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Studies the Interaction Between Anionic Surfactant, Triethanolamine Lauryl Sulphate (Teals) and Gluten (G) (A Seed Protein) by Viscometric Methods

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ABSTRACT : In spite of numerous techniques available for determining the structural organisation, the viscometric method is superior due to its simplicity. The extensive treatment of the dependence of viscosity on the shape and size of the polymers has been reviewed (1). Several notable workers have studied the diverse effects of surfactants on proteins using this method [2-8]. Arora and co-workers have also investigated surfactant protein combinations using viscometric and other related methods [9-12]. However, a literature survey revealed that no such work has been done so far on the interaction of anionic surfactant like triethanolamine lauryl sulphate (ALS) with gluten (from wheat seeds). With a view to extend the existing knowledge of rheological nature of detergent-protein systems, it was thought of interest to investigate flow properties of TE ALS-gluten mixtures. The present research work reports the results of TEALS-gluten system as determined viscometrically. Additional evidences of interaction below isoelectric point are given by measuring the transmittance of detergent-protein mixtures. The effect of pH and temperature on the flow behavior is discussed. A tentative mechanism of combination and unfolding is postulated.

Keywords : VM-Number of moles of metal bound per moles, G-Gluten, TEALS-Triethanolamine lauryl sulphate CD-Current diffusion, CB-Bound molar concenteration, CP-Free molar concentration, CMC-Critical Micelles concentration.

I. INTRODUCTION

A known amount of BDH gluten was soaked in diluted NaOH solution. It was centrifuged to collect the clear alkaline solution. It was precipitated by adding diluted HCl solution. The precipitate was again dissolved in diluted NaOH solution and centrifuged to get the clear solution. The protein solution was dialyzed against distilled water to set a salt free solution. The protein content was estimated by means of a colorimetric biuret method [13]. Triethanolamine lauryl sulphate (TEALS) was a gift from IIICO Products (Pvt.) Ltd., India. Its standard solution was prepared in double distilled water. The critical micelle concentration (CMC) was found out to be 0.0056 mol/L at 25°C by conductance measurements. Buffers of different pH values were prepared from

reagent grade chemicals. The buffers used were acetate, phosphate and carbonate buffers. Potassium chloride (BDH) solution was used for the adjustment of ionic strength of the reaction mixtures.

pH measurement. These were made on an Elico pH meter using a wide range glass electrode. The apparatus was standardized with the help of the standard buffers.

Viscosity measurements. These measurements were made by means of an Ostwald viscometer of relatively long capillary tube (flow time for water was 80 seconds) at different temperatures in a thermostat. Gluten and TEALS stock solutions were centrifuged at 16,000 rpm for 60 min to remove any suspended particles. The density of the solvent and solutions were determined with the help of a density bottle. The viscosity values were calculated from the following expression :

$$\bar{\eta}_{\text{rel}} = \frac{-\eta . t_p}{\eta_0 . t_0 P_0}$$

where, η_{rel}, is the relative viscosity t and p are flow time and density of the solution, while t_0 and P_0 are the time and density of the solvent.

Transmittance measurements. These determinations of TEALS-gluten mixtures, which can be taken as an index of turbidity developed, were made using a Bausch and Lomb Spectronic-20 instrument at a wavelength of 375 nm.

II. PROCEDURE

The following sets of solutions were arranged for various determinations:

1. A fixed amount of gluten (4.0 g/L) was taken in different boiling tubes. Varying amounts of 0.0861 M hydrochloric acid or 0.0650 KOH were added, the total volume was made upto 15.0 ml by adding required amount of distilled water and 1.0 M KCl to

make the ionic strength 0.15. The pH in each case was noted and viscosity recorded.

2. A fixed amount of protein (4 g/L) and TEALS (0.001 M) was taken as in step no.1
3. To a fixed amount of gluten (2.0 g/1), a variable amount of detergent was added. The total volume was made up to 15 ml. The viscosity of such solutions was determined at at different pH levels below as well as at its isoionic point.
4. Fixed amounts of gluten and detergents having the same initial pH values were titrated and viscosity data weak collected.
5. Varaying amounts of gluten were taken along with different amounts of surfactant to determine the intrinsic viscosity values at different pH values and temperatures.
6. The transmittance of mixtures of set 3 were recorded and plotted as transmittance vs surfactant concentrations at varying pH values.
7. A fixed amount of surfactant was titrated against different amounts of gluten at different fixed pH values below the isoctectric. point of. the protein.

III. RESULT AND DISCUSSION

A. Complex Formation from Transmittance Method

Different amounts of TEALS or gluten were mixed with each other at varying pH levels which had been adjusted by adding buffers of required pH values. It was observed that suspensions were produced in a certain range of detergent concentrations. The production of a suspension was regarded as a characteristic of combinations. The binding of TEALS to gluten was evaluated from transmittance vs. concentration curves. The inflexions in such curves exhibited the position where the production of the insoluble protein-detergent complex is completed. Further, if this critical point does not show the completion of the formation of stable suspension, then with the mixing of TEALS or gluten there would be an even greater decrease in the percentage transmittance. From the inflexions in the duties, the number of moles of TEALS bound per mole of gluten was calculated by the usual expression D_B/P where P is the molar concentration of gluten and DA the molar concentration of TEALS bound at the inflexion point. The values of determined from direct and reverse titration compare favourable (Tables 1 and 2). In a reaction involving precipitation, the equilibrium constant may be computed by

$$K_s = 1/(P)^M(D)^2$$

where, K_s is the reciprocal of the solubility product (instability constant), $(P)^M$ and $(M)^2$ represent the molar concentration of protein and detergent at the stoichiometric point (inflexion point). At this point, complete utilization of TEALS or gluten is assumed to involved in the formation of a neutral TEALS-gluten complex. The values of instability constants in terms of log K_s and their corresponding free energy changes ($\Delta G°$) are compiled in Tables 1 and 2.

Table 1 : Binding of TEALS with Gluten by transmittance measurements.

$G = 1.33*10^{-5}$, $\mu = 0.15$, Temperature = 30°

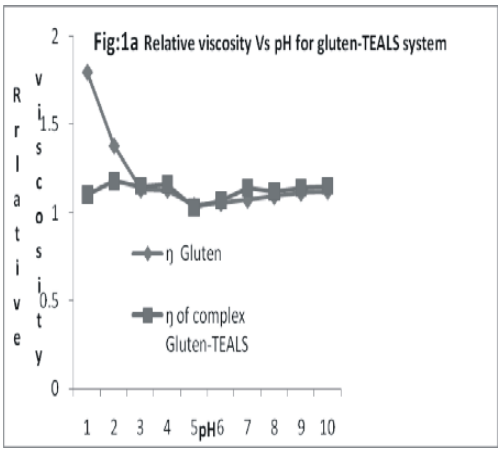
pH	*DB*10⁵*	*Log k*		*Free Energy Change*
3.10	66.5	50	6.935	–10.170
2.50	70.5	53	6.855	–10.875
2.00	81.2	61	6.834	–10.965
1.50	98.5	74	6.798	–10.785
1.09	1117.1	88	6.572	–10.635

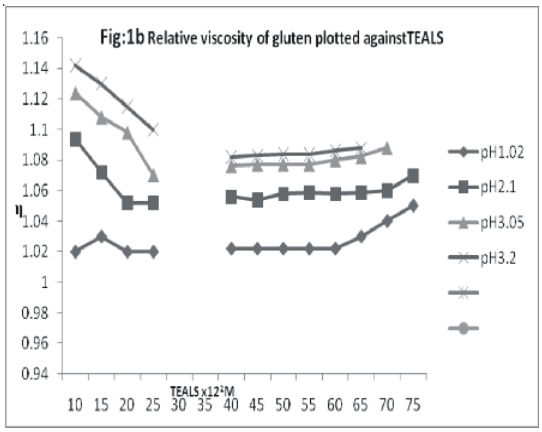
Table 2 : Binding of TEALS with Gluten by transmittance measurements.

Teals = $117.8*10^{-4}$ M, $\mu = 0.15$, Temperature = 30°

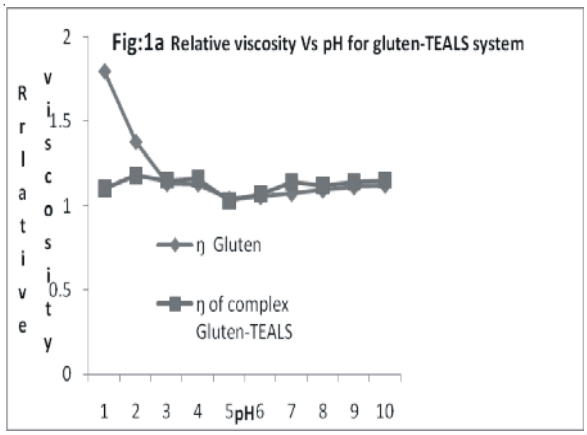
pH	*G*10-5*	*Log k*		*Free Energy Change*
3.09	2.31	51	7.022	–10.550
2.50	2.14	54	7.255	–10.925
2.00	1.82	65	7.354	–11.225
1.50	1.58	75	6.895	–11.225
1.09	1.32	90	6.682	–10.855

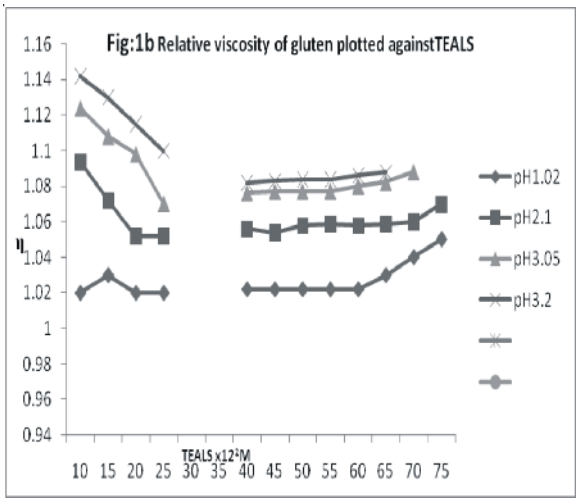
A consideration of the data indicates that the binding increases with decreasing pH of the reaction mixtures. this increased binding may be explained in terms of the electrostatic attraction between protonated gluten and the TEALS anions [14-15]. The similarity in log K_s and DG values shows that the anion binding gluten sites are identical,but their number is different at different pH levels. In the lower pH range, the moles of TEALS bound is found to exceed the total protonated sites on the gluten molecule (16), which could be due to the incorporation of surfactant ions into the swollen protein sphere because of the unfolding of the protein chains. The close similarity among log K_s and $\Delta G°$ at all the pH values also supported the formation of a complex of a constant composition between the TEALS and gluten.





Viscosity results. The viscosity measurements of set1 (Fig. 1) show the variation of the viscosity of gluten with pH. The pH dependence of flow behavior can be theoretically treated on the basis of alterations in the polymer shape caused by the presence of different charged sites on the protein surface. The increase in relative viscosity with the rise in pH appears to be due to the progressive neutralization of the different acidic groups which are known to ionise at different pH levels (Puri and Neelam) [24]. The flow was found minimum at pH 4.90 which is the isoelectric point of gluten. At the point of minimum viscosity, the gluten molecule is in a concentrated form owing to the attractive forces between the balanced positive and negative charges together with possible intramolecular cohesive forces because the Zwitter ion form is more likely to coil up than the charged one, whereas on both sides of this point the gluten molecule possesses a net overall charge which causes the molecule to extend itself by repulsion. However, towards extremely lower and higher pH levels the repulsive forces will be reduced due to increase of tree hydrogen and hydroxyl ions in the mixtures, and therefore, the viscosity would decrease. At higher pH, *i.e.*, 11.2, the viscosity of gluten suddenly falls after attaining the maximum value, it may be partly due to degradation and denaturation effects and partly due to the presence of unreacted alkali which itself has a much lower viscosity. Upon the addition of TEALS, the viscosity vs. pH curve is shifted upwards. These measurements could not be done in the lower pH range due to onset of precipitation. The higher viscosity of gluten-TEALS mixtures has been ascribed to interaction and unfolding. The effect of TEALS concentration on the relative viscosity of a fixed amount of gluten is shown in Fig. 1b. These plots at varying fixed pH levels show that the interaction with an ionic surfactant depends upon the hydrogen ion concentration of the gluten-TEALS system. The surfactant precipitates protonated gluten, *i.e.*, gluten below the isoelectric pH. A decrease in viscosity is observed at each selected pH levels upon the addition of even a very small quantity of TEALS until complete precipitation takes place. The precipitation area is shown by dotted line in the plots. Upon the addition of more TEALS the precipitate initially produced redissolved and an increased relative viscosity was obtained. The gluten molecule which existed in the expanded state below IEP becomes contracted with the progressive interaction with the surfactant anions and, therefore, viscosity decreases. Several workers [17] have reported similar results on the interaction of surfactants with serum albumin and casein (10) which has also been explained on the basis of the concentrations of natural polymers.





The precipitation and dissolution of the neutral surfactants-polymer complexes in excess of the surfactants could also be visualized in the light of the charge reversal phenomenon. Well below the IEP, a protein carrier positive charge, the addition of excess of anionic detergent gives a detergent-protein complex having negative charge all over its surface, while above IEP it has negative charge and addition of excess of anonic detergent yields a species having cationic charge spread all over its surface. On the whole, a neutral polymer may be converted into anionic or cationic species depending upon the nature of surfactants

added to it in excess. It has been shown by any workers that water soluble polymer becomes an association polyelectrolyte in the presence of ionic detergents, dyes, etc. (18-19]. In the situation under consideration, upon adding further amounts of surfactant, a stage is reached where precipitation occurs owing to the orientation of the hydrophobic part of the surfactant ions in solution and the neutral surfactant-protein complex is salted out. With the addition of further amounts of TEALS, a second adsorption layer is produced which makes the molecule hydrophilic and as such the TEALS-gluten complex again disperses causing the viscosity of the solution to increase again. The soluhilization of the precipitate may also he assumed owing to the unequal distribution of charges, in the presence of excess of TEALS anions, the lesser proportion of positive charge on gluten does not permit to reach the solubility product of TEALS-gluten complex to settle down [20-21]. It is observed that the resulting dispersion exhibits nearly the Newtonian flow which was non-Newtonian before insoluble complex formation.

The insoluble complexation limit is highly influenced by the hydrogen ion concentration of the mixed solution, the precipitation range is shifted to a greater TEALS to gluten ratio as the hydrogen ion concentration becomes greater. It can observed that the maximum precipitation and its complete dissolution occurs at different ratio. This fact is in favour of the regular protonation of the gluten sites at the uptake of anionic surfactant would increase. Other workers hi 10111 ihiomettic interactions between micellar compounds and proteins under similar conditions [17]. Such interactions involve two types of linking forces firstly the electrostatic attraction forming salt linkages, and secondly the, non-electrostatic forces which normally link the surfactant ions into micelles. Above isoelectric point of gluten, the viscosity vs. surfactant concentration plots were found to be different than those below isoelectric point of the protein. At every pH in the relative viscosity rises upon the addition of a very little quantity of TEALS, attain a maximum, then falls progressively and ultimately becomes limiting at higher surfactant concentrations. The maximum in each case is shifted towards lesser surfactant to gluten ratio as the pH of mixtures become , higher with a fixed amount of gluten, the molar concentrations of TEALS are 30, 25, 20, 15, 10 and 5 10^{-2} for pH levels 6.40, 7.50, 10.30, 10.60, 10.90 and 11.10 respectively.at the maximum point the relative viscosity are 1.040, 1.0425, 1.055, 1.070, 1.080 and 1.090 at the above mentioned molarity of TEALS. The observed sequence of viscosity with rising pH is an index for the diminishing positive charge and increasing negative charge on gluten, and the corresponding rise in flow property can be either due to repulsion or unfolding or due to a combination of these two factors. Thus, it is clear that the unfolding is maximum if both polymer and surfactant have same sign of charge. It may also be considered that TEALS anions interact with gluten to some extent to provide it with a net charge and consequently cause the molecule to extend itself.

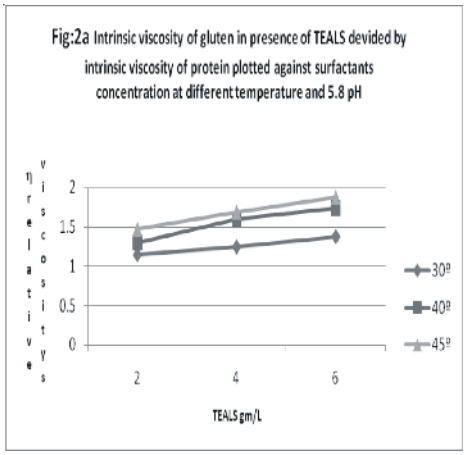
Intrinsic viscosity and molecular shape

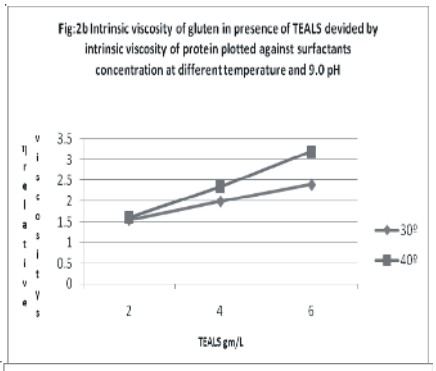
The intrinsic viscosity [η] of gluten in presence of varying quantities of different pH values was determined by plotting viscosity number (reduced viscosity)against gluten concentration and then extrapolating to zero gluten content These values of intrinsic viscosity in absence and presence of different amounts of detergent at different temperatures and pHs are given in Table 3. It may be seen that intrinsic viscosity increases with increasing concentration of detergents as well as pH. The lesser values of intrinsic viscosity at pH 5.80 could be explain by the fact that in the vicinity of the isoelectric point, the gluten molecule exists as a compact species, and additional detergent caused its rapid unfolding due to the co-opcrativity of detergent binding, thus an increasing quantity of detergent may cause solubilization and unfolding of gluten structure. This nature depends on polypeptide length, tightness of packing and number of cross-linkings. With a rising pH, the repulsive effect as well as the hydrophobic interaction enhances, hence much higher values of intrinsic viscosity were obtained. In the start when smaller quantities of TEALS are added, the electrostatic attractions predominate and cause its linking with gluten. In the presence of larger quantities of TEALS, a second type of linking involving non-electrostatic forces is more likely to occur. Lundgren [22] have also proposed two types of likings, identifying the first one as stoichiometric linking and the second one as the secondary association of the extra-detergent in the form of a loose combination due to a polar linking with those which are already electrostatically linked with the gluten. The size mid structure of the apolar portion as well as the presence of ionic group in TEALS are two likely factors controlling such combination. The ionic group contributes to salt-like linking between oppositely charged groups of gluten and the apolar head provides ability- to ionic link and facilitates the linking of extra surfactant. The Huggin's constant (*K*) also support to ionic and a polar linkings in the present investigation.

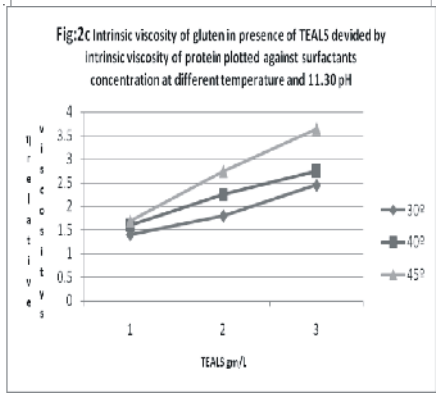
Effect of pH and Temperature on Intrinsic Viscosity

The limiting viscosity number [η] was found to diminish with increasing temperature. The relationship between viscosity of TEALS-gluten and gluten has been plotted in Fig. 2*a*, *b* and *c* as ratio of intrinsic viscosity against added TEALS. All the plots at individual pH and temperature revealed linear specification. These plots go to show that the unfolding phenomenon is dependent on pH, temperature and TEALS concentrations. The intrinsic viscosity in the absence of detergent was found nearly the same upto pH 10.00, an abrupt rise occurred at pH 10.50 and the intrinsic viscosity increased continuously without attaining any limit. The variations in flow property suggest that it is due to

uncoiling or dissociation of gluten molecule into its tractions, probably gliadin and glutenin.







Mechanism of interaction

A probable mechanism of combination between TEALS and gluten may be suggested by means of transmittance and viscosity variations observed above and below the icoelectric point of gluten. At lower pH values the precipitation of the TEALS-gluten complex and then its dissolution may be due to the fact that the more or less expanded gluten molecule becomes hydrophobic by the progressive combination of TEALS ions, and at the stage of complete charge neutralization it is precipitated. On the addition of excess TEALS, however, the second adsorption layer of the TEALS ions would be produced by Vander Waals attraction forces between carbon chains, which makes the molecule hydrophilic and accordingly the precipitate once formed ,dissolved again. The solution thus formed behaves similarly as it was before precipitation. On the other hand. excess of gluten does not solubilise the insoluble complex. Thus, the peculiar characteristic of TEALS of solubilising the TEALS-gluten has been attributed to its micelle and mixed micelle forming property.

From the nature of viscosity variations in gluten and TEALS-gluten mixtures, a tentative mechanism for TEALS linking and consequent uncoiling can also be suggested. The nature of plots at low temperature supported the existence of electrostatic and non-electrostatic types of linkings. on the other hand, the viscosity at higher pH exhibited extensive uncoiling of gluten molecule in the initial stages of TEALS addition which seems to be more rapid with rising pH and temperature. The behavior of linking in lower and higher pH zones has been found to be reversed.The TEALS to Gluten ratio increases with diminishing pH. while. it decreases with lowering The transmittance and viscosity plots in the lower pH revealed analogous behaviour. The onset of precipitation and its complete solubilization took place in a definite ratio of TEALS to gluten. This nature is in line with the similar work of Pankhurst and Smith [14] and Steinhardt *et al.* [23]and others [23] in detergent-protein systems. It may he concluded that TEALS-gluten combination involved ionic, apolar and hydrogen bonding in forming the complexes depending upon the pH and concentration of surtactant.

Table 3 : Viscosity of Gluten - TEALS at varying pH and temperatures.

***TEALS* (*g/l*)**	***pH 5.80***			***pH 7.5***		***pH 9***		***pH 10.40***		***pH 11.40***		***pH 11.30***		
	300	400	450	300	400	300	400	300	400	300	400	300	400	450
0.0	10.12	6.20	9.10	8.60	8.80	11.60	8.20	10.60	12.70	18.80	20.4	15.40	22.00	12.20
2.0	11.30	8.20	10.50	13.20	15.40	17.80	14.40	----	----	----	----	27.50	33.00	20.00
4.0	12.20	10.00	11.70	14.90	18.60	28.60	19.30	----	----	----	----	33.00	46.20	37.60
6.0	12.50	12.20	14.60	20.60	23.60	28.80	26.40	----	----	----	----	39.80	56.00	45.20
8.0	15.50	15.60	18.20	27.50	28.80	32.00	30.30	16.50	18.80	22.00	24.20	44.00	68.00	50.20

Table 4 : Effect of TEALS on reduced viscosity of Gluten at different Temperature and fix pH.

TEALS (g/l)	*pH 5.80*			*pH 7.5*		*pH 9*		*pH 10.30*		
	300	400	450	300	400	300	400	300	400	450
0.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2.0	1.12	1.32	1.15	1.53	1.75	1.53	1. 75	1.78	1.50	1.64
4.0	1.21	1.61	1.27	1.72	1.77	2.46	2.35	2.13	2.10	3.10
6.0	1.24	1.96	1.60	2.40	2.68	2.48	3.22	2.58	2.54	3.70
8.0	1.53	2.51	2.00	3.20	3.15	2.75	3.70	2.85	3.10	4.11

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