Electrometeric Titration Curves of a Few Oilseed Proteins

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Electrometric titration curves and isoelectric points of castor, sunflower, mustard and cottonseed proteins have been reported. By carrying out such titrations at different temperatures and employing Van't Hoff equation, the heats of ionization in different pH regions have been calculated. This enables identification and estimation of various reactive groups ionizing at increasing pH values from a single titration curve. The amino, guanidinium and carboxylic groups have been determined separately from the titration curves in the presence of formaldehyde. The results are in good agreement with those obtained by the complicated analytical methods.

Electrically charged groups of protein molecules play an important role in understanding the pH-dependence of all properties of a protein molecule and in determining the nature of interactions of proteins with other substances. Information concerning the number of these groups and their ionization constants can be obtained from titration curves.The theory and methods of protein titration have been reviewed (1).

Present paper reports a study of the electrometric titration curves of a variety of oil-seed proteins isolated from mature castor, sunflower, mustard and cottonseeds.

EXPERIMENTAL

MATERIALS Castor, sunflower, mustard and cottonseeds were crushed and freed of oil by extraction in a soxhlet extractor with petroleum ether (boiling range 40°-60°C). Whole proteins were isolated from the oil-free seed meals by dilute alkali extraction (pH 9.0) and acid precipitation. Globulin fraction in each case was isolated by extraction with 10% sodium chloride followed by saturation of the clear extract with ammonium sulphate. The precipitated protein was dialyzed against distilled water at 5°C till free of SO_4^{--} and Cl^- ions. Cottonseed β-globulin was isolated by the procedure outlined by Jones and Csonka(2).

METHODS

Isoelectric points of the various proteins were determined by flocculation with anionic detergent (sodium lauryl sulphate)(3)

Freshly prepared wet protein was dissolved in 0.1 M NaCl. Concentration of this solution was determined by drying an aliquot of this solution in an air oven (110°C). 25 ml of 0.25 per cent protein solution was taken in a small beaker and its pH value was determined on a Beckmann pH meter having an accuracy of ± 0.01 pH unit. 0.1 N sodium hydroxide solution was then added from the burette in 0.25 ml portions. After each addition, the contents were allowed to stand for 25-30 minutes so as to attain equilibrium,then the pH was determined. This time interval was found sufficient from a set of preliminary experiments carried out for this purpose. In a similar manner, titration was carried out with another 25 ml portion of the protein solution using 0.1 N HCl as the titrant.

Experiments were also conducted in each case in the presence of 1% (V/V) formaldehyde when NaOH was used as the titrant.

The pH measured at any stage was used to calculate the free (H^+), m_{H^+}, when HCl was used as the titrant, or that of the free (OH^-), m_{OH^-}, when NaOH was used as the titrant, employing expression (1) or (2).

$$pH = - \log m_{H^+} \cdot \nu_{H^+} \quad \ldots\ldots (1)$$

$$POH = - \log m_{H^+} \cdot \nu_{OH^-} \quad \ldots\ldots (2)$$

Subtracting m_{H^+} or m_{OH^-} from the molality of H^+ or OH^- added, the amount of proton or hydroxyl ion bound by protein was calculated. The values of activity coefficients at appropriate concentrations were taken from the tables of Robinson and Harned(4). The pH values were plotted against (H^+) or (OH^-) actully bound by the proteins. The reversibility of the complete titration curve was checked by starting with protein solution in HCl at pH 2.0 and adding increasing amounts of NaOH.

RESULTS AND DISCUSSIONS

Elemental analysis of the various proteins is given in the table 1. Most seed proteins have a relatively high nitrogen content compared with

animal proteins as has been observed by Brohult and Sandegren (5).

TABLE 1

ELEMENTAL ANALYSIS OF THE OIL-SEED PROTEINS

Protein Sample	Carbon (%)	Hydrogaen (%)	Nitrogen (%)
1. Whole Castor seed protein	49.67	6.92	17.50
2. Whole sunflower seed protein	50.01	7.47	16.90
3. Whole Mustard seed protein	48.77	8.43	17.00
4. Globulin fraction of Mustard seed protein.	45.01	6.42	17.85
5. Whole cottonseed protein	47.44	6.52	16.90
6. Globulin fraction of cottonseed	49.80	7.64	18.05
7. Cottonseed-B globulin	49.99	6.25	18.30

Isoelectric points, determined by flocculation with sodium lauryl sulphate (3), given in Table II, are of the same order as reported for various seed proteins, in general, indicating genuineness of the products. Values are higher for the globulin fraction as compared to whole proteins. The isoionic points of these materials in 0.1 M NaCl are also given in Table II.

The plots of pH vs equivalents of acid or alkali bound per 10^5 g protein for whole castor, sunflower, mustard and cottonseed proteins, globulin fractions of mustard and cottonseed proteins and cottonseed β-globulin are shown in Figure 1 (a,b,c). Each curve shows a sharp point of inflexion on the alkaline side in the pH range 7-9.

It is seen that beyond a certain stage no more of acid gets bound as the pH starts falling on the addition of even a small amount of acid. However, the titration curves remain incomplete at the basic end because at high pH values (i) the protein gets denatured and (ii) the calculation of base bound becomes unreliable.

The point of maximum acid-binding capacity corresponds to the point of maximum proton charge at a pH value of about 2 . At this pH value all the acid groups are in the neutral state while the

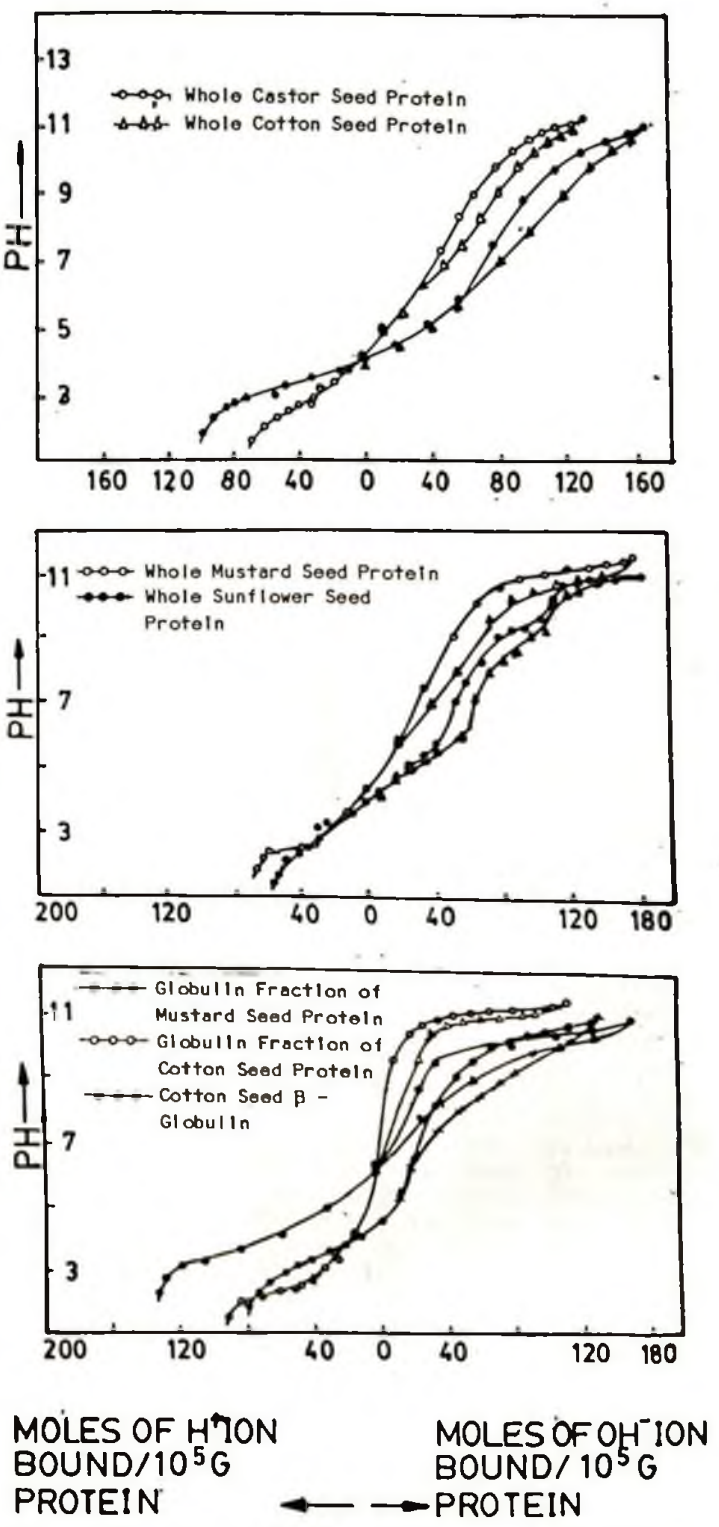


Fig. 1 Electrometric Titration curves of various oil-seed proteins with hydrochloric acid and sodium hydroxide in the presence and absence of formaldehyde.

cationic groups carry positive charge and none of the groups is dissociated. On the addition of alkali, however, various groups get ionized in

TABLE 2

ISOELECTRIC AND ISOIONIC pH VALUES OF VARIOUS OIL-SEED PROTEINS.

Name of Protein	Isoelectric point	Isoionic point
Whole castor seed protein.	4.45	4.20
Whole sunflower seed protein.	4.65	4.00
Whole mustard seed protein.	4.55	4.40
Globulin fraction of mustard seed protein.	4.75	4.60
Whole cottonseed protein.	4.15	4.10
Globulin fraction of cottonseed protein.	4.60	5.85
Cottonseed B-globulin.	4.90	4.65

different pH regions depending upon their pK values. The number of electrically charged groups of protein molecules can be calculated from the electrometric titration curves provided we know the pH range in which these groups ionize. This information can be obtained from measurements of heats of ionization at different pH values (6). For this purpose titrations were carried out at different temperatures (17, 30 and 45°C) and heats of ionization are calculated employingt Van't Hoff equation.

$$Q = -4.579\ T_1\ T_2\ (pH_2 - pH_1)/T_2 - T_1 \quad .....(3)$$

Where pH_2 and pH_1 are the pH values corresponding to the same amount of NaOH at temperatures T_2 and T_1, respectively. The apparent heats of ionization (Q) thus obtained for the temperature intervals 17-30° and 30-45°C were plotted against the mean pH value $(pH_1 + pH_2)/2$. The plots for some of the proteins are shown in Fig 2. The curves show three horizontal zones between pH 2 and 11, and within any one of these flat regions, the value of ordinate (Q) on the curve indicates the apparent heat of ionization of the groups ionizing within that pH range. The groups ionizing in the pH range 2-6.5 have an average apparent heat of ionization of -1500 to -2000 calories. The value is within the range characteristic of heat of ionization of carboxylic groups (1). The groups ionizing between pH 6.5 and 8.0 show an average apparent heat of ionization of +6500 to +7000 calories, the value being consistent with that found for imidazole groups (6). The groups still left

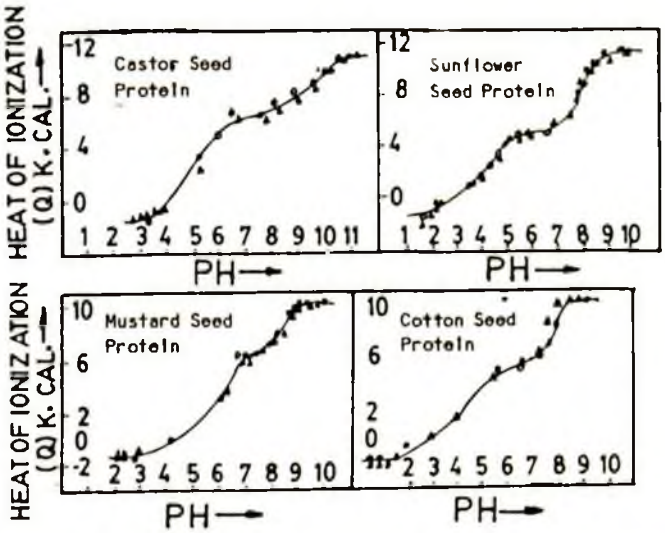


Fig. 2 Plots of apparent heats of ionization (Q) vs. pH for various oil-seed proteins.

unneutralized are ∝-amino, ε-amino, phenolic and sulphydryl groups. These are evidently neutralized in the third and the final stage, represented by the third plateau going right up to pH 11.0.

It was thus possible to calculate the number of carboxyl and imidazole groups from the equivalents of acid/base required to titrate the protein from pH 2-6.5 and 6.5-8.0, respectively. The values as read from the titration curves are given in Table III.

The effect of formaldehyde in modifying the titration curves of proteins has been studied in detail by Kekwick & Cannan (7), and many other investigators (8,9). They have shown that most acid part of the titration curves remains practically unaffected by the formaldehyde, while on the alkaline side the pK values of the dissociating groups are shifted to more acid regions. Figures 1 (a,b & c) show that the curves for water and for 1% formaldehyde are identical up to pH 6-6.5 and diverge widely at more alkaline reactions. It can be inferred that the break at this point represents the end of the region where carboxylic groups are being titrated and the beginning of the titration of imidazole groups. Similar findings have been reported by Lichtenstein (10) for the titration of gelatin in 1% formaldehyde.

In the presence of formaldehyde, the titration of amino groups is essentially complete at pH 9.0 whereas hardly 5 percent would be dissociated at this pH value in the absence of formaldehyde. The difference between the equivalents of alkali bound

TABLE 3

EQUIVALENTS OF VARIOUS IONIC GROUPS PRESENT IN DIFFERENT OIL PROTEINS

Description of the protein	Amounts of groups present (equivalents/10^5 gm protein)*						
	Carboxylic (pH range 26.5)			Imidazole	α-+ϵ-Amino	Guanidinium.	Total
	From titration curve in water	From titration curve in HCHO	Average value.				
Whole castor seed protein.	106	110	108(97)	15(8)	16(18)	40(49)	179(172)
Whole sunflower seed protein.	87	99	93(64)	12(15)	20(23)	20(48)	145(150)
Whole mustard seed protein.	94	92	93(76)	16(18)	20(36)	30(35)	159(165)
Globulin fraction of mustard seed protein.	97	84	91(76)	16(18)	27(36)	34(35)	168(165)
Whole cottonseed protein.	166	170	168	16(20)	25(37)	60(67)	269
Globulin fraction of cottonseed protein.	133	126	130(128)	19(19)	30(34)	81(78)	260(259)
Cotton β- globulin	85	83	84	6	12	66	168
Milk casein	-	-	92	20	56	24	211

* Values in the parenthesis represent analytical values for the respective samples(20-25).

at pH 9.0 in the presence and absence of formaldehyde is thus a measure of the number of amino groups present.

The guanidinium groups are not fully neutralized even up to the maximum pH value since their pK value lies beyond pH 12. These groups were, however, determined as follows. The number of equivalents of acid required to titrate from the isoionic point to pH 2 gives the number of total cationic groups, i.e. the sum of imidazole, amino and guanidinium groups (say n). By subtracting the number of amino + imidazole groups (obtained from the titration data as indicated above) from n, the number of guanidinium groups are calculated.

Cannan et. al(11) have shown that the net charge of the protein molecule near pH 8.5 in water, as determined from the base bound per mole, represents negative charge of all the carboxylic groups minus the cations present at this pH (the sum of the amino and guanidinium groups). Since the guanidinium groups are the only cations at this pH in the presence of formaldehyde, it becomes possible to calculate the value for the carboxyl minus guanidinium groups. As the number of guanidinium groups have been determined separately, the formal titration curve affords an alternative method for calculating the number of carboxylic groups. The values for these groups obtained in this way are included in Table III and are found to agree with those read from the titration curve in the pH range 2-6.5.

The numbers of various ionic groups present in different oil-seed proteins, expressed as equivalents per 10^5 g protein, as obtained from the titration curves, following the procedure discussed above, are given in Table III. Amongst different protein samples, values are seen to be comparable for castor, sunflower and mustard seed proteins. Cottonseed protein and its globulin fraction, however, contain relatively larger number of carboxylic and guanidinium groups. The corresponding values for the number of various ionic groups in milk casein (12) (Which is considered to be of the highest quality as regards its nutritional value) are also given in Table III. It appears that the oil-seed proteins contain relatively larger amounts of arginine but are deficient in lysine.

The values are seen to be fairly close to those obtained by analytical methods, as reported in the literature (13). A better agreement can not to be expected because of ecological and genetic reasons This shows the adequacy of the electrometric titration curves for the estimation of various ionizable groups in proteins. The method is simpler and more elegant than the usual chemical methods generally employed for this purpose.

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