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RESEARCH ACCOUNT

Application of ion–solvent interaction parameter in interpreting the kinetic profiles of Diels–Alder reactions and thermal stability of DNA duplexes in ionic solutions

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Rate enhancement in Diels–Alder reactions and thermal stability of a DNA duplex at several ionic concentrations are the main issues addressed in this account. The proposed quantity, internal pressure of a salt-solvent, which is also a measure of electrostriction effect together with the activation volumes of reactions can describe the impressive rate accelerations of the Diels–Alder reactions carried out in salt solutions. Similarly, obviation of high pressure conditions for the synthesis of organic molecules is described. More importantly, the application of the concept of internal pressure and volume has demonstrated that the thermal stability of several DNA duplexes in the presence of ions can be explained. The pressure dependence of thermal stabilities at constant ionic concentrations is linear in nature. The correlations exposed for the DNA duplexes have strong potential to unravel the forces responsible in stabilizing a DNA duplex or the formation of a duplex from its complementary strands.

MOLECULAR interactions of a solvent molecule with nonionic and ionic solutes play vital roles in governing the static and dynamic behaviour of a system. Various thermodynamic, kinetic and other solution properties of the system represent specific behaviour of these molecules in a given environment. With regard to solvents,

Mother Nature discovered the secrets of water in different biological and other natural processes, whereas nonaqueous solvents continued to gain importance in a variety of man-made processes.

Considering the importance of the solute–solvent interactions in several processes, we have very recently embarked on a programme to investigate their role in controlling the kinetic profiles of the organic reactions, like Diels–Alder (D–A) reactions^{1,2} and the association and dissociation processes involved in nucleic acids³. Some of these results along with the futuristic views have been described here.

Definition of solute–solvent interactions in the present context

Cohesion among molecules in the liquid phase results from intermolecular forces. These forces, in general, include hydrogen bonding, dipole–dipole, multipolar, dispersion interactions and also interactions emerging from the repulsion between two molecules. The cohesion due to intermolecular forces gives rise to a 'pressure' which is experienced by the solvent molecules. This term for pure solvent was initially proposed by Hildebrand and Scott⁴ and subsequently supported by the equation of state⁵. A liquid undergoing a small, iso-

thermal volume expansion does work against the cohesive forces which causes a change in the internal energy, U . The function $(\partial U/\partial V)_T$ is called internal pressure of a liquid. According to Hildebrand and Scott⁴, definition of internal pressure for a non-polar liquid can be transformed into that for polar by a specific factor indicating the polarity of a solvent. Thus, the internal pressure for a non-polar liquid differs from that of a polar. For instance, the internal pressure values for water and *n*-hexane are noted to be 166.4 MPa and 235.7 MPa, respectively.

Internal pressure changes upon the addition of a solute. Such addition of a solute, particularly of ionic nature, brings about a great change in the cohesion of a solvent. While some solutes like NaCl, KCl, etc. enhance the internal pressure of the solvent, salts like of guanidinium decrease it. As seen later, this phenomenon in solution can prove to be a vital tool in unraveling the origins of forces involved in some organic reactions and more importantly, the thermal stabilities of nucleic acids.

Due to the high electric field associated with an ion, water molecules are oriented around it, causing a local collapse of the bulk water structure. Since water molecules are more firmly packed around an ion than in bulk water, the net volume of the system decreases in the region. This process, called electrostriction (ES), is thus a result of ion-water interactions and it mainly depends on the charge and the radius of an ion, and the changes in local dielectric constant around the ion^{6,7}. The ES effect can be described in terms of internal pressure. Internal pressure, P_i of a solution can be estimated from the experimental quantities by the expression $P_i = \alpha T/\beta$; where α and β are the coefficient of thermal expansion and isothermal compressibility, respectively at a temperature T . Both α and β can be measured from experiments on volumetric properties. In the cases where these values are not readily available, the scaled particle theory⁸ can be used. We have employed this theory for the solute and solvent of different sizes⁹. This theory yields P_i to within $\pm 3\%$, when compared to experimental data. In Table 1, the P_i values for some salts in water are represented by a polynomial equation as a function of the salt concentration, M .

Fascinating observations in the kinetic profiles of D-A reactions

These addition reactions result from the reaction of a diene with dienophile and are useful in synthesizing complex organic molecules. Several years ago, Rideout and Breslow¹⁰ demonstrated the application of pure water as a reaction medium in these reactions. Two exciting outcome of this medium were the impressive enhancement in reaction rates by several order of magni-

Table 1. Parameters of the equation $P_i/\text{MPa} = 166.4 + aM + bM^2 + cM^3$ for a few representative salts in water ($P_{iw} = 166.4$ MPa) as function of M (mole l^{-1})

Salts	a	b	c
NaCl	10.792	61.273	0.532
KCl	18.841	70.541	-0.096
NaClO ₄	88.114	-1.565	0.011
KCNS	90.923	-1.734	0.011
CF ₃ COONa	86.464	-1.516	0.007

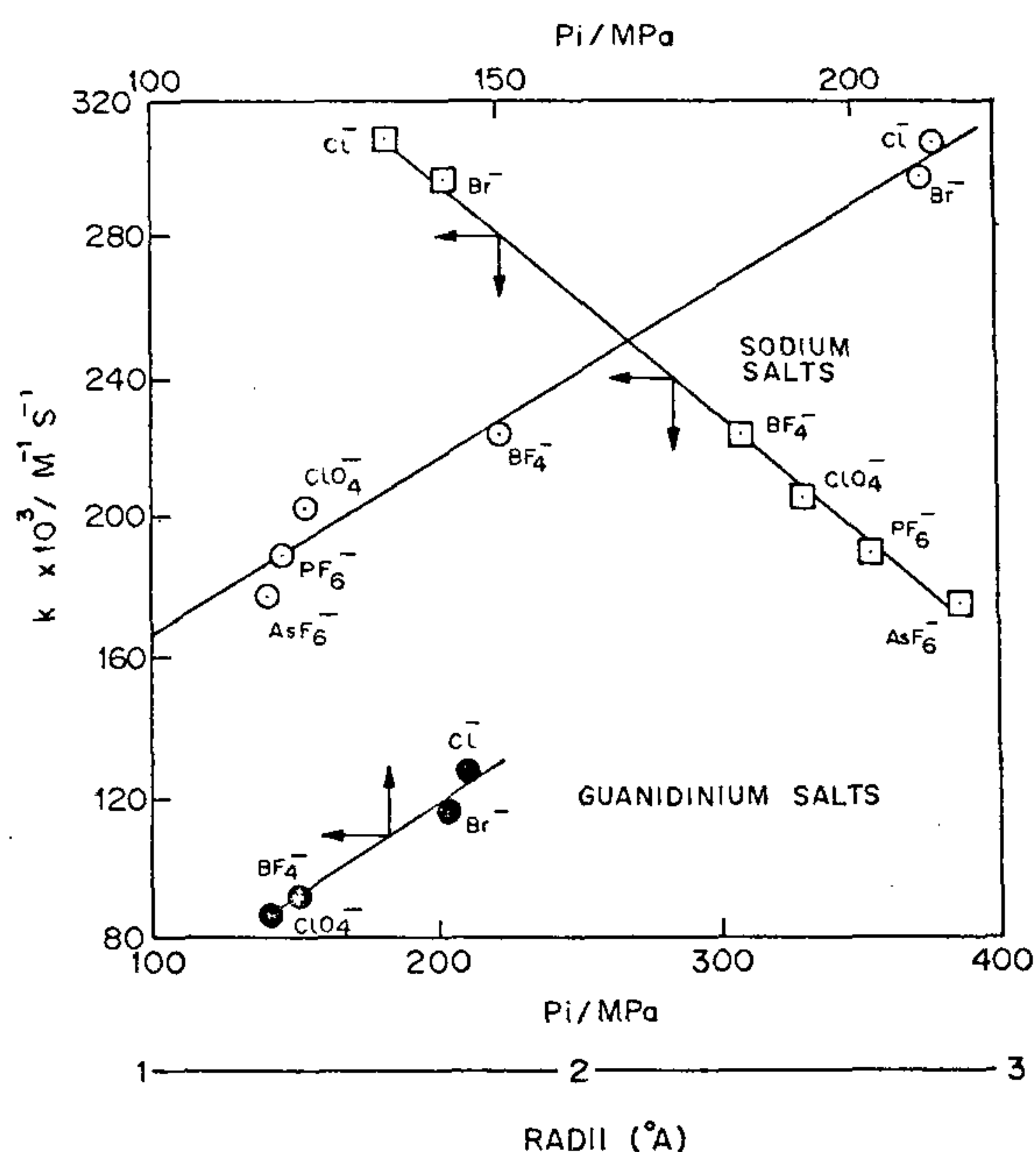


Figure 1. Reaction rates k versus internal pressure P_i (\odot , \bullet) for reaction of *N*-ethylmaleimide with anthracene-9-carbinol in salt solutions of sodium and guanidinium; analogous plots of k versus anionic radii (\square) are also included.

tudes in the cases where the reaction in other solvents was either not possible or very sluggish. Secondly, the stereoselectivities in the reaction products (endo/exo ratios) could be altered to some extent. Success of the reactions in water prompted the application of 'solute-solvent' additives for altering the kinetic profiles of some very cumbersome reactions. One of these reactions is the synthesis of cantharidin, which was conventionally synthesized under an external ultrahigh pressure of 16–18 kbar or 1.6–1.8 GPa (ref. 11). The use of a reaction medium comprising of lithium perchlorate–diethyl ether (LDPE) led to the synthesis of cantharidin with better yields at atmospheric pressure¹². Conversion of a high pressure reaction to an atmospheric reaction using such additives which are comprised of ionic solutes and water or nonaqueous solvent, proved to be a milestone in

synthetic chemistry. Following this work, several sluggish reactions have been successfully conducted in such media²⁻¹⁴. Similarly, the use of other salts like LiCl, NaCl, etc. in water has further enhanced the reaction rates. On the other hand, salts like guanidinium chloride have retarded the reaction rates. Figure 1 depicts a general picture of variation in the reaction rates of such D-A reactions in the pressure of salt-solvent systems. Examples of some D-A reactions in aqueous as well as in nonaqueous solvents with ionic solutes are mentioned in Table 2. Rate enhancement in aqueous media has been ascribed to several effects like hydrophobic interactions, micellar catalysis, hydrogen bonding and solvent effects. An excellent discussion on these effects has been recently documented¹³⁻¹⁴. One of the most potential explanations to describe the impressive rate enhancements is based upon the Lewis acid catalysis of the reaction.

In short, several other contrasting explanations to describe the origin of forces that are responsible for such effects have been put forward. With due credence to all the explanations, we in our recent work, have attempted to correlate and explain the variations in reaction rates with the help of a bulk property of solution, i.e. internal pressure as shown in the following discussion.

Possible explanations of the reaction profiles of the D-A reactions

First, we examine the reactions in aqueous reaction media. Two situations in this case are pertinent, where the reaction rates of the D-A reactions are increased or decreased by different salt-water systems¹³. Among others, a simple reaction of *N*-ethyl maleimide and anthracene-9-carbinol shows enhancement in rates in presence of aqueous NaCl, NaBr, etc.¹⁵. On the other hand, the rates are decreased by the medium of guanidinium salt. An examination of the P_i values in such salts shows that those reaction media which enhance the rates, also exhibit the increase in internal pressure. The same is true

Table 2. Some examples of the Diels-Alder reactions promoted by the salt-solvent systems

Reactions	Systems
1. <i>N</i> -ethylmaleimide + anthracene-9-carbinol	aq., aq. LiCl; GnCl*, LiClO ₄ ; GnClO ₄
2. 9,10-dimethylantracene + acrylonitrile	aq. LiCl
3. Isoprene + <i>N</i> -phenylmaleimide	aq. LiClO ₄
4. Cyclopentadiene + methyl vinyl ketone	aq. LiCl; aq. GnCl
5. 2,3-dimethylbutadiene	LiClO ₄ + acetone
6. Furan + 2,5-dihydrothiophene-3,4-dicarboxylic anhydride	LiClO ₄ + diethyl ether

Gn* = Guanidinium

for the decrease in the rate reactions. It is, thus, possible to correlate the rates with internal pressure for different reactions. In Figure 1 are shown the reaction rates versus P_i for the above reaction in the presence of sodium and guanidinium salts. An excellent linear plot is obtained in this system. Such linear plots between the reaction rates and internal pressures for several other reactions in aqueous environment indicate that the internal pressure can be used as a strong correlating parameter in these reactions. As a matter of fact, the arrangement of water molecules is drastically changed in the presence of salts¹⁶.

This change affects the transition state of a reaction. The increase in internal pressure with increasing concentration of salts like NaCl, NaBr, etc. enhances the ES effect. On the contrary, the ES effect due to the guanidinium salt type decreases with the increase in the ionic concentrations. An important outcome emerging out of such work is the possibility of the role of hydrophobicity¹³.

Both diene and dienophile due to their dislike for water come close enough to react. This hydrophobic effect is further enhanced in the presence of those salts which accelerate the D-A reactions. Obviously, a decrease in hydrophobicity can be considered, when the reaction rates decrease in the presence of salts like those of guanidinium. Similarly, the hydrophobicity increases in the rate enhancing salts¹⁷. We have also noted that the reaction rates also depend upon the size of salt used as a reaction medium. As seen in Figure 1, the reaction rates decrease, if the size of anions increases for the same salt series keeping the cation common. This variation is consistent with the increasing or decreasing hydrophobic effects in the presence of salts and their relations with the reaction rates. These results can also be understood in terms of ion-water interactions.

Though our primary objective in this account is focused on aqueous environment, it is essential to briefly mention ionic interactions in nonaqueous solvents and their implications on the reaction rates. Such interactions, like those available in the lithium salts with diethyl ether, acetone, ethyl acetate, etc. can be used to correlate both the reaction rates and stereoselectivities of the D-A reactions². For obtaining these correlations, activation volume and internal pressure P_i are used to describe the rate data.

Activation volume, an activation parameter is the only appropriate choice for studying a reaction, when pressure of the system is variable. Activation volume (ΔV^\ddagger) data yield valuable information on the kinetics of reaction. The ΔV^\ddagger is defined as the difference of reactant partial volumes and the volume of the transition state. The ΔV^\ddagger can be obtained by the use of liquid phase non-ideality calculations, besides high pressure kinetics¹⁸. In the method used in our work, activity coefficients were deployed to yield liquid phase non-idealities of compo-

nents. We used a well-known model known as Universal Functional Group Contribution model abbreviated as UNIFAC¹⁹, where the interactions between cation, anion and their individual and mutual interaction with solvent are explicitly considered by a combination of extended Debye-Huckel equation and interaction energies of nonideality. In a nutshell, both quantities i.e. internal pressure and activation volume offer us excellent correlations with the kinetic profiles. Thus, it is possible to explain why the reaction rates vary with ionic concentrations and why two reactions show different reaction profiles under specific conditions of a reaction medium. In further work, we have also been able to demonstrate the utility of our proposed concept in dealing with the D-A reactions conducted in different pure solvents and their aqueous mixture²⁰. The parameter on which such correlations are based, requires the internal pressure normalized to a polarity scale.

In the foregoing discussion, we noted that the addition reactions like the D-A reaction, which yield impressive rate enhancement in the presence of salt solutions, can be described using an internal pressure term. In essence, enhanced internal pressure together with activation volume of a reaction prove to be of tremendous use in understanding the kinetic accelerations in the D-A reaction in the ionic solutions described above.

With this development in reaction kinetics, it was intriguing to explore the possibility of explaining melting behaviour in nucleic acids. Melting of a nucleic acid, for example, of DNA duplex involves a dissociation process or reaction, which takes place in ionic environment. On the other hand, the formation of a DNA duplex in ionic environment is an association reaction. In the following discussion we shall attempt to examine the dissociation or association reactions in a similar fashion that was used to correlate the kinetic data of Diels-Alder reactions.

Nucleic acids

Effect of various salts

Although both proteins and nucleic acids are particularly prominent among the molecules essential to life, molecular interactions in nucleic acids, particularly DNA will be the sole focus of our discussion. DNA is a very long biomacromolecule composed of a large number of deoxyribonucleotides. A nucleotide consists of three molecular fragments, i.e. sugar, base and phosphate. Interactions to nucleic acids are highly specific and play crucial roles in controlling the transcription, recombination, replication, etc. An important aspect of a DNA molecule which warrants attention is its stability in aqueous ionic media. This stability can be judged by the melting of DNA solution or adding some ionic sub-

stances. The melting temperature, T_m at which a DNA double helix converts into two complementary strands, depends markedly on the base composition, base stacking and the ionic environment in general²¹⁻²⁶. In fact the very formation of the DNA duplex from its complementary single strands is governed by these parameters. It is established both by experiments and theory that DNA molecules are heavily hydrated. Effect of water on the stabilization of DNA helices via hydration, therefore, assumes a vital role in understanding the energetics of DNA.

As a matter of fact, interactions of counter ion Na^+ with the regular lattice of negatively-charged phosphate groups (PO_4^-) of the sugar-phosphate backbone of the DNA helix can change the degree of hydration and contribute to the stability of DNA helices. A valuable contribution in this regard has been made by Manning²⁷, who used a rigorous approach of counterion condensation, where the statistically averaged properties of mobile cloud of counterions tends to neutralize the net electrostatic charge of the nucleic acid. Later, Record *et al.*²¹ utilized the polyion condensation theory of Manning for the analysis of stability of the DNA helices. In their simple treatment, they introduced a concept of thermodynamic binding, defined on the basis of a suitable combination of several ionic effects. These interactions which are electrostatic in nature have been the subject of intense investigation by theoretical methods. The classical methods of analysing these interactions have largely been based on the Debye-Huckel, Gouy-Chapman, Poisson, Poisson-Boltzmann equations, etc. whereas Monte Carlo simulations, molecular and Brownian dynamics are some of the very recent techniques used to understand the role of ions on the stability, structure, dynamics and related properties of DNA²⁸. The resultant equations have been useful in analysing the stability data of DNA helices particularly in the low ionic concentration range. A reference should be made to the work of Troll *et al.*²⁹ in which the effects of permittivity discontinuity at the boundary between aqueous phase and the low dielectric DNA cylinder were studied in order to understand the stability aspects of the DNA helices.

As seen above, several qualitative and quantitative explanations have been put forward to describe the stabilization of the DNA helix in aqueous medium. In our work, we have been primarily concerned to gain insights into the process of formation of a DNA helix from its complementary single strands or the melting of a DNA helix into two complementary single strands in the presence of an ionic atmosphere. For explaining the thermal stability effects, we have preferred to correlate T_m with the process of electrostriction. It seems to us that the electrostriction effects, if expressed in terms of a measurable thermodynamic quantity, can be used to interpret several features of the thermal stability of a

DNA helix, without recourse to much complex mathematics. A quantity useful in the present analysis of thermal stability of DNA helix is the volume change, ΔV , accompanying the transition process. The hydrated DNA consists of two discrete layers representing primary and secondary hydration shells. In fact, the bases in single strands tend to form complementary hydrogen bonding leading to the formation of hydrophobic core in the DNA helix. The phosphate groups lie exposed to water and impart considerable charge density to single strands and to their double helix. The magnitude and sign of ΔV depend upon the base stacking, the degree of hydration, the charge density parameters of both the single complementary strands and duplex, and the electrostriction of the water molecules^{23,24,26,30,31}.

Both the P_i and ΔV parameters can now be utilized to examine the issue of thermal stability of a DNA duplex in different situations.

Thermal stability – Enhancing behaviour

It is established that the thermal stability of the duplex is greatly influenced by salt concentrations²¹. For instance, higher concentrations of NaCl induce greater stabilization of the duplex (higher T_m), as compared to lower T_m noted at low concentrations data of salt. Our calculations of P_i using the most reliable density and compressibility^{32,33} show that P_i of water increases upon the addition of NaCl. Such a variation in P_i can be indicated by the effective internal pressure ΔP_i , as $\Delta P_i = P_i - P_i^w$ where P_i^w is internal pressure of pure water. The term P_i denotes the change in internal pressure of water occurring upon the addition of a salt. The aqueous ionic environment thus remains under high pressure conditions, which are enhanced almost up to the solubility limit of NaCl in water. The transition of a duplex to single strands is reported to be accompanied with the positive volume changes. The possible reasons for the positive ΔV values and their variations with the salt concentrations, observed in the above transition of a duplex can be understood in terms of electrostatic and stacking effects³⁴⁻³⁶. For instance, in the case of Poly(dA).Poly(dT), the ΔV values are noted to vary from 2.60 to 7.81 cm³ mol⁻¹ in the range of NaCl concentration from 0.02 to 1 M. The ΔP_i value at 1 M is much higher than that at 0.02 M. This high internal pressure suppresses the process of transition of a DNA duplex to single strands since V is positive for the melting process. Thus, in order to obtain transitions at a higher NaCl concentration, a higher temperature needs to be employed. This leads to a higher T_m value which increases with the addition of NaCl. In other words, one may state that the ES or the enhanced P_i resulting from the salt concentrations controls the T_m of a duplex DNA. To illustrate these variations, the relationship between

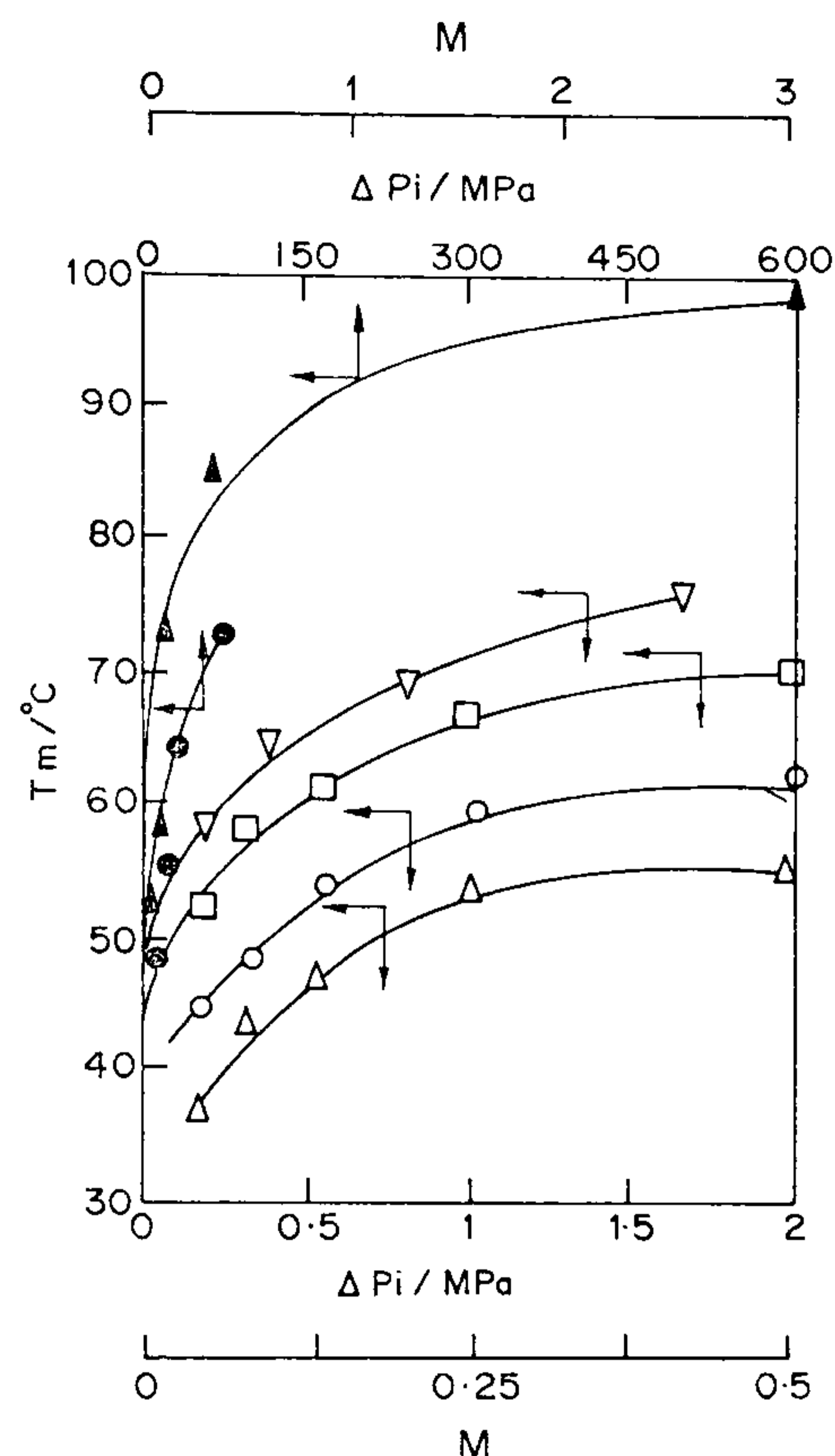


Figure 2. Relationship between transition temperature T_m and effective internal pressure ΔP_i for the double helix transition. \blacktriangle , Poly(dA).Poly(dT); \bullet , Poly[d(A-T)] Data ref. 36; ∇ , Poly(dA).Poly(dT); \square , Poly(rA) · Poly(dT); \circ , Poly(rA).Poly(rU); Δ , Poly(rA).Poly(dU) (data Rentzeperis *et al.*²⁵). First data set at high NaCl concentration; All other sets low NaCl range; corresponding salt concentrations are also shown.

T_m and ΔP_i are shown, in Figure 2 for the transitions of several duplexes of Poly(dA).Poly(dT) or the polymers containing AU base pairs in different NaCl concentrations, ranging from low to high, at atmospheric pressure. The duplexes with GC base pairs are known to have higher T_m values owing to lesser hydration and more number of hydrogen bonds as compared to the AT base pairs. In the case of duplexes³⁷ with high GC contents, curves of T_m versus P_i are shown in Figure 3. The shape of the curves is a typical temperature–pressure plot encountered in transitional reaction kinetics. In the case of higher valent salts like MgCl₂, higher values of P_i are obtained as compared to those for NaCl at corresponding concentrations. In general, values of P_i for MgCl₂ are almost three times as high as those for NaCl up to 4 M, as calculated from literature data^{32,33}. Thus, almost the same stability for a given duplex can be obtained using a much lesser concentration of MgCl₂ as compared to a given concentration of NaCl^{21,38-41}. The ES effect enhances when one moves from uni-, multi-valent ionic salts¹⁸ for which the thermodynamic support comes from the activity coefficient data⁴².

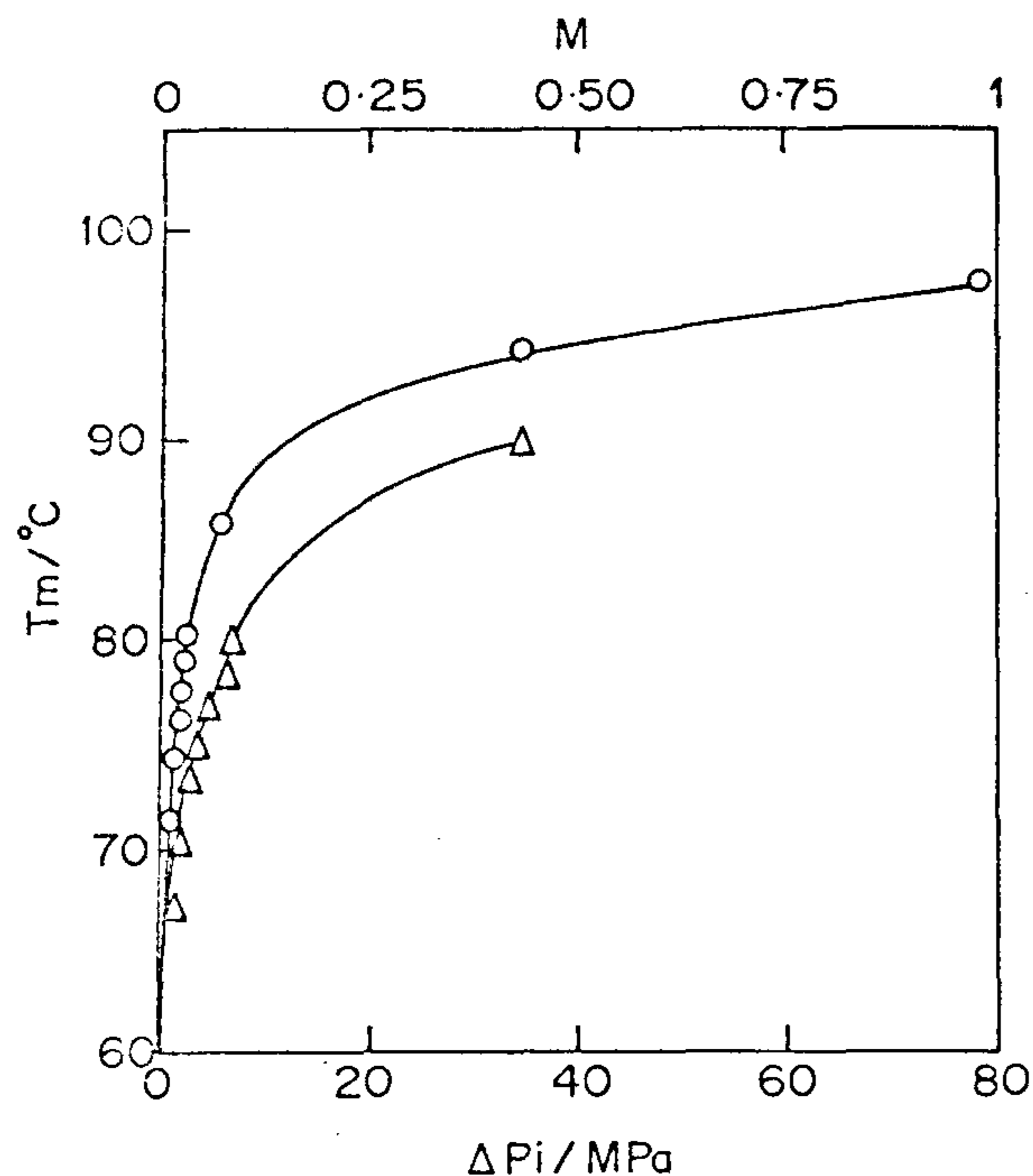


Figure 3. Demonstration of relationship between T_m and ΔP_i for the denaturation of *E. coli* (O) and *D. pneumoniae* (Δ) with high GC contents in KCl. The corresponding concentrations are given in ref. 37.

Thermal stability decreasing behaviour

As seen above, thermal stability of a DNA duplex increases by the addition of certain salts like NaCl, KCl, etc. Contrary to the observation, the DNA duplex is destabilized in the presence of some other salts, such as acetates, perchlorates, etc. As a matter of fact, it is the property of a salt or ionic media, which has a strong correlation with the variations in T_m of a duplex. For instance, the T_m values of a duplex in the presence of NaClO_4 , KCNS and CF_3COONa at several ionic concentrations are reported to be decreasing with increasing concentration⁴³. Explanation of this experimental observation can be found out using the concept of negative changes in ΔP_i upon the addition of salts. Values of ΔP_i in such salts can be calculated from the experimental data or theoretical methods. Such cases of decreasing T_m with increasing salt concentrations is demonstrated in Figure 4, where the T_m values for these salts are plotted as a function of ΔP_i . There are two interesting aspects in this case; (i) the ΔP_i values are negative and (ii) the curves are smooth up to high concentrations of salts. An examination of plots reveals that the proposed correlation is applicable even to a situation where the T_m decreased by 60°C. The ΔP_i values for CF_3COONa

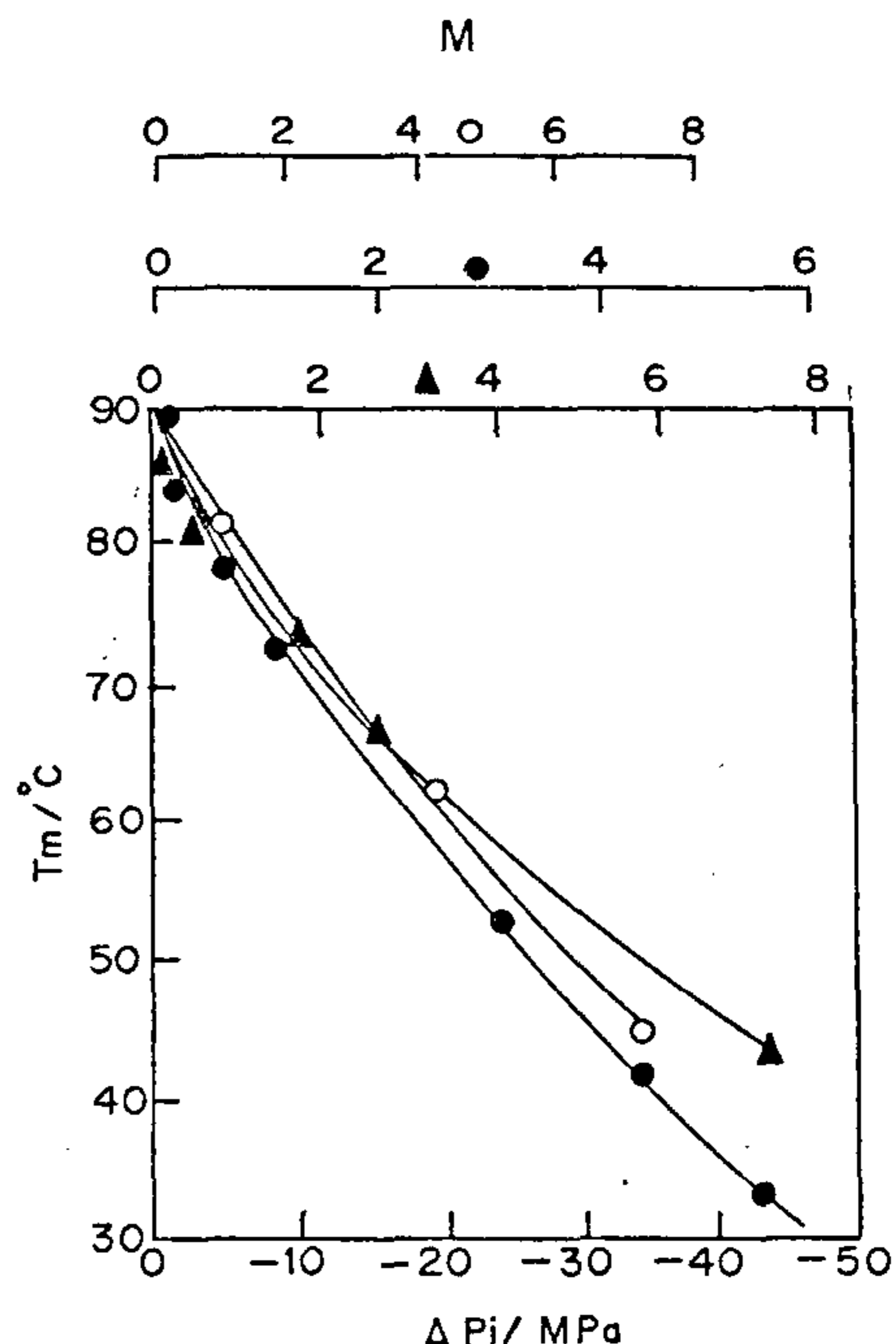


Figure 4. Plot of T_m against ΔP_i for denaturation of DNA duplex in the presence of salts. \blacktriangle , NaClO_4 ; O, KCNS; \bullet , CF_3COONa (ref. 47).

demonstrate the salt's capability as the most powerful denaturant out of the three studied. In such cases, the negative, ΔP_i values are the results of the anti-electrostriction effect caused by salts as indicated above. In other words, the difference between the molar volume of a liquid salt, and its partial molar volume tends to become negative. This situation appears to be analogous to one arising out of the salting-in effect. Salts like those shown herein have very large anions that make the charge density smaller than that of water dipole, and thus decreasing the interactions as compared to those among water molecules. This argument is supported by several theoretical calculations⁴⁴.

Both the thermal expansion and isothermal compressibility, i.e. α and β , respectively are responsible for calculating P_i values of a system. In the above examples both the contrasting cases of T_m -enhancing and reducing thus should be examined in terms of the α and β terms. It was noted that the salts which enhance the thermal stability with increasing concentration, exhibit a noticeable increase in the thermal expansion and a decrease in the isothermal compressibility with an increase in salt concentrations. On the other hand, for the T_m decreasing salts, an opposite trend is observed, i.e. an increase in isothermal compressibility dominates the thermal expansion when one moves from a dilute to a concentrated

salt solution. For these salts, one can think that a situation of internal pressure drop over pure water is created due to ion-solvent interactions, which when coupled with positive ΔV values, tends to destabilize the duplex, with the consequent lowering of T_m . This destabilizing effect continues to dominate with the increasing salt concentration. The anionic size in the salts influences the internal pressure drop in such situations.

The T_m values for different duplexes at the same ionic concentration show variations, which can be analysed on the basis of similar arguments. Since the ΔV values for the duplex depend upon several parameters described earlier, the effect of a given NaCl concentration or the thermodynamic internal pressure can easily explain the differences in T_m values for the different duplexes. For example, the ΔV values for the transitions of Poly(dA).Poly(dT) and Poly[d(A-T)] at 0.2 M NaCl concentration are 4.59 and 2.14 $\text{Cm}^3\text{mol}^{-1}$, respectively³⁶. The internal pressure of the medium being the same (~ 189 MPa) at a fixed NaCl concentration (0.2 M), the transition for Poly(dA).Poly(dT) would, therefore, require a higher temperature than that for Poly[d(A-T)], considering the basic thermodynamic relationship between pressure and volume with respect to the kinetics of two processes. Thus, T_m for Poly(dA).Poly(dT) is higher (73.1°C) than that for Poly[d(A-T)] (64.3°C). Thus, the value of ΔV , an indication of the hydration and other structural properties of any duplex, as described earlier, controls the variation in thermal stability at a definite internal pressure at a given NaCl concentration. Values of ΔV , thus, should be credited due importance in the transition reactions.

Pressure effects

Application of external pressure greatly influences the transition of duplex to single strands³⁶. In general, high pressure has been observed to result in an increase in T_m for several duplex-single strand transitions. The calculations in the present investigation of P_i for NaCl using high pressure data⁴⁵ at each applied external pressure value demonstrate that P_i increases with an increase in the external pressure. Thus, the total internal pressure of the system at a given concentration of NaCl is higher at an elevated external pressure than at atmospheric pressure. This increase in the total internal pressure, coupled with the positive volume of transition, inhibits the transition of duplex to single strands. In order to achieve the transition, therefore, a higher temperature is required when the external pressure is higher than the atmospheric pressure. For explaining our argument, we define two quantities: $T_m^R - 1$, (where T_m^R is the ratio of T_m at any external pressure to that at atmospheric pressure) and $P_i^R - 1$ (with an analogous definition as used for T_m). Some valuable information emerges out of plots

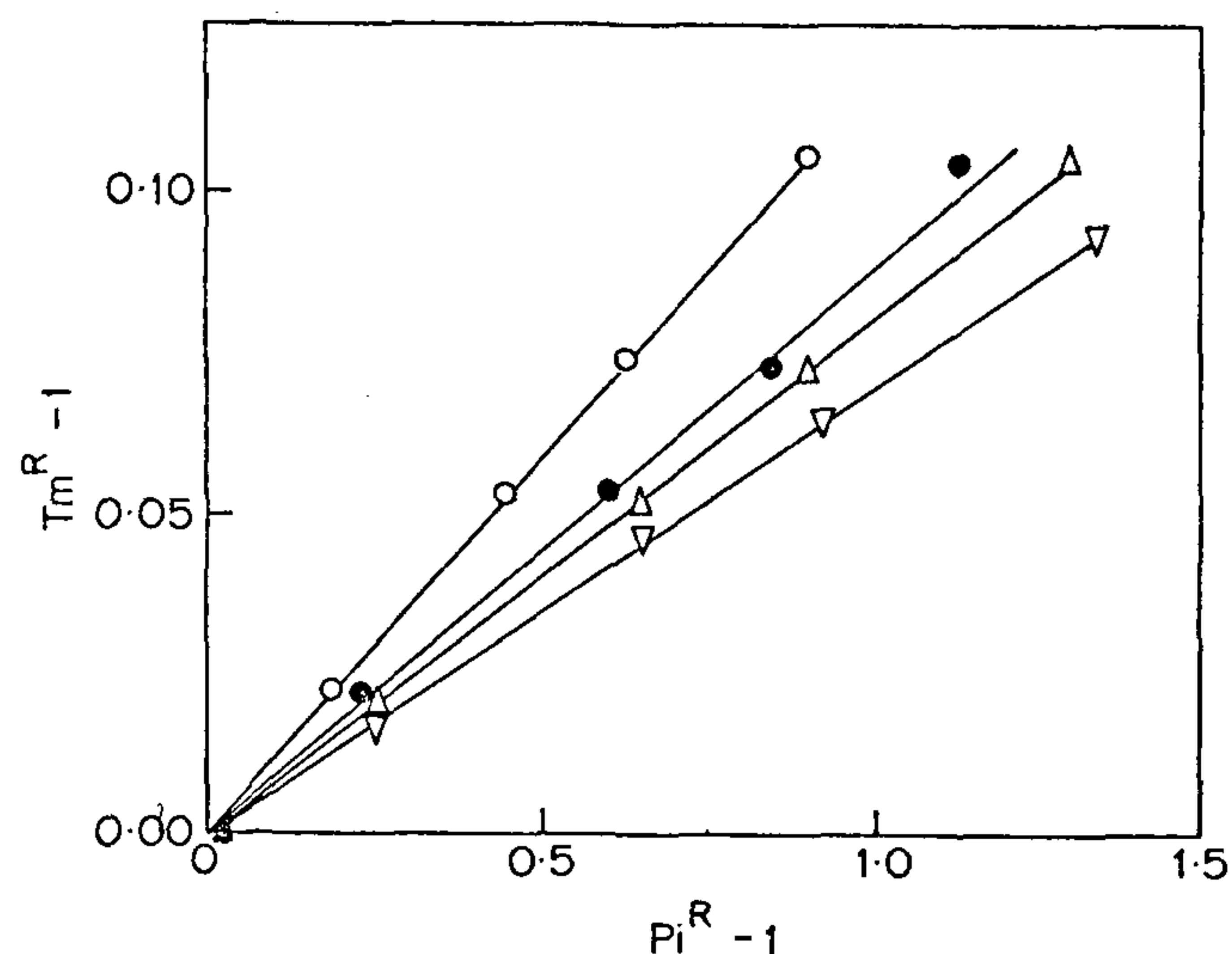


Figure 5. Relative transition temperature at various pressures. $T_m^R - 1$ as a function of relative internal pressures $P_i^R - 1$ at various pressures in several NaCl concentrations. O, 1 M; ●, 0.2 M; Δ, 500 mM; ▽, 200 mM duplex; Poly(dA).Poly(dT), range of external pressure covered 200 MPa; at atmospheric pressure (0.1 MPa) $T_m^R - 1 = P_i^R - 1 = 0$; points in plots are chosen to show extent of linearity, though linearity is observed throughout; experimental data from ref. 36; identical observations for other duplexes also.

(Figure 5) between $T_m^R - 1$ and $P_i^R - 1$ at several NaCl concentrations. For example, in the case of Poly(dA).Poly(dT), for which extensive data are available, the plots $T_m^R - 1$ versus $P_i^R - 1$ are linear at each NaCl concentration in the range from 200 mM to 1 M. The extent of linearity is seen up to 200 MPa with slopes of significant magnitude, suggesting the strong influence of internal pressure on thermal stability. It is, therefore, possible to characterize and correlate the thermal stability of DNA duplexes at several ionic concentrations under elevated external pressure conditions.

An important parameter, as pointed out in the foregoing discussion, is the magnitude and the value of ΔV . Two contributions of prime significance resulting into this property come from the base stacking and electrostatic effects. These effects give rise to negative and positive ΔV in the cases of formation of a duplex and transition of a duplex into two complementary strands, respectively. The base stacking effect originates from dispersion forces and hydrophobic effects, whereas the interactions between Na^+ and PO_4^- of DNA strands yield electrostatic effects. Though the signs of ΔV in the duplexation and the reverse reaction are negative and positive respectively, both positive and negative values are noted for duplexation in some cases. These very small positive values noted at extremely low Na^+ concentration are presumably the result of the combined effect of specific base pairing and the low degree of electrostriction. For example, Rentzeperis *et al.*²⁵ noted

positive ΔV at 16 mM Na^+ for Poly(rA).Poly(rU), which became negative at higher Na^+ concentration of 116 mM. Such variations in sign of ΔV have been described in terms of change in hydration of the DNA duplex as a function of ionic concentration.

Recalling the polyion condensation theory²⁷, thermodynamic binding of counter ion Na^+ with polyion PO_4^- is the major step. First, in presence of a salt, the counterion condenses on the polyion in order to reduce the axial charge density of a strand. This is followed by the second step, in which the remaining unneutralized polyions are shielded (screening effect) by an atmosphere of mobile counterions. It is not yet clear how much difference exists in counterion condensation in single strands and duplex²⁴. In the present approach, internal pressure induced by the salt is a bulk solution property and acts as a driving force for the formation of a duplex from its complementary single strands based upon the volume changes accompanying duplex formation. It appears that both the condensation and screening effects are indirectly accounted for in the internal pressure term of a salt solution. In our recent work, we have noted the magnitudes of both of these effects on the T_m values of the DNA duplexes, where the counterion condensation effects was noted to outcompete the screening effect. Details of these effects in terms of the pressure parameters are being communicated.

Conclusion and futuristic ideas

To summarize, the correlations exposed in the foregoing discussion are significant in many ways. First, the reaction rates and their spectacular enhancement in salt-solvent media can be quantified by a single concept based upon internal pressure and volume changes in reactions. This achievement which is still in the state of infancy needs rigorous testing. Secondly, a plausible explanation can be thought over for obviation of external ultrahigh pressure conditions during the synthesis of a compound. These two major achievements may eventually lead to the preparation of a selection software, which could be of potential use in recommending the reaction conditions to some extent. Thirdly, an alternate explanation is now available to describe the thermal stabilities of DNA duplexes at different ionic concentrations both at ambient and elevated pressures. Fourthly, since the analysis of thermal stabilities of DNA molecules is a radical departure from the presently-available explanations, there exists a new possibility to examine the physical etiology in sea water.

A brief account of the work discussed above, however, does not answer several important questions that a chemist or biochemist is faced with. An issue of both theoretical and industrial importance, which warrants urgent attention of the physico-organic chemists, is to

quantify the kinetic profiles of organic reactions of different types in terms of molecular interactions. Effect of additives, which are observed to alter the reaction rates and stereoselectivities of the products, is being currently investigated in detail.

How do the electrostatic forces or ion-water interactions govern the behaviour and biological functions of a single DNA strand and a triplex? How can we learn *a priori* about the activation parameters for duplex and triplex formation just from the knowledge of available information on the single DNA strands? A systematic study of duplexes made up of Peptide Nucleic Acid (PNA) and DNA as PNA-DNA for their thermal stability looks promising for designing of certain drug molecules following the concept of anti-sense technology. Calculations on these duplexes are yielding some exciting insights into the thermodynamics of nucleic acids, the results of which will be reported elsewhere.

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RESEARCH ARTICLE

Electrical resistivity imaging of Mohand Anticline of Siwalik range: A tectonic appraisal

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The results of a pilot resistivity sounding experiment undertaken in the Siwalik belt and contiguous Indo-Gangetic Foredeep (IGF) are presented and their lithotectonic significance is discussed akin to the Mohand anticlinal structure. The inferred large lateral resistivity contrast near Ganeshpur delineates the concealed Mohand thrust. The observed resistivity doublet is compatible with the transition in the clast-size and clast-matrix ratio of conglomerate

to sandstone. This correspondence provides strong geophysical support to the proposed four stage evolutionary model of the Mohand Siwalik basin on the basis of the facies association and palaeocurrent data. Cyclic pattern of resistivity distribution seen in the Mohand-Sherpur sector of IGF is considered to manifest episodic character of tectonic events in the Himalayan orogeny.

THE Indian Institute of Geomagnetism has been carrying out the natural source electromagnetic investigations in the Himalayas and the adjoining shield region¹⁻³. These studies, employing magnetovariational and magnetotelluric techniques, image the deep crustal structures in terms of electrical conductivity (inverse of resistivity) distribution. The strong dependence of conductivity on

temperature prevailing at deeper depths and the fluid content released and remobilized by the metamorphic and tectonic processes, makes these investigations a sensitive pointer of the geodynamic process controlling seismic and tectonic activity in the collision regime. These visualizations are well illustrated by the noted correlation of the mapped Trans-Himalayan and Garh-