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VISCOMETRIC STUDIES ON THE INTERACTION OF ANIONIC SURFACTANT WITH α – AMYLASE

P. ARORA[*] and R. P. SINGH[a]

Shriram Institute for Industrial Research, DELHI, INDIA
[a]D. A. V. College, MUZAFFARNAGAR (U.P.) INDIA

ABSTRACT

The viscometric studies on α–amylase-TEALS (triethanolamine lauryl sulphate) system were made and the effect of pH and temperature on viscosity behavior was investigated. The variation of viscosity with pH has been interpreted in terms of charged groups on the α–amylase surface. The intrinsic viscosity [η] of α-amylase in absence and presence of varying amounts of TEALS at different pH values was determined by plotting viscosity number vs. protein concentration in g/mL and then extrapolating to zero concentration of the α-amylase. With the help of intrinsic viscosity, molecular weight, hydrodynamic radius [Re], end to end root mean distance (r) and average molecular weight (M) of enzyme-surfactant system were also calculated at different temperatures and pH values.

Key words: Viscosity, Protein, Surfactant, Intrinsic viscosity, Temperature, pH.

INTRODUCTION

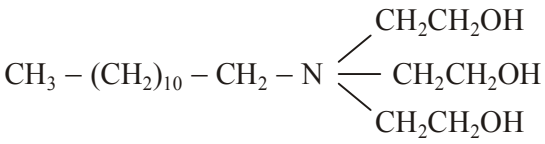
There are many methods to know the shape, size and end to end dimensions and other related topics in chemistry of electrolytes. The notable methods are viscometry, optical rotatory dispersion (ORD), circular dichroism (CD), sedimentation, nuclear magnetic resonance (NMR) etc. Out of the various methods used for studying the complexation and structural determinations, viscosity is the simplest and easily assemblable because viscosity is not only one of the most important measures of the gross conformation, but it reflects particle volume. The measurement of the viscosities of macromolecules needs relatively simple instrumentation. Due to the simplicity of this technique, the number of studies is quite large[1-3]. The concept of intrinsic viscosity has been used in deciding the physico-chemical and structural properties of polymeric systems.

Owing to the unique applicability of the flow technique, numerous references are

[*]Author for correspondence; Ph.: +919412485902, 0131-2443699; E-mail: rp_dav@rediffmail.com

available on this problem in the existing literature. Many workers have studied the thermal denaturation[4], effect of salt and temperature[5] as well as effect of denaturants[6,7] on protein conformations using this technique. It has also been employed to study the detergent protein interactions[8,9] and also to follow the denaturation of proteins by detergents[10,11], urea and other organic compounds[12]. The dissociation of thyroglobulin into subunits[13] and structural changes in bovine serum albumins (BSA) by sodium dodecyl sulphate[14] have also been investigated by this method. Besides surfactant–protein interaction, the effect of alcohols on the conformation of proteins has also been followed viscometrically[15-20]. Viscosity measurements have also been used to identify the subunits (α, β, γ) in casein above pH 10.50[21]. The effect of cationic surfactants on the intrinsic viscosity of transfusion gelatine[22,23] and that of SDS on the viscosity of casein[24] has also studied. The interaction of skin keratin with detergents and the mechanical behaviour of β–lactoglobulin with tween-20 have been studied by dynamic surface tension and surface shear rheological measurements[25,26]. The interaction of soy protein with an anionic SDS and a nonionic surfactant (tween-20) were studied as a function of pH and viscosity[27]. The interactions of bovine serum albumin (BSA) with anionic surfactant sodium dodeyl sulfonate and cationic surfactant decyltricetylammonium bromide were investigated by viscosity measurements[28]. The interaction of sodium caseinate with likely charged anionic surfactant CITREM and the effect of water soluble, globular protein papain with cationic surfactant dodecyl dimethylethyl ammonium bromide (DDAB) in aqueous solution were investigated by using viscosity and other methods[29,30]. The interaction of α–amylase with n-alkyl ammonium bromide and of CTAB was investigated at various experimental conditions[31,32].

A literature survey indicates that very little work has been done so far on the interaction of triethanolamine lauryl sulphate (TEALS) with proteins[33-35]. With a view to extend the present knowledge of rheological behaviour of protein-surfactant systems, the viscometric studies of α-amylase-TEALS, was planned. This paper deals with the results of α-amylase-TEALS system as determined viscometrically. The effect of pH and surfactant concentrations on the viscosity behaviour has been discussed.



EXPERIMENTAL

Reagents and solutions

α–Amylase was purchased from Sigma–Aldrich Chemicals Ltd. and its stock solutions

were prepared by dissolving known weight of double distilled water. Triethanolamine lauryl sulphate (TEALS) was obtained from Hico Products (Pvt.) Ltd. India and its purity was evaluated by a standard method. The buffers were prepared from reagent grade chemicals. Potassium chloride (BDH) solution was used to maintain the ionic strength of reaction mixtures.

Viscosity measurements

These measurements were carried out by means of an Ostwald viscometer of relatively long capillary at different temperatures. α–Amylase and TEALS stock solutions were centrifuged to remove particulate matter. The viscosity values were calculated by the relation -

$$\eta_{rel} = \frac{\eta}{\eta_o} = \frac{t}{t_o} \times \frac{\rho}{\rho_o} \quad \ldots(1)$$

Where η_{rel} is the relative viscosity, t and ρ are the flow time and density of solution, t_o and ρ_o are the time and density of solvent (water), respectively.

Procedure

The following sets of solutions were prepared for the measurements of viscosity-

(i) A fixed amount of α–amylase (2.0 g/L) was added with varying amounts of HCl or KOH. The total volume was kept upto 15.0 mL and the pH and viscosity were recorded in each case. Same compositions were prepared with TEALS (0.001 mole/L) and pH and viscosity were noted.

(ii) To a fixed amount of α–amylase (2.0 g/L), different amounts of TEALS were added keeping the total volume 15.0 mL and the viscosity of this set was recorded at different pH values.

(iii) Fixed α–amylase and surfactant (TEALS) having the same initial pH values were titrated and viscosity values were recorded.

(iv) Varying amounts of α–amylase were taken along with different amounts of surfactant (TEALS) and the results are plotted as reduced viscosity (viscosity number) vs. protein concentration to determine the intrinsic viscosity. The pH was adjusted with different range of buffer solutions.

Treatment of the viscosity data

The viscosity values are calculated by the following relations –

$$\eta_{rel} = \eta/\eta_o = \text{Relative viscosity} \quad \ldots(2)$$

Where η = Viscosity of solution and η_o = viscosity of solvent.

$\eta_{sp} = \eta_{rel}^{-1}$ = Specific viscosity; η_{sp}/C = Viscosity number = Reduced viscosity and η_{sp}/C vs. C at zero concentration = Intrinsic viscosity

The quantity intrinsic viscosity can also be used to determine the molecular weight of protein-surfactant complex by the following relations:

$$[\eta] = 8.69 \times 10^{-5} M_n^{-0.76} \text{ (In benzene)} \quad \ldots(3)$$

$$[\eta] = 8.69 \times 10^{-5} M_n^{-1.0} \text{ (In water)} \quad \ldots(4)$$

Where M_n is average molecular weight of the polymer and polymer-surfactant complex. The slope of log [η] vs. log molecular weight will give an idea about the nature of the polymer. The hydrodynamic radius (Re) was calculated according to the following relation -

$$Re = \left[\frac{3M}{10\pi N}[\eta]\right]^{1/3} \quad \ldots(5)$$

Where M = Molecular weight of polymer and N = Avogadro number. It is also used in calculating the end-to-end root mean square distance $(r^2)^{1/2}$ as follows -

$$(r^2)^{1/2} = (M.[\eta]/\varphi)^{1/2} \quad \ldots(6)$$

Where φ is shape factor, which is independent of the solvent.

RESULTS AND DISCUSSION

Viscosity is one of the important tools to study the conformational and rheological changes in proteins/enzymes during their interaction with ionic surfactants. The results of the present study are shown in the form of well defined curves to visualize the association of surfactant to the reactive sites of α-amylase as well as to elucidate the changes produced in the shape and dissymmetry of the macromolecular system. The system under investigation covers the wide pH range in view to elucidate the mechanism of surfactant-α-amylase system as influenced by pH.

Effect of pH on relative viscosity of α-amylase-TEALS system

The variations in the relative viscosity (η_{rel}) values of α-amylase in the absence or presence of TEALS are shown in Fig. 1, as a function of pH. The increase in relative viscosity with the rise in pH appears to be due to successive neutralization of the various acidic groups which are known to ionize at different pH values[36]. The formation of increasing amounts of negatively charged enzymate ions;

$$(\bar{O}OC - \underset{\displaystyle R}{\underset{|}{C}}H - \overset{+}{N}H_3 + OH^- \longrightarrow \bar{O}OC - \underset{\displaystyle R}{\underset{|}{C}}H - NH_2 + H_2O)$$

results in a greater stretching and uncoiling of the polypeptide chains at the same time, the interaction of the ions with the water dipoles will cause progressive increase in the degree of hydration. Both these effects tend to increase the viscosity of the system. The decrease in viscosity at higher pH may be due to degradation and denaturation effects, and partially due to the presence of unreacted caustic alkali, which itself has a lower viscosity. In lower pH range, the viscosity shows a sharp rise, this is due to the formation of increasing amounts of positively charged ions in the pH range 2-3.

$$(\bar{O}OC - \underset{\displaystyle R}{\underset{|}{C}}H - \overset{+}{N}H_3 + \overset{+}{H} \longrightarrow HOOC - \underset{\displaystyle R}{\underset{|}{C}}H - \overset{+}{N}H_3)$$

On addition of TEALS, the isoelectric point shifts toward lower pH, which may be due to substraction of the positively charged centres of enzyme as a result of surfactant interaction. The smaller relative viscosity of α-amylase-TEALS mixture in lower pH indicates the contraction of enzyme-surfactant complex. However, the viscosity values are found to be very high in enzyme-surfactant mixtures than enzyme alone in lower and higher pH. This may be attributed to the extensive solvation and unfolding of the enzyme in presence of surfactant. It is evident from Fig. 1 that the viscosity is minimum at pH 5.2, which is the isoelectric point (IEP) of the α-amylase. At this point, the macromolecule of enzyme is probably in the coiled form on account of the attractive forces between balanced charges together with possible hydrogen bonding and other cohesive forces; while on both the sides of this pH, the macromolecule possesses a net overall charge, which may cause the molecule to extend itself by repulsion. However, in more acidic or basic solutions, the repulsive forces will be again reduced due to the increase in free ions in these mixtures and hence, viscosity decreases. On the addition of surfactant, the viscosity vs. pH curve is shifted on the upper side of the identical α-amylase curve. The higher viscosity of α-amylase-TEALS mixture has been attributed to protein unfolding as a result of solvation with surfactant.

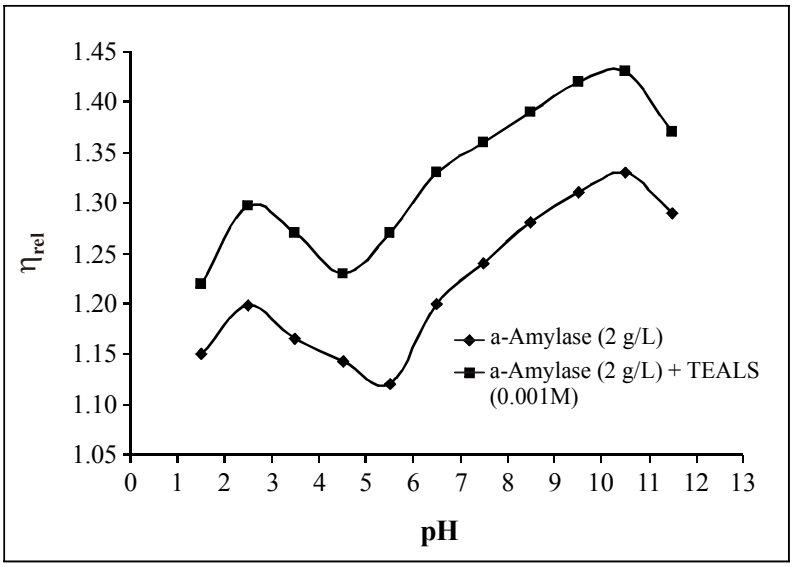


Fig. 1: Effect of pH on the relative viscosity of α-amylase in absence and presence of TEALS at 25°C in acidic pH range

The results of set (II) are shown in Figures 2 to 5 in the form of reduced viscosity (η_{red}) plotted against concentration of the added surfactant at varying pH values and temperatures. These figures go to show that the binding of surfactant to α-amylase depends upon the pH values of the mixed solutions. A decrease in viscosity is seen at the specified pH values upon the addition of very small quantity of surfactant until precipitation takes place. Precipitation regions are represented by dotted lines in curves. The effect of temperature is only to alter the viscosity, but it does not affect much the stoichiometry of the combination. On addition of more TEALS, the precipitate initially formed redissolved and an increased viscosity is obtained. The α-amylase molecule, which existed in the expanded state at the acidic side of IEP, gets (coiled) with contracted the progressive combination of TEALS ions and therefore, the viscosity decreases. This alteration in viscosity has been explained on the basis of the contraction of macromolecule by earlier workers[36]. The addition of more quantity of the surfactant at any specified pH, a stage is achieved, when precipitation occurs due to the orientation of the hydrophobic tail (CH_3- $(CH_2)_{10}$ -CH_2-) of TEALS in solution and TEALS-α-amylase complex is sedimented. With the addition of more TEALS, a second adsorption layer is formed, which makes TEALS-α-amylase complex hydrophilic and therefore, the precipitate gets solubilized; hence, the viscosity increases. It may be again noticed that the dispersed protein-surfactant complex exhibited nearly the Newtonian flow, which was non-Newtonian before the commencement of precipitation (Fig. 2-5).

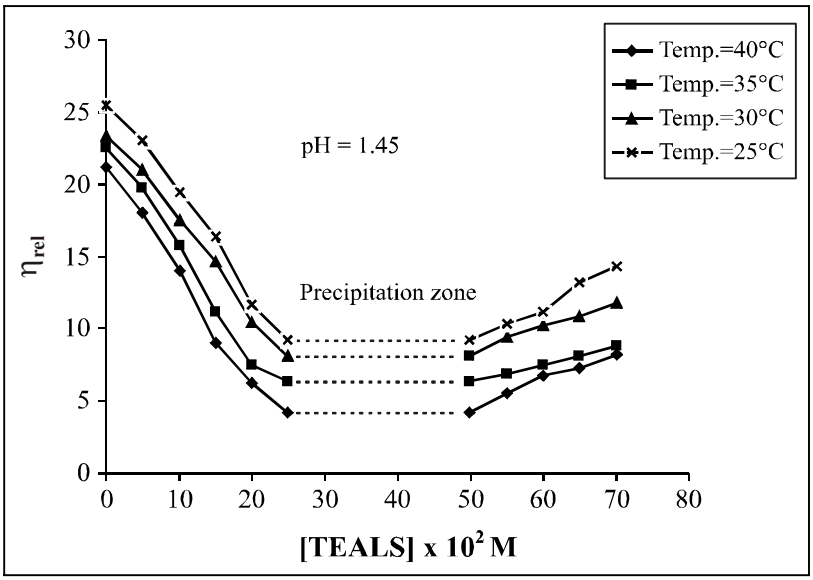


Fig. 2: Plots of TEALS conc. vs. relative viscosity of α-amylase in acidic pH range at different temperatures

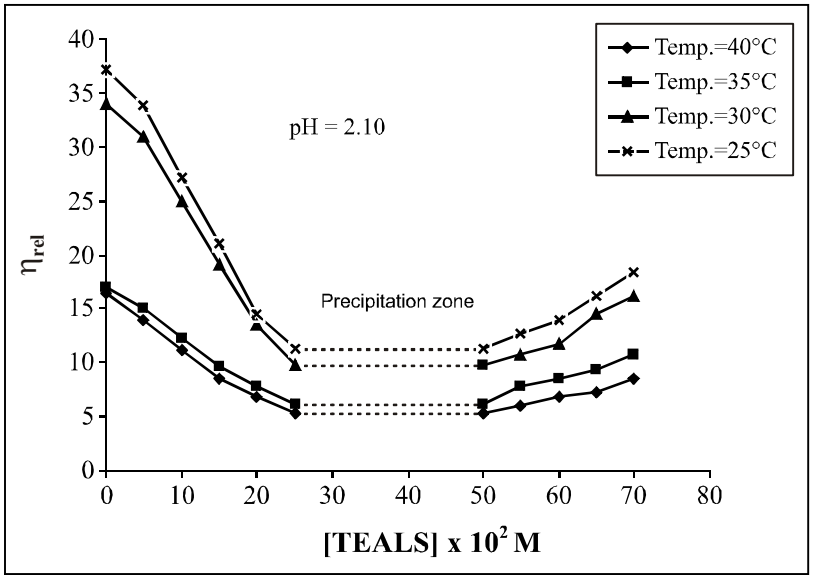


Fig. 3: Plots of TEALS conc. vs. relative viscosity of α-amylase in acidic pH range at different temperatures

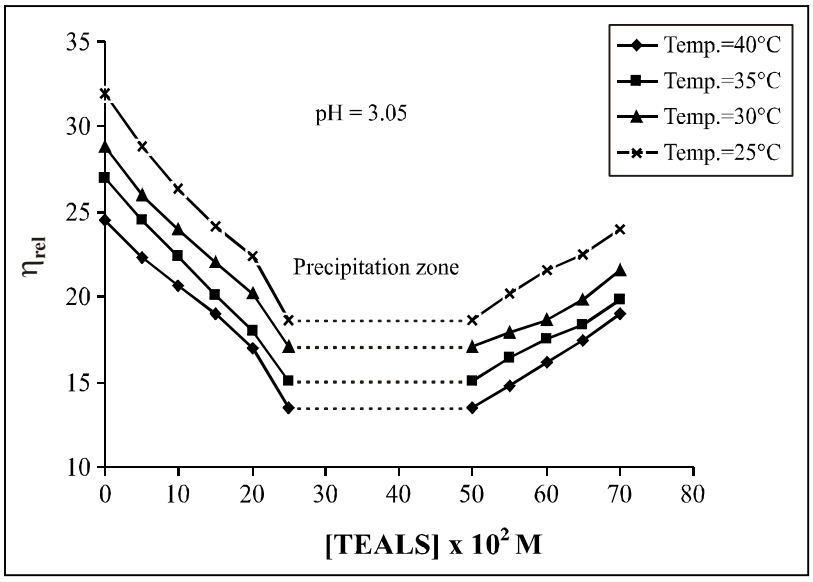


Fig. 4: Plots of TEALS conc. vs. relative viscosity of α-amylase in acidic pH range at different temperatures

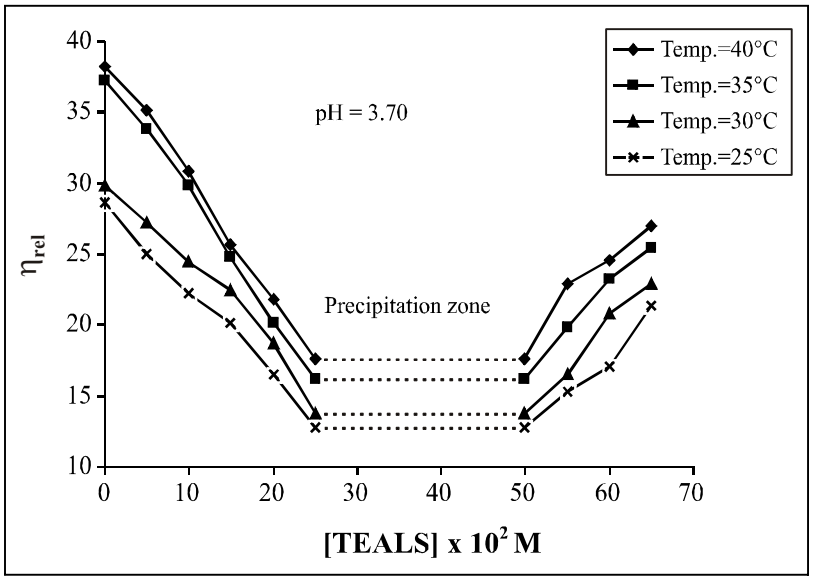


Fig. 5: Plots of TEALS conc. vs. relative viscosity of α-amylase in acidic pH range at different temperatures

Above isoelectric point of the α-amylase, the general nature of viscosity vs. surfactant concentration curve is seen to be quite different from those obtained below isoelectric point of the α-amylase. At all pH values i.e. from 6.4 to 11.40 as given in Figure 6, the relative viscosity increases on addition of even a small quantity of the anionic surfactant, attains maximum and then decreases regularly and becomes constant at higher concentration of TEALS. The point of maximum viscosity is shifted towards lower ratio of surfactant; to α-amylase as the pH of mixed solution becomes greater. The enhanced viscosity and its sequence with increasing pH is an indication of decreasing positive charge and increasing negative charge on α-amylase and the subsequent rise of viscosity could be either due to repulsion or uncoiling or due to the combined effect of both these factors. From these observations, it is evident that uncoiling is greater, when both α-amylase and the surfactant possess the same sign of particle charge. Perhaps the anionic surfactant combined with α-amylase molecule to some extent to give it net charge and consequently, forced the macromolecule to extend itself. The occurrence of maxima and decrease of viscosity with rising TEALS concentration is probably due to the screening effect of counter ions.

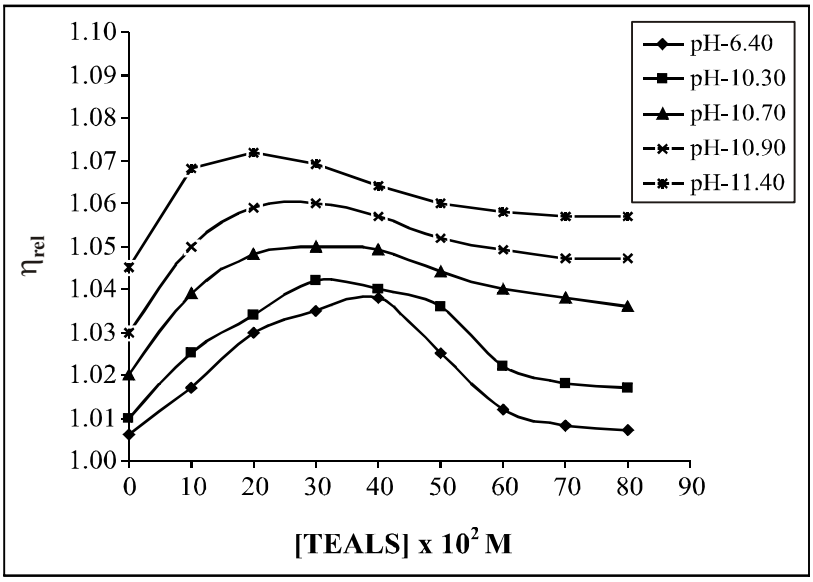


Fig. 6: Plots of TEALS conc. vs. relative viscosity of α-amylase in acidic and basic pH range at different pH values

Effect of pH and concentration of TEALS on intrinsic viscosity of α-amylase

The intrinsic viscosity was found to increase with rising concentrations of TEALS (g/L) as well as pH of the mixed solutions (Tables 1-4). It may be argued that in general the

molecular shape of polymer is directly proportional to hydrogen-ion concentration and the quantity of surfactant present in the mixed solutions. The lesser value of intrinsic viscosity at pH 5.2 could be explained by the fact that in the vicinity of isoelectric point, the macromolecule of α-amylase exists as a compact (coiled) structure and the additional TEALS caused rapid uncoiling owing to the cooperativity of surfactant binding. The cooperativity of combination depends on chain length, tightness of packing and the number of crosslinks. With decreasing hydrogen ion concentration, the repulsive forces as well as the hydrophobic binding is enhanced; therefore, resulting in increased values of viscosity numbers. The increase in intrinsic viscosity is due to a corresponding elongation of the molecule and a consequent increase in the dissymmetry of the macromolecular units.

Table 1: Intrinsic viscosity [η], hydrodynamics radius (Re), end to end distance (r) and average molecular weight (M) of α-amylase-TEALS system at pH 5.20, μ = 0.15 M and different temperatures.

TEALS conc. (g/L)	[η]	Re	r	M
		30°C		
0.0	10.2	2.069	4.72	117376
2.0	11.3	2.141	4.97	130034
4.0	12.1	2.190	5.15	139240
6.0	13.2	2.255	5.37	151898
8.0	16.4	2.424	5.99	188722
		35°C		
0.0	9.1	1.992	4.46	104718
2.0	10.6	2.096	4.82	121979
4.0	11.7	2.166	5.06	134637
6.0	14.6	2.332	5.65	168009
8.0	18.1	2.505	6.29	208285

Cont…

TEALS conc. (g/L)	[η]	Re	r	M
		40°C		
0.0	6.2	1.573	3.68	71346
2.0	7.9	1.900	4.16	90909
4.0	9.9	2.049	4.65	113924
6.0	12.1	2.190	5.14	139240
8.0	15.4	2.374	5.80	177215

Table 2: Intrinsic viscosity [η], hydrodynamics radius (Re), end to end distance (r) and average molecular weight (M) of α-amylase-TEALS system at pH 7.50, μ = 0.15 M and different temperatures

TEALS conc. (g/L)	[η]	Re	r	M
		30°C		
0.0	8.6	1.955	4.34	98964
2.0	13.2	2.255	5.37	151898
4.0	14.9	2.348	5.71	171461
6.0	16.2	2.414	5.95	186421
8.0	27.5	2.880	7.76	316455
		35°C		
0.0	10.5	2.089	4.79	120828
2.0	16.6	2.424	5.99	191024
4.0	26.6	2.848	7.63	306098
6.0	29.7	2.955	8.06	341772
8.0	31.1	3.001	8.25	357882

Cont…

TEALS conc. (g/L)	[η]	Re	r	M
		40°C		
0.0	8.3	1.932	4.26	95512
2.0	15.4	2.374	5.80	177215
4.0	19.3	2.559	6.50	222094
6.0	24.4	2.767	7.31	280782
8.0	30.3	2.975	8.14	348676

Table 3: Intrinsic viscosity [η], hydrodynamics radius (Re), end to end distance (r) and average molecular weight (M) of α-amylase-TEALS system at pH 11.8, μ = 0.15 M and different temperatures.

TEALS conc. (g/L)	[η]	Re	r	M
		30°C		
0.0	22.0	2.674	6.94	253164
2.0	33.0	3.061	8.50	379746
4.0	46.2	3.424	10.06	531645
6.0	56.1	3.653	11.08	645569
8.0	66.7	3.870	11.81	767548
		35°C		
0.0	15.4	2.374	5.80	177215
2.0	27.5	2.880	7.76	316455
4.0	33.0	3.061	8.50	379746
6.0	39.6	2.252	9.31	455696
8.0	44.0	3.369	9.82	506329

Cont…

TEALS conc. (g/L)	[η]	Re	r	M
		40°C		
0.0	12.5	2.214	5.23	143843
2.0	20.0	2.590	6.62	230149
4.0	37.5	3.194	9.06	431530
6.0	45.1	3.396	9.94	518987
8.0	49.5	3.504	10.41	561720

Table 4: Intrinsic viscosity [η], hydrodynamics radius (Re), end to end distance (r) and average molecular weight (M) of α-amylase-TEALS system at different pH and temperature, μ = 0.15 M

TEALS conc. (g/L)	[η]	Re	r	M
		pH 10.4 & 35°C		
0.0	12.7	2.226	5.27	146144
8.0	18.8	2.537	6.41	216340
		pH 10.4 & 40°C		
0.0	10.5	2.089	4.79	120828
8.0	16.5	2.429	6.01	189873
		pH 11.0 & 35°C		
0.0	20.4	2.607	6.68	234752
8.0	24.2	2.760	7.28	278481
		pH 11.0 & 40°C		
0.0	18.7	2.533	6.40	215189
8.0	22.0	2.674	6.94	253164

Effect of temperature on the intrinsic viscosity [η] and other binding parameters

The effect of temperature on intrinsic viscosity and other parameters of α-amylase-

TEALS system have been examined. The intrinsic viscosity of α-amylase in absence and presence of TEALS decreases with rising temperature (Tables 1-4). The surfactant appears to open the helixes; the extent of helix opening is being facilitated owing to the breaking off interchain hydrogen bonding and other cohesive forces, responsible for the stability of enzyme structure. The increasing temperature forces the helixes (random coils) to become more and more compact and hence, the intrinsic viscosity and hydrodynamic radius decreases. The decrease in viscosity with rising temperature either indicates the helix coil transition as also reported in the case of phosphogluccomutase[38] or could be due to increasing chain flexibility with increasing temperature[39]. Jirgensons[40] observed that the protein possessing high α-helical contents became slightly disorganized after treatment with anionic surfactants, while those devoid of helical contents get converted to partly α-helical conformation on treatment with anionic surfactants. From the higher [η] value, it is probable to suppose that protein in presence of varying amount of TEALS forms either highly asymmetric particles or else highly solvated while reverse process occurred at higher temperatures, which may be due to "collapsing". The hydrocarbon part of TEALS may wound over the hydrocarbon part of enzyme with rising temperature.

Molecular weight of α-amylase-TEALS complex

The slope of log [η] vs. log molecular weight is found to be unit (Figure 7), which is

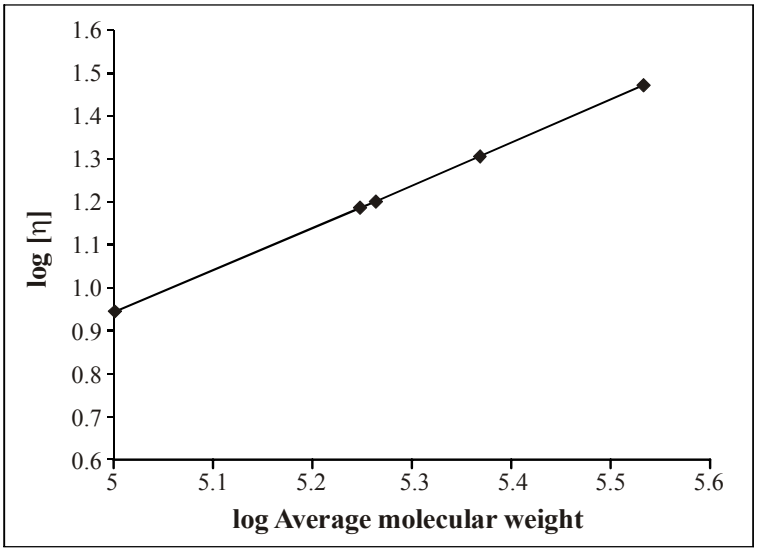


Fig. 7: Plot of log [η] vs. log molecular weight at pH 7.50 and 30°C

characteristic for polymer with random coils. This indicates that α-amylase and its TEALS

complexes are randomly coiled. This again suggests that TEALS caused unfolding of α-amylase and consequently binds to polypeptide chains.

CONCLUSION

The pH, temperature and surfactant concentration dependence of flow property of protein/enzyme may be used to explore the nature of surfactant-protein/enzyme interaction. The general shape of curves at low temperature may be attributed to the existence of electrostatic and non-electrostatic modes of binding, but at the higher temperature, it is in favour of enhanced uncoiling of polypeptide chains even in the initial stages of surfactant addition.

Ionic surfactant (TEALS) possesses the property of breaking ionic bonds, dissociate macroionic complexes, and complexes with acidic and basic compounds, both synthetic and natural. This is also evident from the value of binding parameters such as hydrodynamic radius (Re), end to end distance (r) and average molecular weight. The sudden change in viscosity and other parameters would suggest that viscosity behaviour of α-amylase in TEALS is cooperative, and the changes are certainly due to change of the asymmetry of the molecule.

At higher surfactant concentration, if the interaction was due to non-polar attraction, there should be less of particle-particle interaction and increase in the values of binding parameters should not be as much as is observed in the initial stages of surfactant addition. The pH-dependent flow behaviour of protein and protein-TEALS mixtures is in line with those suggested by earlier workers[41-43] in synthetic surfactant-macromolecular systems. However, at a fixed pH value above isoelectric point of the biopolymer, the viscosity continuously rises and this increasing flow may be attributed to the hydrophobic kind of interaction. The binding data are in line with this mode of linking with rising concentration of the anionic surfactant. In view of the viscosity changes one has to predict uncoiling of α-amylase by added surfactant and these combinations may involve either of (a) electrostatic repulsion between the charges of the bound species including the net charge of the enzyme or (b) penetration of the non-polar chain into a polar region of the enzyme and replacing the conformation stabilizing enzyme segment-segment interaction by ligand-segment interactions, or both the above factors may operate simultaneously inducing the uncoiling with in the macromolecule of enzyme. It may therefore be concluded that α-amylase-TEALS interaction involved ionic, hydrophobic and hydrogen bonding in forming the

complexes depending upon the pH, temperature and concentrations of enzyme and that of the surfactant.

The specific feature of TEALS binding to α-amylase is complicated in nature but some interesting conclusions about the combination could be made on the basis of the structural organisation of the anionic surfactant. The presence of three aliphatic hydroxyl groups in TEALS makes it a more effective denaturant than the earlier known sodium lauryl sulphate, it is because the aliphatic -OH in TEALS would form powerful hydrogen bridges with ionised nitrogen groups on enzyme surface.

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