in literature[8]. For Cu^{2+} discharge reaction from aqueous sodium sulphate, there is no $k_{s,a,\theta=0}$ value reported in literature, but from 1M KNO_3 solution, a value of $4{\cdot}50 \times 10^{-2}$ cm sec^{-1} has been reported[9] The present value for Cu^{2+} discharge reaction is found to be of the correct order. It is therefore concluded from the above discussions that SAS investigated here does not alter the mechanism of the three discharge reactions.

Aramata and Delahay[10] showed that for Zn^{2+} discharge reaction in the presence of *n*-amyl alcohol, the values of I° (corrected for diffused double-layer potential, ϕ_2) plotted against surface coverage (θ) of alcohol gave a 45° line for $\theta < 0{\cdot}50$, while at higher coverages, the corrected values of I° were lower than the values predicted by the 45° line. On the basis of this, they concluded that the inhibition of Zn^{2+} discharge reaction by *n*-amyl alcohol can be explained by a simple "blocking effect" (*i.e.*, reduction in the true area available for reaction due to the adsorbed SAS) at lower coverages. Inhibition by strongly adsorbed SAS and by SAS at high coverages is stronger than that expected by simple "blocking effect"[11]. An examination of the results of the present work depicted in Figs. 1–3 as a plot of $k_{s,t,\theta}$ versus θ, reveals that in the three systems studied, the plots deviate from 45° line. This suggests that the mechanism of inhibition of Cu^{2+}, Cd^{2+} and Zn^{2+} discharge reactions cannot be explained by a simple "blocking effect" and the observed variations in $k_{s,t,\theta}$ with θ cannot be accounted on the basis of the change in ζ_2 due to changes in surface charge density (q) with θ. Moreover, the contribution of oriented dipoles of various SAS used in the present work to ϕ_2 has been neglected. Such a contribution could be significant at high surface coverages[12]. The other factor which should be taken into account is the surface coverage (θ) which is generally obtained from Γ/Γ_s, where Γ_s is the saturated surface excess of the SAS. This definition of θ implies two main assumptions, namely, monolayer formation of SAS and constant covered area per molecule, which may not be justified at high surface coverages[13].

January 5, 1981.

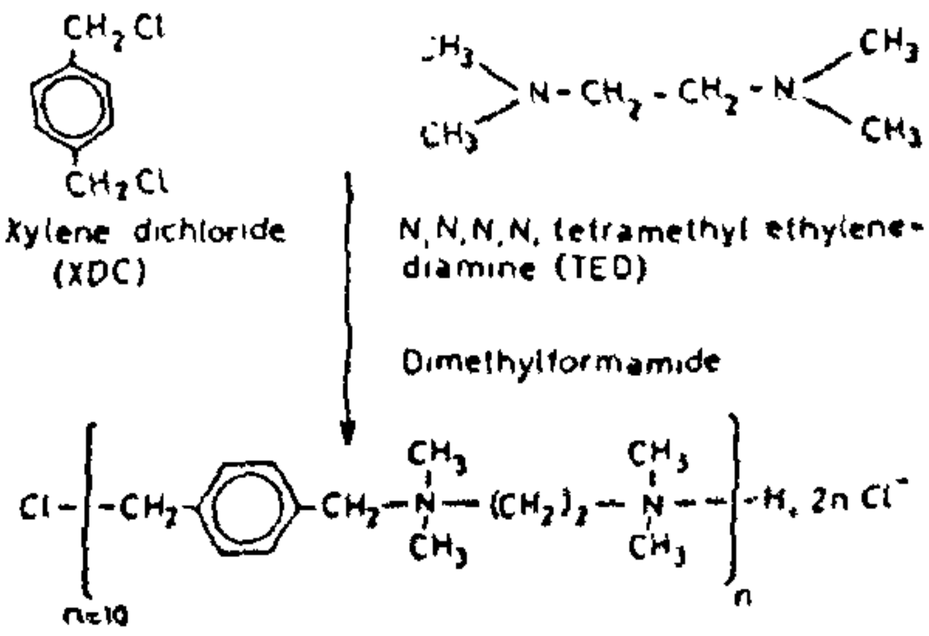
1. Venkatachalam, C. S., Rajagopalan, S. R. and Sastri, M. V. C., *Electrochim. Acta*, 1980, **25**, 695.
2. —, — and —, *Ibid.* (in press).
3. Satyanarayana, S., *J. Electroanal. Chem.*, 1965, **10**, 119.
4. Venkatachalam, C. S., Rajagopalan, S. R. and Sastri, M. V. C., *Trans. S.A.E.S.T.*, 1978, **13**, 109.
5. — and —, *Proc. Indian Acad. Sci.*, 1968, **67A**, 115.
6. —, *Ph.D. Thesis*, 1974,
7. Nurnberg, H. W. and von Stackelberg, M., *J. Electroanal. Chem*, 1961, **2**, 350.
8. Randle, J. E. B., *Progress in Polarography*, ed. by Zuman, John Wiley, 1962, **1**, Chapter 6.
9. Tanaka, N. and Tamamushi, R., *Electrochim. Acta*, 1964, **9**, 963.
10. Aramata, A. and Delahay, P., *J. Phys. Chem.*, 1964, **68**, 880.
11. Delahay, P., *Double Layer and Electrode Kinetics*, Interscience, 1966, p. 233.
12. Satyanarayana, S., *J. Electroanal. Chem.*, 1974, **51**, 195.
13. Frumkin, A. N. and Damaskin, B. B., *Modern Aspects of Electrochemistry*, 1965, **3**, 149.

INHIBITION OF ALPHA AMYLASE BY POLYCATIONIC IONEN

V. H. Mulimani and R. A. Day*
Department of Chemistry
Division of Biochemistry, Karnatak University
Dharwad 580 003, India
*Department of Chemistry, University of Cincinnati
Cincinnati, Ohio 45221, USA

Polycationic ionens are linear and contain aromatic aliphatic and quaternary salt units. The simpler ionen polymers are structurally related to spermine, spermidine and their methylated derivatives. It is well established that various types of polycations interact strongly with nucleic acids which are polyanions[1–3]. High molecular weight quaternary ammonium compounds, cationic dyes and sodium lauryl sulphate *inactivate alpha amylase of fungal and pancreatic* origin[4]. The effect of polycationic ionen on alpha amylase is not known; its effect on alpha amylase is *now investigated*.

The following polycationic ionen was synthesised according to the procedure of Rembaum and coworkers[5,6].



Chloro-poly (ethylene-p-phenylenemethylaminoethylene dimethylamino dichloride)

Alpha amylase (Bacillus subtilis temp. resistant) was obtained from Calbiochem, San Diego, California,

USA. The enzyme activity was measured according to the method of Bernfeld using starch as substrate[7]. The reaction mixture contained 0·5 ml of substrate and different concentrations of ionen and incubated for exactly three minutes at room temperature (27° C). The reaction was stopped by adding 1 ml of 3,5-dinitrosalicylic acid. The tubes are heated in boiling water bath exactly for 5 minutes, cooled and then 10 ml of water added. Absorbance of each solution containing the brown reduction product was measured at 540 nm using Carl-Zeiss spectrocalorimeter. A blank was prepared in the same manner without adding enzyme. A calibration curve established with maltose was used to convert the reading of optical density into miligram of maltose. The nature of alpha amylase inhibition by ionen was evaluated according to the method of Lineweaver Burk[8]. Initial velocity was determined at varying concentration of starch at fixed concentration of ionen in the presence of 0 01 M phosphate buffer containing 0·006 M sodium chloride. Enzyme concentration was 100 μg/ml.

Alpha amylase activity is expressed in terms of micromole of reducing sugar liberated by 1 ml of enzyme per minute at room temperature.

In Fig. 1, it is indicated that polycationic ionen decreases the alpha amylase activity. With increase in concentration of ionen there is corresponding decrease in alpha amylase activity reaching constant at 8 mM. The type of inhibition by ionen was found to be non-competitive, *i.e.*, the inhibitor had no effect on K_m (Fig. 2).

The effect of high molecular quaternary ammonium salts on alpha amylase was studied by Pomeranz[4]. The electrostatic component of the interaction between quaternary polycationic ionen and alpha amylase is responsible for enzyme inactivation. Ionen contains positive quaternary nitrogen group which presumably interacts electrostatically with essential anionic site of enzyme. Positive quaternary nitrogen group is specifically adapted to the substrate binding region of the alpha amylase by electrostatic interaction. Hydrophobicities in the domains of several ionen polymers were measured by utilising the properties of 1-anilino 8-naphthalene sulphonic acid (ANS) which is a fluorescent probe for hydrophobic region[9]. The affinity of the quaternary salts for the anionic sites of the enzyme has been shown to depend on the spatial arrangement of ionen and on the chemical structure, *i.e.*, presence of long chain alkyl group.

It is therefore feasible that mechanism of the polycationic ionen involves an electrostatic interaction between the polar groups and an interaction between the hydrophobic portion resulting in binding of large amount of ionen and profound denaturation of alpha amylase.

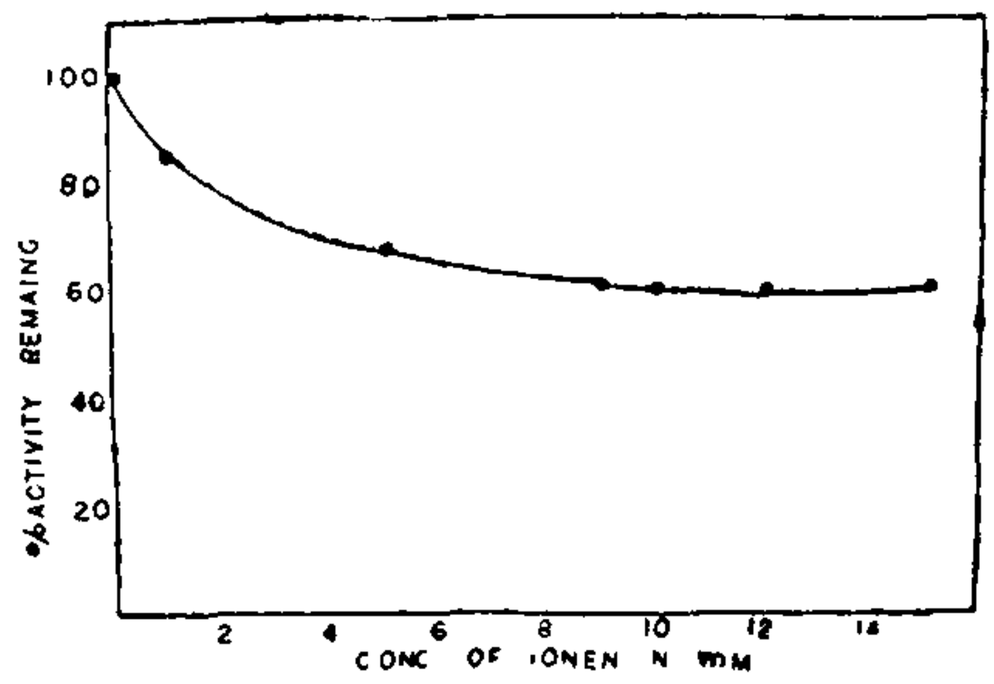


FIG. 1. Effect of polycationic ionen on alpha amylase activity.

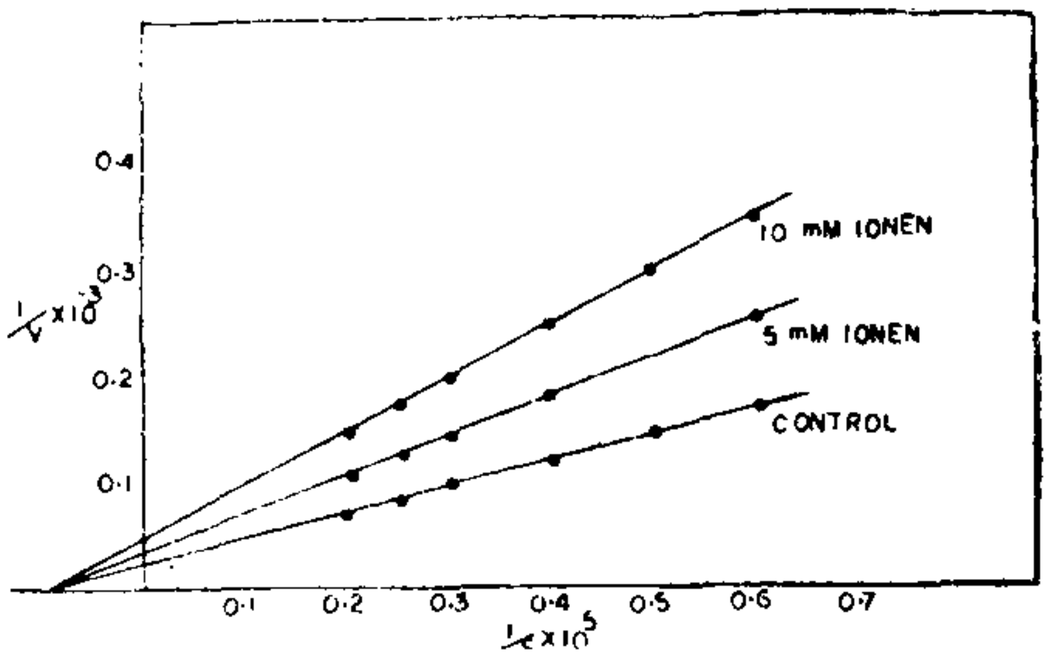


FIG. 2. Double reciprocal plot for action of ionen on alpha amylase.

The author expresses the sincere thanks to Professor and Head of Department of Chemistry, Karnatak University, Dharwar, for providing necessary laboratory facilities.

August 27, 1980.

1. Bhat, G., Roth, A. C. and Day, R. A., *Biopolymers*, 1977, **16**, 1713.
2. Day, R. A., Bhat, G., Lin, L. C., Roth, A. C., Mulimani, V. H. and Krueger, R. C., *Biochem. Biophys. Res. Commun.*, 1978, **84**, 969.
3. Mulimani, V. H. and Day, R. A., *Indian J. Biochem. Biophys.* (in press).
4. Pomeranz, Y., *Biochim. Biophys. Acta*, 1963, **73**, 105.
5. Rembaum, A., Baumgartner, W. and Eisenberg, A. J., *Polymer. Sci.*, 1968, **B6**, 159.
6. Noguchi, H. and Rembaum, A. J., *Ibid.*, 1969, **7**, 383.
7. Bernfeld, P., In *Methods in Enzymology*, S. P. Colowick and N. P. Kaplan (eds.), 1955, **1**, 149.
8. Linwear, H. and Burk, D. J., *J. Am. Chem. Soc.*, 1934, **75**, 3132.
9. Abe, K., Koida, M. and Tsuchida, E. J., *Polymer Sci.*, 1977, **15**, 2469.