

## Ionic solutions and their pivotal roles in organic and biological systems

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**Abstract:** Ionic solutions are known to affect the static and dynamic behaviour of organic and biological systems through ion-solvent interactions. With respect to organic systems, spectacular enhancement in both the reaction rates and stereoselectivities in the Diels-Alder (D-A) reactions are noted in presence of ionic solvent media. Several D-A reactions otherwise were not possible or were very sluggish. Application of ultrahigh external pressure could be obviated by using the ionic solutions. In the case of biological systems, the thermal stabilities of the DNA duplexes have been noted to vary with the type of counterions available in system. Variations in the rates of D-A reactions and thermal stability of DNA duplexes in ionic solutions have been discussed in terms of internal pressure, a ion-solvent interaction quantity and a volume parameter. Useful correlations are noted between internal pressure and reaction rates of the D-A reactions. More significantly, the correlations exposed in connection with the thermal stability of DNA duplexes are simple, thought-provoking and present radical departure from our current understanding on the ion-DNA interactions.

### INTRODUCTION

A large number of phenomena pertain to and are conducted in liquid phase involving ionic species (1). Millions of years ago, Mother Nature discovered the secrets of water molecule in different biological and other natural processes. Sea-water present in the oceans, a striking example from Nature, is a multicomponent salt solution reflecting the distant marine origin of life on earth together with the composition of physiological fluids. The ionic solutions play roles in several industrial and geological processes in addition to their deep impact on the biological molecules, in general. This enormous power of ionic solutions is based on the interactions of ion with solvent. In this work, we present some interesting results with far-reaching implications on the application of ion-solvent (i-s) interactions on organic reactions and thermal stability of DNA duplexes.

### ION-SOLVENT INTERACTIONS

In general, cohesion among molecules in the liquid phase results from intermolecular forces. These forces 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 (2). A liquid undergoing a small, isothermal 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 as internal pressure ( $P_i$ ) of a liquid and is supported by the equation of state. Internal pressure increases upon the addition of some solutes like NaCl, KCl, etc. and decreases by salts like of guanidinium. This change in internal pressure upon addition of ionic solute is given by  $\Delta P_i$  or  $P_{i,\text{eff}} = P_i - P^0$ , where  $P^0$  is  $P_i$  of pure solvent. 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 mainly depends on the charge, radius of an ion, and the changes in local dielectric constant around the ion (4). The ES effect can be described in terms of internal pressure, which can be estimated from the experimental quantities by the expression  $P_i = \alpha T / \beta$ ; where  $\alpha$  and  $\beta$  are the coefficient of thermal expansion and isothermal compressibility, respectively at a temperature  $T$ . Both  $\alpha$  and  $\beta$  can be measured from experiments on volumetric properties or by theoretical methods (5,6) to within  $\pm 3\%$ , when compared to experimental data. In Fig. 1 are plotted the  $\Delta P_i$  values for some salts in solvents as a function of the salt concentration,  $M$ . One can easily note the high internal pressures in the plots.

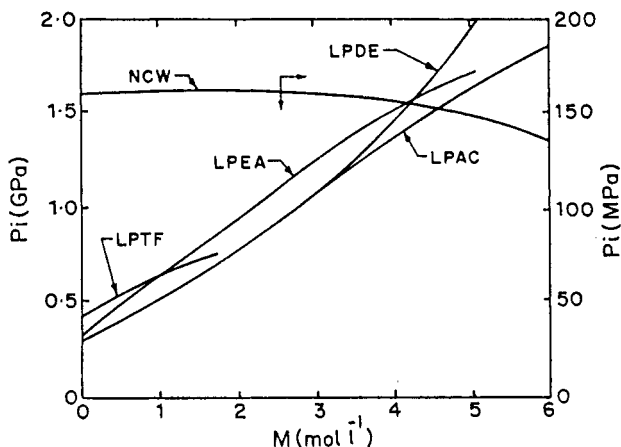


Fig. 1:  $P_i$  as a function of salt concentration Lithium perchlorate in acetone(AC),ethylacetate(EA), diethyether (DE), tetrahydrofuran (TF)and sodium perchlorate in water (NPW)

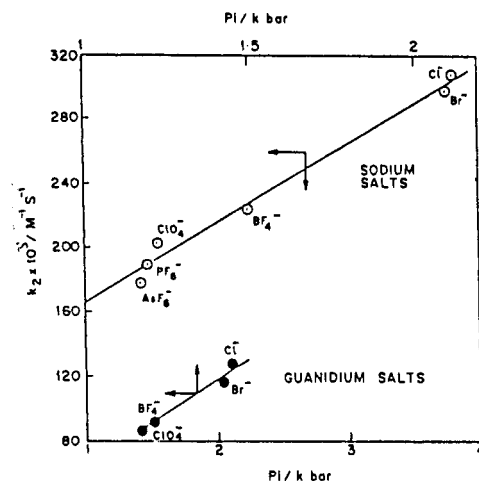


Fig. 2: Rate constant,  $k$  versus  $P_i$  for reaction of N-methylmaleimide and anthracene-9-carbinol in the sodium and salt solutions (ref. 11)

## I-S INTERACTIONS AND KINETIC PROFILES OF ORGANIC REACTIONS

Diels-Alder (D-A) reactions occupy a significant place in the synthetic organic chemistry. Several years ago, Rideout and Breslow (7) demonstrated an exciting outcome of using ionic solutes as reaction media by reporting impressive enhancement in their reaction rates by several order of magnitudes in the cases where the reaction in other solvents was either not possible or very sluggish. Stereoselectivities in the reaction products (endo/exo ratios) could be altered to some extent. The use of an ionic reaction medium i.e. lithium perchlorate - diethyl ether (LDPE) led to the synthesis of cantharidin with better yields at atmospheric pressure (8) as compared to the conventional method operating at 16-18 kbar or 1.6-1.8 GPa (9). It is seen that salts like LiCl, NaCl, etc. in water enhance the reaction rates, while some other salts like guanidinium chloride retard the reaction rates. Examples of some D-A reactions in aqueous as well as in nonaqueous solvents with ionic solutes are mentioned elsewhere (8). Rate enhancement in aqueous ionic media has been ascribed to several effects like hydrophobic interactions, micellar catalysis, hydrogen bonding and solvent effects and Lewis acid catalysis (10). In view of controversial explanations on the origin of forces responsible for such effects we have attempted to correlate and explain the variations in reaction rates with the help of internal pressure.

In the case of the reactions in aqueous reaction media, for example, a simple reaction of N-ethyl maleimide and anthracene-9-carbinol shows enhancement in rates in presence of aqueous NaCl, NaBr, etc. (11) and decrease by guanidinium salt. An examination of the  $P_i$  values in such salts show that those

reaction media which enhance the rates, also exhibit the increase in internal pressure. Similar is true for the decrease in the rate reactions. It is, thus, possible to correlate the rates with internal pressure for different reactions. In Fig. 2 are shown the reaction rates,  $k$  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 noted by us indicate that the internal pressure can be used as a strong correlating parameter in the kinetic profiles of the reactions (12). In fact, the arrangement of water molecules is drastically changed in the presence of salts (1) affecting 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 hydrophobicity (10). 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 salts. In a similar fashion one can understand the effect of anionic sizes on the reaction rates.

In the D-A reactions with salt-nonaqueous solvent (e.g. lithium salts with DE, AC, EA, etc.) the reaction rates and stereoselectivities can be correlated using the activation volume,  $\Delta V^\ddagger$  and  $P_i$  values (13,14).  $\Delta V^\ddagger$  is defined as the difference of reactant partial volumes and the volume of the transition state. The D-A reactions are accompanied with negative  $\Delta V^\ddagger$ . As seen in Fig. 1 lithium salts with the above organic solvents yield vary high  $P_i$  values. Thus, it is easy to understand the rate enhancement of the D-A reactions, as higher  $P_i$  is made available to reaction having negative  $\Delta V^\ddagger$ . For example, reaction of 2,3-dimethylbutadiene with 1,4-naphthoquinone ( $\Delta V^\ddagger = -21.9 \text{ cm}^3 \text{ mol}^{-1}$ ) in presence of LPAC by about 50 times faster, when  $\Delta P_i$  increases from 100 to 600 MPa). Based on the analysis of the kinetic data on the D-A reactions, it is possible to define the salting-in and -out zones for demonstrating the effects of salts.

With this development in reaction kinetics, it was intriguing to explore the possibility of explaining melting behaviour in nucleic acids as melting of a DNA duplex involves a dissociation process or reaction in an ionic environment. On the other hand, the formation of a DNA duplex in ionic environment is an association reaction.

## I-S INTERACTIONS AND THERMAL STABILITY OF DNA DUPLEXES

In the present work, we shall focus on the thermal stability of the DNA duplexes in aqueous ionic media. 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 general (15,16). In fact the very formation of the DNA duplex from its complementary single strands is governed by these parameters. The DNA molecules are heavily hydrated and thus, effect of water on the stabilization of DNA helices via hydration assumes a vital role in understanding the energetics of DNA.

Effect of ions on the thermal stability of DNA has been discussed by Manning (17) in the form of counterion condensation theory (C-CT) where the statistically averaged properties of mobile cloud of counterions tend to neutralize the net electrostatic charge of the nucleic acid. Later, Record et. al. (15) utilized the C-CT for developing operational equations for the analysis of the  $T_m$  data. Several theoretical methods, like the Debye-Huckel, Gouy-Chapman, Poisson, Poisson-Boltzmann equations, Monte-Carlo simulations, Molecular, Brownian dynamics, etc. (18) have been employed to understand the electrostatic interactions present in the DNA duplex. Due to apparent failure of these methods to deal with concentrated salt solutions, we have preferred to look for an alternate method to correlate  $T_m$  with the process of electrostriction (21). Similar to the above organic reactions the volume change  $\Delta V$ , accompanying the transition process is useful in the present analysis of thermal stability of a DNA helix. The magnitude and sign of  $\Delta V$  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 (16,19). The possible reasons for the positive  $\Delta V$  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 effects (20-22). These effects give rise to negative and positive  $\Delta V$  in the cases of formation of a duplex and transition of a duplex into two complementary strands, respectively. We can now examine the issue of thermal stability of a DNA duplex in different situations using  $\Delta P_i$  and  $\Delta V$  parameters.

### T<sub>m</sub>-Enhancing and Decreasing Behaviour

Higher concentrations of NaCl induce greater stabilization of the duplex (higher T<sub>m</sub>), as compared to lower T<sub>m</sub> noted at low concentrations of the salt. Since internal pressure of water increases upon the addition of NaCl, the aqueous ionic environment remains under high pressure conditions enhancing 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. For instance, in the case of Poly(dA).Poly(dT), the  $\Delta V$  values are noted to vary from 2.60 to 7.81 cm<sup>3</sup> mol<sup>-1</sup> in the range of NaCl concentration from 0.02 to 1 M (23). The  $\Delta P_i$  value at 1 M is much higher than that at 0.02 M. This high  $P_i$  value suppresses the process of transition of a DNA duplex to single strands, since  $\Delta V$  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<sub>m</sub> value which increases with the addition of NaCl (20,23). In other words, one may state that the ES or the enhanced  $\Delta P_i$  resulting from the salt concentrations controls the T<sub>m</sub> of a duplex DNA. To illustrate these variations, the relationship between T<sub>m</sub> and  $\Delta P_i$  are shown in Fig. 3 for the transitions of several duplexes of Poly(dA).Poly(dT) or the polymers containing A.U base pairs in different NaCl concentrations, ranging from low to high, at atmospheric pressure. Similar trend is observed in the case of duplexes with high G.C contents. In the case of higher valent salts like MgCl<sub>2</sub>, higher values of  $\Delta P_i$  are obtained as compared to those for NaCl at corresponding concentration.

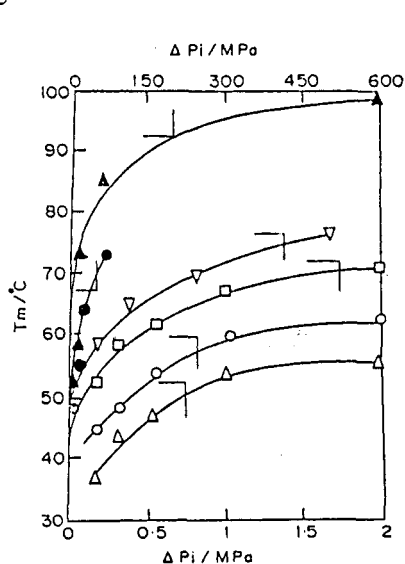


Fig. 3: T<sub>m</sub> vs.  $\Delta P_i$  for the double helix transitions  
 $\Delta$ Poly(dA).Poly(dT); O Poly[d(A-T)] ref. 20;  
 Poly(dA).Poly(dT);  
 Poly(rA).Poly(dT);  
 Poly(rA).Poly(dU); Poly(rA).Poly(dU)

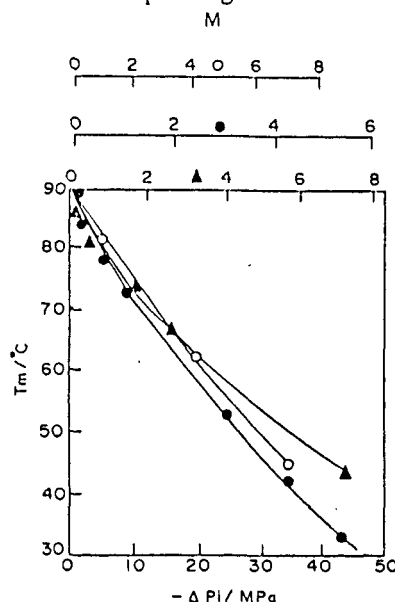


Fig. 4: Plot of T<sub>m</sub> against  $\Delta P_i$  for denaturation of DNA duplex in the presence of salts  $\Delta$  NaClO<sub>4</sub> O KCNS O CF<sub>3</sub>COONa Ref.(24)

Contrary to the above observation, the DNA duplex is destabilized in presence of some other salts, such as acetates, perchlorates, etc. (24). Explanation of this experimental observation can be found out using the concept of negative changes in  $\Delta P_i$  upon the addition of salts. The plots between T<sub>m</sub> and  $\Delta P_i$  shown in Fig. 4 are smooth upto high concentrations of salts and up to a temperature drop in T<sub>m</sub> by 60°C. In such cases, the negative  $\Delta P_i$  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. In the case of the  $T_m$  reducing salts, a situation of internal pressure drop over pure water is created due to ion-solvent interactions, which when coupled with positive  $\Delta V$  values, tends to destabilise the duplex, with the consequent lowering of  $T_m$ . This destabilizing effect continues to dominate with the increasing salt concentration. The anionic size of the salts influence the internal pressure drop over pure water, which together with positive  $\Delta V$  values, tends to destabilise the DNA duplex, with the consequent lowering of  $T_m$ . This destabilizing effect continues to dominate with the increasing salt concentration. The anionic size of the salts influence the internal pressure drop in such situations. The  $T_m$  values for different duplexes at the same ionic concentration show variations. The  $\Delta V$  values for the duplex depend upon several parameters described earlier and therefore, the effect of a given NaCl concentration or the thermodynamic internal pressure can easily explain the differences in  $T_m$  values for different duplexes. For example, the  $\Delta V$  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  $\text{cm}^3 \text{mol}^{-1}$ , respectively (20). The internal pressure of the medium being the same ( $\sim 189 \text{ MPa}$ ) 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 ( $73.1^\circ\text{C}$ ) than that for Poly[d(A-T)] ( $64.3^\circ\text{C}$ ). The value of  $\Delta V$ , 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  $\Delta V$ , therefore should be credited due importance in the transition reactions.

### Pressure Effects

Increase in the  $T_m$  values of a DNA duplex on the application of external pressure (20) as a result in an increase in total internal pressure of the system at a given concentration of NaCl. 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:

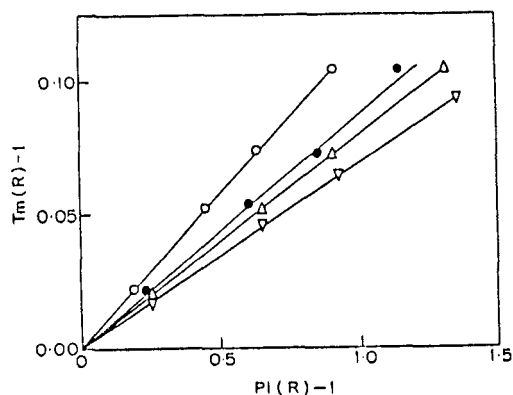


Fig.5 :  $T_m^R - 1$  vs.  $P_i^R - 1$  at various pressures in different M of NaCl  $\circ$  1 M;  $\circ$  0.2 M;  $\Delta$  500 mM;  $\nabla$  200mM Poly(dA).Poly(dT) ref. 20

$T_m^R - 1$ , (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  $T_m$ ) and plot them at several NaCl concentrations in the case of Poly(dA).Poly(dT). These plots (shown in Fig. 5)  $T_m^R - 1$  versus  $P_i^R - 1$  are linear at each NaCl concentration in the range from 200 mM to 1M. The extent of linearity up to 200 MPa with slopes of significant magnitude suggests the strong influence of  $P_i$  on  $T_m$ . Analogous results are noted for other DNA duplexes at several ionic concentrations under elevated external pressure conditions.

## CONCLUSION

To summarize, the reaction rates and their spectacular enhancement of the D-A reactions and thermal stability of the DNA duplexes in salt-solvent media can be quantified by a single concept based upon internal pressure and volume changes in reactions. In view of radical departure from the currently available explanations of thermal stabilities of DNA molecules, there exists a new possibility to examine the physical etiology in sea water. Several issues regarding the electrostatic forces or ion-water interactions governing the behaviour and biological functions of DNA molecule need to be addressed (25)? Our preliminary results on the variations of the ES effect in single complementary strands and duplex look promising, which will be published in near future.

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