INTRODUCTION
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Casein has been defined by ADSA (1) as a heterogeneous group of phosphoproteins precipitated from skim milk at pH 4.6 and 20°C. The term casein, strictly speaking, designates a product isolated from milk rather than a biochemical substance occurring in it. The proteins left behind in milk after isolation of casein are known as whey proteins. These consist mainly of lactalbumin and lactoglobulin. Casein constitutes nearly 80% of total milk proteins while the remaining 20% or so are the whey proteins.

Casein is known to be heterogeneous in character. It consists of a number of fractions. These fractions differ in molecular size, shape, hydration and electrical charge. Such differences are reflected in the rates of sedimentation and diffusion as well as solubility and electrophoretic behaviour. The heterogeneous character was revealed for the first time by Osborne and Wakeman (2). They were successful in isolating a small amount of alcohol-soluble fraction from the original product. Linderstrom-Lang and Kodama (3,4), a few years later, were able to isolate as many as seven fractions, by extracting the original product with 60% alcohol acidified with hydrochloric acid and precipitating by the addition of sodium hydroxide. Three of these fractions were major
and constituted 54.2, 20.2 and 14.3 per cent while the remaining four were minor. The fractions differed appreciably in their phosphorus and nitrogen contents as well as physico-chemical properties mentioned above. Cherbuliez et al. (5,6) obtained four fractions of casein from alkaline ammonium chloride solution by alternate addition of hydrochloric acid and acetone. Mellander (7), while working with electrophoretic behaviour of casein, reported the presence of three different fractions moving with three different mobilities. He designated them as $\alpha$, $\beta$- and $\gamma$- caseins in the decreasing order of their mobilities. Warner (8), utilising solubility differences of the various fractions in water at 2°C and pH 4.4, was able to separate casein into $\alpha$- and $\beta$- fractions. Hipp et al (9) isolated $\gamma$- casein, the third major component, a few years later by precipitating sodium caseinate solution at 2°C and pH 7.0 with 50% ethanol. This fraction was found to be identical with the alcohol-soluble casein of Osborne and Bakeman (2). The $\alpha$- and $\beta$- fractions can be separated by isoelectric precipitation. Thus if the isoelectric point is approached slowly from the acid side, $\alpha$- fraction gets preferentially precipitated at pH 4.4. Some alternative methods, including fractional precipitation from alcohol and urea solutions,
have also been suggested (10) for the fractionation of casein into \( \alpha \)-, \( \beta \)- and \( \gamma \)-fractions. These methods involve conditions under which mutual interactions of various fractions are minimum.

Waugh and coworkers (11—17) by employing a technique based upon ultracentrifugation showed that \( \alpha \)-casein, far from being homogeneous, was made up of subfractions, called \( \alpha_5 \)-casein, which can be precipitated under certain conditions in the presence of calcium ions and is also frequently referred to as calcium sensitive casein, and \( \kappa \)-casein (calcium insensitive casein). They offered evidence to show that \( \kappa \)-casein acts as a "key micelle stabilizing factor" as it protects \( \alpha_5 \)-casein against precipitating action of calcium ions. They also showed that \( \kappa \)-casein constitutes the primary sites of rennin action. Waugh (14) found that \( \alpha_3 \)-casein itself was made up of two components called \( \alpha_{3,1} \)- and \( \alpha_{3,2} \)-caseins. Annan and Manson (18) have further isolated several minor fractions of \( \alpha_5 \)-casein and have used the following terminology: \( \alpha_{5,0} \)-, \( \alpha_{5,2} \)-, \( \alpha_{5,3} \)-, \( \alpha_{5,4} \)- and \( \alpha_{5,5} \)- to distinguish them from one another as well as from their parent fraction which is still designated as \( \alpha_5 \)-casein. Hoagland et al (19) have shown that \( \alpha_5 \)-casein can be converted, in the presence of reducing agents, into \( \alpha_{5,4} \)- and \( \alpha_{5,3} \)-caseins.

Aschaffenburg (20,21) discovered genetic polymorphism in \( \beta \)-casein. By using simple paper
electrophoresis, he was able to show that $\beta$-casein exists in three forms: A, B, and C. This opened up a vast area of research in the chemistry of casein. Aschaffenburg's observation was supported by Thompson et al. (22, 23). They showed that $\alpha_S$-casein is also genetically variable. Its genetic variants were termed as $\alpha_{S_1}$, $\alpha_{S_2}$, and $\alpha_C$-casein (in the order of decreasing electrophoretic mobility). Neelin (24) and Woychik (25) suggested that $\kappa$-casein is also genetically variable. The use of paper electrophoresis and starch gel electrophoresis techniques developed and used in recent years have indicated remarkable complexity of casein. Thus Wake and Baldwin (26) have reported the presence of about 20 distinct components, most of which are found in $\alpha$-casein.

Aschaffenburg and coworkers (27, 28) have shown that $\alpha$-fraction of buffaloes' casein migrates more slowly than that of cows' casein. These observations were confirmed by Ganguli and Bhalerao (29) as well as Singhal and Ganguli (30), who, by employing paper disc electrophoresis, showed that not only $\alpha$- but $\beta$- and $\gamma$-fractions of buffaloes' casein also had smaller mobility than the corresponding fractions of cows' casein.

**Casein Micelles**: Waugh (13) has shown that casein occurs
in milk at body temperature in two distinct states, i.e., as micelles, which are easily removed by high speed centrifugation, and as monomers and small polymeric units, which cannot be removed by centrifugation. One of the striking properties of natural casein micelles is their extraordinary stability. For example, milk may be cooled to 0°C or boiled for a considerable period without obvious micelle degeneration. Further, the micelle system can be appropriately dried and reconstituted on addition of water (17).

Casein micelle diameter size has been determined by various workers by using different techniques. Thus the size range, by using ultramicroscopy, has been reported to be 50-1200 Å (31), by light scattering as 800-1100 Å and 860-1230 Å (32,33), by ultracentrifuge as 200-1400 Å (34) and 1000-2000 Å (35) and by electron microscopy as 400-2800 Å (36), 400-2400 Å (37), 400-2000 Å (38) and 800-1000 Å (39).

A number of workers have used various techniques to obtain molecular weights of casein and its fractions. Thus the value for whole casein in 6.6 M urea has been determined by osmotic pressure measurements by Burk and Greenberg (40) to be 33,600. The value for α-casein has been reported by Sullivan et al (41) to be 121,800. They
employed sedimentation and diffusion techniques for the purpose. \( \alpha \)-casein molecules were not completely disaggregated in these studies. Perlmann (42) suggested a minimum molecular weight of 31,000 for \( \alpha \)-casein on the basis of its phosphorus content. The values for \( \lambda \)-casein have been found to be 27,000 as reported by Dreizen et al (43), 31,000 by Kalan et al (44) and 25,500 by McKenzie and Wake (45). These workers have used light scattering, carboxypeptidase data, assuming a single polypeptide chain, and diffusion and sedimentation rate techniques, respectively. Molecular weight of \( \beta \)-casein has been reported to be 24100 (41) and 19,800 (45) by diffusion and sedimentation techniques, respectively. The values from amino acid analysis and by Archibald sedimentation method have been found to be 24,200 (46) and 25,000 (47). The molecular weight of \( \kappa \)-casein has been reported to be 26,000 (45) by Archibald method and 19,500 (48) by sedimentation equilibrium method.

Choate et al (49) differentially centrifuged micelles from milk and found the proportion of \( \alpha \)- and \( \beta \)-caseins to be independent of micelle size. This observation, however, was not confirmed by Rose (50) who reported that larger micelles contain, relatively, a higher percentage of \( \beta \)-casein. Similarly Sullivan (51) showed that smaller
micelles contain, relatively, a larger percentage of $\kappa$-casein. The proportions of $\alpha_S$-, $\beta$- and $\kappa$-caseins were found by Waugh (12) to be 55, 30 and 15 per cent and by Brunner et al (52) for $\alpha_S$-, $\beta$- and $\gamma$-caseins to be 59-73, 25-33 and 4-8 per cent by weight of micellar casein. McKenzie and Wake estimated $\alpha_S$-casein plus $\kappa$-casein to be 77 per cent of the micellar casein (53).

According to Rose (54) micelles occur in milk in various size ranges and their size is influenced by soluble calcium, $\kappa$-casein and citrate contents.

With regard to the shape of the molecule Waugh (13) has suggested, on the basis of his calculations of frictional ratios and sedimentation constants, that each polypeptide chain exists in a coil form and may be represented by a cylinder of diameter equal to 16 Å and of length varying with the molecular weight of the protein. This was found to be 210 Å for $\alpha_S$-casein, 215 Å for $\beta$-casein and 150 Å for $\kappa$-casein. Waugh and Noble (16), later on, showed that casein micelles have a composition which changes from the surface to the centre. The surface is richer in $\kappa$-casein and the interior is richer in $\alpha_S$- and $\beta$-caseins.

**Chemical Composition:** The elementary composition of the unfractionated casein and its major fractions has been
reported by several workers (46, 55-60). Some of the results are given in Table I.

The amino acid analyses of the various fractions have been published by Gorden et al, Hipp et al, Mellon et al and some other workers (46, 55-57, 61-64). The major differences between the unfractionated casein and its fractions have been found to be in their proline, tyrosine, tryptophan, cystine, methionine and arginine contents.

According to Kassel and Brand (65) and Gorden et al (46), sulphur in casein is present mainly in the form of two amino acids, namely methionine and cystine. Springer and Quentin, however, did not agree with this view (66). They found $\beta$-casein and $\gamma$-casein, each containing an appreciable amount of sulphur, to be completely free of cystine (46). The whole amount of cystine was found to be present in $\alpha$-casein. Further fractionation of $\alpha$-casein showed that cystine was predominant in $k$-casein (14).

Phosphorus appears to be incorporated in casein and its fractions as ester of phosphoric acid with hydroxy amino acids serine and threonine (67-70). This view received support from the fact that phosphate is readily
Table I

Elementary composition of casein and its major fractions

<table>
<thead>
<tr>
<th>Description of casein</th>
<th>Elementary composition 9/100g</th>
<th>Nitrogen</th>
<th>Phosphorus</th>
<th>Sulphur</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole casein</td>
<td></td>
<td>15.40(46)</td>
<td>0.79(46)</td>
<td>0.78(56)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.63(56)</td>
<td>0.86(56)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.19(a)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.98(b)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>α-casein</td>
<td></td>
<td>15.58(10)</td>
<td>0.99(10)</td>
<td>0.72(10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.25(a)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.12(b)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>δ-casein</td>
<td></td>
<td>15.10(60)</td>
<td>1.01(60)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.00(59)</td>
<td>112</td>
<td>-</td>
</tr>
<tr>
<td>k-casein</td>
<td></td>
<td>13.50(58)</td>
<td>0.35(58)</td>
<td>-</td>
</tr>
<tr>
<td>β-casein</td>
<td></td>
<td>15.33(10)</td>
<td>0.55(10)</td>
<td>0.86(10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.95(a)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.84(b)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>γ-casein</td>
<td></td>
<td>15.40(10)</td>
<td>0.11(10)</td>
<td>1.03(10)</td>
</tr>
</tbody>
</table>

(a) Our work for cows' caseins.
(b) Our work for buffaloes' caseins.
released by mild alkaline hydrolysis (71). On the basis of proteolytic digestion of casein with enzymes, various types of phosphorus ester linkages have been suggested some of which are as shown below.

- **Monoester**
  \[
  - CH_2 - O - P - OH
  \]

- **Diester**
  \[
  - CH_2 - O - P - O - CH_2
  \]

- **Pyrophosphate**
  \[
  - CH_2 - O - P - O - P - O - CH_2
  \]

- **Phosphamide**
  \[
  - N - P - OH
  \]

- **Phosphamide ester**
  \[
  - N - P - O - CH_2
  \]

Perlmann (72), on the basis of her experiments, connected with proteolytic hydrolysis & dephosphorylation of casein, suggested
that \( \alpha \)-casein contains 40 per cent of the phosphorus as monoester, 40 per cent as phosphamide diester and 20 per cent as pyrophosphate while \( \beta \)-casein contains considerable portion of the phosphorus as diester. However, later work (73, 74 & 75) showed that the same type of phosphorus linkage is present in whole casein as in casein fractions and that this linkage is of the type of a monoester (76-82). The presence of phosphorus as well as the manner in which it is incorporated is important in determining the properties of a protein.

During the isolation of casein from skim milk by acidification, the inorganic calcium and phosphorus associated with casein micelles dissolve progressively with the lowering in pH of milk. At the isoelectric point (pH - 4.6), the casein that separates out contains about 0.85 per cent phosphorus, mostly organic phosphorus, in combination with casein. Calcium caseinate phosphate complex, however, gets coagulated without much change in composition by the action of rennet. The pH of the system in this case does not change much and, therefore, the colloidal calcium and phosphate remain attached to casein as in the original state. The product thus obtained is known as rennet casein. This is to be distinguished from paracasein (83) which is the name given to the product obtained by the action of rennet on casein.
Physico-Chemical Properties of Casein:

A number of publications have appeared in recent years on various physico-chemical properties of casein. Since the main emphasis in the present thesis is on physico-chemical behaviour of casein and casein fractions, it may be desirable to review some of the work that has been reported in this field in recent years.

Solubility: Casein is very slightly soluble in water at its isoelectric point (84,85). The value is reported to be 0.05 g/litre at 5°C (86) and 0.11 g/litre at 25°C (87,88). The solubilities of some of the major fractions of casein at their respective isoelectric points at 25°C are given in the following table (89).

<table>
<thead>
<tr>
<th></th>
<th>α-casein</th>
<th>β-casein</th>
<th>γ-casein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoelectric pH</td>
<td>4.70</td>
<td>4.90</td>
<td>5.07</td>
</tr>
<tr>
<td>Solubility(mg/100g) in</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>water</td>
<td>0.05</td>
<td>0.41</td>
<td>1.00</td>
</tr>
<tr>
<td>50% alcohol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% water</td>
<td>0.135</td>
<td>1.90</td>
<td>1.45</td>
</tr>
</tbody>
</table>

Casein is even much less soluble in most of the organic solvents. However, it dissolves more readily in mixtures
of water with organic liquids, like ethanol and pyridine (85,90). The solubility also increases in aqueous solutions of salts, such as chlorides of sodium, potassium, calcium, barium, strontium, magnesium; bromides of sodium, potassium, calcium and thiocyanates of lithium, sodium, potassium and calcium (91-95).

The solubility in water increases on altering the pH on either side of the isoelectric point i.e., in acidic and alkaline solutions. It dissolves more readily in alkaline than in acidic solutions.

**Hydrolysis:** Casein solutions do not undergo hydrolysis in the pH range 2.5–8.5, ordinarily, at room temperature (96,97). However, if the temperature is allowed to rise, hydrolysis sets in rapidly (98). Hydrolysis also sets in through the action of certain enzymes and micro-organisms if the solution is allowed to stand at the room temperature for several days. This is important in the manufacture of cheese from the point of view of its texture, flavour and keeping quality. Hydrolysis above pH 10 results in the evolution of ammonia (99,100) and splitting off of phosphorus and sulphur (101, 102).

**Acid-Base Combining Capacity:** Casein being an ampholyte, like other proteins, can bind acids as well as bases. The capacity to do so varies with the method employed in the
isolation of casein from milk. For example, micellar casein and rennet casein have much lower acid and base binding capacities than the acid casein (103). The values can be estimated by potentiometric titrations and conductivity measurements (104-108) or by determining the minimum amount of acid or base required to dissolve a given weight of casein (109-112). The values also depend, as expected, upon the pH of casein solution at the time of the determination as well as upon the nature of the acid or base used for the purpose. Sandelin (107), for example, reported binding of 67 equivalents of sodium hydroxide and 75 equivalents of calcium hydroxide as the base per 10^5 g casein at pH 7.0.

Cohn and Bergrenn (96) used acid and base binding capacities for calculating the total number of dissociating groups and tried to correlate them with amino acid composition of casein. The maximum acid and base binding capacities of α-, β- and γ-caseins were also determined by Hipp et al (113) using potentiometric titrations. The values reported by them are 78, 66 and 85 equivalents for acid binding (hydrochloric acid) and 198, 150 and 96 equivalents for base binding (sodium hydroxide) per 10^5 g of each of these products. Recently acid (Hydrochloric acid) and base (sodium hydroxide) binding capacities have
been reported by Ho and Waugh (114) for $\alpha$-casein to be $-73$ and $210$ equivalents per $10^5$ g and by Creamer (115) for $\beta$-casein to be $-62$ and $137$ equivalents per $10^5$ g.

**Viscosity and Surface Tension**: Casein solutions in aqueous alkalies like sodium hydroxide, calcium hydroxide and ammonium hydroxide and aqueous acids like hydrochloric acid are truly viscous as they obey Poiseullie's Law (41, 113, 116-125). These are more viscous than water even at comparatively low concentrations. As the concentration increases, the relative viscosity increases more rapidly (126), giving ultimately a product known as casein glue (127-129). The viscosity decreases, more rapidly than that of water, with rise in temperature (126). The plot of viscosity against the log of reciprocal of temperature ($^K$) has been found to be linear (128). The acid solutions are less viscous than the alkaline solutions (116). The viscosity generally, increases with rise in pH till a maximum is obtained in the pH range 10.5-11.5 and then begins to decrease (118, 179). Kuntzel and Doehner (130) observed liberation of ammonia at high pH. Similar maximum is also obtained in the acid range between pH 2.2-2.8. The viscosity in alkaline solutions, however, decreases in the presence of neutral salts. This decrease has been attributed to electroviscous effect caused by
the adsorption of cations on the negatively charged caseinate ions (131-133).

Casein being a protective colloid concentrates more at the interface and, therefore, the surface tension of its solutions is invariably lower than that of the solvent (134, 135). It has been shown that the surface tension of freshly formed solutions of casein in an acid or alkali remains the same as that of water for about 6 seconds after which it continues to decrease systematically for several hours (88). The casein micelles are considered to be oriented with polar -CONH- group oriented towards water (135).

**Vapour Sorption:** A large amount of work has been reported on the sorption of water vapour by proteins (136-147) other than casein. It is generally believed (143, 147, 148), that the sorption of water by proteins takes place through hydrogen bonding with the polar side chain groups of amino acids such as lysine, arginine, tyrosine, glutamic acid and aspartic acid. Bull and Shaw's (136, 149) data on a number of proteins (casein not included), as interpreted by Pauling (150), indicate that peptide bonds show little attraction for water molecules. It is believed that these bonds are held together by hydrogen bonding to such an extent that the residual attraction for
water is almost negligible. Bull and Breese (147) showed that the presence of amides may actually inhibit water binding by other polar groups. Mellon et al (151-154), however, were of the view that peptide bonds from 2-6 units in length can adsorb water vapour.

Data on the sorption of water vapour by casein are relatively fewer than those on other proteins (155-159). Berlin, Anderson and Pallansch (160), however, have shown that casein, dried directly from the wet state in vacuum, differs remarkably in its capacity to sorb water vapour at room temperature and nitrogen at \(-195^\circ C\) from the product dried by exchange of solvents of decreasing polarity. The hygroscopicity of casein is considered to be important from the point of view of manufacture and storage of dry milk products. Desorption studies (160-164) have revealed that the sorption process is not completely reversible. An appreciable amount of hysteresis has been observed. According to some of the workers (160, 164), a part of the water cannot be desorbed even on evacuating the system for several days at the same temperature at which sorption had been studied. Berlin et al (165) reported appreciable sorption of benzene vapour as well but only if casein had been dried by exchange of solvents of decreasing polarity and not if it had been dried
directly from wet state in vacuum.

Swelling: Casein, like all other proteins and high polymers, is capable of swelling in various liquids which can cause its wetting. It is believed that some of the liquid molecules penetrate into the intimate structure of the micelles. This results in swelling. Heat is evolved during the swelling of casein by water particularly during the early imbibition of water (166). The heat evolved in the saturation of one gram of casein has been found to be about 25 calories. Swelling in aqueous solutions of various salts and alkalies has also been observed (167). The extent of swelling has been reported to be 31 per cent and to be completed in 90 minutes. The maximum swelling in one per cent solutions of salts, like calcium thiocyanate, ammonium thiocyanate, cobalt ammoniate, potassium tartarate and ammonium chloride has been found to be comparatively less. However, it is completed in relatively shorter time, i.e. in about 60 minutes. Pauli and Mandovsky (168) have shown that the ions which cause maximum swelling can bind minimum amount of water. Thus the order in which the capacity of ions to cause swelling decreases follows the reverse order of the well known lyotropic series. The extent of swelling in 0.1 per cent aqueous ammonia is
reported to be 200 per cent, and in 5 per cent ammonia to be 250 per cent. The swelling in aqueous sodium hydroxide is much less (167).

Optical Activity: Casein solutions are optically active, being laevorotatory (169, 170). The values reported are -105° for unfractinated casein, -81.4 to -90.5° for \( \alpha \)-casein (depending on its method of preparation), -125° for \( \beta \)-casein and -132° for \( \gamma \)-casein (10). The complete removal of fat gives a higher value of specific rotation (171). The value also increases with pH but only initially. Beyond a certain maximum the value falls off slightly (172). At a given pH, specific rotation increases with decrease in concentration of the solution (173).

Cation Binding: Casein in alkaline solution is able to bind a number of cations (174-186); the capacity to bind polyvalent cations is much more than that to bind monovalent cations. The binding has been shown to be pH dependent. Thus the value for the binding of sodium ion has been shown by Carr et al (178) to be 18 moles/10⁵ g casein at pH 7.5 and zero at pH 5.7. According to Dickson and Perkins (184), who employed equilibrium dialysis technique, the binding of cations varies with the concentration of free cations, pH and ionic strength of the medium. Complex formation of \( \alpha \)- and \( \beta \)-caseins with copper and cobalt have also been
reported (187, 188).

Oxidation: Casein is oxidised by many different reagents to different degrees determined by the severity of the conditions employed. The sulphhydryl group is most susceptible to oxidation, being oxidised under mild conditions. More drastic oxidation is caused by such reagents as oxygen, bromine, potassium permanganate and dichromate. The phenol and indole groups of tyrosine and tryptophan are also susceptible next only to the sulphhydryl groups.

**SCOPE OF THE PRESENT WORK**

The perusal of the literature shows that a number of physico-chemical properties of a variety of proteins have been investigated in recent years. The study has been useful in characterising these materials and understanding their behaviour in different environments. Similar work on casein, and its major fractions (α- and β-fractions) in particular, however, has not received sufficient attention. Moreover, in view of compositional differences between cows' and buffaloes' milk, it is interesting to compare physico-chemical behaviour of caseins isolated from both varieties of milk under similar conditions.
In the present work casein was isolated from fat-free milk of each variety by the gradual addition of hydrochloric acid to lower the pH value to the isoelectric point. The product was also resolved into its major fractions, namely \( \alpha \)- and \( \beta \)-caseins. The details of the exact procedure are given in the experimental portion of the thesis (pages 151 to 153). The nitrogen contents of the dried samples were determined by Kjeldahl's method, and the values marked as (a) and (b) are included in Table I (page 9).

The thesis has been divided into six chapters, followed by a separate part which gives details of the experimental techniques and methods used in the investigations.

The first chapter describes electrometric titration curves of unfractionated casein and its major (\( \alpha \)- and \( \beta \)-) fractions using sodium hydroxide, calcium hydroxide and ammonium hydroxide as the titrants in the alkaline range, and hydrochloric acid in the acid range. These curves provide information regarding the number of various groups ionising in different pH ranges. Titration with sodium hydroxide has also been carried out in the presence of formaldehyde in order to estimate the number of amino groups by an alternate method.

The second chapter relates to measurements of viscosities and surface tensions of casein and
its $\alpha$- and $\beta$- fractions in aqueous solutions of sodium hydroxide, calcium hydroxide and ammonium hydroxide. The data can be useful for characterising these materials as well as for calculating their base binding capacities and axial ratios of the molecules.

The next chapter describes adsorption isotherms of water vapour at 30°C by casein and its fractions. The data have been analysed by using B.E.T., Harkins and Jura and Frankel, Halsey and Hill equations. The values for specific surface area obtained by applying various equations have been found to be in fair agreement. The thermodynamic functions like free energy change, enthalpy change and entropy change of the process have been calculated. The freezing point of water held by casein in equilibrium with different relative vapour pressures has also been determined.

The fourth chapter deals with adsorption of certain organic polar vapours, such as methanol, ethanol and butanol, of increasing molecular dimensions. The isotherms have been analysed by applying the same equations as in the case of water isotherms. The t-plots of the various adsorbate-adsorbent systems, following the work of De Boer (189) and Hagymassy, et al (190), have been discussed. The differential free energy, enthalpy and entropy changes accompanying the adsorption of these vapours have also been calculated.
The fifth chapter relates to the hysteresis effect in the sorption-desorption isotherms of water, methanol and ethanol vapours at 35°C on casein and its major fractions in the native as well as denatured state. The data have been discussed in the light of various theories of hysteresis.

The sixth and last chapter deals with the flocculation of casein sols at pH 7 and 10 at 0.5 and 1.0 per cent concentrations by the addition of various electrolytes. The binding of a few transition metal cations to different casein sols at pH 7 has also been studied in detail by following equilibrium dialysis technique. The equilibrium binding constant, intrinsic association constant as well as standard free energy and entropy changes for the binding of first metal cation to various caseins have been calculated.
REFERENCES


34. Svedberg, T., Kolloid, Z., 51, 10 (1930).


69. Lipman, F., Biochem. Z., 262, 3 (1933).


170. Robertson, T.B., 'Physical Chemistry of the Proteins', New York, Longmans Green, Ch. 14 (1918).


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