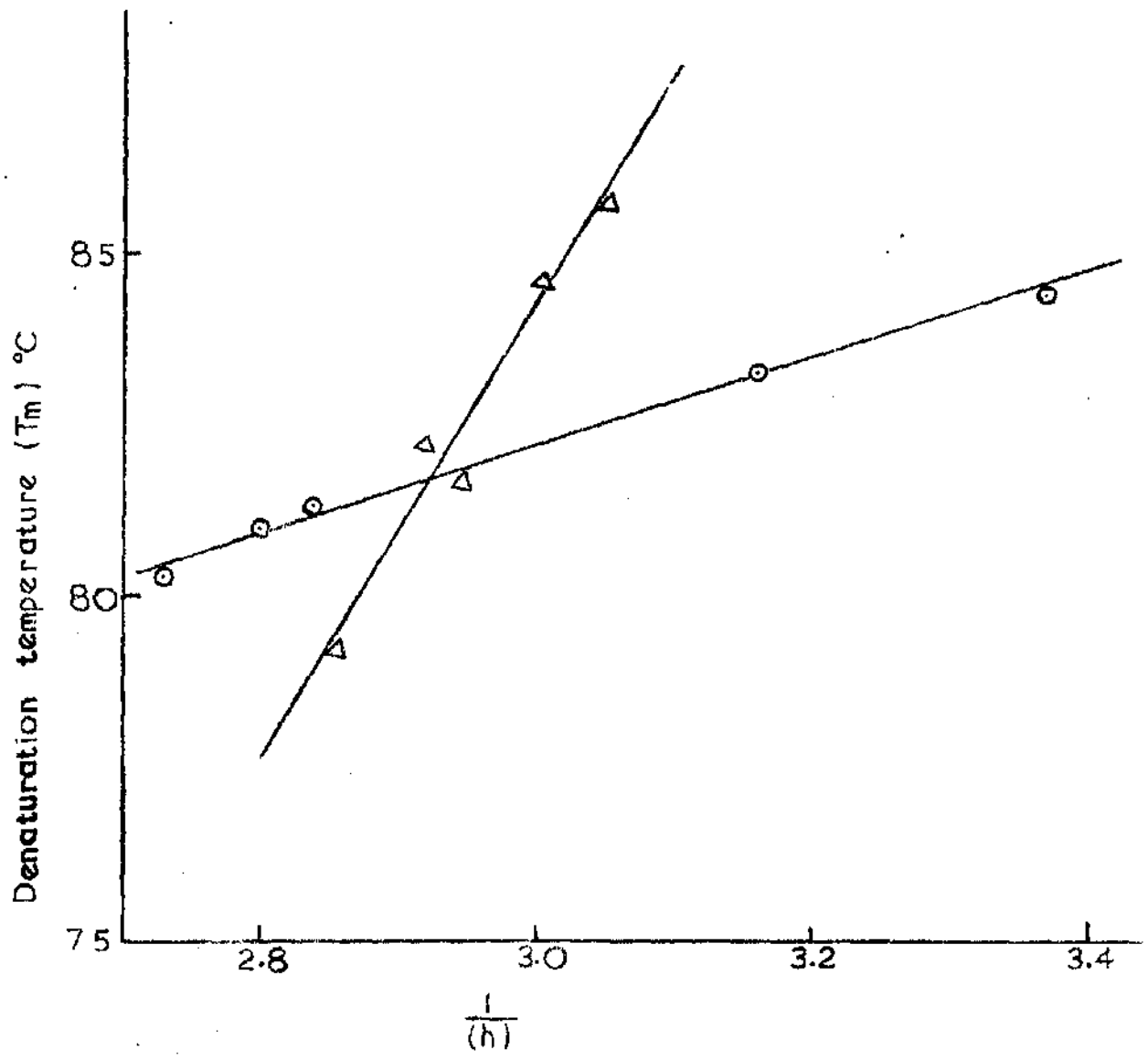


Fig. 38 : The variation of (Tm) with the hyperchromic rise (h) at 260mu for partly denatured (dialysed) DNA: (⊙), DNA–Cd^{2+} and (Δ), DNA–Mg^{2+}.

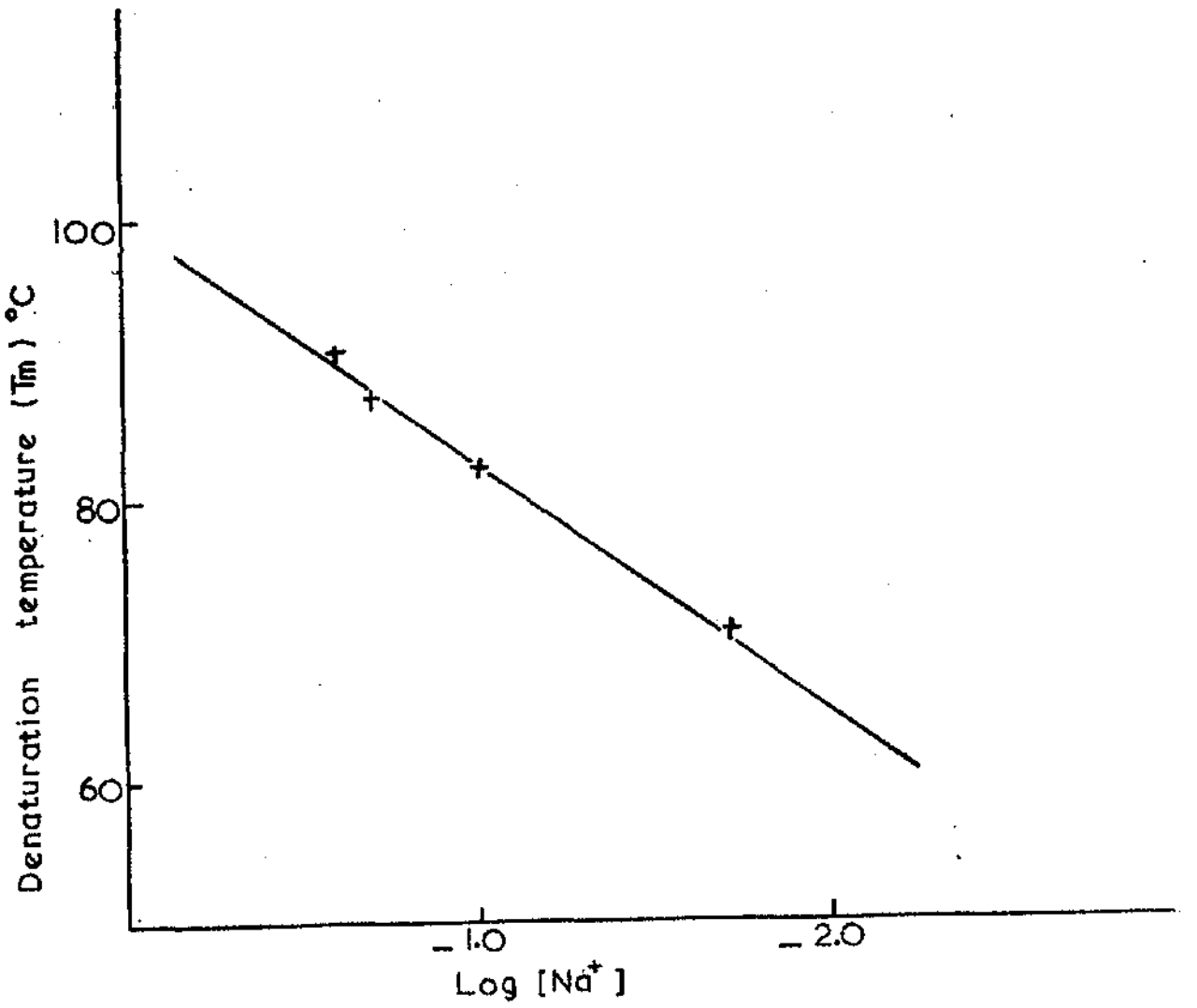


Fig. 39 : The dependence of (Tm) of thymus DNA on the concentration of sodium ions in the aqueous solution at 260mu.

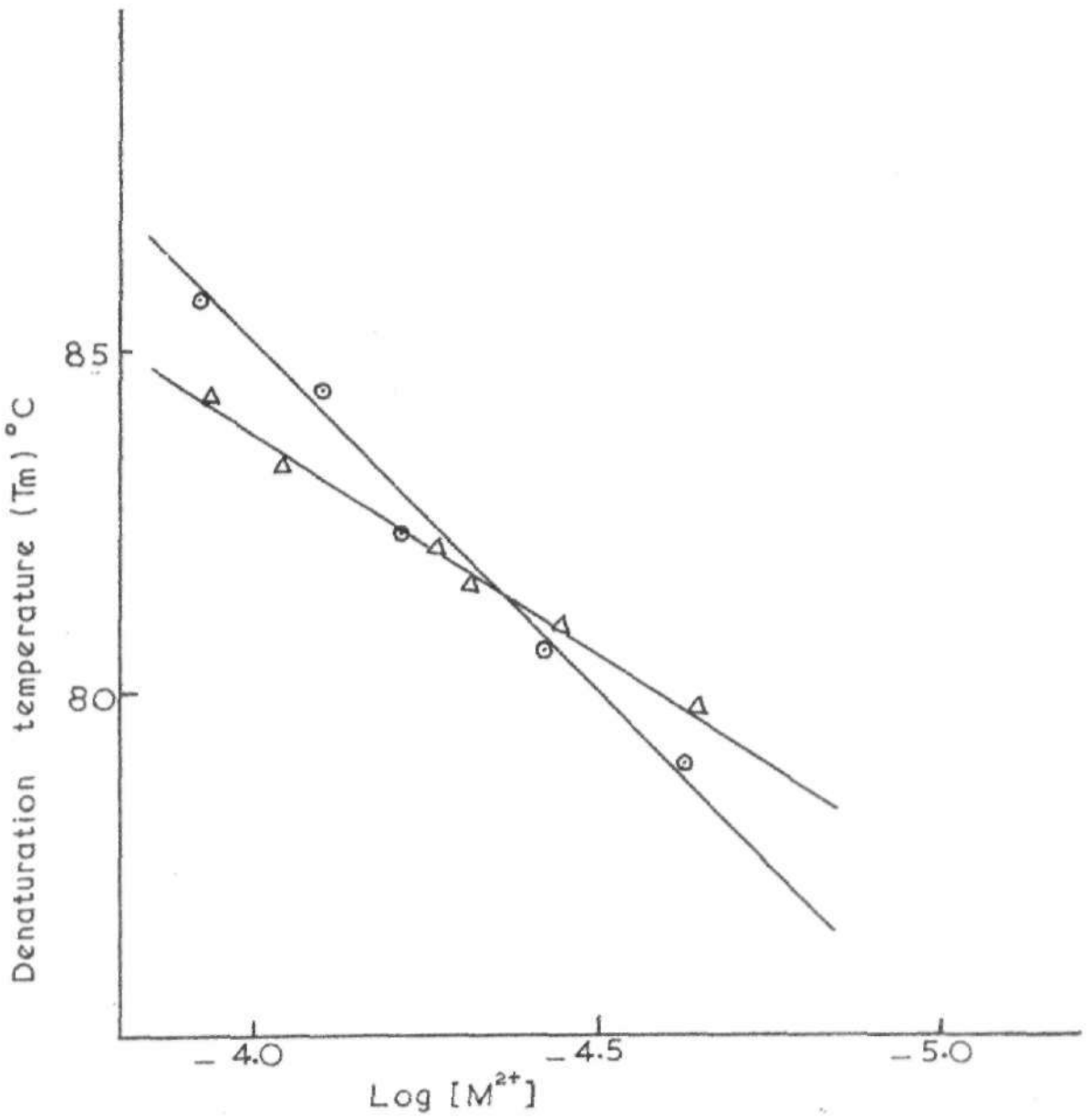


Fig. 40: The dependence of (Tm) of thymus DNA on the concentration of calcium ions (Δ); and magnesium ions (⊙), in the aqueous solution at 260mu.

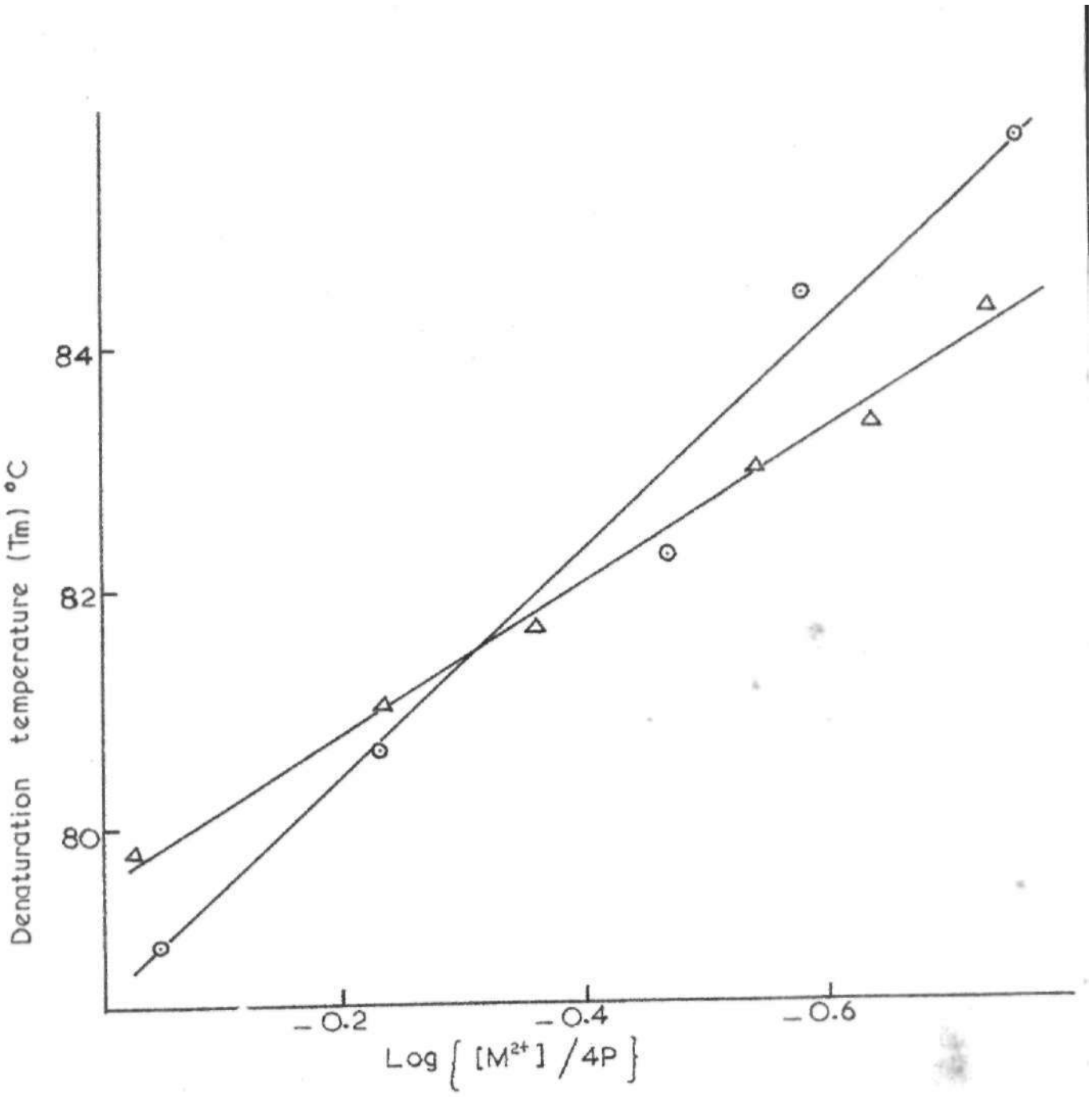


Fig. 41: The dependence of (Tm) on the [cation]/nucleate phosphorus ratio at 260mu. (Δ), DNA-Ca^{2+} ; (⊙), DNA-Mg^{2+}

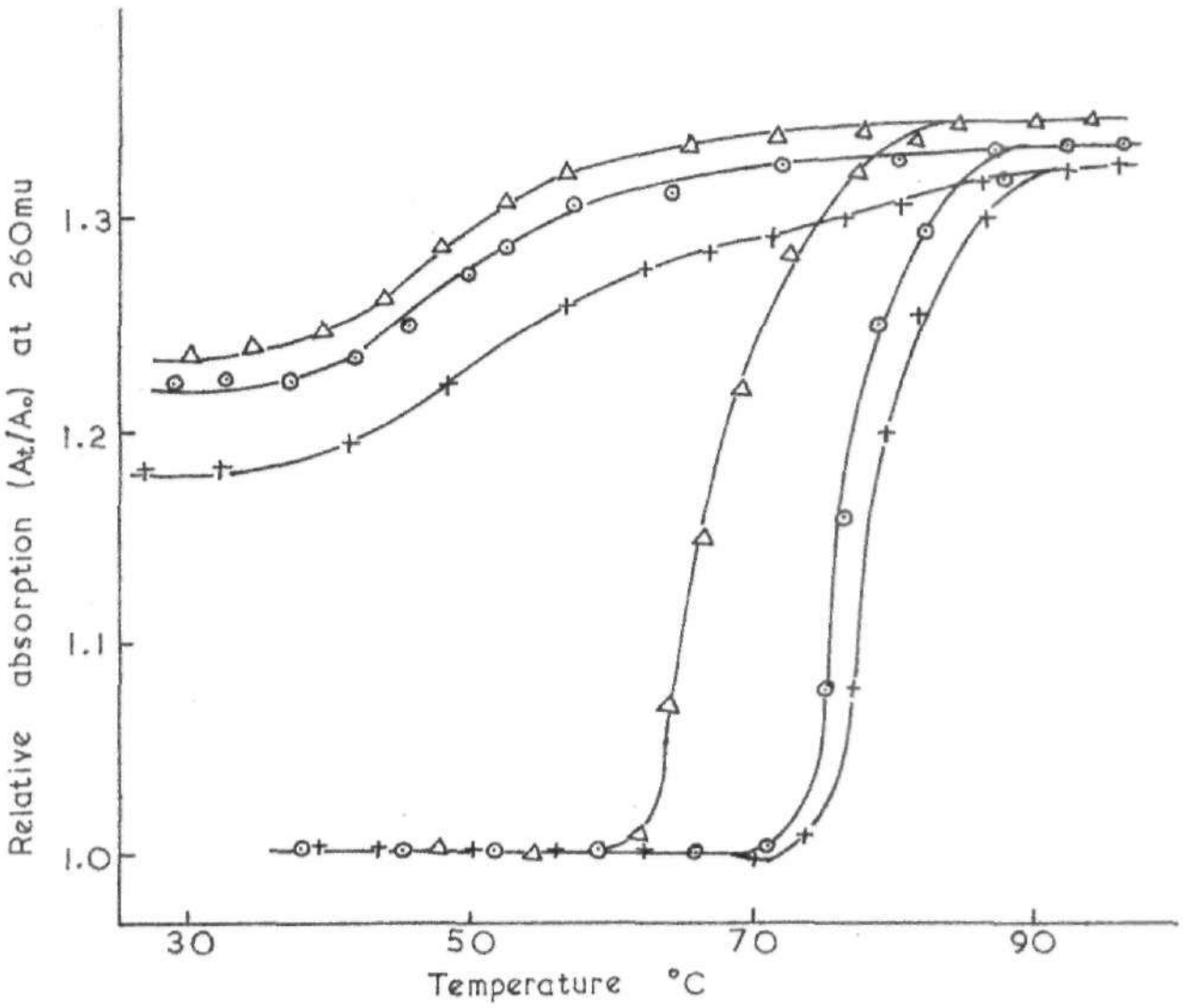


Fig. 42: Denaturation and renaturation of thymus DNA in aqueous salt solutions of: (Δ), 6.39×10^{-5}; (⊙), 8.10×10^{-5}; (+), 12.34×10^{-5} mole of $MgCl_2$ per litre.

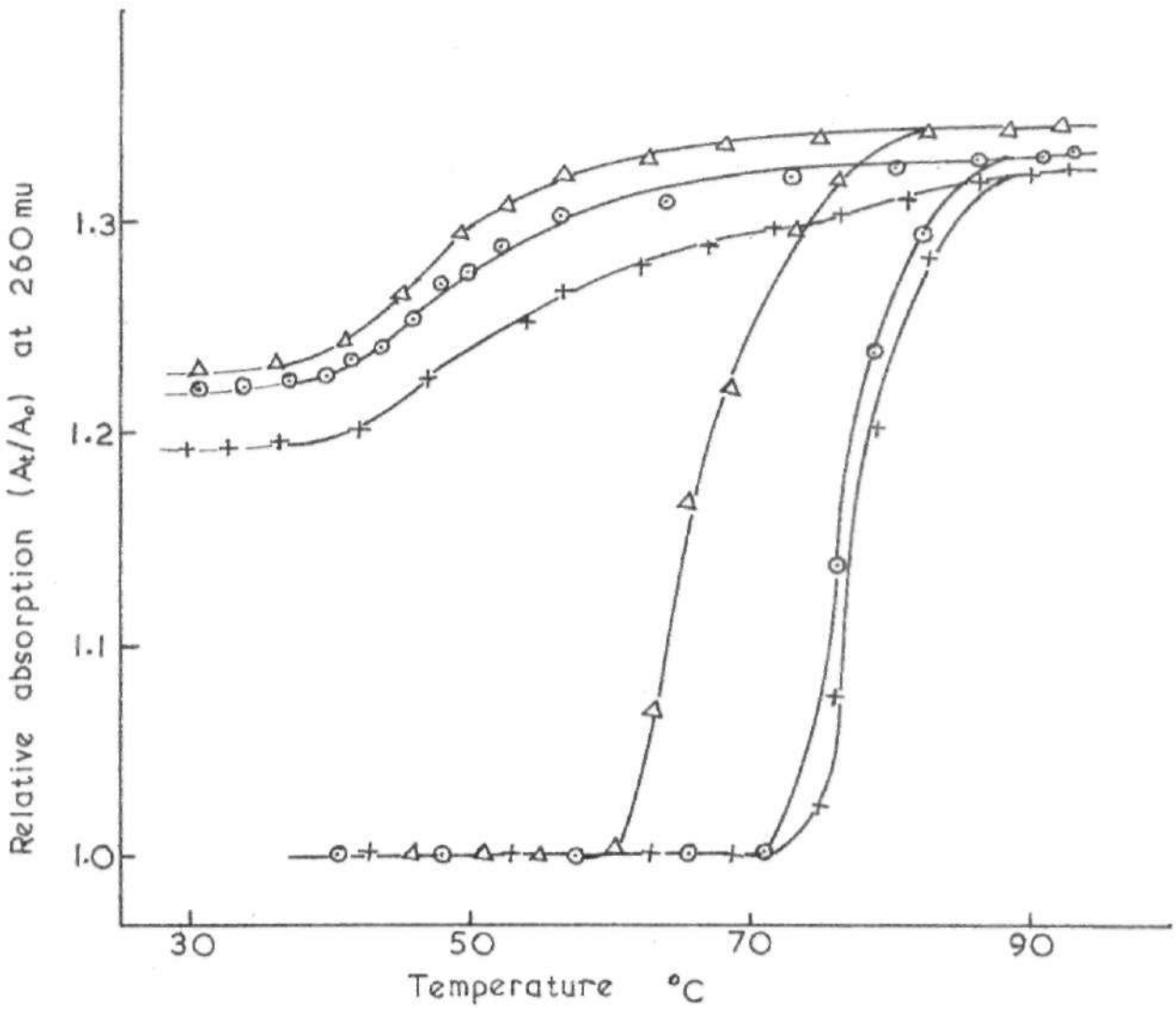


Fig. 43: Denaturation and renaturation of thymus DNA in aqueous solutions of: (△), 5.77×10^{-5}; (⊙), 7.38×10^{-5}; (+), 9.15×10^{-5} mole of $CaCl_2$ per litre.

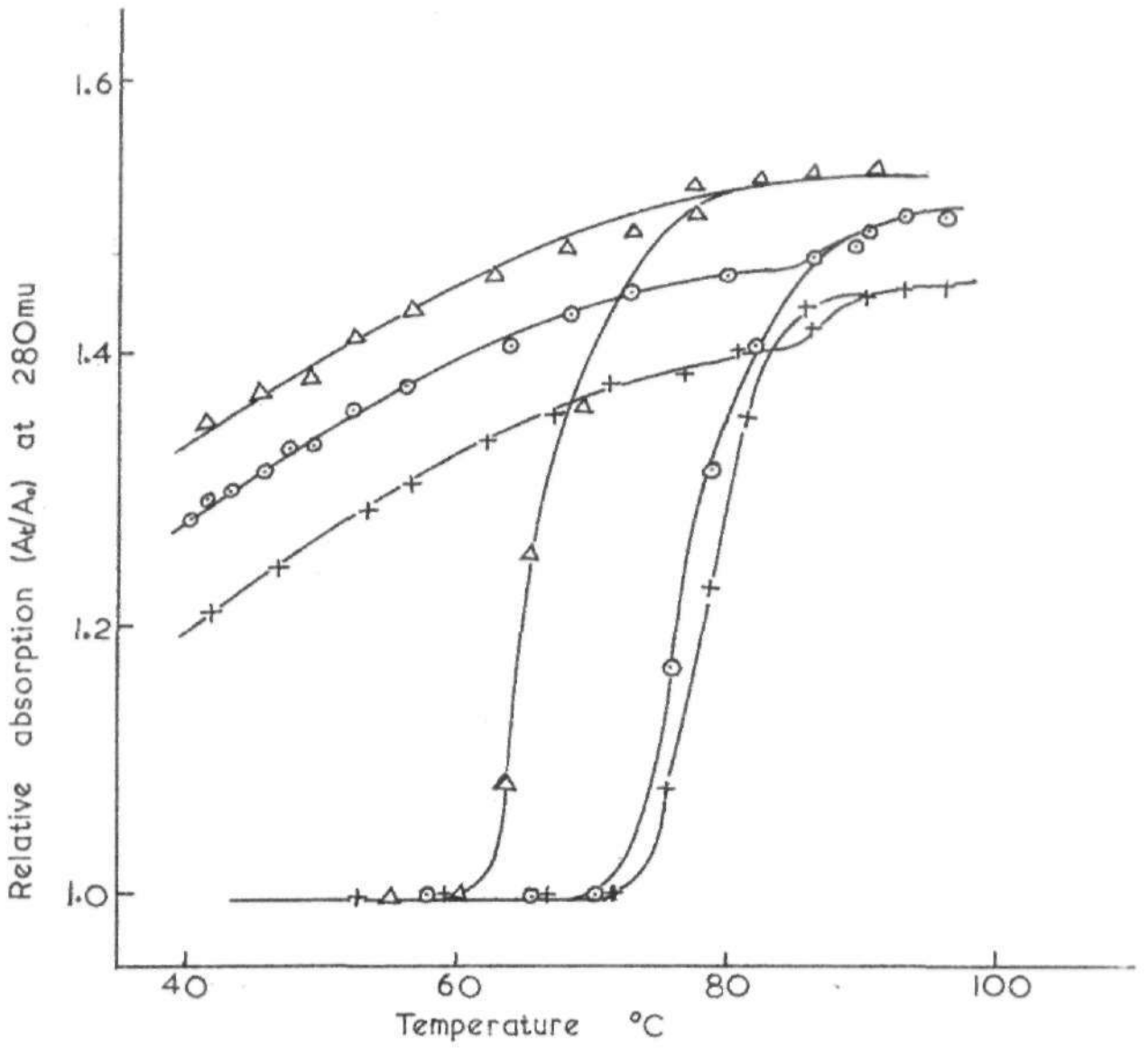


Fig. 44: Denaturation and renaturation of thymus DNA in aqueous solutions of: (Δ), 6.39×10^{-5}; (⊙), 8.10×10^{-5}; (+) 12.34×10^{-5} mole of $MgCl_2$ per litre.

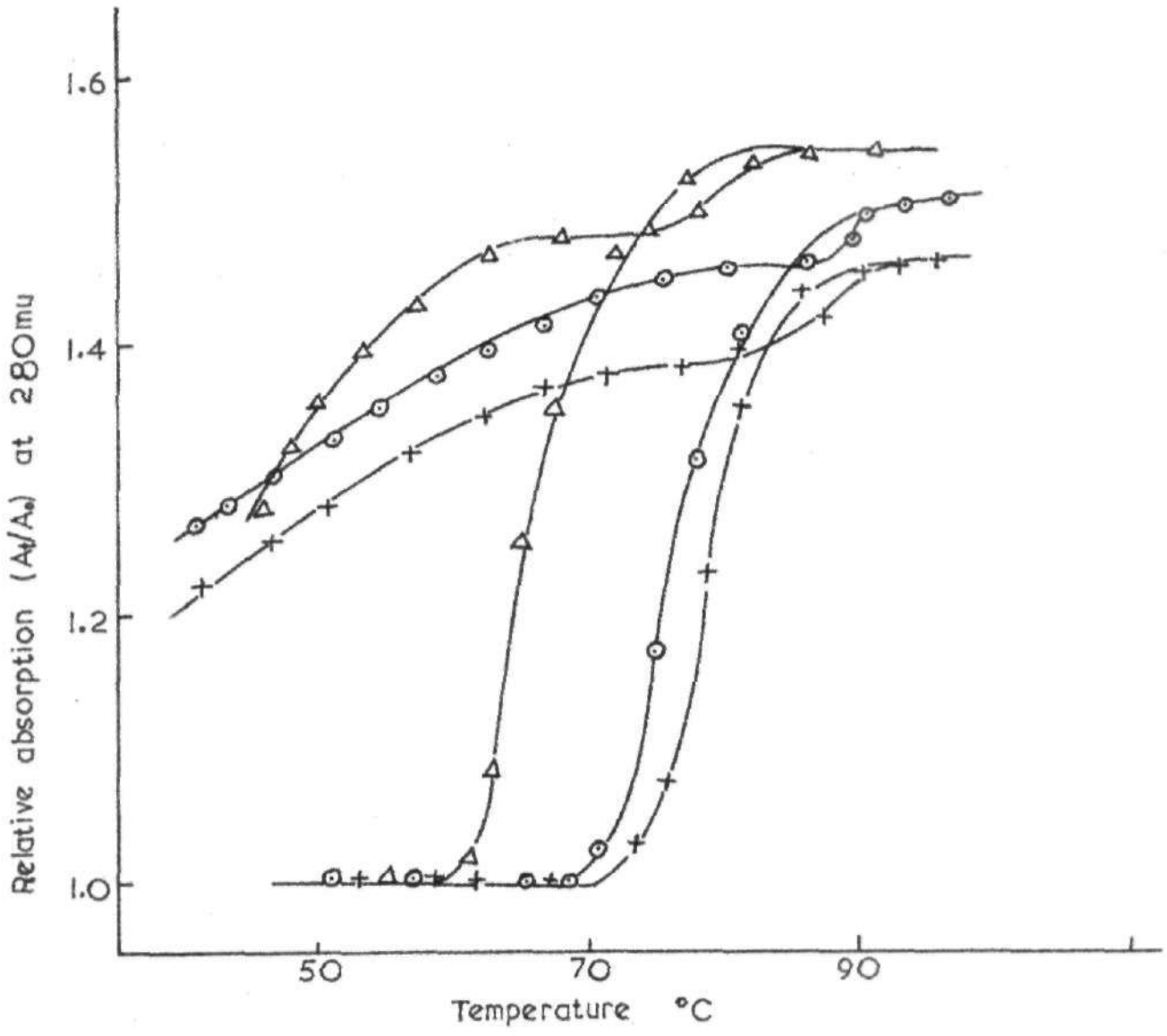


Fig. 45: Denaturation and renaturation of thymus DNA in aqueous solutions of: (Δ), 5.77×10^{-5}; (⊙), 7.38×10^{-5}; (+), 9.15×10^{-5} mole of $CaCl_2$ per litre.

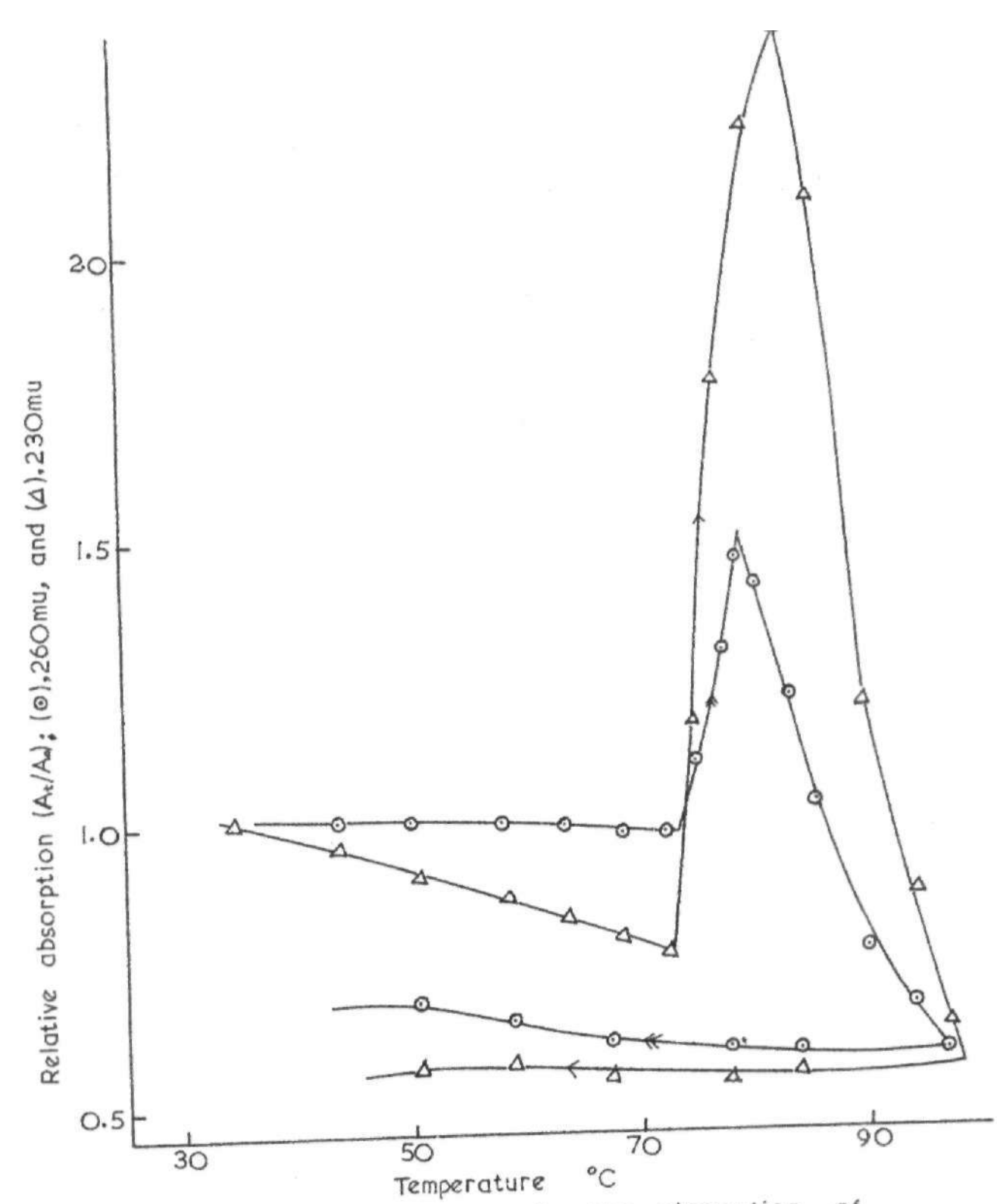


Fig. 46: Heating and cooling curves for the absorption of DNA–Ca^{2+} system to which $CaCl_2$ was added to a total concentration of 0.05M.

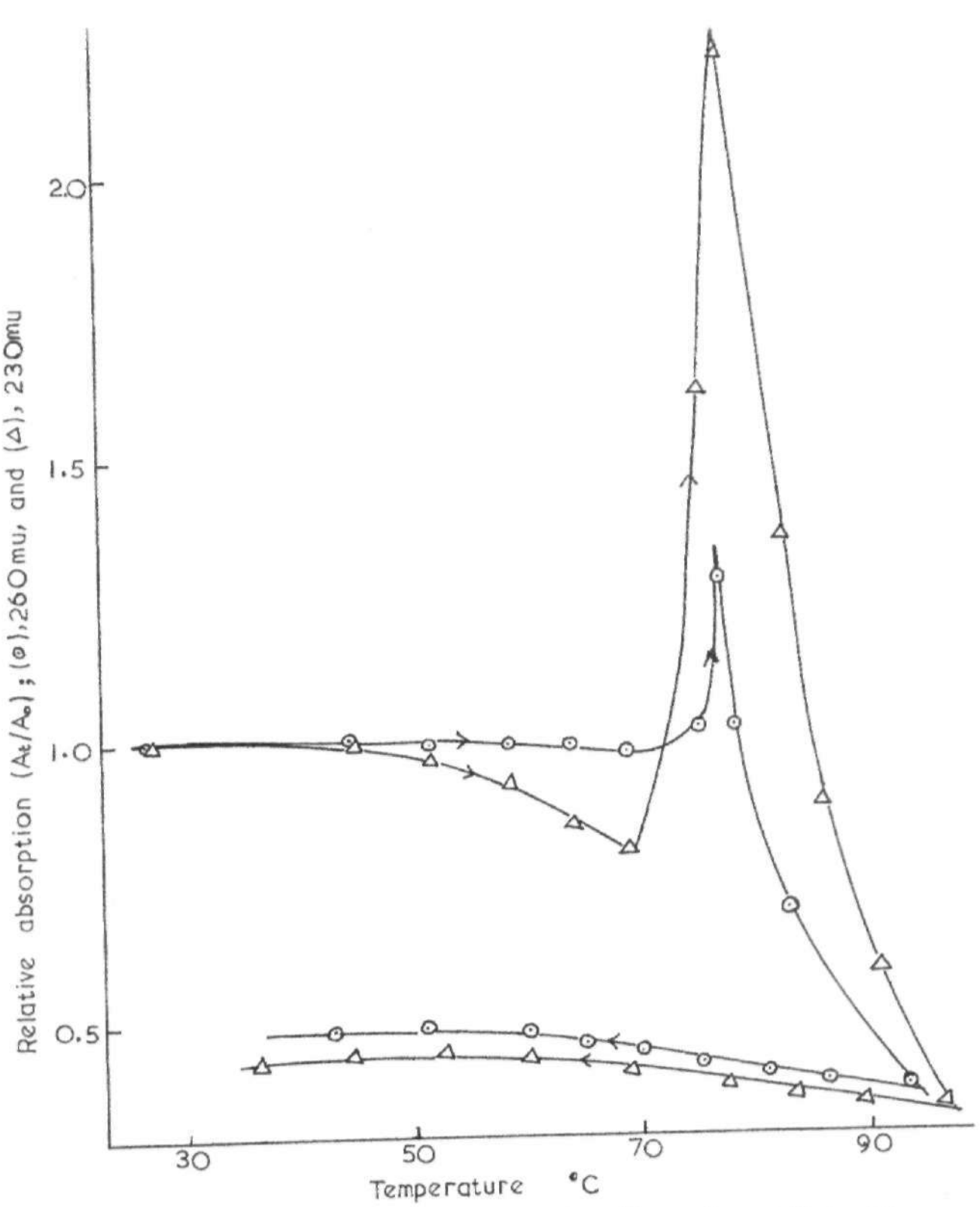


Fig. 47: Heating and cooling curves for the absorption of DNA-Ca^{2+} system to which $CaCl_2$ was added to a total concentration of 0.075M.

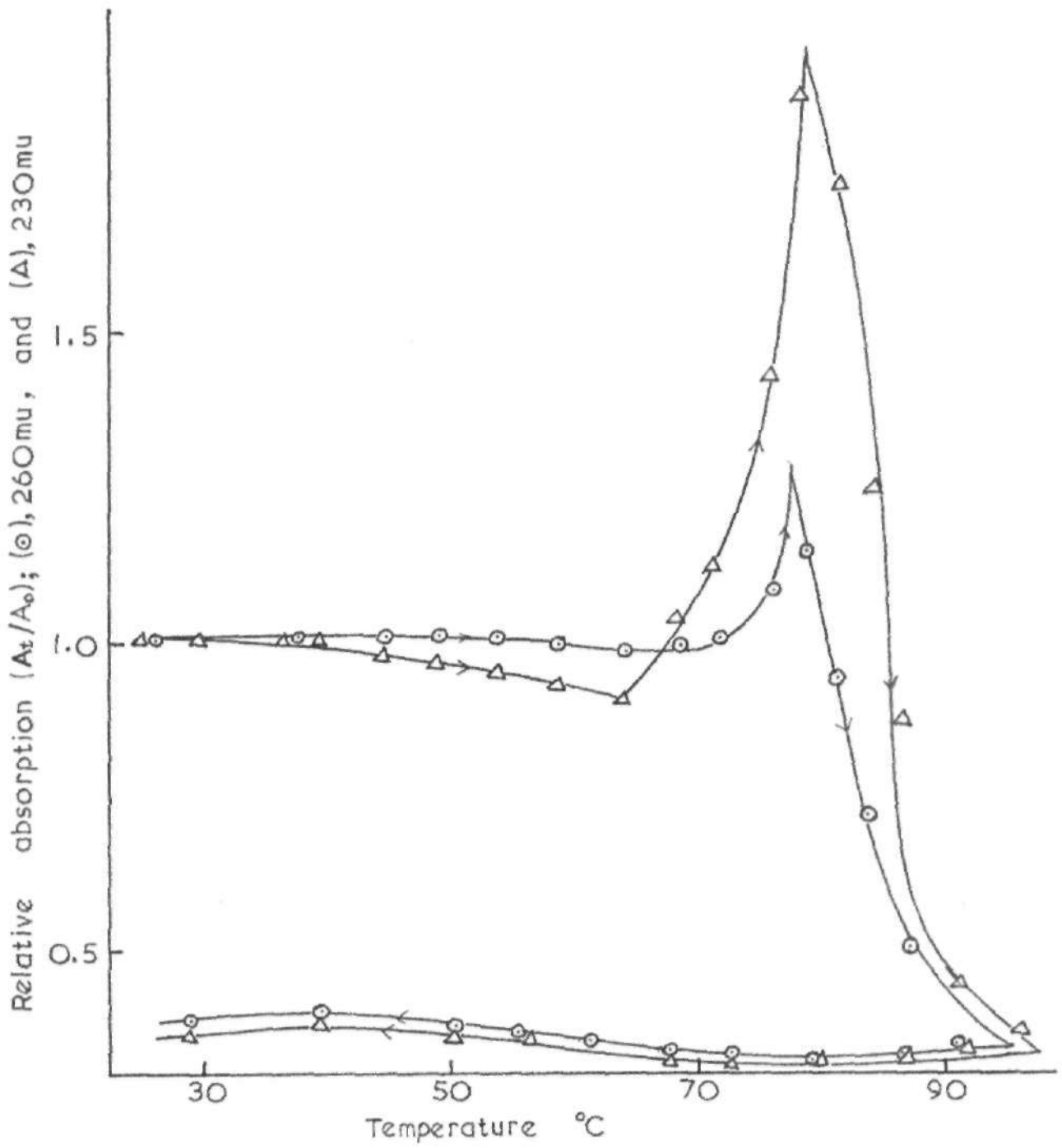


Fig. 48: Heating and cooling curves for the absorption of DNA–Ca^{2+} system to which $CaCl_2$ was added to a total concentration of 0.10M.

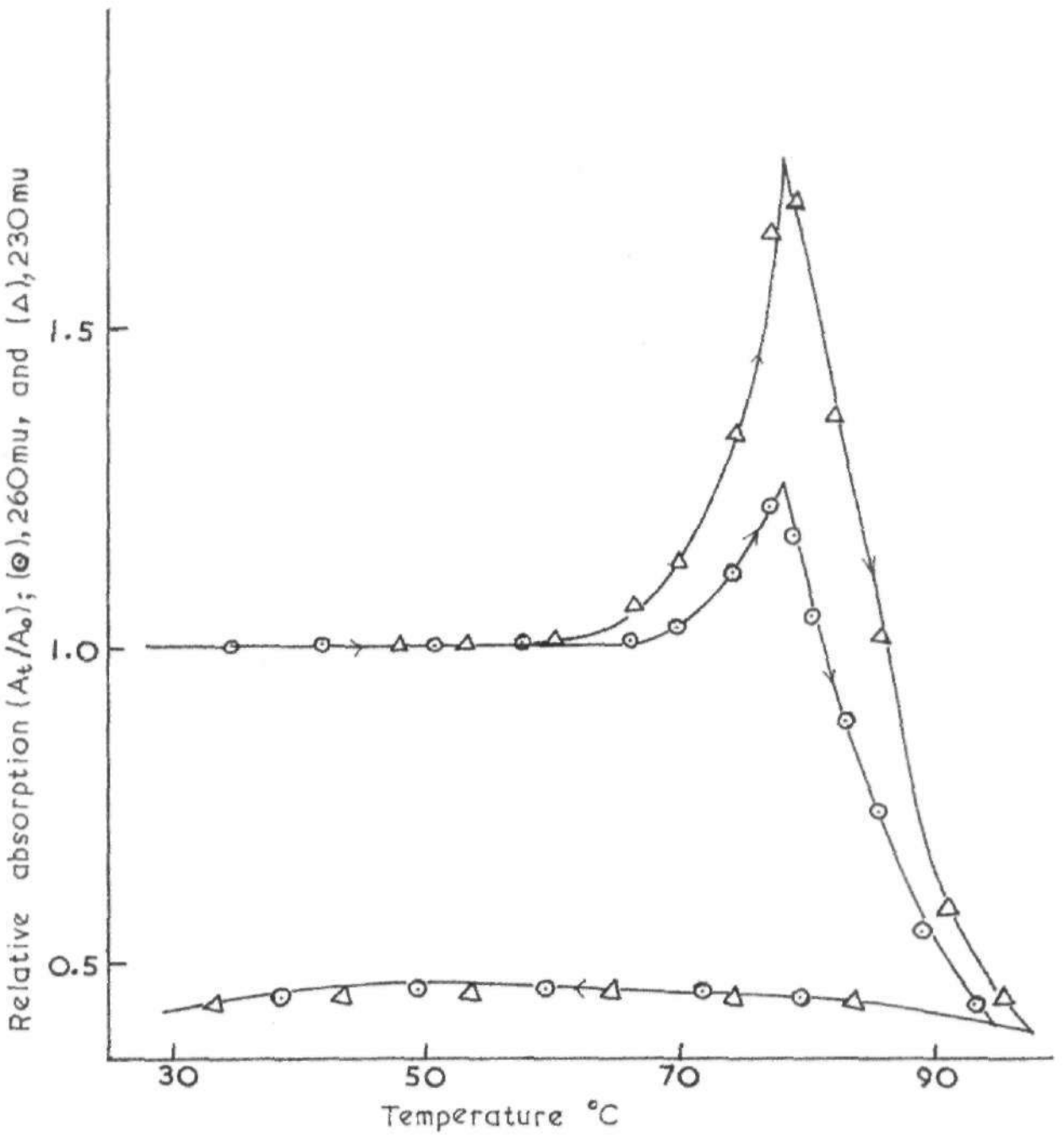


Fig. 49 : Heating and cooling curves for the absorption of DNA–Ca^{2+} system to which $CaCl_2$ was added to a total concentration of 0.20M.

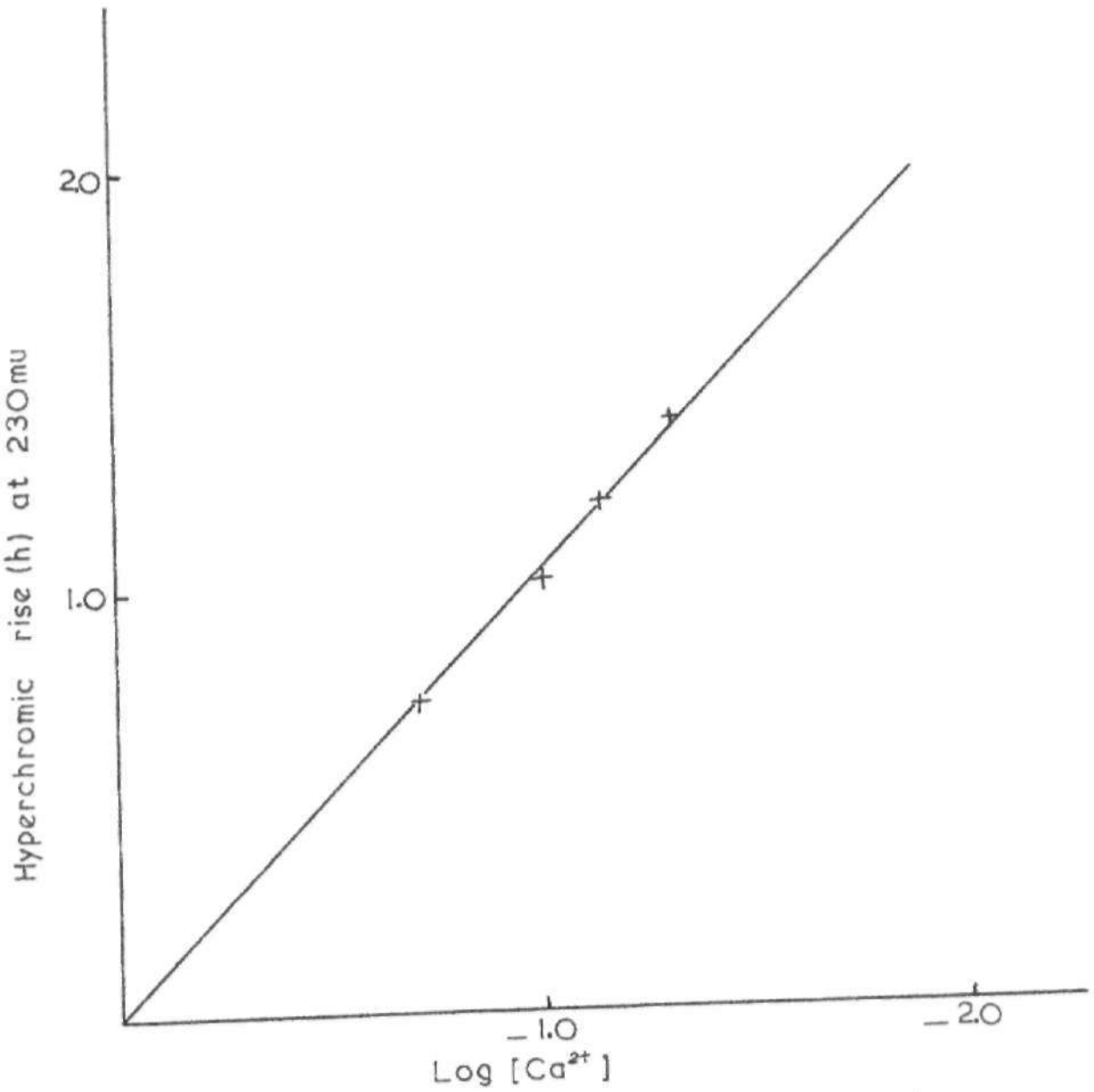


Fig. 50: The dependence of hyperchromic rise (h) on the concentration of $CaCl_2$ added to DNA–Ca^{2+} system.

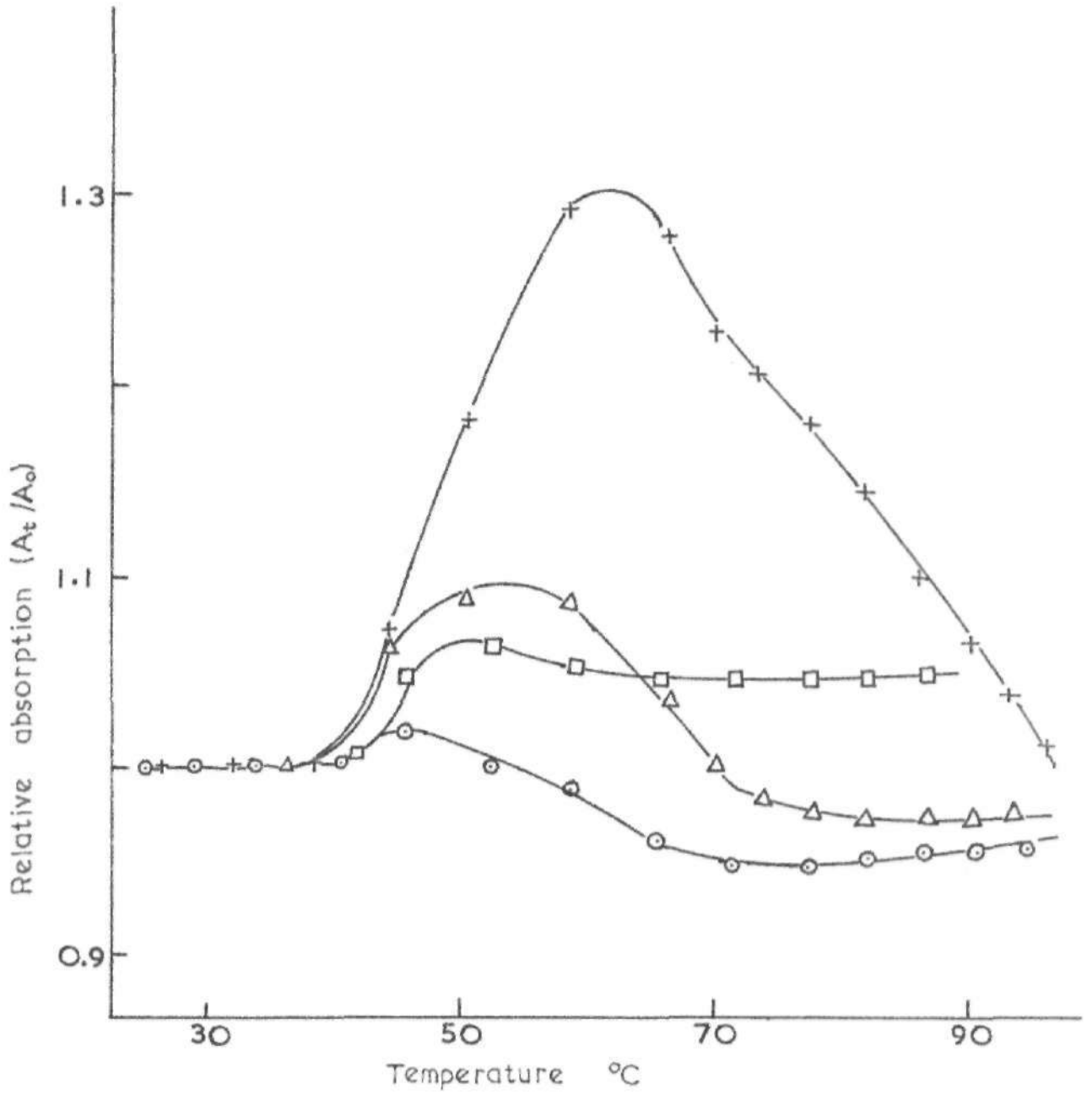


Fig. 51: Absorption–temperature profiles for preheated DNA in the presence and absence of Ca^{2+} ions.
zero conc: (⊙) 230mu, (□) 260mu
0.10M Ca^{2+}: (+) 230mu, (△) 260mu

II.3 RESULTS AND DISCUSSIONS

For each of the spectra of the nucleate studied, there was an absorption maximum at about 260 mμ and a minimum at 230 mμ. The concentrations of the nucleate solutions are expressed in moles of phosphorus per litrer of solution. The molar absorptivity $\varepsilon(P)$ of the nucleate was found to be 6,600 at 260 mμ and about 3,000 at 230 mμ for a preparation in 0.2M sodium chloride and pH 7.05. The value of $\varepsilon(P)$ recorded here compares very well with those reported earlier [21]. This shows that no appreciable amount of degradation into the separate nucleotides had taken place during the extraction processes, and during the solution preparation, as much higher values of $\varepsilon(P)$ would be expected if the preparation was a mixture of mononucleotides or oligonucleotides [22 - 24].

Figures (27 - 51) represent the results of the UV studies of the interaction of DNA with sodium, calcium and magnesium. In general strong interactions of the divalent cations with DNA have been observed. As shown by the figures, thermal stabilisation of the nucleate by sodium ion was not as strong as those of calcium and magnesium, as seen in the concentration of the divalent cations required to raise the denaturation temperature (T_m) of the nucleate by an amount, ΔT_m, which is only about one thousandth that for sodium ions.

Again, the phosphate groups have been assumed to be the only sites of interaction with the cations at pH 7.05 investigated. Four types of DNA have been studied:

(i) DNA partly denatured during dialysis to remove all counterions.

(ii) Native (undenatured) DNA.

(iii) DNA - M^{2+} + MCl_2 System.

(iv) Heat-denatured DNA.

II.3.a DNA denatured during dialysis

In order to study the effect of very low salt concentration on the UV absorption and the thermal characteristics of DNA, the preparation in its stock form, was dialysed against changes of conductivity water to remove all counterions. This, diluted to 9×10^{-5} mole phosphorus per litre of solution in the absence of supporting electrolyte, was found to have been denatured to some extent as evidenced by its low denaturation temperature of about 75°C, as compared to 86.5°C for the native molecule.

The various investigations conducted on this denatured sample gave the results summarised below.

(a) The variation of the absorption ratio A_i/A_o with the concentration of the added electrolyte is shown in figs. (31-33). Here, A_i is the absorption of a nucleate of a given concentration i (in mole/l) of simple electrolyte NaCl, $CaCl_2$ or $MgCl_2$, and A_o is the absorption of nucleate of same concentration, but zero concentration of simple electrolyte.

The difference in absorption spectrum of denatured DNA in the presence and absence of added electrolyte are also shown in figs. (28 - 30).

The denatured sample had an $\epsilon(P)$, i.e. molar absorptivity approaching 7500, which decreased as the salt concentration in sodium chloride, calcium chloride or magnesium chloride was increased figs. (28 - 33). There was a much greater decrease of the absorption with salt concentration than was anticipated had the nucleate not been denatured, compare figs. 32 and 33 with table 5.1 This occurred only for calcium chloride and magnesium chloride, but not for sodium chloride. For sodium chloride it could be concluded therefore that the increase in absorption at low salt concentrations was irreversible and it was perhaps due to some irreversible structural changes in the DNA molecule, as previously suggested by Thomas [1,28]. The lowering of absorption in the presence of added salt might be due to some compression of the extended nucleate ion. This compression could have resulted from the removal of any expansive force, e.g. electrostatic force of repulsion on the charged phosphates, on the nucleate thus allowing the normal amount of base stacking expected in a native nucleate.

The extent of lowering of absorption at first increased with concentration of added calcium and magnesium salts up to a concentration of one mole of divalent cation to two of nucleate phosphorus. This finding is in harmony with the results published by Shack, Jenkins and Thompsett. These authors found that the quality of any divalent cations, particularly magnesium, needed to bring about the transformation was about the same order of magnitude as the quantity of the nucleate phosphorus [29] . No such relation has been established for sodium ions, as it has been observed that for the concentration of Na^+ ions investigated, the absorption continued to decrease. A reason for this might be that the sodium ion, in the state of only a sort of electrostatic interaction with the negatively charged phosphate sites only reduced gradually the electrostatic expansive forces between nearest neighbour phosphate sites as its concentration was increased. At very high concentration of the salt, all expansive interactions would be expected to have been eliminated and the absorption measured, e.g. in 0.2M Nacl and in higher concentrations as have been shown, could be taken as equal to that observed for the minimum amount of calcium and magnesium required to produce the largest decrease in absorption as obtained from figs. 32 and 33.

The results observed for the divalent cations suggest that the optical changes could be associated with a tight and possibly specific binding between the DNA and the divalent cations.

(b) The absorption of UV radiation at 260 mμ by DNA was found to remain constant with temperature up to a temperature value which was dependent on the concentration of added sodium chloride, calcium chloride or magnesium chloride. After this there was a steep rise (or hyperchromic rise) of absorption with temperature to a constant and maximum value of absorption which was dependent on the salt concentration.

The thermal transition breadth decreased with salt concentration for all the three neutral salts studied.

Both the hyperchromic rise and the transition breadth can be used to define the denaturation temperature (T_m) which has been known to depend on the (G + C) content of the nucleic acid, among other things. For a particular nuclease preparation, the (G + C) content is the same, and (T_m) therefore varies only with the salt concentration as the external condition, and in a manner represented in equations (5-9) and (5-17).

As indicated in figs. (34 - 36), the denaturation temperature increased with salt concentration for all the three DNA-salt systems. The manner in which (T_m) varied with salt concentration is shown in figs. (39 - 41), in a plot of (T_m) versus log [salt]. These results have revealed that (T_m) varied linearly with the logarithm of the salt concentration for both monovalent and divalent electrolytes.

Equation (5-17) is therefore obeyed. References on the dependence of (T_m) on salt concentration are found in this chapter [30 - 37].

Although (T_m) increased with salt concentration for all the three DNA-salt systems, it increased at a faster rate for DNA-divalent salt system than for DNA-NaCl. This implies that magnesium and calcium ions are more effective in increasing (T_m) at far lower concentrations than sodium ions. Both magnesium and calcium have proved to be more than one thousand times as effective as sodium ions, as judged by the results shown in figs. (39 - 41). Results similar to these have also been published by Thomas for magnesium [1, 38] .

Figures (34 - 36) represent the variation with temperature t, of A_t/A_o (the relative absorption where A_t is the nucleate absorption at temperature t°C, and A_o is its absorption at 25°C, both of which are measured at 260° mμ). A sigmoid-shaped graph was obtained in each case. Each graph is characterised by

(i) a hyperchromic rise (h), given as (h) = $A_t/A_o - 1$,

(ii) a thermal transition breadth (δ) which is the range or increase of absorption from $(h)_o$ to $(h)_{max}$, and

(iii) the denaturation temperature (T_m) which has been defined as the temperature where (h) = $\frac{1}{2}(h)_{max}$.

Each of these three parameters shows some dependence on the salt concentration of the nucleate solution.
In general, (h) decreased with salt concentration, and generally at a faster rate with increasing concentration of magnesium and calcium than of sodium ions. It varied inversely as the logarithm of salt concentration or as denaturation temperature, as shown in figs. 37 and 38.

The slopes of the plot of (1/h) versus log $[M^{2+}]$ for magnesium and calcium have confirmed that calcium ions have a greater influence on the structure (particularly the secondary structure and the longitudinal dimension) of the nucleate ion than magnesium ions have. The hyperchromic rise (h) decreased at a faster rate in the presence of calcium than in the presence of magnesium, indicating a stronger binding of the former than of the latter to DNA.
The maximum hyperchromic rise for the DNA preparation was about 0.37 at 260 mμ.

Figures 38 and 37 indicate that calcium is more effective in stabilising DNA against thermal denaturation at lower concentrations than magnesium is, up to one mole of cation to two moles of nucleate phosphorus. At higher concentrations, magnesium appears to stabilise the nucleate against thermal denaturation more than calcium. This might be on the account of the difference in the ionic radii of the two (unhydrated) cations.

On the one hand, calcium ions being larger, would shield stereochemically the stabilising hydrogen bonds from ~~thermal energy~~ water molecules more effectively at lower concentrations than magnesium ions would, hence they would increase (T_m) at a faster rate in their low concentrations than magnesium ions would. On the other hand, after the quantitative binding of one mole of divalent cation to two of nucleate phosphorus, a cluster of ions could be assumed to be formed round the nucleate. In this, many more magnesium ions could be associated with the polyion-cation complex already formed. Consequently, a more effective ~~shielding~~ stabilisation of ~~thermal energy from~~ the hydrogen bonds against thermal denaturation could be possible.

Figure 39 gives a (T_m) of about 86.6°C for a native DNA in 0.2M NaCl and pH 7.05. This gives a value of 41.7% (G+C) from the equation [T_m = 69.3 + 0.41 (G + C)], as compared to the value of about 42.2% given previously [38a - 38d]. It also gives a slope of approximately -18.5, in agreement with equation (5-9), and as reported earlier by Kotin and Nagasawa [11], and by Ohnishi [12]. From this plot, a minimum amount of sodium chloride of about 10^{-4}M would be required to maintain the secondary structure of DNA which would give rise to a (T_m) of 25°C. This in other words, is the minimum concentration of sodium chloride required to prevent the denaturation of the nucleate at the temperature of 25°C.

The same concentration (10^{-4}M) of calcium and magnesium would produce a (T_m) of 84° and 85° respectively.

From the slopes of the plot of (T_m) versus log [salt concentration] it has been possible to estimate the change ΔSo in the entropy of the helix-to-coil transition of DNA in the presence of the three cations, sodium, calcium and magnesium from equations (5-8) and (5-9). From figure 39, (in which T_m = 359.6°K and the slope = - 18.5), and taking C_2 as equal to 0.60, as used by Walker [11], the change in the entropy of helix-to-coil transition in 0.2 M sodium chloride has been estimated to be -23.17 cal. deg^{-1} per mole base-pair, as compared to -22.5 cal. deg^{-1} in 0.18M sodium chloride reported earlier [9].

For magnesium and calcium systems, in the presence of 1.41×10^{-4} mole of the cations, the quantity ΔSo has been found to be respectively - 41.58 and - 67.78 Cal. deg^{-1} per mole base-pair. These values of ΔSo have indicated that calcium, magnesium and sodium ions tend to increase the order in a nucleate during its thermal denaturation in the order of calcium > magnesium >> sodium ions.

Generally, the results given in this report are in close agreement with others reported earlier. A hyperchromic effect in nucleic acids has already been reported by a number of workers [39 - 43]. As defined more fully here, it is a measure of the percentage of fractional increase in the UV absorption at a given wavelength on degradation or denaturation of the nucleate ion.

Generally, the magnitude of the hyperchromic effect on thermal denaturation is a function of the (G + C) content of the acid, varying from about 44 percent for DNA of 38 percent (G + C) to 34 per cent for one of 67 per cent (G + C), [2].

The decrease of (h) with concentration of salt can probably be explained by the greater ease with which DNA strands which have been separated by heating can re-associate on cooling at higher ionic strengths. At low ionic strength, the strands associate less readily with consequently less interaction between the bases, and a lower hyperchromicity.

The transition breadth (δ) for a helix-to-coil transition depends on molecular heterogeneity of the preparation. It reveals the extent of physical or chemical heterogeneity in the preparation. For a homogeneous preparation it would be a minimum, and depends on the conformation of the DNA preparation. The greater rate of decrease of (δ) with increasing concentration of magnesium and calcium might imply a greater change in the conformation of the DNA molecule in the presence of these divalent cations than in the presence of sodium ions. The length of the molecule in particular, would be expected to decrease during the binding with these two divalent cations, thus approaching the length of the native nucleate.

The effect of varying the ionic strength on (T_m) has been considered in terms of its effect on the differences in the free energy ΔG between the helical and the denatured forms of a sequence as discussed by Walker and Peacocke [39].

The change in (T_m) with ionic strength would depend on the change of the free energy ΔG with ionic strength as shown in equation (5-12). Thus a relation between ionic strength and (T_m) could be derivable by considering the effect of ionic strength on the free energies of helical-coiled sequences. This type of relation between (T_m) and ionic strength is given in an empirical equation (5-17) for monovalent salt. The same expression has been found to hold for divalent salts.

(c) In a graph of absorption-temperature on heating above the denaturation temperature of the nucleate and on cooling slowly, the absorption was found to decrease with decreasing temperature, figs. (42 - 45). The denaturation of DNA has been known not to be a completely reversible process [1]. The extent of reversibility is a function of the rate of cooling of a heat denatured sample, and more importantly it is a function of the salt concentration. With the condition of cooling held constant, the degree of renaturation (or the extent of reversibility) becomes a function of only the salt concentration for a particular DNA preparation. This is shown in figures (42 - 45). In these, the effect of low concentrations of calcium and magnesium on the forward (sigmoid) curve and the reversed curve are shown in the plot of A_t/A_o against temperature °C, for both 260 mμ and 280 mμ.

Two wavelengths were used for the purpose of dispersion studies of the absorption by the nucleate sample. The results indicate that there was a difference in absorption at these two wavelengths and there was a much greater hyperchromic rise at 280 mμ for any particular DNA-M^{2+} system. The rate of decrease of the hyperchromic rise with increase of salt concentration was higher at 280 mμ than at 260 mμ. This might mean that two distinct groupings present in blocks in different amounts might be mainly responsible for the absorption at these two wavelengths. The ratios of the rise at 260 mμ to that at 280 mμ is 41.59 which is just about the same ratio of (G + C) to (A + T) composition (i.e. 41.5 : 48.5) of the nucleate sample. In the same way as the hyperchromic rise is greater at 280 mμ, its fall is also greater than at 260 mμ during the cooling. At the two wavelengths the extent of renaturation of the DNA-M^{2+} system on cooling increased with the salt concentration. The transition breadth (δ) at both wavelengths are the same, so is the denaturation temperature, (T_m) for the heating curve.

The following deductions have been made from these results: that the bigger hyperchromicity at 280 mμ, assuming that hyperchromicity generally arises from the extension of chain, might have been due to, (i) same type of groupings which occurred in blocks, which could undergo different amounts of expansion during denaturation, or (ii) that there was an unequal amount of two types of groupings occurring in blocks along the polymer chain.

In either case, the idea is that there must be at least two types of groupings present in the polymer chain. If identified with the (G + C) and (A + T) groupings, then the problem boils down to finding which absorbed more strongly at 280 mμ. The suggestion of an unequal capability of expansion of these types of grouping is inadequate here since it is known that all bases in DNA are separated by the same distance (3.4 A). On the other hand the suggestion of unequal amounts of the groupings has been known and reported in this section. Hence the higher hyperchromicity could be attributed to the (A + T) regions. It is also necessary that the two groupings should occur in blocks along the polynucleotide chain. This would also mean that the preparation could be either chemically homogeneous with each separate molecule containing about 41 per cent (G + C) or could be heterogeneous with molecules having an average of (G + C) of 41 per cent. The question could however be asked as to why the denaturation temperatures of the heating curves are the same for the two wavelengths. Although (T_m) is the same for the two heating curves, at 280 mμ, the cooling curve first dropped sharply and then continued with a gradual fall. These results, together with the fact that the rate of decrease of (h) is bigger at 280 mμ than at 260 mμ would suggest that the grouping more easily denatured is more easily renatured.

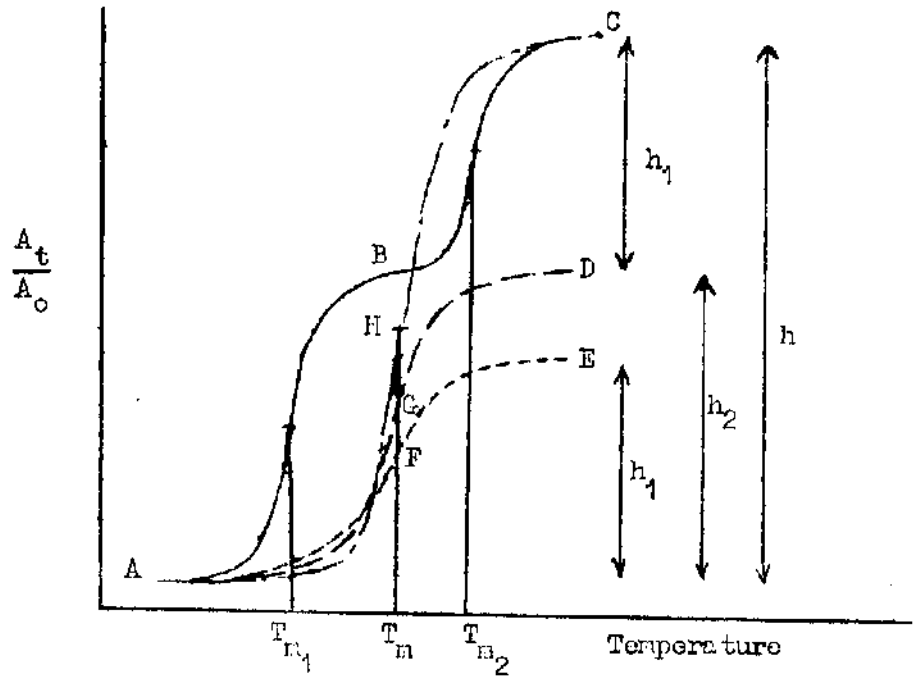
However, the reasons for not observing any difference in (T_m) at the two wavelengths are given in the following illustration. Two types of preparations have been considered, one with a high compositional heterogeneity and the other homogeneous.

The two preparations could be assumed to satisfy the necessary conditions that

(a) there must be blocks of (A-T) and (G-C)

(b) there should be a greater overall percentage of (A+T) than (G - C)

with the main difference being only the arrangement of the base pairs along the chain.



The two cases can be represented as follows:

Case one

A	A	A	A	T	T	T	T	T	G	G	C	C	C	C
T	T	T	T	A	A	A	A	A	C	C	G	G	G	C

block (A - T) block (G - C)

Case two

A	A	A	A	G	G	G	T	T	T	T	T	C	C	C
T	T	T	T	C	C	C	A	A	A	A	A	G	G	C

alternating block (A - T) and block (G-C)

If it were possible to construct a polynucleotide chain of the structure in case one, and its denaturation temperature determined, one would likely find that there would be a dispersion of absorption either at 260 mμ or at 280 mμ as shown in the diagram in curve ABC. Characteristic of this curve are two denaturation temperatures T_{m1} and T_{m2}, where $T_{m2} > T_{m1}$. In this curve, AB is longer than BC, in other words, $h_2 > h_1$ (i.e. the hyperchromic rise from A to B and from B to C). It is most likely that the separate transition breadths would be equal however. The portion BC of the curve could be attributed to transition of the (G - C) regions since this has a higher T_m value.

The ratio of the hyperchromic rise from the two regions AB and BC to the total hyperchromic rise for the molecule would then be

$\frac{h_2}{h}$ and $\frac{h_1}{h}$ respectively.

The discussion suggests that if the DNA preparation consisted of blocks (A - T) and block (G - C) (non-alternating) then measurements of the absorption at 280 mμ and 260 mμ would lead to two different values of T_m.

As observed in these series of studies, this is not so. Measurements at these two wavelengths gave the same T_m values, but different values of hyperchromic rise, but same value of breadth of transition. These observations are explained by the diagram.

Curve A G D could represent results at 280 mμ, with curve A F E representing those at 260 mμ. They both have the same T_m value, but different values of hyperchromic rise. The ratio h_1 to h_2 has been found to be 41 to 59 which is the same order as the ratio of percentages of (G + C) to (A + T) of the preparation.

The fact that the quantity h has not been observed experimentally would mean that the helix-to-coil transition must be cooperative, and this is possible only if the preparation is chemically homogeneous. The (A-T) regions whose hydrogen bonds are more easily ruptured must alternate as much as possible with the (G - C) regions so as to be able to induce the helix-to-coil transition of the latter thus making the process cooperative.

Should the transition take place only in the (A - T) region of case one, regions of order and disorder would result and this would lead to an instability of the molecule. Thus either a complete order must be regained (which might be possible only during cooling), or a complete disorder is accomplished by the breaking of the hydrogen-bonds of the (G - C) regions.

Suggestions for the possible sequential arrangement of the nucleotides along a polynucleotide chain (and in a nucleic acid molecule) have been made from the studies of the denaturation temperatures by a number of workers [44 - 54]. In particular, Gool and Montroll [41] have considered the case of breaking the hydrogen bonds of the (A - T) and the (G - C) types and have,

through some arguments based on statistics concluded that there is likely to be present in the particular nucleato sample studied, alternating block (A - T) and block (G - C) with some occurrences of alternating mononucleotides of either types. They studied the melting curves of DNA molecules at two wavelengths, one at which pure (A - T) and pure (G - C) homopolymers have the same absorption coefficient (i.e. 260 mμ), and the other at which they have a different absorption coefficient (e.g. 280 mμ). They were able to show that the (G - C) homopolymer had a higher melting point value than the (A - T), and there was a dispersion in the absorption curve when the two were mixed together. This would correspond to curve ABC in the diagram. For a homogeneous preparation however, a single value of (T_m) has been reported at both wavelengths. This is higher than (T_m) for homopoly -(A - T), but lower for homopoly -(G - C). All these observation are still adequately explicable by the diagram included here.

II.3.b Native DNA

The results obtained by the denatured DNA (i.e. DNA denatured during dialysis) have led to the temptation of concluding that the absorption at room temperature 25°C, of any nucleate preparation would depend not only on the nucleate concentration in mole phosphorus per litre, but also on the salt concentration (in simple neutral salts like NaCl, $CaCl_2$ and $MgCl_2$) The limitations to this conclusion was determined in a number of experiments with the native DNA. This was prepared undialysed and of 10^{-4}M of sodium chloride supporting electrolyte. The relative absorption A_i/A_o at 260 mμ was determined for different concentrations of added calcium chloride and magnesium chloride. The results are shown in table 5.1. The relative absorption at 260 mμ was found to be almost constant for all the salt concentrations examined. The absorptivity of the nucleate solution was also found to be 6600 at 260 mμ. It is therefore conclusive that native DNA solution of a given concentration at room temperature has a constant absorption at different concentrations of added electrolytes. These results help in explaining those observed in figs. (28 - 40), where $(A_i/A_o)_{260}$ varied with salt concentration, as due to the denaturation of the nucleate ion. These cations were probably engaged not necessarily in renaturating the nucleate, but almost certainly in eliminating the extension of the polymer by reducing the electrostatic repulsion between the phosphates. The binding of the cations to the phosphate site has led to a greater base stacking of the nucleate and a shortening of the

the polymer with a consequent decrease in absorption. The divalent cation required to cause a minimum value of $(A_i/A_o)_{260}$ is that amount required to bind quantitatively to the phosphate sites in a 1:1 ~~mole~~ eqv. ratio. The fact that a minimum was observed for (A_i/A_o) for DNA-M^{2+} system but not for DNA-NA^{+} system is an evidence of stronger binding of DNA with magnesium or calcium than with sodium ions. Similar results have been reported by Shack, Jenkins and Thompsett [29].

II.3.c The DNA-M^{2+} + MCl_2 Systems.

The purpose of this study was to examine the effect of calcium and magnesium on the system of DNA-M^{2+} in which (M^{2+}) was calcium or magnesium. Nucleate solutions of a total of 0.05M, 0.075M, 0.10M and 0.2M in calcium chloride were made by the addition of a weighed amount of dry calcium chloride to the system of DNA-Ca^{2+} which originally contained 9.27×10^{-5} mole of calcium per litre. The absorption - temperature profile of each nucleate solution of high concentration of calcium was determined at 230 mμ and 260 mμ. The results are presented in figs. (46-49).

Quite different from the types of curves observed for low concentrations of added electrolyte with temperature increase, the relative absorption first dropped below its initial value, (this being marked at 230 mμ), and then rose steeply to a maximum and then dropped sharply to a value much below its initial.

On cooling, a final value of relative absorption still much lower than the initial was obtained.

What could be regarded as the hyperchromic rise in absorption here was bigger at 230 mμ than at 260 mμ and decreased with increasing salt concentration at both wavelengths. An emprical equation of (h) = a log $[M^{2+}]$, where a is a constant, was obeyed by the absorption at 230 mμ. Results at 260 mμ did not fit so well into this equation. These results would lead to the hasty conclusion that the hyperchromicity at 230 mμ would vanish at a concentration of calcium equal to one mole, fig. 50. The results obtained at 260 mμ have shown that (h) approaches a minimum constant value with increasing ionic strength of calcium.

All curves at 230 mμ have the same (T_m) value as the curves at 260 mμ for all the salt concentrations examined. This suggests that increasing salt concentration on a system of DNA-Ca^{2+} has no effect on (T_m). With the information of higher hyperchromicity and same (T_m) value at 230 mμ, it is reasonable to conclude that at least two main regions are distinct in a nucleate ion. The absorption hyperchromicity at 260 mμ would be made up largely by the denaturation of that region which is present in a smaller quantity and that at 230 mμ, which is present in a larger quantity so as to give rise to bigger hyperchromicity.

If the nucleate were composed of alternating purine and pyrimidine mononucleotides, the hyperchromic rise at the two wavelengths would be expected to be of the same value.

However, this has been found to be otherwise. Hence the nucleate would most likely consist of alternating block purine and pyrimidine oligonucleotides with the two main regions separated from each other by some random occurrences of mononucleotides:

A - T
G - C

A - T
T - A
T - A
A - T

G - C
A - T

G - C
C - G
C - G

A - T
A - T
T - A
G - C

block (A - T)

block (G - C)

Again it could be assumed that the (A - T) region was the same one contributing significantly to the absorption at 230 mμ with a higher value of hyperchromicity. The fact that this DNA is richer in (A + T) than in (G + C) justifies this bigger hyperchromicity at 230 mμ. The transition breadth (δ) which is bigger at 230 mμ than at 260 mμ could also be a proof of the higher degree of heterogeneity of the preparation.

For example, since the preparation is richer in (A + T) than in (G + C), it could be assumed that there would be a greater heterogeneity in the (A - T) sequence than there would be in the (G - C) sequence as can be explained by the following arrangement as in this specific case:

A A A A G G A A G G A A A A G G

Here there is 60% (A + T) and 40% (G + C) in the preparation. In each case there is the same number of interruption (as typical of this example), but a greater heterogeneity of distribution of (A - T) than of (G - C).

A particularly interesting observation made here is that the relative absorbance first dropped below its initial value before the hyperchromic rise commenced. The fact that this is particularly so at 230 mμ suggests that the behaviour of the two regions (A - T) and (G - C) at the two wavelengths are different on heating and particularly with the salt concentration used. It might possibly indicate the greater flexibility of the longer (A - T) region. This flexibility, results have indicated, decreased with increasing salt concentration. This is not unexpected.

The unusual results observed on continuing the heating after the hyperchromic rise can be attributed to

(i) coiling up of the separate strands of the denatured acid, since the bases are no longer stacked one on top of the other, but are all packed together, or

(ii) a possibility of precipitation of insoluble nucleotides.

As observed, the absorption was approaching a zero value as heating was continued, and this makes the suggestion of precipitation more probable although the posibility of the occurrence of aggregation could not be entirely ruled out.

The fact that on cooling, the absorption was still much lower than its initial value suggests that a permanent change in the structure of the polyion must have occurred. Such permanent changes in structure of the polyion could be aggregation or degradation followed by precipitation.

But whether the nucleate was precipitated in its insoluble salt as mononucleotides or possibly as dinucleotides, and whether the metallic ions were specific at breaking the covalent bonds of the nucleate back bone are problems yet to be solved. All that could be concluded at this stage of the study is the aggregation or precipitation of the nucleate in high concentration of magnesium or calcium.

II.3.d Heat-denatured DNA

A nucleate solution (9×10^{-5} mole P/litre) was heated at 95°C for one hour to ensure an appreciable amount of denaturation. It was cooled rapidly in an ice-bath.

The absorption-temperature profile of the nucleate solution was studied at 260 mμ and 230 mμ to investigate the action of divalent cations on denatured nucleate, and whether the absorption was going to show any dispersion at these two wavelengths.

The following results have been noted and are contained in fig. 51.

The denaturation temperature T_m, had the same value for the two wavelengths for the two concentrations of calcium ions studied.

The hyperchromic rise at 230 mμ is bigger for high concentration of calcium than at 260 mμ. The reverse is true for zero concentration of cations. Surprisingly, the transition breadth at the two wavelengths increased with increasing concentration of calcium, and is bigger at 230 mμ for high cation concentration than at 260 mμ. Again the reverse is true for zero cation concentration.

The results indicate that calcium does not have significant effects on the denaturation temperature (T_m) of a preheated nucleate possibly because most of the hydrogen bonds were not reformed on cooling prior to the second heating.

This would not mean that the cations were not bound to the nucleate ion however. It might only imply that the processes of binding to the nucleate phosphates to form stoichiometric complexes and that of thermally stabilizing the polyanion are different and take place together only for a native nucleate. For a denatured nucleate, only the binding process would be freely possible. It is believed that no tenable formula can be established for the extent to which the process of thermal stabilisation of a partially denatured nucleate could take place unless the degree of denaturation is known precisely.

Nevertheless, it could be deduced that the probability that this process would occur would likely diminish with the degree of denaturation.

Thus the presence of calcium, and possibly magnesium does not produce a renaturation of a denatured nucleate, but enhances some order in the molecule possibly by causing an alignment of the strands by binding to the phosphate groups, thus making hydrogen bond formation possible. If the base in the two strands are arranged such that complementariness is still maintained, then partial re-naturation could be possible. Where appreciable complementariness could not be accomplished, as in the case of coiled strands, no renaturation of the nucleate would be anticipated.

These results again suggest that at least two main types of major groupings must be present in the nucleate and in an unequal amounts too.

The higher hyperchromicity at 230 mμ suggests that the groupings mainly responsible for absorption at this wavelength must be present in a larger quantity than that at 260 mμ. This grouping again could be block (A - T) within the polyanion.

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CHAPTER SIX

LIGHT SCATTERING STUDIES OF THE BINDING OF SODIUM, CALCIUM AND MAGNESIUM IONS TO DEOXYRIBONUCLEIC ACID.

SECTION I. The theory and practice of light scattering

I.1. Introduction

The method of light scattering has become a standard technique for studying the size, shape and degree of aggregation of natural and synthetic polymers. It has been used for studying solutions in which molecules interact, and for determining the molecular weight of macromolecules. References [1 to 26] deal with the problems of light scattering by particles of various sizes, shapes and molecular weights, but only a brief discussion of the relevant theories has been included. However, emphasis has been laid on the scattering of light by macromolecules, such as proteins and nucleic acids, in their aqueous solutions. The method of light scattering has been applied to the problem of binding of sodium, calcium and magnesium ions to DNA.

It has been concluded from the conductance, the equilibrium dialysis and UV absorption of nucleic acid and its cation systems (Chapters 4 and 5), that the shape and configuration of native DNA is affected by the presence of divalent cations such as calcium and magnesium. If calcium and magnesium bind intermolecularly to DNA, as suggested in these two chapters, it might be possible to establish the stoichiometry of DNA-cation complexes with a knowledge of the

molecular weight of the nucleic acid and its cation complex. The aims of the studies here also include investigating how various concentrations of sodium, calcium and magnesium ions can affect the length and radius of gyration of the DNA, and the role of the cations in the problem of polymer-solvent interaction and extent of stabilisation of the doubly stranded DNA in aqueous solution.

I.2. Debye and Zimm Equations

The scattering of light by small independent particles arises from the polarisability of the constituent electrons of such a system. As a consequence of this electronic polarisability, such a particle will behave like an oscillating dipole when exposed to a periodically varying electric and magnetic field, such as occurs in a beam of light. The basic theory of electromagnetism predicts that such an oscillating dipole must emit, or scatter radiation in all directions [1,8].

Different systems of scattering particles have been considered. For example, for a system of completely independent scattering particles, e.g. a gas at low pressure, the intensity of the scattered light from the various particles has been shown to be additive and directly proportional to the number of particles in unit volume [27]. At the other extreme, a completely ordered system, such as a perfect crystal at absolute zero temperature, will scatter no light as a result of complete destructive interference of light dispersed from different scattering centres. However, ordinary solutions can be taken as representing an intermediate case.

The theory of light scattering by independent particles was developed mainly by Lord Rayleigh [2]. It predicts that the intensity of light (i_θ) scattered in a direction forming an angle (θ) with the direction of the incident beam is given as

$$(i_\theta) = \left[\frac{2\pi^2 (dn/dc)^2}{No\ \lambda^4} \right] \frac{I_o}{r^2} \cdot \bar{M}o\ (1 + \cos^2 \theta) \qquad (6\text{-}1)$$

In expression (6-1), (I_o) is the incident intensity, (r) is the distance between the illuminated volume and the point of observation, (λ) is the wavelength of the light in vaccuo, (No) is Avogadro's number, ($\bar{M}$) is the average molecular weight of the particle, and (c) is the concentration (in g/ml) of the particle in solution, while (dn/dc) is the refractive index increment of the solution.

Equation (6-1) is generally written as

$$\frac{i_\theta\ r^2}{(1+\cos^2\theta) I_\theta} = \frac{K'}{\lambda^4} = \frac{R_\theta}{(1+\cos^2\theta)} \qquad (6\text{-}2)$$

where (R_θ) is known as Rayleigh's ratio, or the reduced intensity, which is the parameter which is determined experimentally [1].

For molecules whose size is very small compared to the wavelength of the light, (R_θ) has been found to be independent of (θ), [6-9]. However, if the dimension of the particle scatterer is greater than ($\lambda/20$), a destructive interference of the light scattered from different parts of the same molecule will result [6-9], with the decrease of (R_θ) as (θ) increases.

For the case of small particles and sometimes for large particles, (R_θ) is determined only at 90°. Usually, for large particles, (R_θ) is determined at different angles, generally angles between 30° and 135°.

In solutions there are local and continual fluctuations in concentration in any small region of the solution. These fluctuations occur as a result of the thermal, or the Brownian motion of the solute molecules, although the long-time average of the concentration of the solution remains the same. These fluctuations explain the light scattering by such a system of solution which would otherwise be considered condensed phases. These fluctuations have been considered in deriving the equations for light scattering.

I.2.a Debye equation

For small, non-interacting and optically isotropic particles in solution, the fluctuation theory [8] gives that

$$\left(\frac{Kc}{R_{90}}\right)(1+\cos^2 90) = \left(\frac{Kc}{R_{90}}\right) = \frac{1}{M} \qquad (6\text{-}3)$$

where $K = 2\pi^2 n_o^2 (dn/dc)^2/No\ \lambda^4$ for an unpolarised light, and n_o is the refractive index of the solvent.

However, where particles are not completely non-interacting, it has been shown that

$$\left(\frac{Kc}{R_{90}}\right) = \frac{1}{M} + 2Bc \qquad (6\text{-}4)$$

$$\text{and} \quad \left(\frac{Hc}{\tau}\right) = \frac{1}{M} + 2Bc \qquad (6\text{-}4a)$$

Here, $H = 16\pi/3$, and τ is the turbidity of the solution, and is equal to $16\ \pi\ R_{90}/3$.

Thus the average molecular weight ($\bar{M}$) of the particle scatterer is obtained from the intercept of the graph of (Kc/R_{90}) or (Hc/τ) versus concentration (c). The term (B) in equations (6-4, 4a) indicates the extent of the particle-particle interaction (or the degree of non-ideality of the solution).

I.2.b Zimm equation

Where the particle scatterers are bigger than ($\lambda/20$), (R_θ) has been found always to depend on (θ). This is as a result of light scattered from different parts of the same molecule, which always has the effect of reducing the intensity of light scattered with increasing angle of scattering [1,8]. A simple approach of keeping the concentration of the solution constant at an infinitely small value and examining the dependence of (R_θ) on (θ) has often been adopted. This usually leads to an equation simply expressed as

$$R_\theta = Kc\ P(\theta)\ \bar{M}$$

$$\text{or} \quad \frac{1}{\bar{M}\ P(\theta)} = \left(\frac{Kc}{R_\theta}\right)(1 + \cos^2\theta) \qquad (6\text{-}5)$$

where $P_{(\theta)}$ is the internal interference factor and contains the angular dependence of (R_θ), [1]. The term $P_{(\theta)}$ depends on the shape and dimension of the particle scatterer and has a limiting value of unity at (θ) equals zero and decreases with increasing (θ), for ($0 \leq \theta \leq \pi$).

At finite concentration of the scatterer, equation (6-5) becomes

$$\left(\frac{Kc}{R_\theta}\right)(1 + \cos^2\theta) = \frac{1}{\bar{M}\, P(\theta)} + 2\,Bc \qquad (6\text{-}6)$$

in which the term (B) is again the particle-particle interaction constant. The reciprocal of the particle scattering factor which appears in equation (6-6) has been expressed as

$$P^{-1}_{(\theta)} = 1 + \frac{16}{3}\pi^2 \left(\frac{R_g}{\lambda'}\right)^2 \sin^2(\theta/2) + \ldots\ldots \qquad (6\text{-}7)$$

and neglecting powers of $(\theta/2)$ higher than two in equation (6-7), equation (6-6) has been written as

$$\left(\frac{Kc}{R_\theta}\right)(1+\cos^2\theta) = \frac{1}{\bar{M}}\left\{1 + \frac{16\pi^2}{3}\left(\frac{R_g}{\lambda'}\right)^2 \sin^2(\theta/2)\right\} + 2\,Bc \qquad (6\text{-}8)$$

where λ' is the wavelength of the light in the solution and is equal to (λ/n_o), and (R_g) is the radius of gyration of the scatterer [12-26] .

I.2.c <u>The molecular weight average $\bar{M}$</u>

Different types of molecular weight averages are known for polydisperse molecular systems, such as the number average $(\bar{M}n)$, the weight average $(\bar{M}w)$, and the z-average, $(\bar{M}z)$ [1]. The molecular weight average $(\bar{M})$ of the particle scatterer that appears in equations (6-4) to (6-8) has been identified as the weight average molecular weight $(\bar{M}w)$, and is given by the expression

$$\bar{M}w = \Sigma_i\, n_i\, \Sigma_i^2/\Sigma_i\, n_i\, M_i = \Sigma_i c_i M_i/\Sigma_i c_i \qquad (6\text{-}9)$$

where n_i is the number of molecules of molecular weight M_i, and c_i is its concentration.

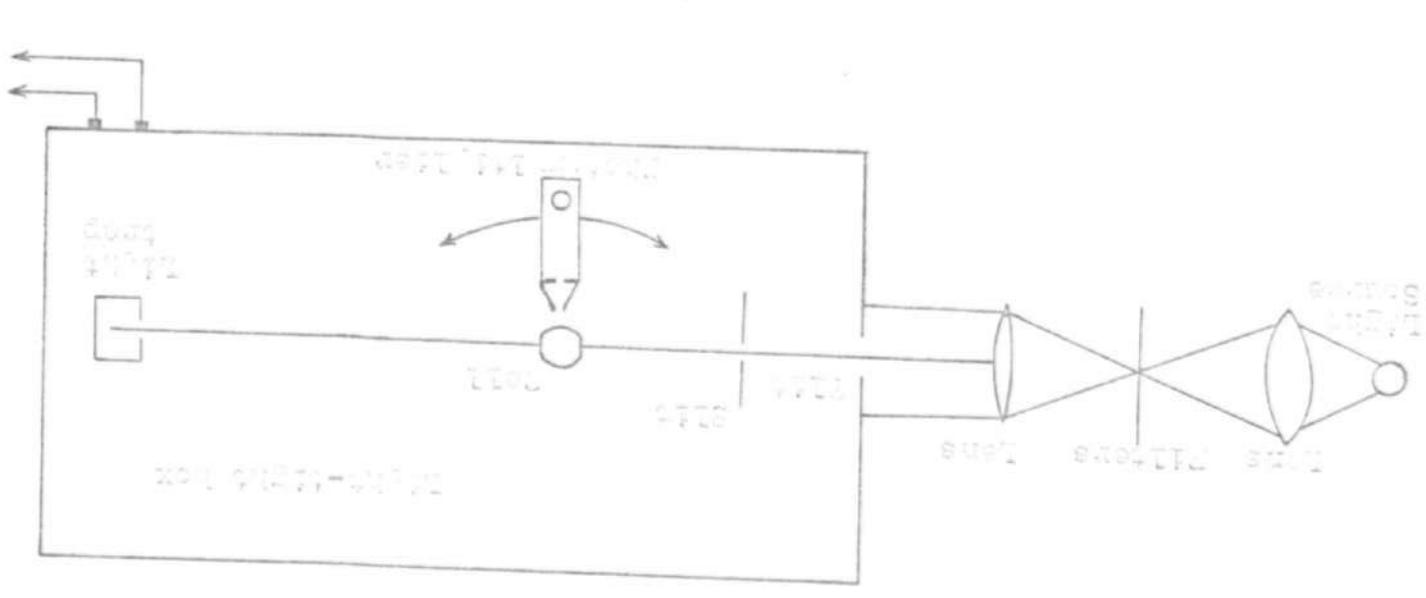
Generally, the ratio of $\overline{M}n$: $\overline{M}z$ is an indication of the polydispersity of a molecular system, and the nearer $\overline{M}w$ approaches $\overline{M}n$, the less polydisperse is the system. For a monodisperse sample, $\overline{M}n = \overline{M}w = \overline{M}x$. The light scattering method which leads to the evaluation of $\overline{M}w$ has always been found most suitable for studying systems of particles of large size and polydispersity.

I.2.d The particle scattering factor $P_{(\theta)}$

The radius of gyration (R_g) is the only shape parameter which can be obtained from light scattering studies without making assumptions as to the form of the particle, whether coil, rod or sphere. Equations (6-7 and 8) provide the basis for obtaining the radius of gyration from the angular dependence of the reduced intensity (R_θ). To obtain (Rg), a double extrapolation to zero angle and zero concentration of the plots of (Ko/R_θ) against $\sin^2(\theta/2) + kc$, where k, is a constant chosen to make the concentration term comparable with the $\sin^2(\theta/2)$ term, has been used by a number of authors, including Zimm [9].

Apart from equation (6-7), various expressions have been formulated for $P_{(\theta)}$, [12-26], particularly by Debye [12], Sidney [18], Rayleigh [25] and Gauss [26]. From these expressions it seems possible to evaluate $P_{(\theta)}$, and hence (R_g) using equation (6-8). This however, is usually difficult in practice, so the special extrapolation procedure mentioned above and discussed later is normally used [19].





I.3 The Practice of light scattering

Light scattering apparatus, in general, has been discussed by staoey [1], Debye [12] and Zimm [19]. The particular type used in the studies reported in this paper is described in the manual of the Phoenix Precision Instrument Company [20], a photograph of which is shown. They are the same in principle, but vary in design.

The light scattering instruments consist essentially of a powerful source of monochromatic polarised or unpolarised light (546mμ or 436mμ), an arrangement of lenses and slits, a cell holding the experimental solutions (this is of various designs), and a photomultiplier. They all allow for the determination of the relative intensity (R_θ) of light scattered from a solution for different angles of observation.

Measurements of scattered intensity are made at various angles of scattering, particularly angles between 30° and 135°, for the study of the angular dependence of light scattering, and at 90° for the turbidity of the solution. Details of such measurements are found in the references, particularly in [20].

However, one of the essential steps in the use of the light scattering method to study solutions of particles is the clarification of the experimental solvents and solutions. This seems to be the greatest problem facing any worker using this method of study.

It is essential that all extraneous scatterers must be removed from the solvents and solutions and from all apparatus to be used. Many articles have appeared on the solution of this problem [18, 22, 23, 28, 29, 30, 31, 32, 33, 34]. A particular approach to the problem has been used in the work reported in this thesis [34]. Photographs of some of the apparatus used have also been included here, such as the millipore filter apparatus and the P.C.L. cell cleaner.

I.3.a Treatment of data

Of the two methods of studying the parameters such as the molecular weight of a particle scatterer, namely, by measuring the turbidity of solutions of different concentrations of the scatterer in solution at 90°, and by the method of Rayleigh's ratio (R_θ), determined for different angles of scattering, the latter has been found to be more informative [19], while the former is almost restricted to small particles. However, the treatment of the results obtained from both methods have been disoussed here.

(i) Turbidity method

From equation (6-4a) and following which the turbidity (τ) of a solution has been given as equal to ($16\ \pi\ R_{90}/3$), it is obvious that the knowledge of (R_{90}) would give (τ). The term (Hc/τ) in equation (6-4a), plotted against the concentration (c) of the scatterer (in g/ml) is expected to be linear.

The intercept of this graph gives the reciprocal of the weight average molecular weight of the scatterer, and the slope gives the particle-particle interaction constant (B). This is almost the only information obtainable from the turbidity study as based on equation (6-4a).

(ii) Rayleigh's ratio method

The results obtained from the measurement of (R_θ) has been usefully represented graphically as explained by Zimm [19]. This requires the simultaneous extrapolation of (Kc/R_θ) versus $\sin^2(\theta/2)$ + kc to zero angle and zero concentration.
Smoothed lines are drawn through the experimental points at constant angle and at constant concentration, thereby forming a series of grid lines.
The Zimm plot therefore, consists of two groups of curves i.e. the curves for ($Kc/R\theta$) against kc and those for (Kc/R_θ) against $\sin^2(\theta/2)$. The two sets of curves have the same intercept of ($1/\bar{M}w$) along the (Kc/R_θ) axis.

The limiting slope of the graph of $[Kc\ (1 + \cos^2\theta)/R_\theta]$ as a function of $\sin^2(\theta/2)$ at zero angle and for zero concentration is given by limiting slope = $\frac{16\pi^2}{3\bar{M}w}\ (\frac{Rg}{\lambda})^2$
and it provides the basis of evaluation of the radius of gyration (Rg). To obtain a reliable value of (Rg), it is important to extend measurements to as low angles as possible, since curvature is sometimes present which may make the limiting slope at ($c = 0$) and ($\theta = 0$) difficult to determine.

The slope of the line of zero angle gives the value of the interaction constant (B).

If the shape of the particles is known or assumed, it is possible to compute its characteristic dimension from the radius of gyration, if the shape can be approximated to one of several models of simple geometry such as thin and rigid rod, for which $L = \sqrt{12}\ Rg$, where L is the length of the rod; or sphere, for which $r = \sqrt{5/3}\ Rg$, where (r) is the radius; or random coil, for which $\overline{r}^2 = 6\ Rg^2$, where $\overline{r}^2$ is the average squared value of the separation of the ends [18].

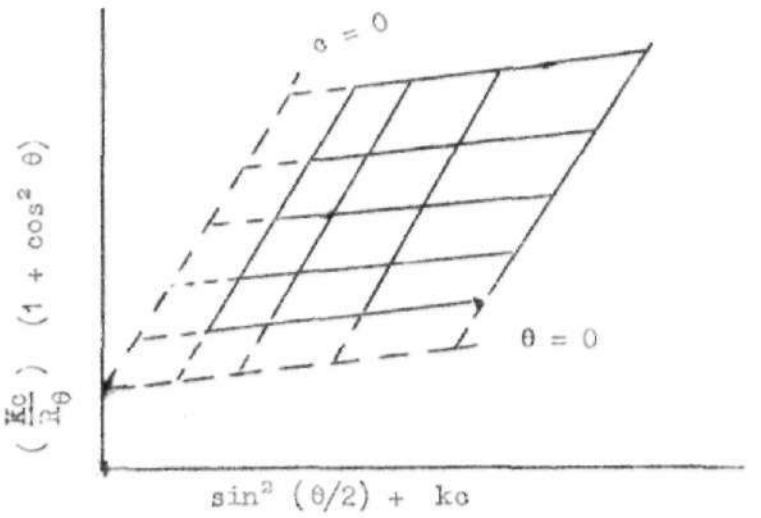


Fig. 6.4 : A typical Zimm plot.
The lines of constant angle are extrapolated to zero concentration first before extrapolating the lines of constant concentration to zero angle because of the uncertainty of the results of experiments at lower angles.

In the two methods i.e. the turbidity and the (R_θ) methods, the reciprocal of the intercept along the ordinate is equal to the molecular weight ($\bar{M}w$) if the incident light is vertically polarised or is twice the molecular weight if an unpolarised light has been used [35].

I.3.b. Yang's method [40]

For particles having very high molecular weight, such as DNA, the double extrapolation of Zimm plot has been found to be frequently difficult and uncertain. This is because the group of (Kc/R_θ) versus ko lines and (Kc/R_θ) versus $\sin^2(\theta/2)$ lines are sometimes found to be curved. This in effect might make the extrapolation to zero concentration and zero angle difficult, and the value of ($1/\bar{M}w$) obtained uncertain. It could also make the limiting slope of the (Kc/R_θ) versus $\sin^2(\theta/2)$ line for zero concentration difficult to determine accurately.

Realising these difficulties in Zimm's treatment, Yang [40] modified the equation from which the Zimm plot was derived and applied it particularly to the data obtained from the scattering by very large particles. Equation (6-8) had been rewritten as

$$\left(\frac{Ko}{R_\theta}\right)(1 + \cos^2\theta) = m \sin^2(\theta/2) + \frac{1}{\bar{M}w} + 2Bc \qquad (6\text{-}9)$$

in which $m = (16\pi^2 Rg^2/3\bar{M}w\,\lambda^2)$, and $\frac{1}{\bar{M}w} = \left(\frac{Kc}{R_\theta}\right)_{\substack{c=0\\ \theta=0}}$

from the Zimm plot.

Equation (6-9) shows that a constant angle, the graph of (Kc/R_θ) against (c) will be linear and has an intercept of $\{m \sin^2(\theta/2) + 1/\bar{M}w\}$ along the (Kc/R_θ) axis, and at zero angle of scattering the intercept becomes equal to $(1/\bar{M}w)$.

By dividing equation (6-9) through by $\sin^2(\theta/2)$, Yang obtained

$$\left\{ \frac{Kc\ (1+\cos^2\theta)}{R_\theta \sin^2(\theta/2)} \right\} = m + \frac{1}{\sin^2(\theta/2)} \left(2\,Bc + \frac{1}{\bar{M}w}\right) \qquad (6\text{-}10)$$

The plot of $\{Kc(1 + \cos^2\theta)/R_\theta \sin^2(\theta/2)\}$ versus $\{1/\sin^2(\theta/2)\}$ from equation (6-10) is linear, giving an intercept of (m) which has been identified with the limiting slope of the $\{Kc(1+\cos^2\theta)/R_\theta\}$ versus $\sin^2(\theta/2)$ plot for $(c = 0)$ in the Zimm plot, and has a slope of $(2Bc + 1/\bar{M}w)$, which becomes equal to $1/\bar{M}w$ for the $(c = 0)$ line.

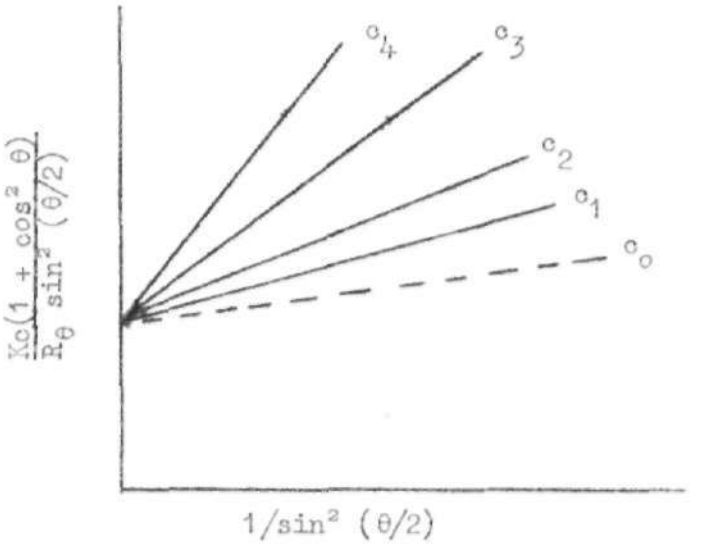


Fig. 6.5 Yang's plot

It has been shown that (m) is equal to $16\pi^2 R_G^2/3\bar{M}w\ \lambda'^2$, where ($R_G$) and ($\lambda'$) have their usual meanings. The term (m) can be determined quite accurately and (R_G) evaluated from the knowledge of $\bar{M}w$ which is first determined from the slope of the ($c = 0$) line, obtained by any of the following methods suggested by Yang.

(i) The graph of $\{Kc\ (1 + \cos^2\theta)/R_\theta \sin^2(\theta/2)\}$ versus concentration (c) is plotted for chosen values of $1/\sin^2(\theta/2)$, and the intercept along the $\{Kc\ (1 + \cos^2\theta)/R_\theta \sin^2(\theta/2)\}$ axis, (c_o) is read off.

(ii) Yang has shown that at any given value of $1/\sin^2(\theta/2)$, we have

$$[Kc\ (1 + \cos^2\theta)/R_\theta \sin^2(\theta/2)]_{c=o} = \{[Kc(1+\cos^2\theta)/R_\theta \sin^2(\theta/2)]_{c_1} c_2 - [Kc(1+\cos^2\theta)/R_\theta \sin^2(\theta/2)]_{c_2} . c_1\}/(c_2-c_1) \qquad (6\text{-}11)$$

and this can be used to obtain the values of $[Kc(1+\cos^2\theta)/R_\theta \sin^2(\theta/2)]_{c=o}$. Six values of $[Kc(1+\cos^2\theta)/R_\theta \sin^2(\theta/2)]_{c=o}$ are obtained for four concentrations and at any given value of (θ), in the following combinations: (c_1 and c_2); (c_2 and c_3); (c_3 and c_4); (c_1 and c_3); c_1 and c_4) and (c_2 and c_4). The average of the six values is taken as $[Kc(1+\cos^2\theta)/R_\theta \sin^2(\theta/2)]_{c=o}$ for the chosen value of $1/\sin^2(\theta/2)$ and is plotted on the graph of $[Kc(1+\cos^2\theta)/R_\theta \sin^2(\theta/2)]$ versus $1/\sin^2(\theta/2)$. Several values of $1/\sin^2(\theta/2)$ are chosen and the corresponding values of $[Kc(1+\cos^2\theta)/R_\theta \sin^2(\theta/2)]_{c=o}$ are calculated and plotted to define the ($c = 0$) line.

Yang applied this method to the data obtained by Benoit, Holtzer and Debye [41] on cellulose nitrate and obtained results very close to those reported for $\bar{M}w$ and $\bar{M}n$ by Benoit and co-workers. Benoit and co-workers, and Yang have shown that the asymptote at high angles ($\geq 80°$) of the Zimm plot gives 1/2 $\bar{M}n$ as the intercept on the ordinate, but it is usually very difficult to evaluate.

I.3.c A further modification of Zimm's method

When equation (6-8) is divided by the concentration term (c), it gives

$$\frac{K(1 + \cos^2\theta)}{R_\theta} = \left\{ \frac{1}{\bar{M}w} + \frac{16\pi^2}{3\ \bar{M}w\ \lambda'^2} Rg^2 \sin^2 (\theta/2) \right\} \frac{1}{c} + 2B \qquad (6\text{-}12)$$

and the plot of $\{K(1+\cos^2\theta)/R_\theta\}$ against $(\frac{1}{c})$ for different values of (θ) have a common intercept of 2B along the ordinate and a slope of $\{\frac{1}{\bar{M}w} + 16\pi^2\ Rg^2\ \sin^2\ (\theta/2)/3\bar{M}w\ \lambda^{i2}\}$, which becomes equal to $(\frac{1}{\bar{M}w})$ at (θ) equals zero.

To determine the radius of gyration of the particle and hence, its dimensions, the graph of the 90° angle of scattering can be used in conjunction with the value of $(\bar{M}w)$, where

$$(\text{slope})_{\theta=90°\text{line}} = \frac{1}{\bar{M}w} \left(1 + \frac{8\pi^2\ Rg^2}{\lambda^{i2}}\right) \qquad (6\text{-}13)$$

Again it is preferable to use the 90° angle since the effect of the extraneous scatterers in solution is a minimum at this angle.

To obtain the value of $[K(1 + \cos^2\theta)/R_\theta]$ at zero angle and for various values of $(1/c)$, the expression

$$[K(1 + \cos^2\theta)/R_\theta]_{\theta=0} = \{[K(1 + \cos^2\theta)/R_\theta]_{\theta_1} \cdot \sin^2(\frac{\theta_2}{2}) - [K(1 + \cos^2\theta)/R_\theta]_{\theta_2} \cdot \sin^2(\frac{\theta_1}{2})\}/[\sin^2(\frac{\theta_2}{2}) - \sin^2(\frac{\theta_1}{2})] \quad (6\text{-}14)$$

has been used. For each value of $(1/c)$ the corresponding values of $[K(1 + \cos^2\theta)/R_\theta]_{\theta=0}$ are calculated for all the angles investigated and then averaged, and then plotted on the graph of $[K(1+\cos^2\theta)/R_\theta]$ versus $(\frac{1}{c})$. A straight line is drawn through the points defining the $(\theta = 0)$ line.



Fig. 6.2

Glass filter apparatus

using millipore filters

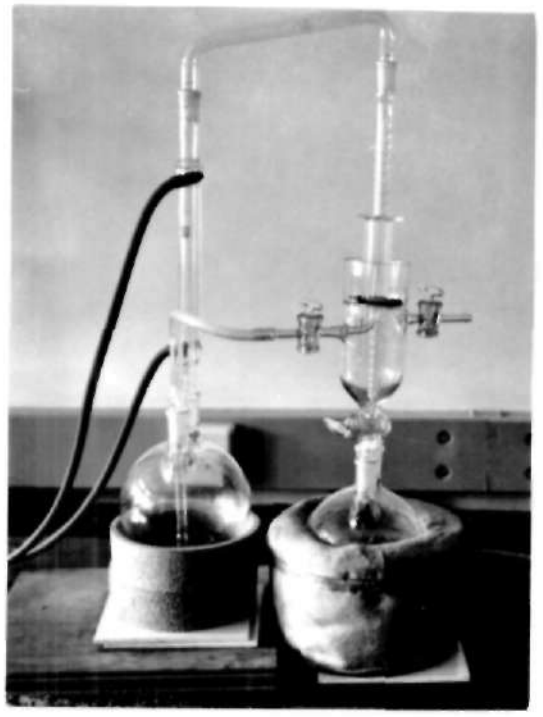


Fig. 6.3

The P.C.L. Cell Cleaner

SECTION II. EXPERIMENTAL

II.1 Cleaning of glassware and clarification of experimental solvents and solutions

All glassware used for handling (except the glass cells) was cleaned with chromic acid and rinsed thoroughly with doubly distilled water, and finally kept in a dessicator which was similarly treated. The glass cells were washed with detergent solutions, rinsed with distilled water which was filtered through 0.22μ millipore filter paper and flushed with a jet of acetone (analytical grade) distilled from an all-glass distillation apparatus with no air space, (figs. 6.2 and 6.3).

All aqueous solutions, except the nucleate solutions, were clarified by filtration under pressure through 0.22μ millipore filters.

The stock nucleate solution was prepared by dissolving about 50 mls of clarified trisbuffer of pH 7.05 and making it up to 150 mls with buffered 0.004M sodium chloride solution which had been clarified by filtration through 0.22μ millipore filters. Three methods were tried for the clarification of the nucleate solutions:

(i) Centrifugation at 21,000 r.p.m. at about 10°C for sixteen hours as recommended by Reichman and Doty [28]. This was found unsatisfactory because the light scattering measurements were not reproducible.

(ii) The method of filtering the stock nucleate through 0.22μ millipore filters which had been washed free of particles [29] was also tried. It was found that the filtration was very slow and there was a big loss of the nucleate on the filter.

(iii) Centrifugation of the stock nucleate at 20,000 r.p.m. at room temperature for sixteen hours, as recommended by Peacocke and Walker [34] was also attempted. This was found satisfactory in that the measurement of light scattering were reproducible and no more nucleate was precipitated after the sixteen hours of centrifugation. A small pellet of nucleate and dust was always left at the bottom of the centrifuge tube. The clarification was found to be improved when nucleate solution contained some salt. This method was therefore used throughout the investigation. Only three quarters of the nucleate solution in each centrifuge tube was removed (with a clean pipette) and used as the final stock solution.

Dilutions of the stock nucleate were made to contain the required strength of sodium chloride (0.2M), or calcium chloride (2.5×10^{-3}M), or magnesium chloride (2.5×10^{-3}M). Each portion of the stock solution diluted was initially of about 0.003M in sodium chloride (also for the divalent salt system). In each case, clarified buffered solution of the salt of the required strength was used for the dilution. The pH was 7.05.

In all cases, the concentrations of the nucleate solutions were determined spectrophotometrically with the aid of calibration curve as explained in Chapter 5 of this thesis. Nucleate concentrations of between 2.0×10^{-5} and 12×10^{-5} g/ml of the 'dry' weight were used throughout the experiments.

The nucleate solutions employed in the turbidity measurements for different cation/nucleate phosphorus ratios were made by dissolving weighed amounts of the nucleate in clarified buffer of pH 7.05 and of the required strength of salt (NaCl, $CaCl_2$ and $Mg\ Cl_3$), without centrifuging. Clear solutions were obtained which gave reliable and reproducible results at 90° angle of scattering. (Here it is recalled that the effect of dust on the light scattering is least at 90° angle of scattering).

II.2. The light scattering measurements

Unpolarised monochromatic light of wavelength 546mμ (in vacuo) was used for all measurements, and all experiments were conducted at room temperature of 26 ± 2°C.

II.2.a. The turbidity measurements

(i) The determination of the weight average molecular weight ($\overline{Mw}$) of the nucleic acid in high concentrations of sodium chloride, calcium chloride and magnesium chloride.

What can be regarded as the weight average molecular weight of the nucleic acid sample in its native form was determined in 0.2M

sodium chloride solution, table 6.2 and figure 52. Its value was also determined in the presence of calcium chloride and magnesium chloride of 2.5×10^{-3}M, tables 6.2 and 6.3; and figs. 56 and 61. A square glass cell was used.

The turbidity (τ) of each of the solutions of different nucleate concentrations was determined by measuring the light scattering at 90° and comparing it with that transmitted at 0° angle. The graph of (Hc/τ) against the nucleate concentration (in g/ml) was plotted for each nucleate-cation system. (As before, H is the optical constant obtained from knowledge of the refractive index and its increment for the solvent). The measurement of the refractive index increment is described in section II.2.o of this chapter.

(ii) The investigation of the dependence of the molecular weight average ($\overline{M}w$) on the cation/nucleate-phosphorus ratio (r), for sodium chloride, calcium chloride and magnesium chloride. A square glass cell was used.

Here, measurement of the turbidity (τ) of nucleate-cation solutions were made for nucleate solutions of different cation/nucleate-phosphorus ratios for each of sodium, calcium and magnesium cations. The graph of (Hc/τ) against (c) was plotted for each system of the same cation/phosphorus ratio to obtain the corresponding molecular weight average of the nucleic acid in the presence of the cations; tables 6.4 and 6.5; and figs. 57 and 62.

II.2.b. The angular dependence of light scattering

Measurement of light scattering at angles between 30° and 135° to the incident beam were made to determine the relative scattering ratio R_θ, for each angle of scattering as described in details in reference [20]. Systems of DNA-sodium, DNA-calcium and DNA-magnesium were studied: figs. 53, 54, 55, 58, 59, 60, 63, 64 and 65. In each case, the scattering at each angle was compared to the intensity transmitted at zero angle. A cylindrical glass cell was used for these measurements.

II.2.c. Measurement of the refractive index increment dn/dc.

The value of the term (dn/dc) that appears in equation (6-1) was determined with a P.C.L. differential refractometer using different concentrations of nucleate of 2 to 22 $\times 10^{-5}$ g/ml. At 546mμ, (dn/dc) was found to be 0.168 ± 0.002 ml/g, and at 436mμ, it was found to be 0.190 ± 0.002ml/g. The calibration constant (K) for the differential refractometer for each of the spectral lines was determined using a standard solution of sucrose (analytical grade). The refractive index of the solvent was determined with an Abbe refractometer.

The value of (dn/dc) for 546mμ reported here compares very well with those recorded by some previous workers. At 546mμ, Cohen and co-workers [42] reported a value of 0.168 ml/g whereas values of 0.160 ml/g and 0.166 ml/g were reported by Tennent

and co-workers [43] and Krasna [44] respectively. For the 436 mμ wavelength however, the value of 0.190 ± 0.002 ml/g reported here for (dn/dc) agrees very well with the values of 0.201 ml/g; 0.188 ml/g and 0.190 ml/g reported by Northrop et al [45], Reichmann et al [46] and Alexander and co-workers [47] respectively.

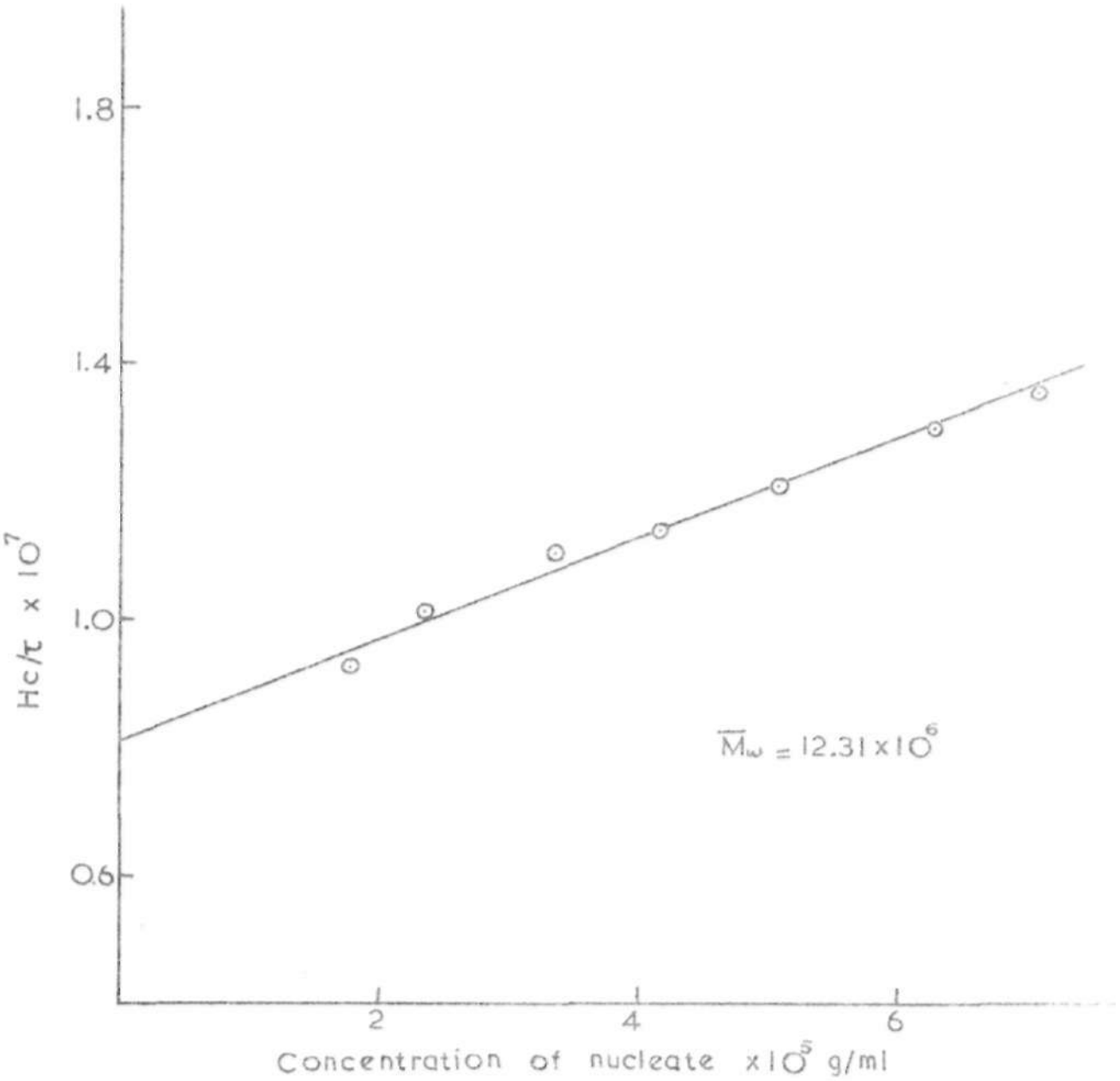


Fig. 52: Determination of the weight average molecular weight of DNA in 0.2M.NaCl, at pH 7.05

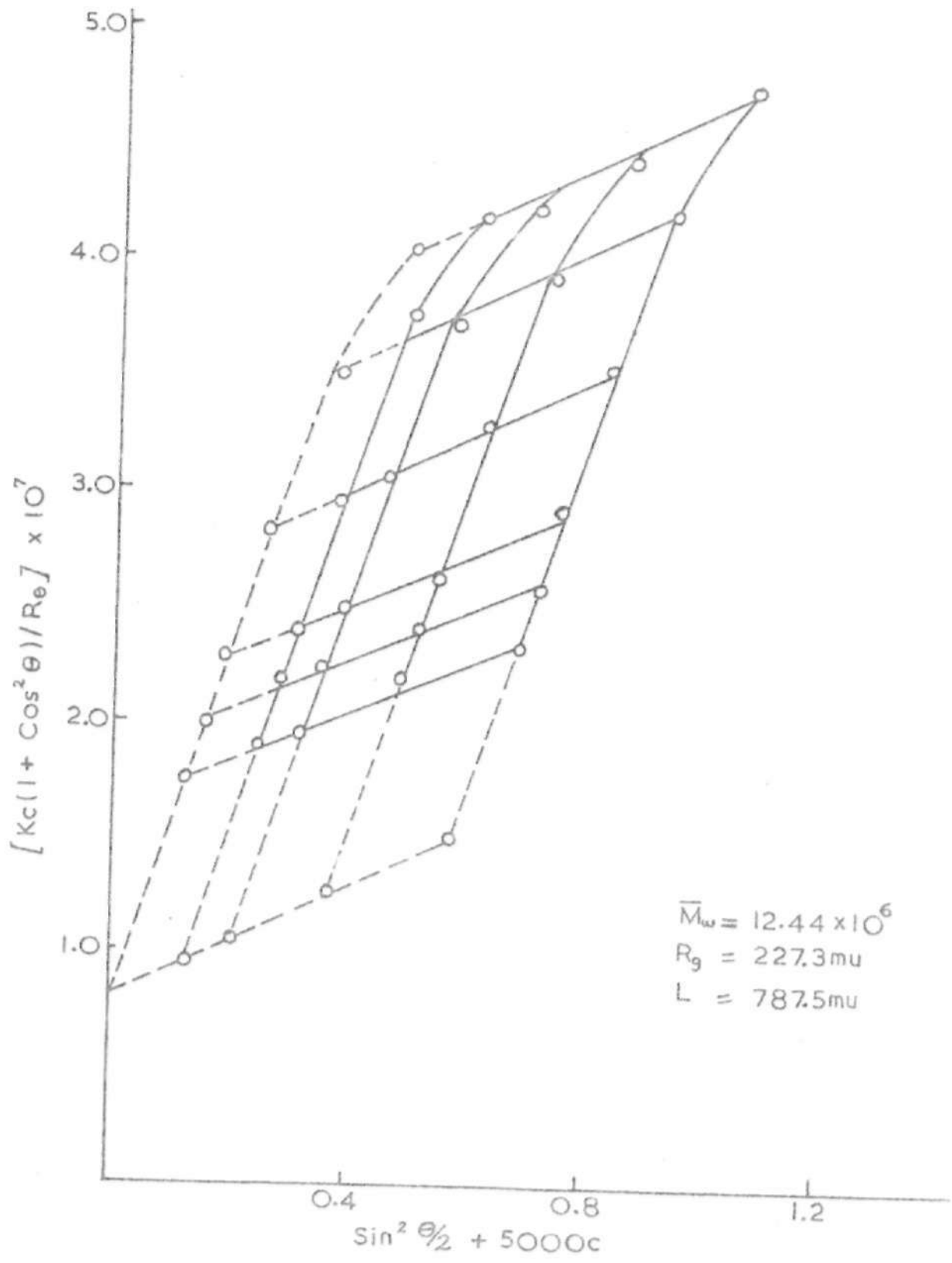


Fig. 53: Zimm plot for DNA in 0.2M-NaCl, at pH 7.05

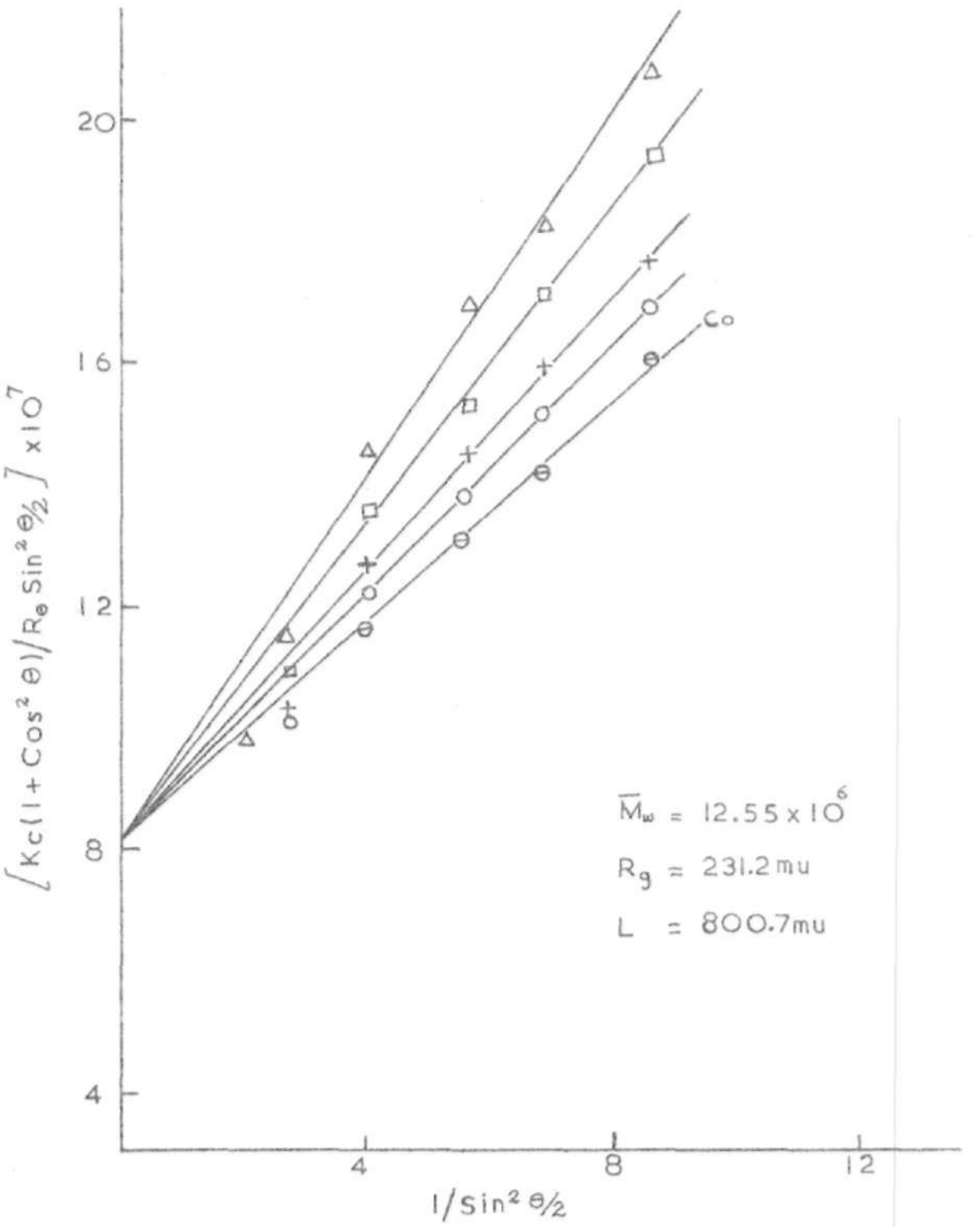


Fig. 54: DNA in 0.2M-NaCl, at pH 7.05

Conc$^{n.}$ of DNA of (Δ; 11.41 : □; 7.31 : +; 4.00 :

○; 2.50) $\times 10^{-5}$ g/ml

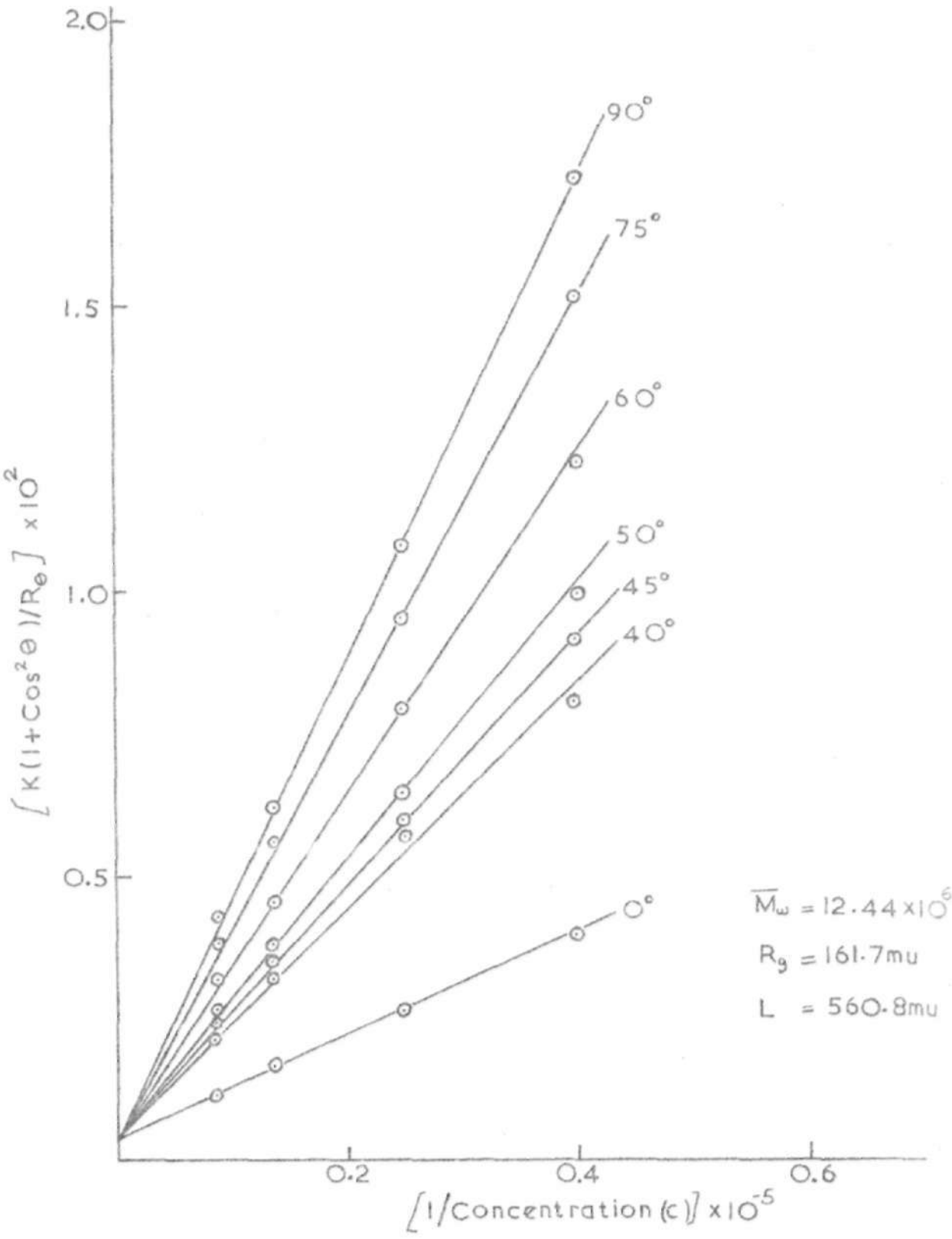


Fig. 55 : DNA in 0.2M-NaCl, at pH 7.05

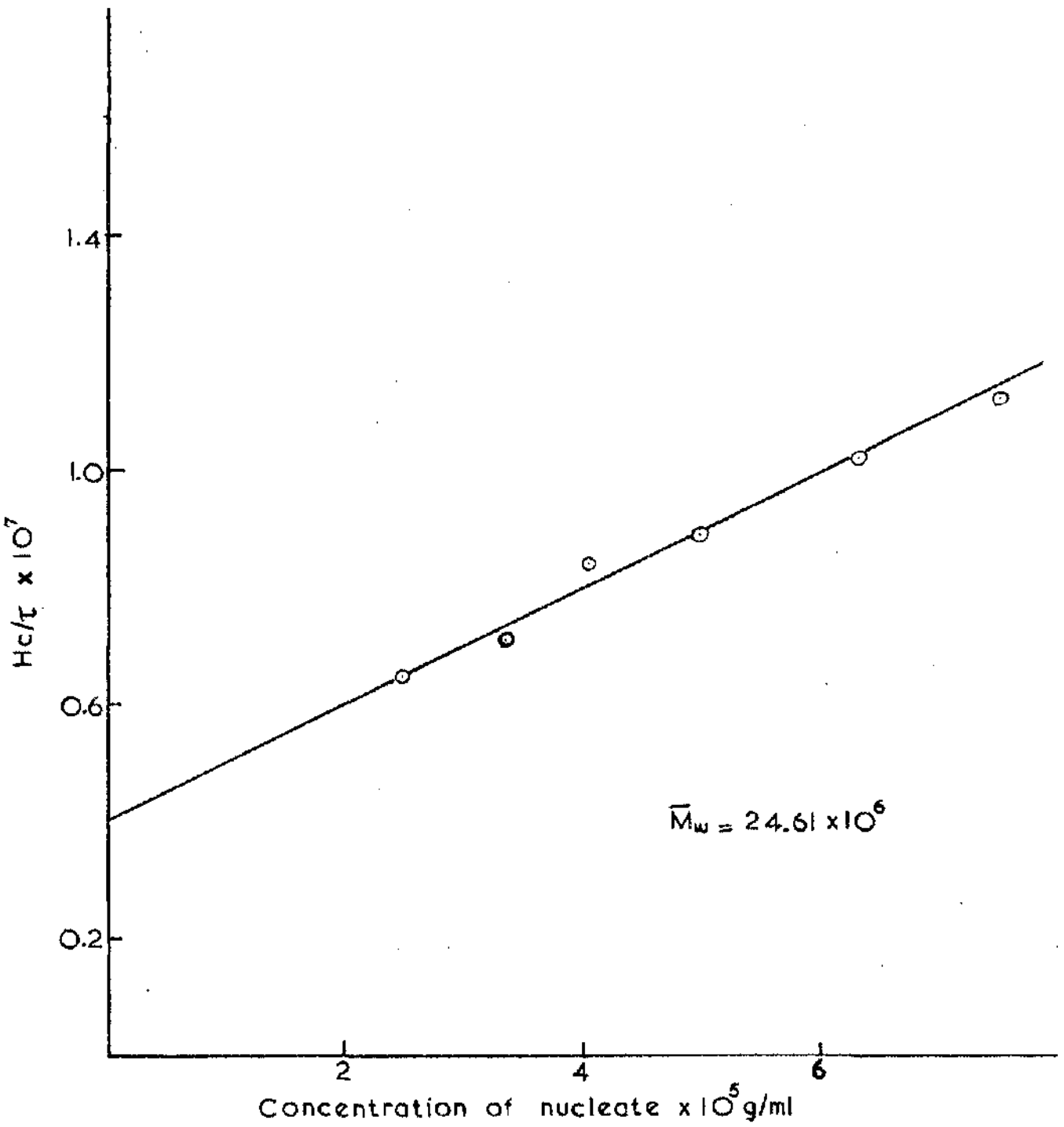


Fig. 56: Determination of the weight average molecular weight of a DNA sample in 2.5×10^{-3}M $CaCl_2$, at pH 7.05

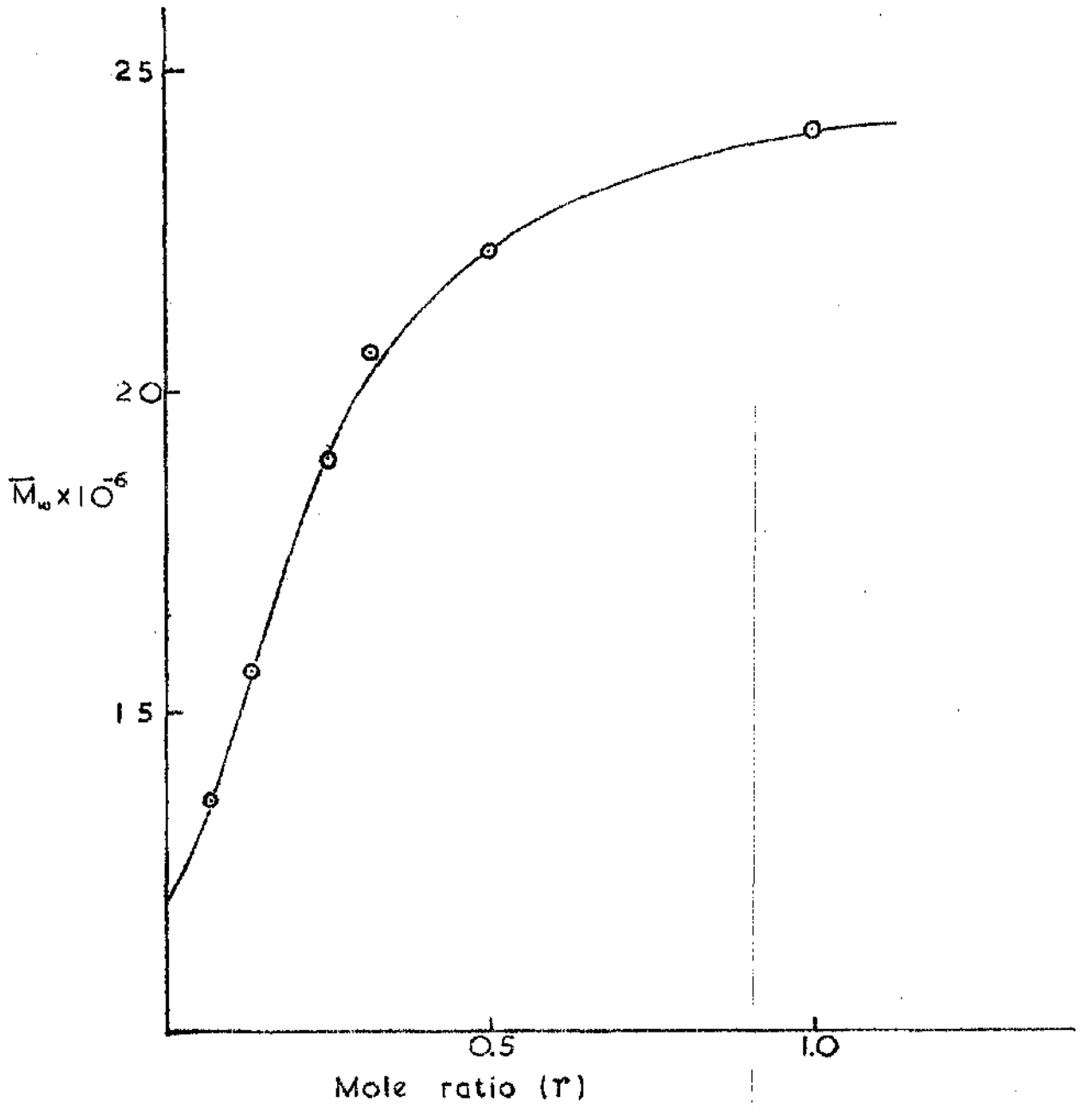


Fig. 57: The dependence of ($\overline{M}_w$) of DNA on the calcium/nucleate-phosphorus ratio (r)

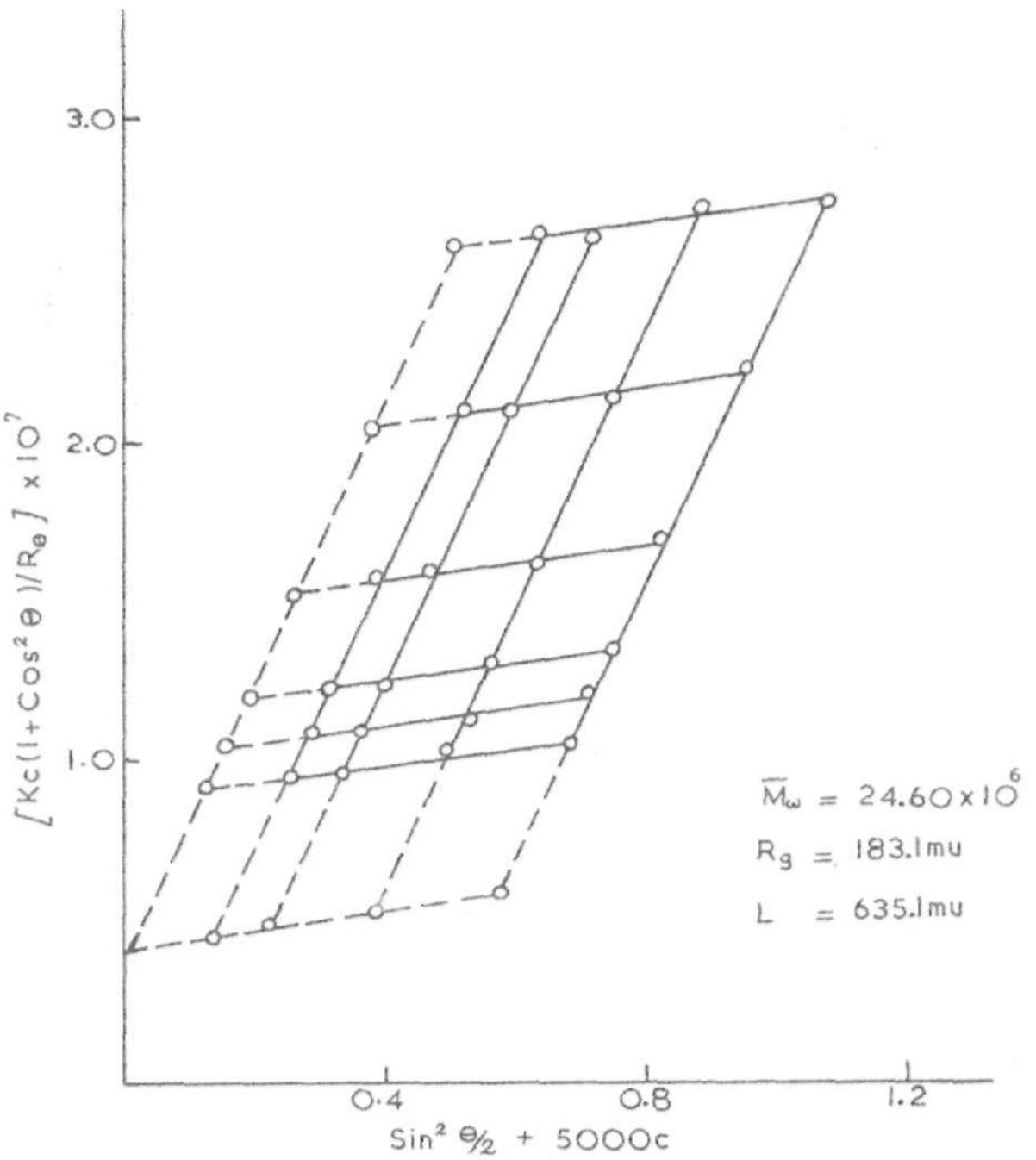


Fig. 58: Zimm plot for DNA in 2.5×10^{-3} M.$CaCl_2$, at pH 7.05

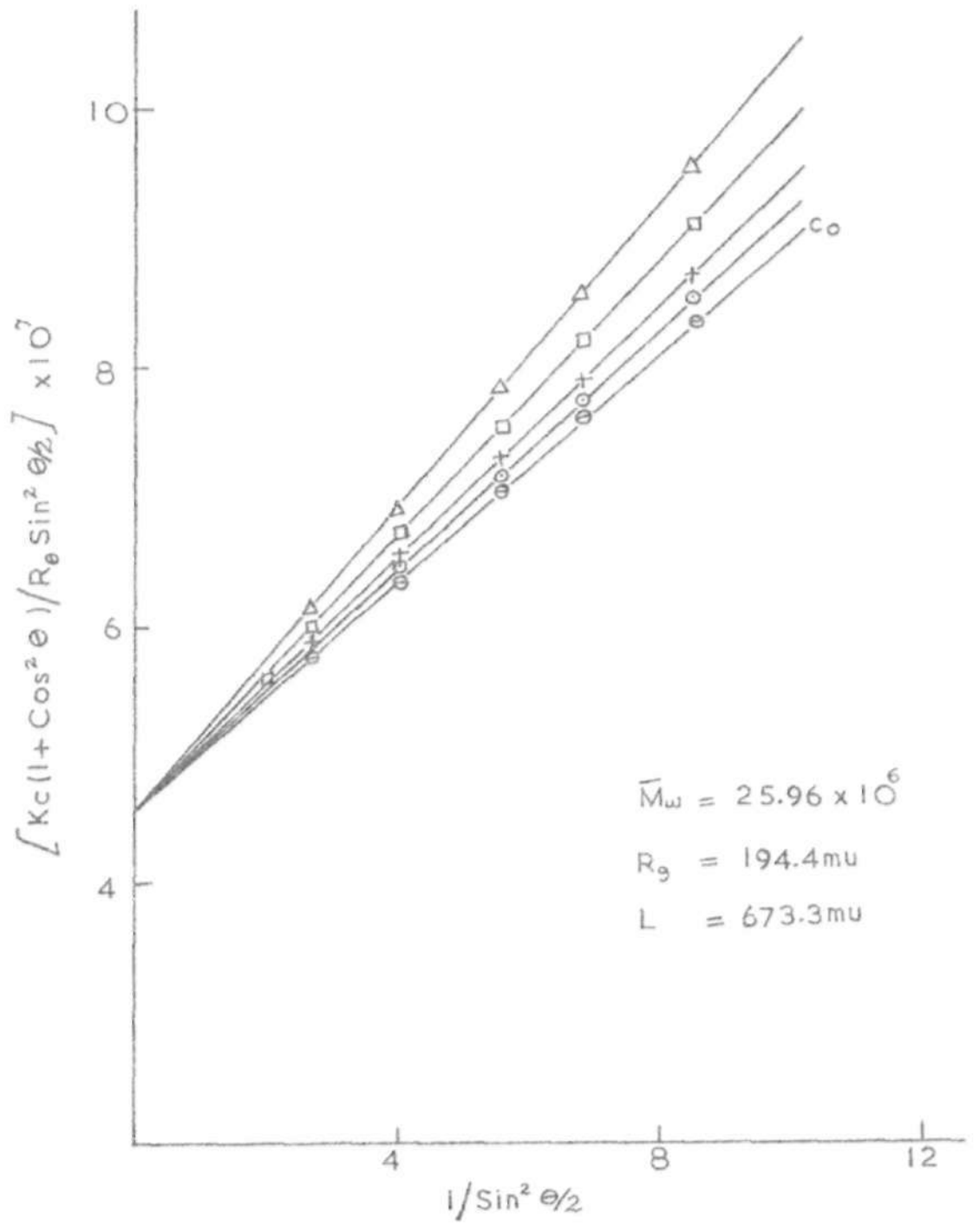


Fig. 59: DNA in 2.5×10^{-3}M.$CaCl_2$, at pH 7.05

Conc$^{n.}$ of DNA of: (Δ; 11.32 : $\square$; 7.50 : +; 4.21 : $\odot$; 2.63) $\times 10^{-5}$ g/ml

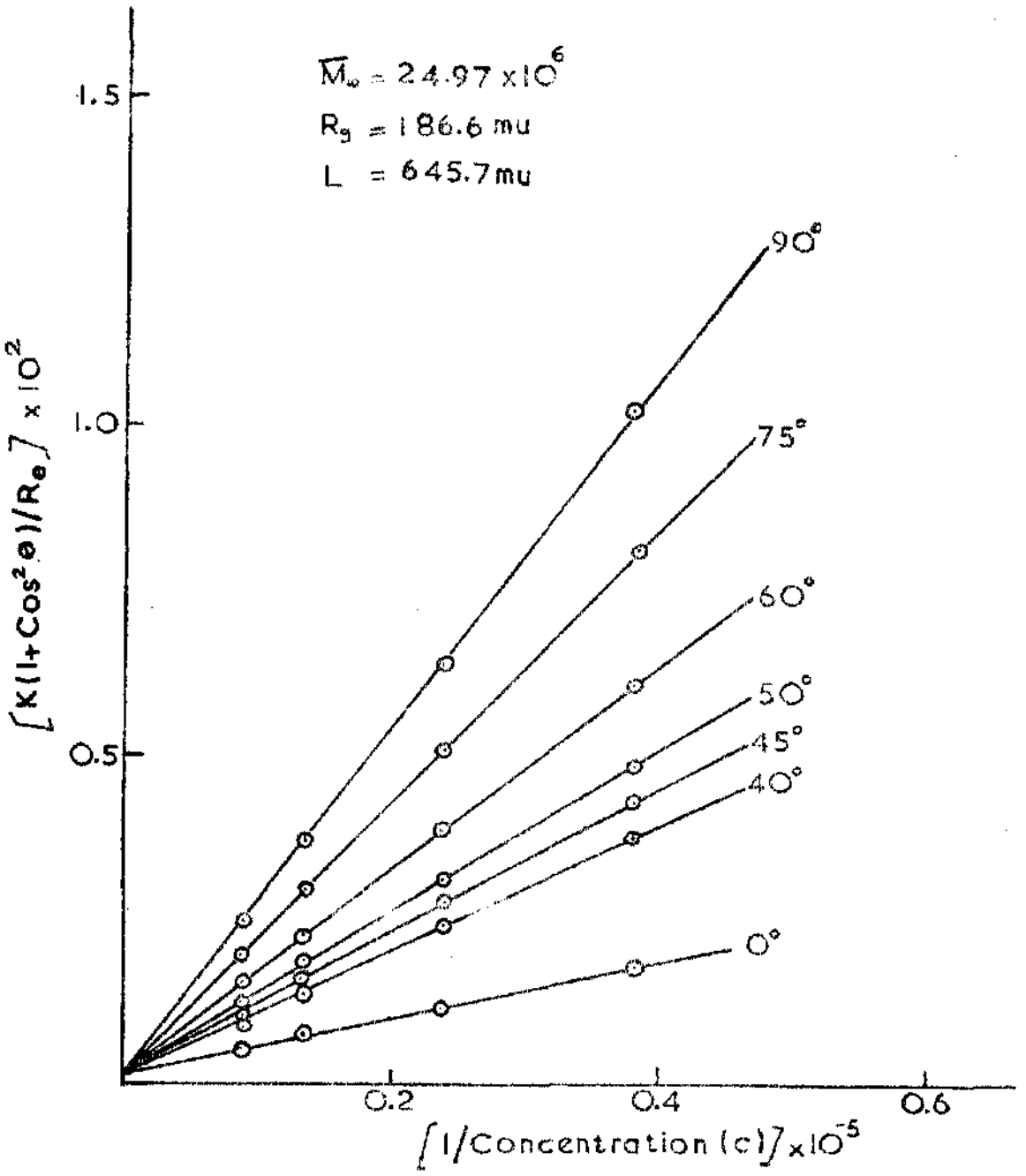


Fig. 60 : DNA in 2.5×10^{-3} M-$CaCl_2$, at pH 7.05

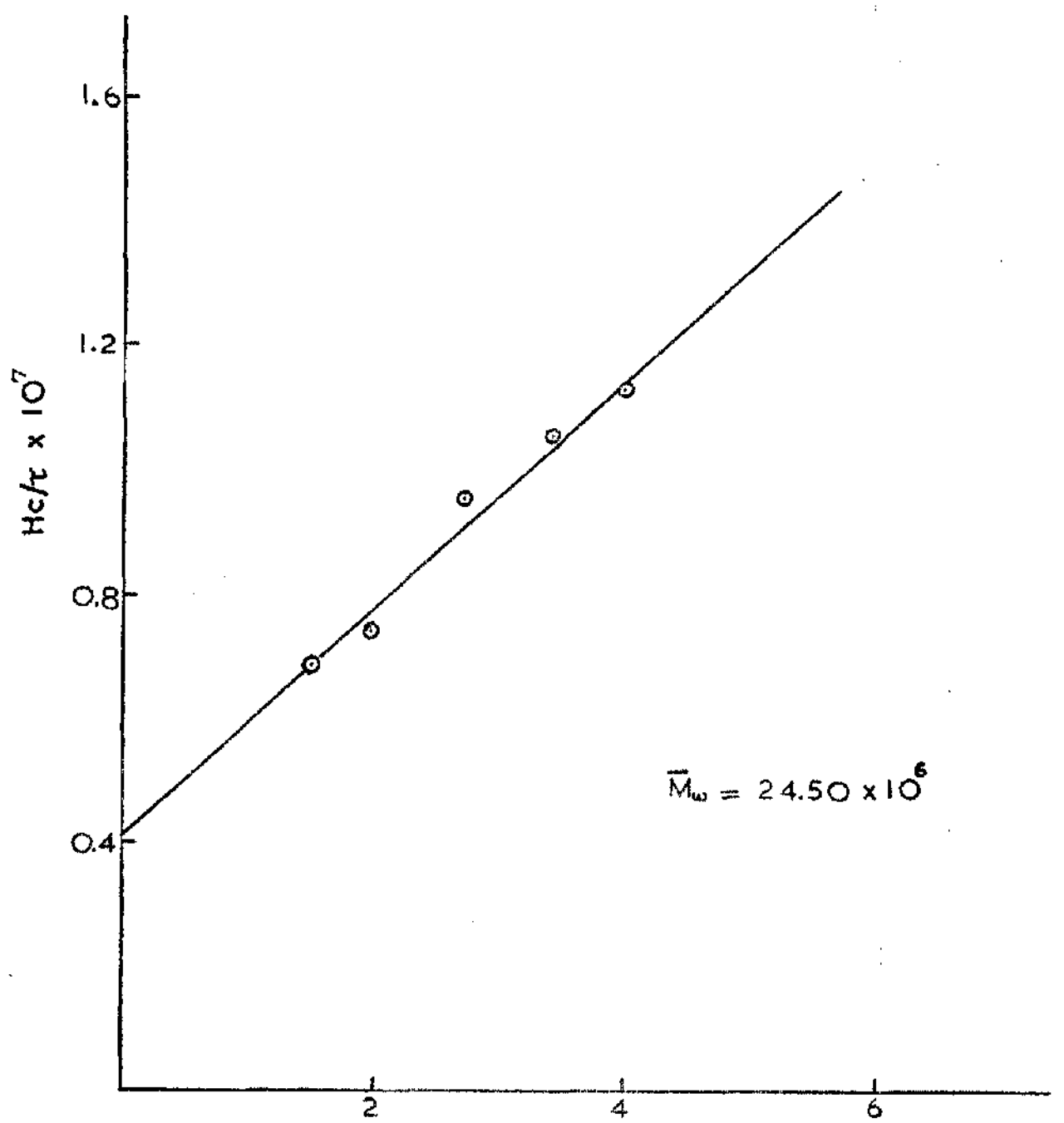


Fig. 61: Determination of the weight average molecular weight of a DNA sample in 2.5×10^{-3} M $MgCl_2$, at pH 7.05

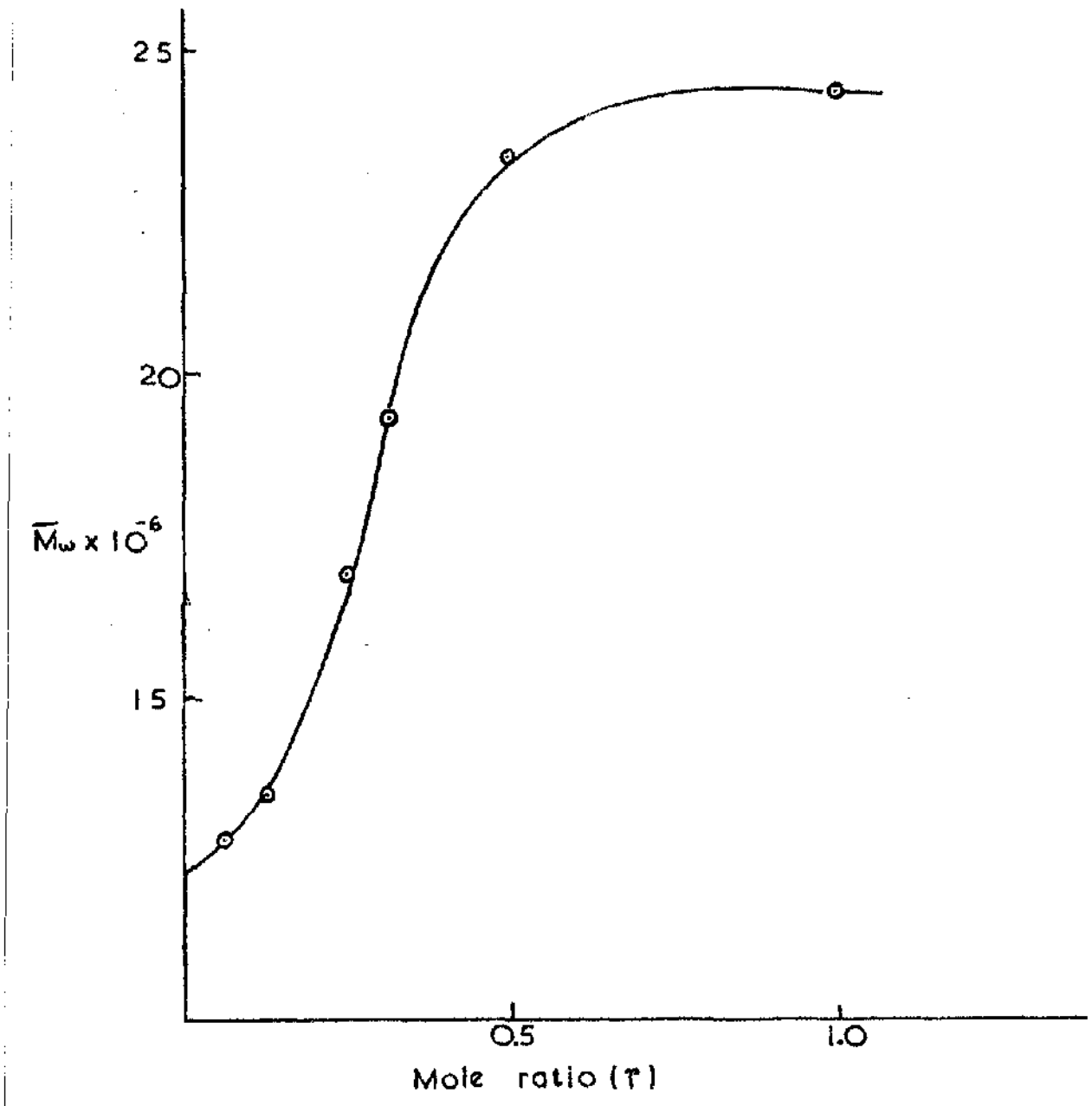


Fig. 62: The dependence of ($\bar{M}_w$) of DNA on the magnesium/nucleate-phosphorus ratio (r)

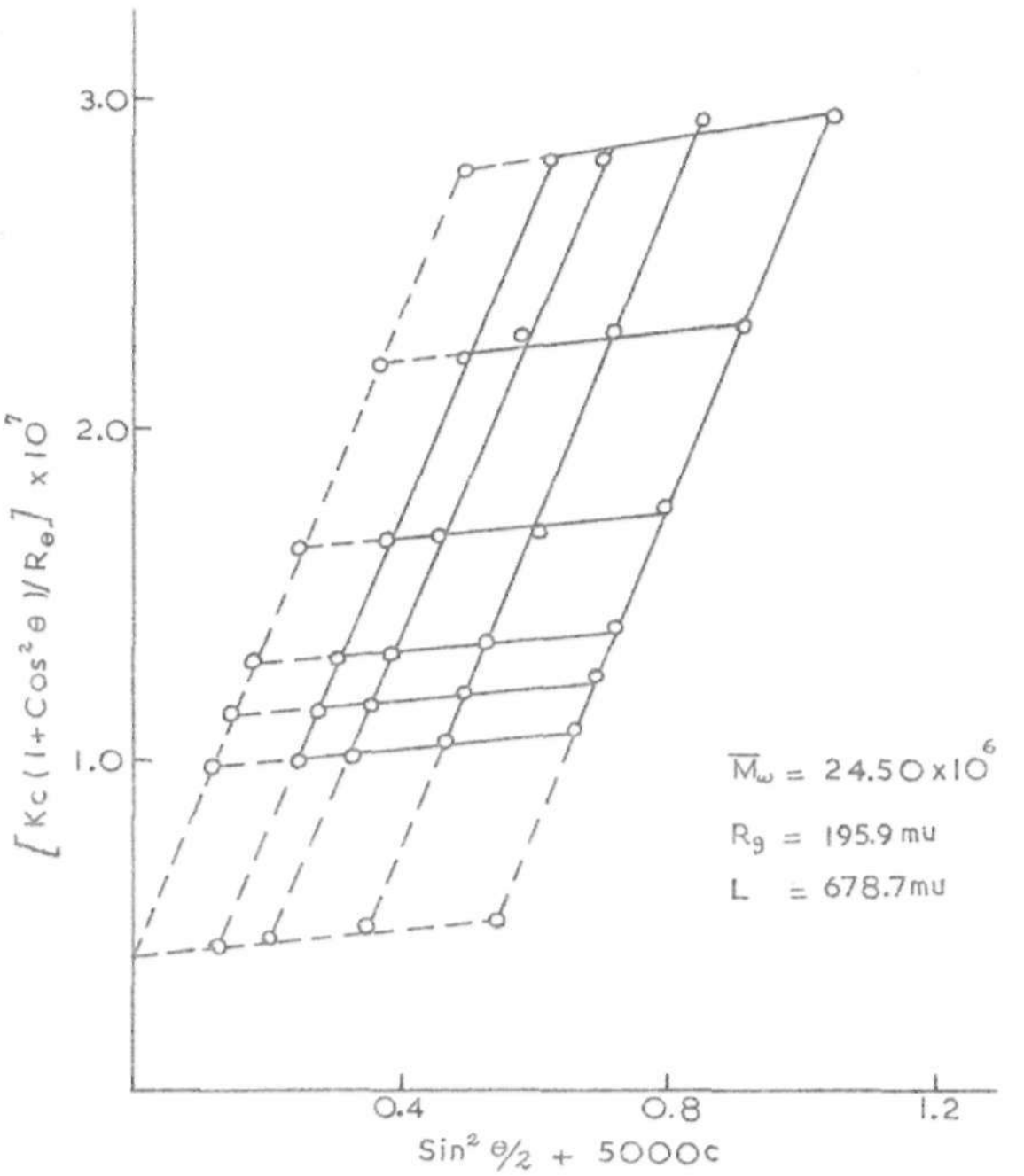


Fig. 63: Zimm plot for DNA in 2.5×10^{-3} M-$MgCl_2$, at pH 7.05

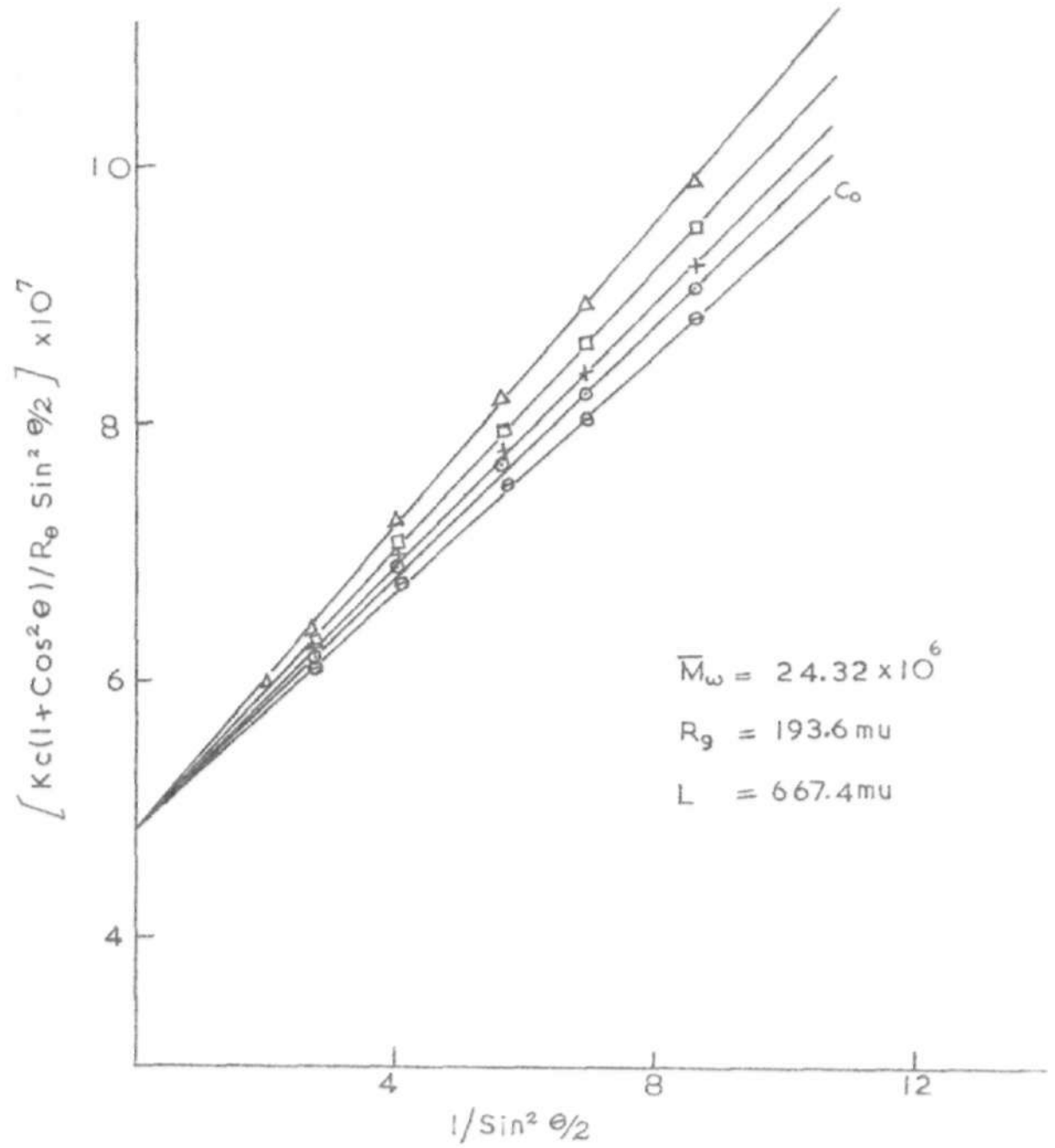


Fig. 64: DNA in 2.5×10^{-3} M-$MgCl_2$, at pH 7.05

Conc^n of DNA of (Δ; 10.99: □; 5.00: +; 4.17: ⊙; 2.58) $\times 10^{-5}$ g/ml

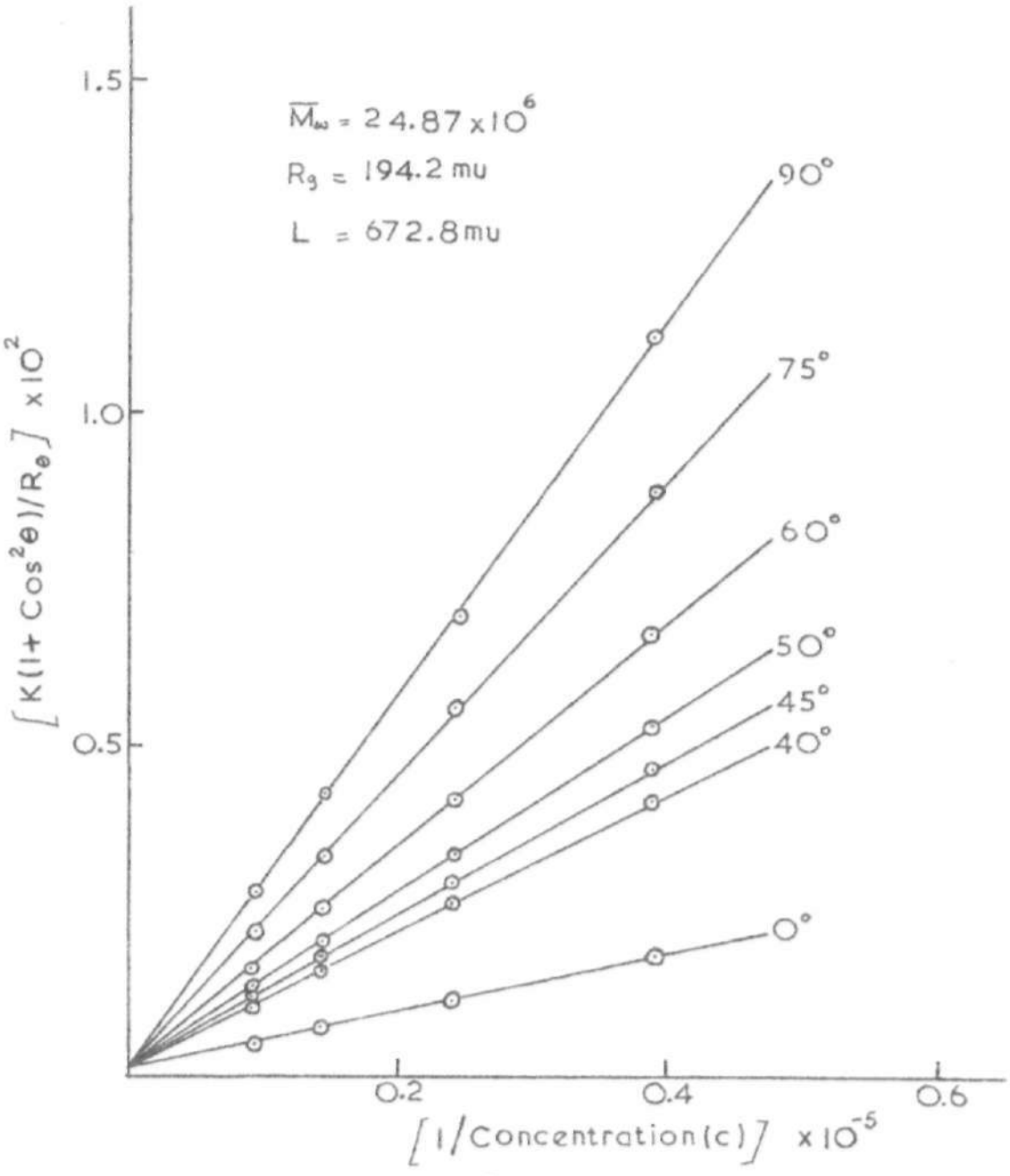


Fig. 65: DNA in 2.5×10^{-3} M-$MgCl_2$, pH 7.05

II.3 RESULTS AND DISCUSSION

II.3.a. RESULTS

Table 6.1 to 6.3: Data for determination of molecular weight of DNA in sodium chloride, calcium chloride and magnesium chloride (Turbidity method)

Table 6.1. DNA in 0.2M sodium chloride

Nucleate Concentration $\times 10^5$ g/ml	Turbidity (τ) $\times 10^4$ cm^{-1}	Hc/τ $\times 10^7$ g.cm
1.780	7.10	0.92
2.340	8.00	1.02
3.347	10.50	1.11
4.199	12.81	1.14
5.072	15.00	1.21
6.279	17.00	1.30
7.071	18.30	1.35

Table 6.2. DNA in 2.5×10^{-3}M calcium chloride

Nucleate Concentration $\times 10^5$ g/ml	Turbidity (τ) $\times 10^4$ cm^{-1}	Hc/τ $\times 10^7$ g.cm
2.498	13.45	0.65
3.372	16.70	0.71
4.039	15.63	0.85
5.002	19.64	0.89
6.337	21.93	1.02
7.521	24.10	1.12

Table 6.3 DNA in 2.5×10^{-3}M magnesium chloride

Nucleate concentration $\times 10^5$ g/ml	Turbidity $(\tau) \times 10^4$ cm^{-1}	Hc/τ $\times 10^7$ g.cm
1.510	8.95	0.69
1.970	9.34	0.73
2.666	9.63	0.96
3.362	11.05	1.06
4.005	13.16	1.13
5.160	16.98	1.06

Table 6.4: Dependence of weight average molecular weight ($\overline{M}w$) of DNA on the divalent-cation/nucleate-phosphorus ratio (r) for calcium and magnesium (Turbidity method)

Mole ratio (r) $M^{2+}/2P$	$\overline{M}w \times 10^{-6}$	
	DNA-Ca^{2+}	DNA-Mg^{2+}
0.063	13.60	12.86
0.125	15.66	13.49
0.250	18.92	16.96
0.313	20.65	19.31
0.500	22.65	23.42
1.000	24.08	24.34

DNA-cation System	Fig. No.	Conc^{n}. of Cation(M)	$\overline{M}_W$ $\times 10^{-6}$	Rg (mμ)	L mμ
DNA-Na^{+}	52	0.2	12.31	-	-
	53	0.2	12.44	227.3	787.5
	54	0.2	12.55	231.2	800.7
	55	0.2	12.42	161.7	560.0
Mean values			12.44±0.05	229.3±2.0	794.1±6.6
		$\times 10^{-3}$M			
DNA-Ca^{2+}	56	2.5	24.61		
	58	2.5	24.60	183.1	635.1
	59	2.5	25.96	194.4	673.3
	60	2.5	24.97	186.6	645.7
Mean values			25.03±0.10	188.0±3.3	651.3±5.5
		$\times 10^{-3}$M			
DNA-Mg^{2+}	61	2.5	24.50		
	63	2.5	24.88	195.9	678.7
	64	2.5	24.32	193.6	667.4
	65	2.5	24.87	194.2	672.8
Mean value			24.63±0.14	194.6±0,7	672.9±3.3

Table 6.5 Summary of results obtained from the study of the angular dependence of light scattering in determining the molecular parameters ($\overline{M}_W$), Rg, and L of the molecule (assuming a rigid rod).

II.3.b Discussions

The molecular weight average for the nucleic acid sample used was found by the method of light scattering to be $(12.44 \pm 0.05) \times 10^6$ in 0.2M sodium chloride. The turbidity measurement and the Rayleigh's ratio methods gave comparable results in the same concentration of the cations, figs. 52 and 53.

As observed in the turbidity studies, the weight average molecular weight ($\overline{M}w$) of the nucleic acid was independent of the concentrations of the sodium ions in the aqueous solution of the nucleic acid. In short, the results have indicated that in sodium chloride of any given strength, provided there was no denaturation of the nucleic acid secondary structure (i.e. NaCl $> 10^{-4}$M), the weight average molecular weight of nucleic acid was the same.

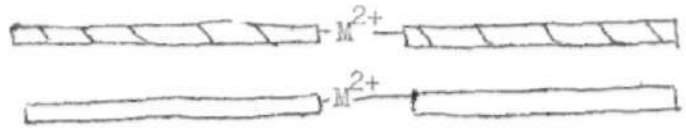
In the presence of calcium and magnesium ions the weight average molecular weight of the nucleic acid was found to depend on the concentrations of the divalent cations in the aqueous solutions as shown in figures 57 and 62 for DNA-calcium and DNA-magnesium systems respectively. The molecular weight average ($\overline{M}w$) increased from about the value observed for DNA-sodium to about twice this value as the concentration of either calcium or magnesium was increased.

Figures 57 and 62 show the dependence of the weight average molecular weight ($\overline{M}w$) of the nucleic acid sample on the molar ratio (r) of divalent cation to two nucleate phosphorus. In the two cases, ($\overline{M}w$) was found to increase to a maximum at about (r) equals unity. The molecular weight average corresponding to (r) equals zero is about 12.30×10^6 for the two divalent cation systems. At the upper limit of the investigation it approached about twice the value for (r = 0).

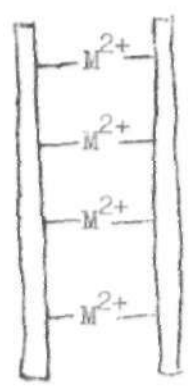
The results observed here for the DNA-M^{2+} (M^{2+} is Ca^{2+} or Mg^{2+}) can be explained in terms of dimerisation of the nucleic acid molecules in the presence of the divalent cations. Dimerisation here means the pairing up through divalent cations of two native DNA molecules.

These results have been used to predict the mode of binding of calcium and magnesium ions to the nucleic acid molecules, though it has not been possible to employ them in concluding anything on the mechanism of dimerisation. However, as seen in figs. 57 and 62, the upper limit of the molecular weight average ($\overline{M}w$) of the nucleic acid seemed to have been attained at about one mole of divalent cation to two moles of nucleate-phosphorus, a result which suggests that there was a quantitative binding of the divalent cations to the nucleate phosphorus.

Suggested mode of dimerisation



(a) Dimerisation of nucleic acid by the divalent cations binding to the terminal phosphate of the nucleic acid molecules.



(b) Dimerisation of nucleic acid by the divalent cations binding along the length of the molecules

Two modes of binding of the divalent cations to the nucleic acid molecules have been suggested above. The first suggestion (a) is improbable, though apparently it could lead to the dimerisation of the nucleic acid molecules. This is because the molar ratio (r) that would cause the doubling of the molecular weight average ($\overline{M}w$) would be much less than unity. This however would be in conflict with the result observed, where ($r \geq 1.0$) for complete dimerisation.

The alternative suggestion (b), seems to be the only possibility for the doubling of the molecular weight of the nucleic acid at ($r \geq 1.0$). Here the divalent cations are found bound to the nucleate in such a way as to allow for the pairing up of the nucleic acid molecules with as many divalent cations as half the number of nucleate phosphorus found between any pair of molecules.

This result is in agreement with those of the conductance studies, the UV studies and the dialysis equilibrium studies already reported in the preceding chapters, all of which suggest that two molecules of nucleic acid pair up by the intermolecular linkage via the divalent cations.

A general comment on figures (52, 53, 56, 58, 61 and 63)

The plots of (Hc/τ) versus the concentration (c) from equation (6-4a) for all the DNA-cation systems have been found to be linear, as expected, figs. 52, 53, 56. The Zimm plot for the DNA-sodium chloride system has been found to be slightly curved at high angles of scattering, as shown for 90° angle in fig. 53. For the DNA-divalent cation systems no such curvature has been observed in the Zimm's plot figs. 61 and 63.

Curvature is generally observed for very long molecules as reported by Yang [40], and this might be the explanation for the curvature here, since the molecule is longer in sodium chloride than in calcium or magnesium chloride.

The number average molecular weight of the molecule and polydispersity.

As explained by Yang [40], the number average molecular weight and the weight average molecular weight of a system of large particles (molecules) can be estimated from the plots of $Kc\ (1 + \cos^2\theta)/R_\theta \sin^2(\theta/2)$ versus $[\ 1/\sin^2\ (\theta/2)]$. The intercept along the ordinate for low angles of scattering ($\leq 75^\circ$) gives $(1/\overline{M}w)$ while the asymptote at high angles (here $> 75^\circ$) of the Zimm plot gives $(1/2\overline{M}n)$ as the intercept on the ordinate.

This therefore means that the degree of polydispersity of a system can be estimated in terms of the ratio $(\overline{M}n/\overline{M}w)$, and it implies that as $(\overline{M}n/\overline{M}w)$ tends to unity, the system becomes less polydispersed or more monodispersed.

The estimation of $\overline{M}n$ from the graph of $Kc(1+\cos^2\theta)/R_\theta \sin^2(\theta/2)$ versus $1/\sin^2(\theta/2)$ is usually difficult in practice and the value of $\overline{M}n$ obtained may not be accurate. Hence, the value of $\overline{M}n/\overline{M}w$ will be inaccurate, and any conclusion about the dispersity of the polymer system based on the $\overline{M}n/\overline{M}w$ value will be uncertain. In this investigation therefore, it is difficult to make comparisons between the influence of sodium ions and divalent cations (Ca^{2+} and Mg^{2+}) on the dispersity of the nucleic acid sample studied.

Summary of results

From the experiments reported it has been concluded that:

(i) (DNA) binds divalent cations (calcium and magnesium ions) and much more strongly than it binds sodium ions.

(ii) The binding of (DNA) to calcium and magnesium ions generally leads to the dimerisation of the nucleic acid molecules, and this is manifested in the gradual increase of the molecular weight average ($\overline{Mw}$) as the concentrations of the divalent cations are increased. There is an increase of molecular weight to a maximum value which is about twice that for zero concentration of divalent cations.

(iii) The binding of (DNA) to sodium ions does not produce any change in the molecular weight of the nucleic acid.

(iv) The length of the nucleate ions (assuming a rigid rod) is decreased in the presence of calcium and magnesium ions below its value in the presence of sodium ions. This is also true of the radius of gyration of the molecule.
The decrease in length of the molecule might have been as a result of the compression of the rod-like nucleic acid molecule when bound to calcium and magnesium cations.

This would mean an increase in the radius of the α-helix of the nucleic acid molecule in the presence of calcium and magnesium ions above its value in the presence of sodium ions.

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APPENDIX 1

A suggested Donnan effect approach to the dialysis study of the binding of divalent cations to DNA

Here, n_{3B} has been defined as the total mole of divalent cation bound, and n_2^o, n_3^o, n_4^o and n_5^o , are respectively the total moles of phosphorus, divalent cations, sodium ions and anions (excluding phosphorus).

The moles of ions of either signs inside and outside the dialysis bag at equilibrium are given as follows:

Inside the bag, we have n_2, n_3, n_4 and n_5; and outside, we have n_2', n_3', n_4' and n_5', while the total kilogrammes of water is $g = (g_1 + g_1')$, in which g_1 and g_1' are respectively the kilogrammes inside and outside the bag at equilibrium.

At thermodynamic equilibrium, and outside the bag, the relations

$$n_5' = 2n_3' + n_4' \quad \text{or} \quad n_4' = n_5' - 2n_3' \qquad \ldots\ldots\ldots \quad (1)$$

must be obeyed for neutrality.

Correspondingly, inside the bag,

$$n_5 + n_2 = 2n_3 + n_4 \qquad \ldots\ldots\ldots \quad (2)$$

must be obeyed.

If it is assumed that there is no binding of anions, then Donnan equilibrium gives

$$[Na^+]' [Ca^{2+}]' [\text{anions}]'^3 = [Na^+] [Ca^{2+}] [\text{anions}]^3 \qquad \ldots\ldots\ldots \quad (3)$$

Here, $\frac{[\text{anions}]'}{\mathcal{E}_1'} = (2n_3' + n_4')$

and $\frac{[\text{anions}]}{\mathcal{E}_1} = (2n_3^o - 2n_3' + n_4^o - n_4')$, where the anions refer to only the chloride ions in NaCl and $CaCl_2$.

On substitution for [anions]' and [anions] into equation (3), the expression obtained is

$$\left\{\frac{n_5' - 2n_3'}{\mathcal{E}_1'}\right\}\left\{\frac{n_3'}{\mathcal{E}_1'}\right\}\left\{\frac{2n_3' + n_4'}{\mathcal{E}_1'}\right\}^3 = \left\{\frac{n_4^o - n_5' + 2n_3' - n_{4B}}{\mathcal{E}_1}\right\} \left\{\frac{n_3^o - n_3' - n_{3B}}{\mathcal{E}_1}\right\}\left\{\frac{2n_3^o - 2n_3' + n_4^o - n_4'}{\mathcal{E}_1}\right\}^3 \quad \ldots\ldots (4)$$

and the final expression is

$$n_{3B} = n_3^o - n_3' - \left[\frac{\mathcal{E}_1}{\mathcal{E}_1'}\right]^5 \left\{\frac{(n_5'n_3' - 2n_3'^2)(2n_3' + n_4')^3}{(n_4^o - n_5' + 2n_3')(2n_3^o + n_4^o - 2n_3' - n_4')^3}\right\} \quad \ldots\ldots (5)$$

Equation (5) contains quantities which are experimentally determinable, and from it the moles of divalent cation bound n_{3B} can be calculated.

Before arriving at equation (5) however, assumption has been made that in equation (4) thequantity n_{4B} which represents the moles of sodium ions bound is negligibly small and could be taken as zero.

Limitations to the use of equation (5)

It is important to note that unless the term n_{4B} which appears in equation (4) is zero, equation (5) may not be strightly applicable to the problems of binding of divalent cations like calcium and

magnesium ions to (DNA) in the presence of sodium chloride supporting electrolyte. It is also possible that equation (5) has been over simplified in a number of ways. Firstly, its derivation has been based on the assumption that the anions (like chloride ions) are not bound to the nucleate. Secondly, it does not give consideration to the cations and anions bound to the dialysis tubing, although these can be allowed for experimentally. Also the use of equation (5) will be adequate only where analytical methods which are sensitive to low concentrations of the ions present in the system have been used.

In this work therefore, equation (5) has only a theoretical significance because of the various limitations and restrictions already mentioned. It has therefore not been used to investigate the problem set out for in this thesis for the following reasons:

(i) the analytical methods for determining the concentrations of the cations and anions present were not sensitive enough for the concentrations of ions involved.

(ii) the assumption that the anions did not bind to the nucleate could not be justified, and

(iii) it had not been possible to prove that sodium ions did not bind to the nucleate ions in the dialysis tubing.

APPENDIX 2.a : RESULTS OF THE CONDUCTANCE STUDIES OF THE INTERACTION OF MAGNESIUM IONS WITH DNA IN 0.001M SODIUM CHLORIDE

Concentration of DNA $\times 10^4$ mole P/litre	Conc. of $Mg^{2+} \times 10^4$ Eqv./litre	Eqv. Mg^{2+} per phosphorus	Specific conductance $\times 10^3$ (mho) pH 3.63	pH 7.05	pH 9.10
8.227	0.2940	0.0360	0.0080	0.0102	0.0113
8.194	0.5856	0.0715	0.0155	0.0204	0.0219
8.162	0.8750	0.1072	0.0242	0.0297	0.0326
8.130	1.1619	0.1429	0.0337	0.0400	0.0441
8.098	1.4468	0.1787	0.0433	0.0498	0.0535
8.066	1.7293	0.2144	0.0532	0.0596	0.0639
8.035	2.0093	0.2501	0.0635	0.0698	0.0749
8.004	2.288	0.2859	0.0745	0.0799	0.0864
7.973	2.5635	0.3215	0.0859	0.0915	0.0979
7.942	2.8374	0.3573	0.0969	0.1016	0.1105
7.912	3.1092	0.3930	0.1079	0.1125	0.1230
7.882	3.3789	0.4287	0.1189	0.1223	0.1365
7.852	3.6465	0.4644	0.1303	0.1325	0.1500
7.823	3.9122	0.5001	0.1413	0.1428	0.1597
7.792	4.1760	0.5359	0.1526	0.1528	0.1736
7.763	4.4370	0.5716	0.1648	0.1660	0.1882
7.734	4.6970	0.6073	0.1751	0.1759	0.1995
7.705	4.9550	0.6431	0.1868	0.1859	0.2028

Concentration of DNA $\times 10^4$ mole P/litre	Conc. of $Mg^{2+} \times 10^4$ Eqv./litre	Eqv. Mg^{2+} per phosphorus	Specific conductance $\times 10^3$ (mho) pH 3.63	pH 7.05	pH 9.10
7.677	5.2110	0.6788	0.1978	0.1959	0.2230
7.648	5.4650	0.7145	0.2088	0.2086	0.2360
7.620	5.7170	0.7502	0.2198	0.2186	0.2490
7.592	5.9670	0.7860	0.2308	0.2251	0.2610
7.564	6.2150	0.8217	0.2418	0.2376	0.2740
7.537	6.4620	0.8574	0.2528	0.2489	0.2871
7.509	6.7070	0.8931	0.2638	0.2586	0.2990
7.482	6.9500	0.9289	0.2747	0.2688	0.3132
7.455	7.1910	0.9646	0.2842	0.2787	0.3251
7.428	7.4300	1.0003	0.2493	0.2887	0.3310
7.401	7.6680	1.0360	0.3043	0.2972	0.3471
7.375	7.9040	1.0718	0.3143	0.3064	0.3633
7.349	8.1390	1.1075	0.3243	0.3476	0.3790
7.323	8.3710	1.1432	0.3343	0.3580	0.3950
7.297	8.6020	1.1789	0.3420	0.3698	0.4111
7.271	8.8320	1.2146	0.3520	0.3796	0.4280
7.246	9.0600	1.2504	0.3616	0.3883	0.4440
7.220	9.2860	1.2861	0.3720	0.3976	0.4590
7.195	9.5110	1.3218	0.3820	0.4068	0.4753
7.170	9.7340	1.3576	0.3907	0.4159	0.4910
7.145	9.9550	1.3933	0.3992	0.4252	0.5070

APPENDIX 2.b. RESULTS OF THE CONDUCTANCE STUDIES OF THE INTERACTION OF CALCIUM IONS WITH DNA IN 0.001M SODIUM CHLORIDE

Concentration of DNA $\times 10^4$ mole p/litre	Conc. of $Mg^{2+} \times 10^4$ Eqv./litre	Eqv. Ca^{2+} per phosphorus	Specific conductance $\times 10^3$(mho) pH 3.63	pH 7.05	pH 9.10
8.227	0.1356	0.0165	0.0020	0.0030	0.0030
8.194	0.7151	0.0873	0.0140	0.0141	0.0180
8.162	1.0683	0.1309	0.0211	0.0211	0.0278
8.130	1.4181	0.1745	0.0290	0.0271	0.0376
8.098	1.7665	0.2181	0.0355	0.0340	0.0461
8.066	2.1115	0.2618	0.0421	0.0410	0.0541
8.035	2.4538	0.3054	0.0519	0.0490	0.0650
8.004	2.7935	0.3490	0.0610	0.0591	0.0751
7.973	3.1305	0.3926	0.0700	0.0681	0.0871
7.942	3.4650	0.4363	0.0800	0.0771	0.0970
7.912	3.7969	0.4799	0.0893	0.0860	0.1060
7.882	4.1265	0.5236	0.0980	0.0950	0.1180
7.852	4.4535	0.5672	0.1075	0.1042	0.1281
7.823	4.7775	0.6107	0.1180	0.1150	0.1380
7.792	5.0994	0.6544	0.1280	0.1271	0.1482
7.763	5.4189	0.6981	0.1380	0.1382	0.1590
7.734	5.7376	0.7419	0.1473	0.1492	0.1690
7.705	6.0508	0.7853	0.1581	0.1592	0.1801
7.677	6.3632	0.8289	0.1670	0.1700	0.1900

Concentration of DNA $\times 10^4$ molo p/litre	Conc. of $Mg^{2+} \times 10^4$ Eqv./litre	Eqv. Ca^{2+} per phosphorus	Specific conductance $\times 10^3$(mho) pH 3.63	pH 7.05	pH 9.10
7.648	6.6733	0.8726	0.1780	0.1811	0.2000
7.620	6.9812	0.9161	0.1881	0.1920	0.2100
7.592	7.2867	0.9598	0.1982	0.2020	0.2191
7.564	7.5900	1.0034	0.2070	0.2130	0.2310
7.537	7.8911	1.0470	0.2181	0.2251	0.2431
7.509	8.1900	1.0907	0.2281	0.2350	0.2540
7.482	8.4867	1.1343	0.2390	0.2452	0.2650
7.455	8.7813	1.1779	0.2481	0.2570	0.2750
7.428	9.0738	1.2216	0.2570	0.2671	0.2860
7.401	9.3642	1.2653	0.2670	0.2780	0.2958
7.375	9.6525	1.3088	0.2781	0.2890	0.3080

APPENDIX 3.a. DATA OBTAINED FROM DIALYSIS EXPERIMENTS AND USED FOR COMPUTING THE STABILITY CONSTANTS FOR DNA-Mg^{2+} SYSTEMS

i) CONSTANT IONIC STRENGTH OF 0.02 IN SODIUM CHLORIDE DNA-magnesium

pH	$\bar{v}$	$\frac{1}{\bar{v}}$	$\log \frac{\bar{v}}{1-\bar{v}}$	$[L] \times 10^3$	$\frac{1}{L} \times 10^{-3}$	log L
9.25	0.090	11.111	-1.005	0.500	2.000	-3.301
	0.185	5.405	-0.644	1.000	1.000	-3.000
	0.276	3.628	-0.420	1.540	0.649	-2.813
	0.360	2.778	+0.250	2.000	0.500	-2.699
	0.459	2.181	-0.072	2.970	0.339	-2.527
	0.502	1.992	0.0035	5.034	0.199	-2.298
	0.826	1.211	0.675	7.252	0.138	-2.140
8.20	0.255	3.922	-0.466	1.500	0.667	-2.824
	0.293	3.418	-0.383	1.784	0.561	-2.749
	0.345	2.899	-0.278	2.500	0.400	-2.602
	0.435	2.299	-0.114	3.000	0.333	-2.523
	0.463	2.161	-0.065	3.414	0.293	-2.467
	0.500	2.000	0.000	5.065	0.197	-2.296
	0.687	1.456	0.342	7.377	0.136	-2.132
	0.862	1.160	0.796	12.570	0.080	-1.901
	0.904	1.106	0.974	17.260	0.058	-1.763
7.05	0.270	3.699	-0.431	1.844	0.542	-2.734
	0.370	2.703	-0.231	2.500	0.400	-2.602
	0.420	2.381	-0.140	3.000	0.333	-2.523
	0.462	2.166	-0.067	3.583	0.279	-2.446
	0.472	2.117	-0.048	5.488	0.182	-2.261
	0.509	1.967	0.015	7.551	0.132	-2.122
	0.596	1.679	0.168	12.960	0.077	-1.887
	0.628	1.594	0.227	17.580	0.057	-1.755

pH	$\bar{v}$	$\frac{1}{\bar{v}}$	$\log \frac{\bar{v}}{1-\bar{v}}$	$[L] \times 10^3$	$\frac{1}{L} \times 10^{-3}$	log L
5.00	0.120	8.333	-0.865	1.000	1.000	-3.000
	0.187	5.342	-0.637	1.447	0.691	-2.839
	0.190	5.263	-0.630	2.000	0.500	-2.700
	0.223	4.476	-0.541	2.920	0.343	-2.535
	0.244	4.098	-0.491	4.000	0.250	-2.398
	0.255	3.917	-0.465	4.915	0.204	-2.309
	0.284	3.521	-0.402	7.626	0.131	-2.118
	0.319	3.133	-0.329	12.240	0.082	-1.912
	0.404	2.473	-0.168	17.440	0.057	-1.759
3.60	0.126	7.962	-0.843	1.267	0.789	-2.897
	0.170	5.882	-0.689	2.000	0.500	-2.699
	0.190	5.263	-0.630	2.500	0.400	-2.602
	0.200	5.000	-0.602	2.931	0.341	-2.533
	0.228	4.392	-0.530	5.043	0.198	-2.297
	0.242	4.141	-0.497	7.575	0.132	-2.121
	0.277	3.610	-0.417	11.550	0.087	-1.937
	0.362	2.764	-0.246	17.270	0.058	-1.763

APPENDIX 3.a DNA-Magnesium

ii) CONSTANT pH OF 7.05

Ionic Strength	$\bar{\nu}$	$\frac{1}{\bar{\nu}}$	$\log \frac{\bar{\nu}}{1-\bar{\nu}}$	[L] x 10^3	$\frac{1}{L} \times 10^{-3}$	log L
0.02	0.270	3.700	-0.431	1.844	0.542	-2.734
	0.370	2.703	-0.231	2.500	0.400	-2.602
	0.420	2.381	-0.140	3.000	0.333	-2.446
	0.462	2.166	-0.067	3.583	0.279	-2.523
	0.472	2.117	-0.048	5.488	0.182	-2.261
	0.509	1.967	0.015	7.551	0.132	-2.122
	0.596	1.679	0.168	12.960	0.077	-1.800
	0.628	1.594	0.227	17.580	0.057	-1.755
0.10	0.095	10.537	-0.979	0.8024	1.246	-3.096
	0.180	5.555	-0.658	1.500	0.667	-2.824
	0.235	4.255	-0.513	2.000	0.500	-2.699
	0.285	3.509	-0.399	2.771	0.361	-2.557
	0.380	2.632	-0.278	3.897	0.257	-2.409
	0.435	2.299	-0.114	7.178	0.139	-2.144
	0.530	1.887	0.052	11.480	0.087	-1.940
	0.575	1.739	0.131	16.900	0.059	-1.772
0.16	0	5.263	-0.628	2.000	0.500	-2.699
	0.958	5.107	-0.614	2.527	0.396	-2.597
	0.330	3.030	-0.308	3.987	0.251	-2.399
	0.335	2.985	-0.298	4.815	0.208	-2.318
	0.398	2.516	-0.181	7.152	0.140	-2.146
	0.425	2.353	-0.131	9.945	0.101	-2.002
	0.470	2.128	-0.052	16.570	0.060	-1.780

APPENDIX 3.b. DATA OBTAINED FROM DIALYSIS EXPERIMENTS AND USED FOR COMPUTING THE STABILITY CONSTANTS FOR DNA-Calcium SYSTEMS

i) CONSTANT IONIC STRENGTH OF 0.02 IN SODIUM CHLORIDE

pH	$\bar{\nu}$	$\frac{1}{\bar{\nu}}$	$\log \frac{\bar{\nu}}{1-\bar{\nu}}$	$[L] \times 10^3$	$\frac{1}{L} \times 10^{-3}$	log L
9.25	0.197	5.126	-0.611	1.050	0.952	-2.979
	0.290	3.448	-0.389	1.500	0.667	-2.267
	0.365	2.740	-0.241	2.000	0.500	-2.824
	0.465	2.159	-0.061	3.102	0.322	-2.699
	0.511	1.963	-0.018	5.401	0.185	-2.508
	0.888	1.126	0.899	7.656	0.131	-2.268
8.20	0.085	11.760	-1.032	0.500	2.000	-3.301
	0.212	4.224	-0.570	1.210	0.826	-2.917
	0.340	2.941	-0.288	2.000	0.500	-2.699
	0.452	2.212	-0.084	3.250	0.308	-2.488
	0.484	2.066	-0.028	5.327	0.188	-2.274
	0.713	1.403	0.396	7.705	0.129	-2.110
	0.835	1.198	0.704	10.891	0.093	-1.963
	0.873	1.145	0.837	16.350	0.062	-1.787
7.05	0.150	6.667	-0.753	1.000	1.000	-3.000
	0.255	3.927	-0.465	1.700	0.588	-2.770
	0.350	2.857	-0.269	2.300	0.435	-2.638
	0.429	2.340	-0.125	3.165	0.317	-2.500
	0.463	2.169	-0.065	5.456	0.184	-2.263
	0.511	1.963	0.018	7.505	0.133	-2.125
	0.598	1.672	0.173	12.351	0.082	-1.908
	0.639	1.565	0.248	17.330	0.058	-1.761

pH	$\bar{v}$	$\frac{1}{\bar{v}}$	$\log \frac{\bar{v}}{1-\bar{v}}$	[L] $\times 10^3$	$\frac{1}{L} \times 10^{-3}$	log L
5.00	0.150	6.667	-0.753	1.000	1.000	-3.000
	0.182	5.495	-0.653	1.421	0.705	-2.847
	0.215	4.651	-0.563	2.200	0.455	-2.658
	0.250	4.000	-0.477	3.405	0.295	-2.468
	0.266	3.759	-0.441	5.111	0.196	-2.292
	0.299	3.365	-0.370	8.324	0.120	-2.080
	0.343	2.929	-0.283	13.050	0.077	-1.885
	0.394	2.549	-0.188	16.750	0.061	-1.776
3.60	0.113	8.865	-0.896	0.953	1.049	-3.021
	0.155	6.452	-0.737	1.500	0.667	-2.824
	0.203	4.938	-0.595	2.500	0.400	-2.602
	0.216	4.630	-0.560	3.133	0.319	-2.504
	0.236	4.234	-0.510	4.957	0.202	-2.305
	0.243	4.122	-0.494	8.154	0.123	-2.089
	0.287	3.481	-0.395	12.040	0.083	-1.919
	0.356	2.806	-0.257	16.720	0.060	-1.777

APPENDIX 3.b. DNA-Calcium

ii) CONSTANT pH OF 7.05

Ionic Strength	$\bar{v}$	$\frac{1}{\bar{v}}$	$\log \frac{\bar{v}}{1-\bar{v}}$	[L] x 10^3	$\frac{1}{L} \times 10^{-3}$	log L
0.02	0.150	6.667	-0.253	1.000	1.000	-3.000
	0.255	3.927	-0.465	1.700	0.588	-2.770
	0.350	2.857	-0.269	2.300	0.435	-2.638
	0.429	2.340	-0.125	3.165	0.317	-2.500
	0.463	2.169	-0.065	5.456	0.184	-2.263
	0.511	1.963	-0.018	7.505	0.133	-2.125
	0.598	1.672	0.173	12.351	0.082	-1.908
	0.639	1.565	0.248	17.330	0.058	-1.761
0.10	0.200	5.000	-0.602	1.942	0.515	-2.712
	0.270	3.704	-0.432	3.127	0.320	-2.505
	0.365	2.740	-0.241	6.396	0.156	-2.194
	0.410	2.439	-0.158	8.726	0.115	-2.059
	0.478	2.092	-0.039	14.700	0.068	-1.833
	0.490	2.041	-0.017	17.550	0.057	-1.756
0.16	0.156	6.431	-0.735	1.891	0.529	-2.723
	0.195	5.128	-0.616	2.500	0.400	-2.602
	0.213	4.695	-0.568	3.000	0.333	-2.523
	0.240	4.167	-0.501	3.924	0.255	-2.406
	0.276	3.630	-0.420	5.637	0.177	-2.249
	0.295	3.390	-0.378	8.222	0.122	-2.085
	0.331	3.026	-0.307	12.310	0.081	-1.910
	0.350	2.857	-0.269	18.030	0.055	-1.744

APPENDIX 4

DATA OBTAINED FROM THE ANGULAR DEPENDENCE OF LICHT SCATTERING FOR DNA/CATION SYSTEMS

(i) DNA-sodium chloride 0.2MNaCl

	Concentration of nucleate x 10^5 g/ml			
	2.50	4.00	7.31	11.41
0°	Rayleigh's ratio x 10^5 cm^{-1} (R_θ)			
40	4.06	6.36	10.10	15.13
45	3.36	5.20	8.90	13.02
50	2.90	4.43	7.71	10.80
60	2.08	3.21	5.49	7.96
75	1.44	2.28	3.92	5.84
90	1.18	1.87	3.27	4.76
105	1.02	1.79	3.19	4.12
135	6.09	3.73	4.99	5.68

(ii) DNA-calcium chloride 2.5 x 10^{-3} M $CaCl_2$

	Concentration of nucleate x 10^{+5} g/ml			
	2.63	4.21	7.50	11.32
0°	Rayleigh's ratio (R_θ) x 10^{+5} cm^{-1}			
40	8.52	13.36	22.77	32.73
45	7.08	11.13	19.05	27.55
50	5.94	9.29	15.96	23.19
60	4.15	6.56	11.34	16.60
75	2.67	4.26	7.41	10.80
90	1.98	3.17	5.47	7.99
105	1.90	2.90	5.28	7.99
135	2.39	4.24	6.22	8.98

(iii) DNA-magnesium chloride 2.5×10^{-3}M mgCl.

	Concentration of nucleate $\times 10^{+5}$ g/ml			
	2.58	4.17	7.31	10.99
θ°	Rayleigh's ratio (R_θ) $\times 10^5$ cm^{-1}			
40	7.85	12.71	20.40	30.63
45	6.51	10.40	17.04	25.61
50	5.40	8.89	14.22	21.49
60	3.80	6.43	10.07	15.37
75	2.46	4.56	6.48	10.03
90	1.82	3.75	4.76	7.39
105	1.80	3.73	4.61	7.22
135	2.24	4.25	5.21	7.84

APPENDIX 5

Materials used for the studies

A)

Sodium chloride	(Analytical grade)
Calcium chloride	(Analytical grade)
Magnesium chloride	(Analytical grade)
Deoxyribonucleic acid	(Crude product kindly supplied by Dr. S. Mathieson, and purified by J. Y. Olayemi)

Description of DNA: (CTA)

Obtained from calf thymus (from the U.K.)

Percentage water of wet weight = 26%

Percentage phosphorus of dry weight = 9.15%

Molecular weight in 0.2M NaCl = 12×10^6

B)

Sodium citrate	(Reagent grade)
EDTA	(Analytical grade)
Tris buffer	(Reagent grade)
Sucrose	(Analytical grade)