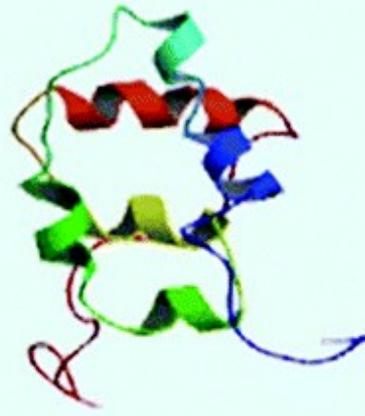


$\alpha_5$ -Casein

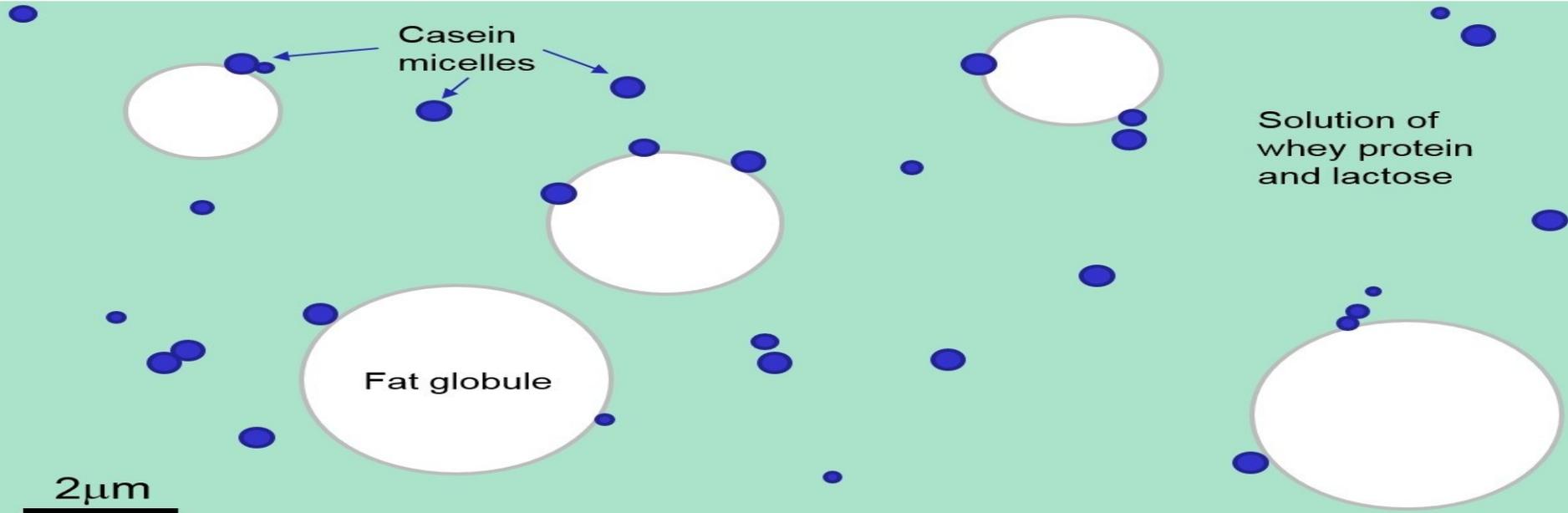


$\beta$ -Casein

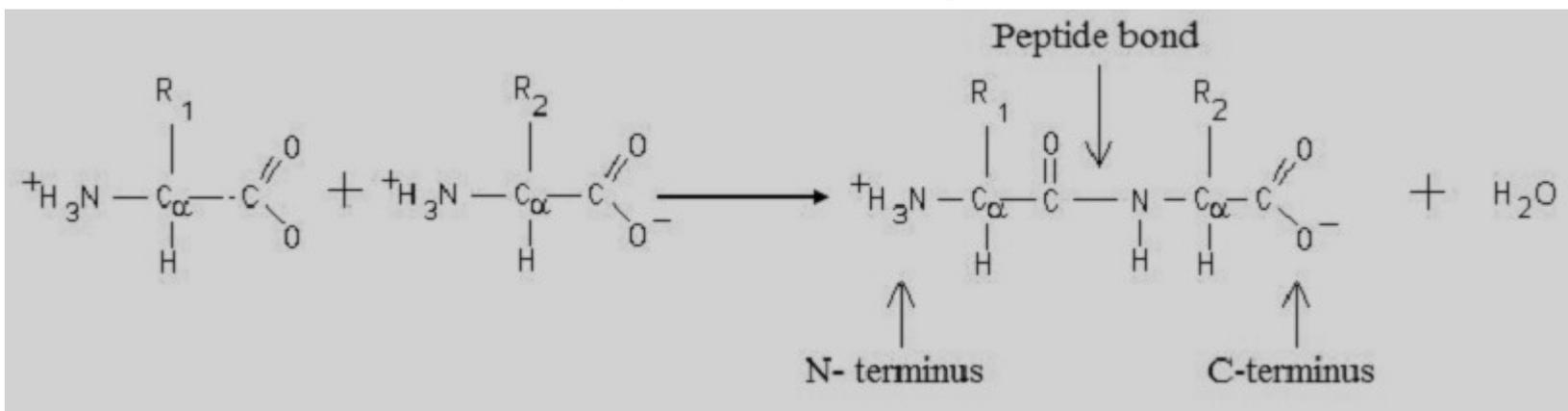


$\kappa$ -Casein

# Milk Protein-Casein



1. Two-thirds of all cells consist of proteins and they have functional and structural roles in human body such as catalyst (enzymes), regulator (hormones), protection (Immunoglobulins), carrier proteins (haemoglobin, lactoferrin, etc.) and as structural proteins (collagens).
2. Milk Protein is widely consumed human food infants and children.
3. Casein is unique due to their characteristic physical properties which are different from globular proteins.
4. Proteins are defined as high molecular weight polymers of  $\alpha$ -amino acids that are formed by living organisms. All these amino acids have L-configuration except glycine.
5. The primary structure of proteins consists of a polypeptide chain of amino acids residues joined through peptide bonds.



# Nomenclature of Milk Proteins

- ✓ The Committee on Milk Protein Nomenclature, Classification, and Methodology of the Manufacturing Section of American Dairy Science Association (ADSA) suggested suitable nomenclature to the milk proteins.
- ✓ Nomenclature is suggested on basis on the resolution of this protein on the free boundary electrophoresis. The proteins which are having similar electrophoretic characteristics at pH 8.6 were actually heterogeneous mixtures of proteins.
- ✓ Waugh and Von Hipp et al. (1956) have shown that  $\alpha$ -casein could be separated into calcium sensitive  $\alpha$ s-casein and calcium insensitive crude k-casein based upon its dissociation and differential solubility in the presence of calcium ions.

✓ According to ADSAC system of classification (American Dairy Science Associations committee on milk protein nomenclature, classification and methodology) a Greek letter with or without a numerical precedes the class name when necessary to identify the family of proteins. The genetic variant of the protein is indicated by an upper case Arabic letter with or without a numerical superscript immediately following the class name. Post translational modifications are added in sequence:  $\beta$  casein B 5P (f1 to 105) indicates that:

- ✓ a. Class of the protein is caseins
- ✓ b. The family is  $\beta$
- ✓ c. The genetic variant is B
- ✓ d. There are 5 post translational phosphorylations
- ✓ e. The amino acid sequence from N-terminal amino acid through residue 1 through residue 105.

# Classification and Distribution of Milk Proteins:

Proteins in milk are classified on the basis of their fractionations and their behaviour during electrophoresis, difference in their solubility in various solutions, difference in their sedimentation rate etc.

**Genus Bos (30-35 g/L )**

**a. Caseins (24-28 g/L)**

**$\alpha_{s1}$ -Caseins (12-15 g/L)**

1.  $\alpha_{s1}$ -Casein X-8P ( X = Genetic variants-A, B, C, D-9P, and E)
2.  $\alpha_{s1}$ -Casein X-9P (X = Genetic variants-A, B,C, D-10P, and E)
3.  $\alpha_{s1}$ -Casein fragments'

**$\alpha_{s2}$  caseins (3-4 g/L)**

1.  $\alpha_{s2}$ -Casein X-10P ( X =Genetic variants-A, B, C-9P, and D-7P)
2.  $\alpha_{s2}$ -Casein X-11P (X =Genetic variants-A,B, C-10P, and D-8P)
3.  $\alpha_{s2}$ -Casein X-12P (X =Genetic variants-A, B, C-11P, and D-9P)
4.  $\alpha_{s2}$  Casein X-13P (X =Genetic variants-A,B, C-12P, and D-10P)

## **β -Casein (9-11 g/L)**

- **βCasein X-5P (X=Genetic variants-A<sup>1</sup>,A<sup>2</sup>,A<sup>3</sup>,B,C-4P,D-4P, and E)**
- **β-Casein X-IP (f 29-209) (X=Genetic variants- A<sup>1</sup>,A<sup>2</sup>,A<sup>3</sup> and B)**
- **β-Casein X-(f 106-209)(X =Genetic variants- A<sup>2</sup>, A<sup>3</sup> and B)**
- **β-Casein X-(f 108-209) (X=Genetic variants-(Fragments from A<sup>1</sup>,A<sup>2</sup>,A<sup>3</sup> and B)**
- **β - Casein X-4P (f 1-28)**
- **β -Casein X-5P (f 1-105)**
- **β -Casein X-5P (f 1-107)**
- **β -Casein X-IP ( f 29-105)**
- **β Casein X-IP (f 29-107)**

## **κ -Caseins (2-4 g/L)**

- 1.κ -Casein X-IP (X = Genetic variants-A and B)**
- 2. Minor κ -caseins X -1, -2, -3, etc. ( x = Genetic variants-A and B)**

**b. Whey proteins (5-7 g/L)**

**$\beta$  Lactoglobulins (2-4 g/L)**

**$\beta$  -Lactoglobulins X ( X = genetic variants-A,B, C, D, Dr, E, F, and G)**

**$\alpha$ -Lactalbumins (0.6-1.7g/L)**

**$\alpha$ -Lactalbumin X ( X = Genetic variants-A and B)**

**Minor  $\alpha$ -Lactalbumins**

**Bovine serum albumin (0.2-0.4 g/L)**

**Immunoglobulins (0.5-1.8 g/L)**

**IgG immunoglobulins**

**IgG1, Immunoglobulins**

**IgG2, Immunoglobulins**

**IgG fragments**

**IgM immunoglobulins**

## **IgA immunoglobulins**

**IgA Immunoglobulins**

**Secretory IgA Immunoglobulins**

**IgE Immunoglobulins**

**J-chain component**

**Free secretory component**

## **c. Milk Fat Globule Membrane (MFGM) Proteins**

- 1. Six major proteins readily stained with Coomassie brilliant blue are XDH/XD; CD36, BTN,ADPH, PAS 6/7 And FABP.**
- 2. Two stained by Periodic Acid/ Schiff (PAS) or silver stain are Muc-1 and PAS III.**

## **d. Minor proteins**

**Serum transferrin**

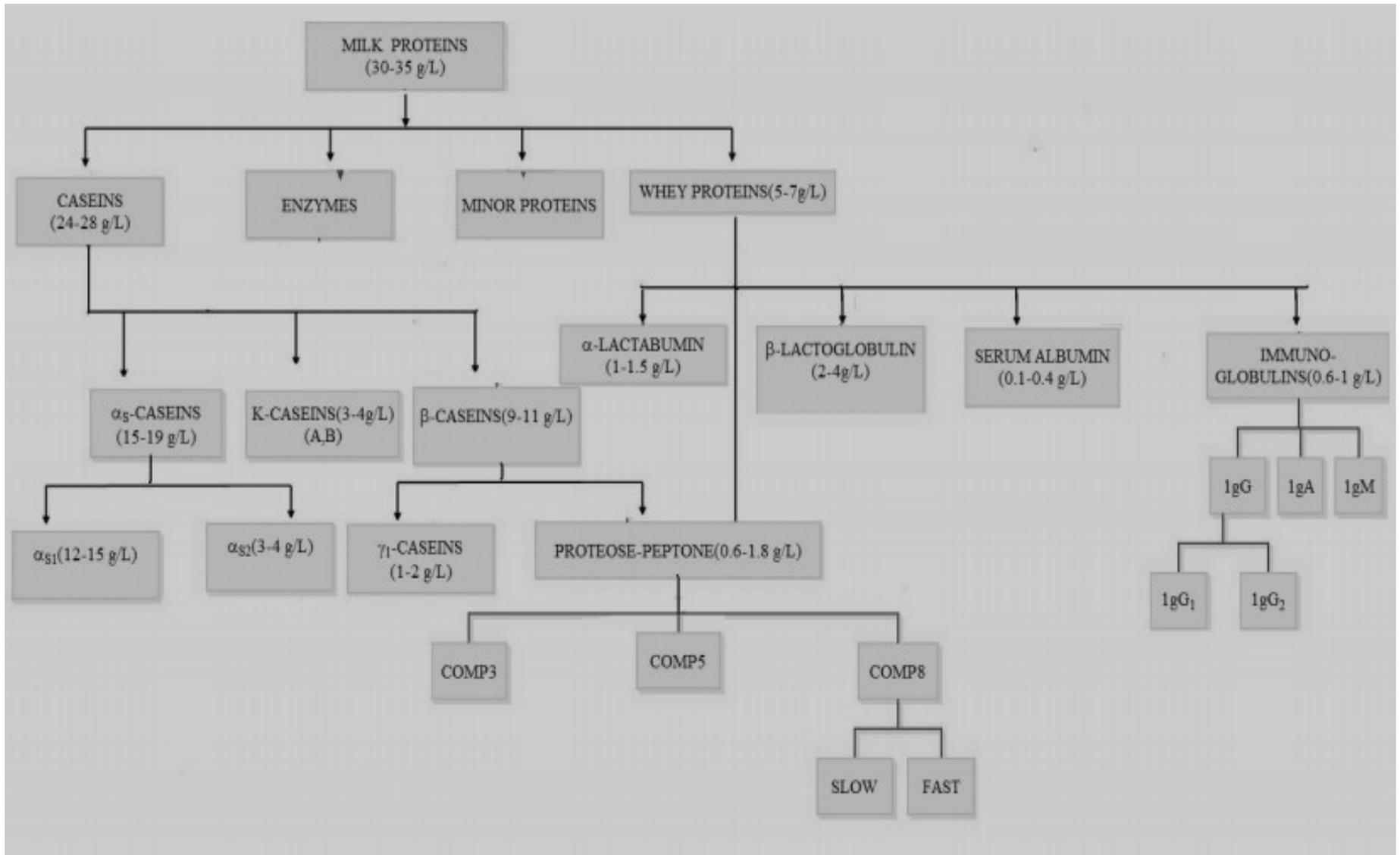
## Polymorphism in Milk Proteins

1. In 1956, Chaffenburg and Drewry discovered that the  $\beta$  lactoglobulin exists in two forms A and B which differs from each other by only a few amino acids.
2. The milk of few individual animal may contain  $\beta$  IgA or  $\beta$  IgB or both and the milk is indicated as AA, BB or AB with respect to  $\beta$  Ig. This phenomenon is referred as **genetic polymorphism** and has since been shown to occur in all the milk proteins.
3. A total of about 30 variants have been demonstrated by Polyacrylamide Gel Electrophoresis (PAGE). Since PAGE differentiates on the basis of charge and molecular weight, only polymorphs which differ in charge alone are detected.

- 4. The frequency with which certain genetic variant occur is breed specific and hence genetic polymorphism has been useful in phylogenic classification of cattle and other species.**
- 5. Various technologically important properties of the milk proteins are related to the genes that occur in the primary polypeptide chain of milk proteins, which will result in variation of the behaviour of that protein either on electrophoretic behaviour or its gel filtration pattern. This is usually due to the changes occurring in genetic code for that protein. This deviation or change is referred as polymorphism. There are several such variations observed in the milk proteins.**

## Distribution of Milk Proteins

1. Proteins in milk are chiefly the caseins, whey proteins and some minor proteins.
2. Since enzymes are protein in nature they are also grouped along with other proteins. Caseins being the major fraction of the entire milk proteins are present in micellar form while the whey proteins being considered as soluble proteins.
3. Enzymes play a significant role in the raw milk storage and processing of the milk and also found to have industrial importance.
4. Minor proteins include vitamin binding protein, lactoferrin, metallo-protein, MFGM protein, Ceruloplasmin, etc.



# Casein

1. Low solubility at pH 4.6 and at this pH, all of the caseins except some of the proteolytic derivatives precipitate. As the solubility of caseins is much less and the whey proteins are having better solubility than casein the separation of the casein has become possible by lowering the pH value to 4.6.
2. The ion exchange chromatography using DEAE-cellulose or hydroxyapatite columns would give satisfactory fractions. However, it is necessary to control the interaction of casein molecules.
3. The unique feature of the caseins is their ester-bound phosphate. All of the casein polypeptide chains have at least one such group per molecule; whereas, none of the whey proteins have any ester bound phosphate.
4. The  $\alpha_1$  and  $\beta$ -caseins contain no cysteine residues, while  $\alpha_2$  and  $\kappa$ -caseins each have two cysteine residues.
5. Proline contents of caseins are rather high ( $\alpha_1$ -,  $\alpha_2$ -,  $\beta$ -, and  $\kappa$ -caseins contain 17, 5, 17 and 12 mol%, respectively).

6. They have short lengths of  $\alpha$ -helix or  $\beta$ -sheet structure in them. Their ionizable groups of the amino acids are accessible to titration and are also involved in several other side chains to reaction.
7. Denaturing agents and heating seems to have no effect on the secondary structure of these proteins. Thus their conformation appears to be much like that of denatured globular proteins.
8. As the protein is having large proportion of proline residues, closely packed orderly secondary conformation is being prevented in this protein molecule.
9. The four caseins differ greatly from each other in charge distribution and the tendency to aggregate in the absence and presence of  $\text{Ca}^{2+}$  ions.

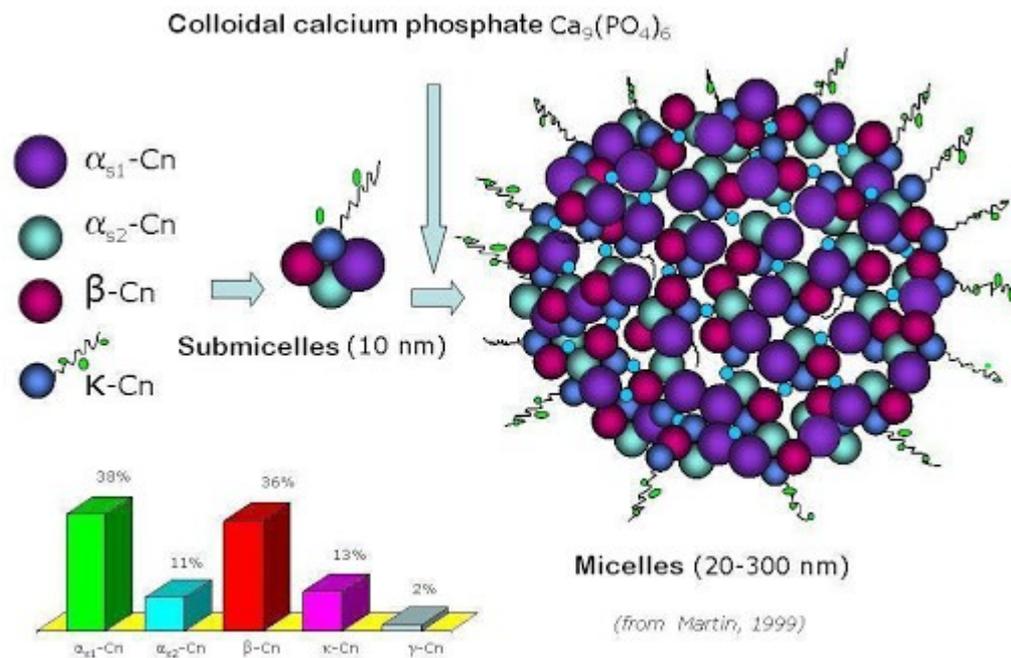
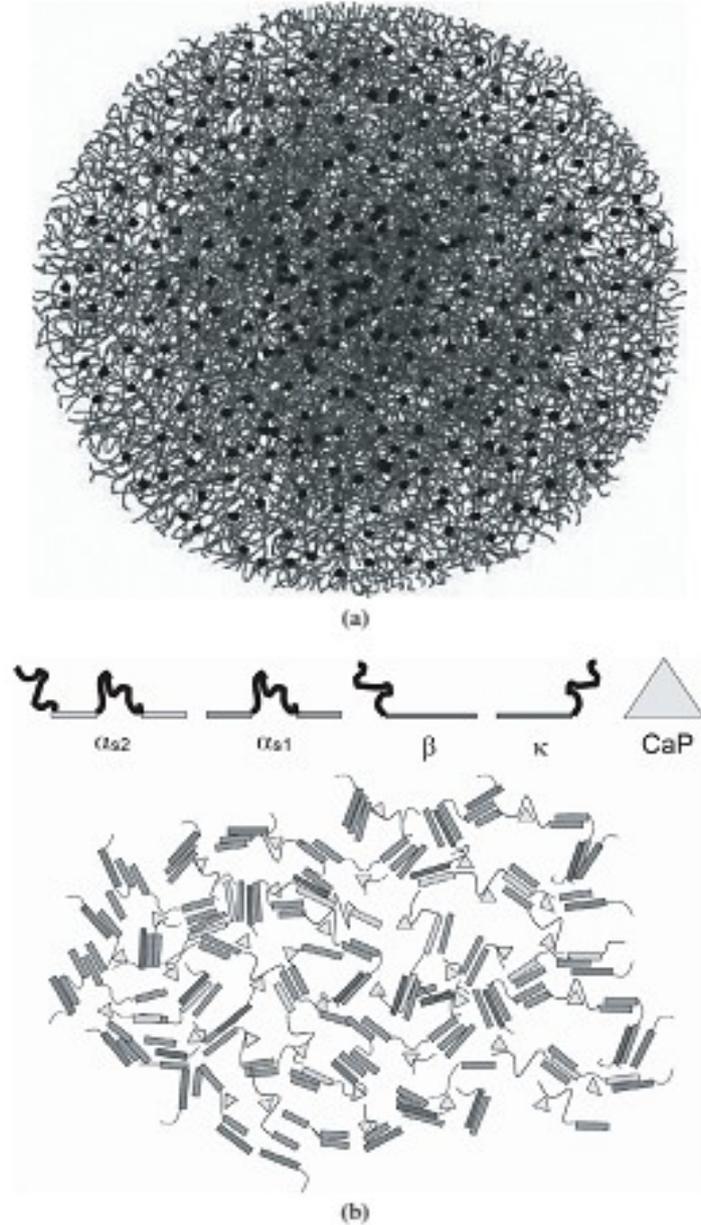
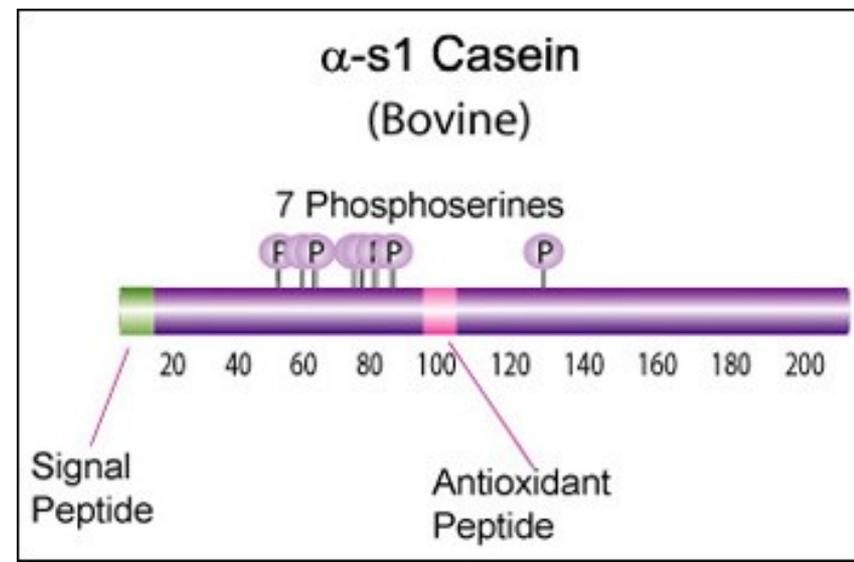


Figure 2. (a) The nanocluster model for casein micelles. Casein monomers are thread-like, while the dark circles represent calcium phosphate nanoclusters; reprinted from [12] with permission of Springer, NL. (b) The model of Horne – dual bonding model of casein micelles using casein monomers as indicated. Protein-protein interactions occur between hydrophobic regions (rectangular bars) while the protein hydrophilic regions (loops) bind to calcium phosphate clusters (triangles).  $\kappa$ -Casein is monomeric and on the surface; reprinted with permission from Elsevier.

# $\alpha$ 1-casein

1. The polypeptide chain of  $\alpha$ 1-casein consists of two predominantly hydrophobic regions (residues 1-44, and 90-199) and a highly charged polar zone (residues 45-89).
2. All but one of the phosphate groups is in the 45-89 residues segment, and prolines are distributed at intervals in the hydrophobic segments. Thus, this protein can be visualized as a rather loose flexible polypeptide chain.
3. Self-association of  $\alpha$ 1-casein depends markedly on its concentration and on the pH, ionic strength, and kind of ion in the medium, but it is relatively independent of temperature.



4. Physical measurements such as light scattering, sedimentation, and viscosity indicate that  $\alpha_1$ -casein is completely dissociated to a flexible random chain monomer of MW= 24,000 at pH 12, in 3M guanidinium chloride and at neutral pH and 0.01 ionic strength, it associates at neutral pH and higher ionic strength, the extent of association depending on protein concentration, it binds about 8 moles  $\text{Ca}^{2+}$  per mole near pH 7, probably to the ester phosphate groups.
5. It aggregates and precipitates at very low concentrations of  $\text{Ca}^{2+}$  (7 mM  $\text{Ca}^{2+}$  28 mM NaCl).
6. A small amount of peptides, sometimes called A-casein, is present in milk; these appear to originate from proteolysis of  $\alpha_1$ -casein.

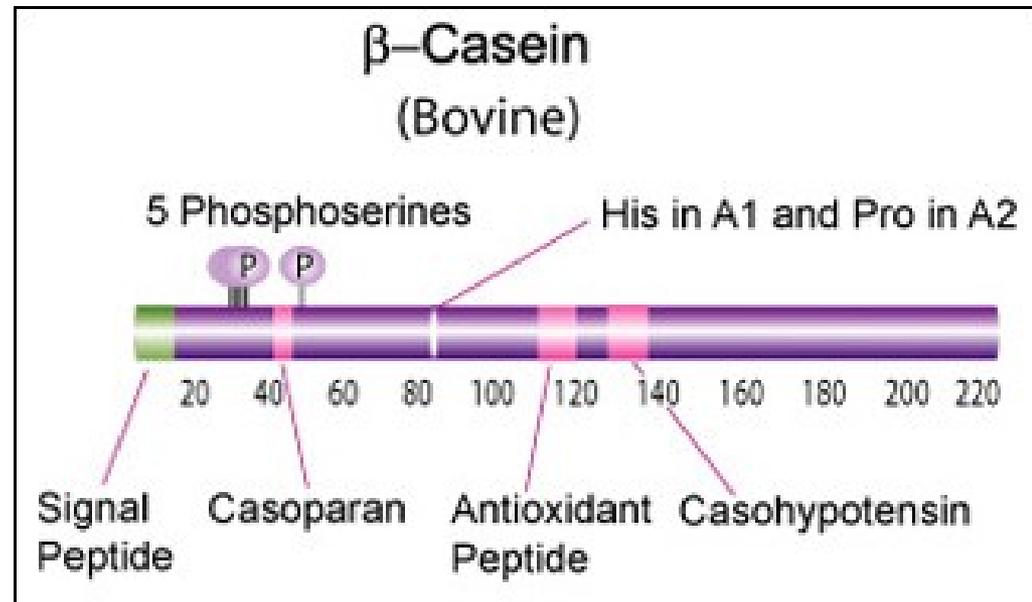
## **$\alpha$ s2-casein**

- 1.  $\alpha$ s2-casein has a remarkable dipolar structure with a concentration of negative charges near the N-terminus and positive charges near the C-terminus.**
- 2. Its properties have not been investigated as thoroughly as those of the other caseins, but certainly it binds Ca strongly and is even more sensitive to precipitation by  $\text{Ca}^{2+}$  than  $\alpha$ s1-casein.**
- 3. It self-associates at neutral pH in the absence of  $\text{Ca}^{2+}$  and the association depends markedly on ionic strength and is at a maximum at an ionic strength of about 0.2.**

# $\beta$ -casein

1.  $\beta$ -casein has a strong negatively charged N-terminal portion.
2. The net charge of the 21-residue N-terminal sequence is 12 at pH 6.6, and the rest of the chain has virtually no net charge.
3. The large number of Pro residues effectively precludes extended helix formation. Thus, the  $\beta$  casein molecule is somewhat like that of an anionic detergent with a negatively charged head and an uncharged essentially hydrophobic tail.
4. The outstanding characteristics of the association of  $\beta$ -casein in both the absence and the presence of  $\text{Ca}^{2+}$  are its strong dependence on temperature.
5. In the absence of  $\text{Ca}^{2+}$ , only monomer is present at 4°C, but large polymers (20-24 monomers) are formed at room temperature.

6. Removal of the 20-residue C-terminal sequence destroys the ability of  $\beta$ -casein to associate, suggesting that specific hydrophobic interactions may be involved.
7.  $\beta$ -casein tightly binds about 5  $\text{Ca}^{2+}$  per mole, consistent with its ester phosphate content.



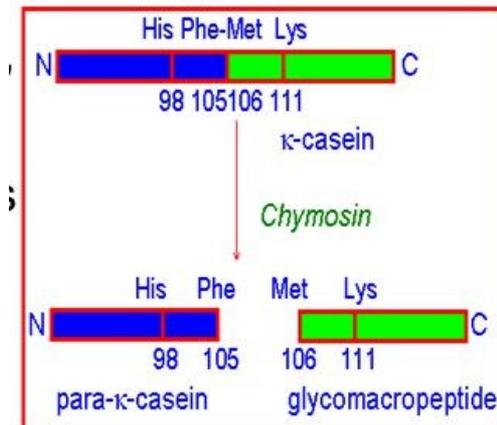
## $\gamma$ -casein

- 1. Group of caseins designated as  $\gamma$ -caseins have been known for some time to correspond to C terminal portions of the  $\beta$ -casein sequence. These are formed by cleavage of  $\beta$ -casein at positions 28/29, 105/106, and 107/108 by the enzyme plasmin.**
- 2. The fragments 29-209, 106-209, and 108-209 constitute the  $\gamma$ -caseins. The smaller fragments resulting from the cleavage appear in the whey when casein is precipitated by acid and constitute part of what has long been designated as the proteose-peptone fraction of the whey. Thus, fragments 1-105 and 1-107 were called as whey component 5, fragment 1-28 is whey component 8-fast, and fragments 29-105 and 29-107 were named as whey component 8-slow.**

## **κ-casein**

- 1. About one-third of the κ casein molecules are carbohydrate-free and contain only one phosphate group (SerP-149).**
- 2. They have varying numbers of N-acetylneuramic acid (NANA) residues and one, at least, appears to have a second phosphate (SerP-127).**
- 3. Three different glycosyl oligomers linked to Thr-133 have been identified.**
- 4. The N-terminal residue of κ-casein is glutamic acid. In the isolated protein it is present as the cyclic derivative pyroglutamic acid.**
- 5. κ -casein consists of a mixture of polymers probably held together by intermolecular disulfide bonds; these polymers range in molecular weight from about 60,000 (trimers) to more than 150,000.**

- $\kappa$ -casein is rapidly hydrolyzed at the Phe (105)-Met (106) bond by the enzyme chymosin (EC 3.4.23.4) yielding N-terminal fragment called para- $\kappa$  casein, which contains the two cysteine residues, a C-terminal fragment of 64 residues called the macropeptide, containing all of the carbohydrate and phosphate groups.  $\kappa$ -casein binds about 2 moles  $\text{Ca}^{2+}$  per mole of protein at neutral pH but differs markedly from the other caseins in its solubility.
- The naturally occurring mixture of bovine  $\kappa$ -casein variants polymerizes through -S-S- linkages to subunits containing three to eight monomers. These further polymerize by non covalent association to particles of about 6,50,000 D. This polymerization is insensitive to concentration of  $\text{Ca}^{2+}$  and to temperature.

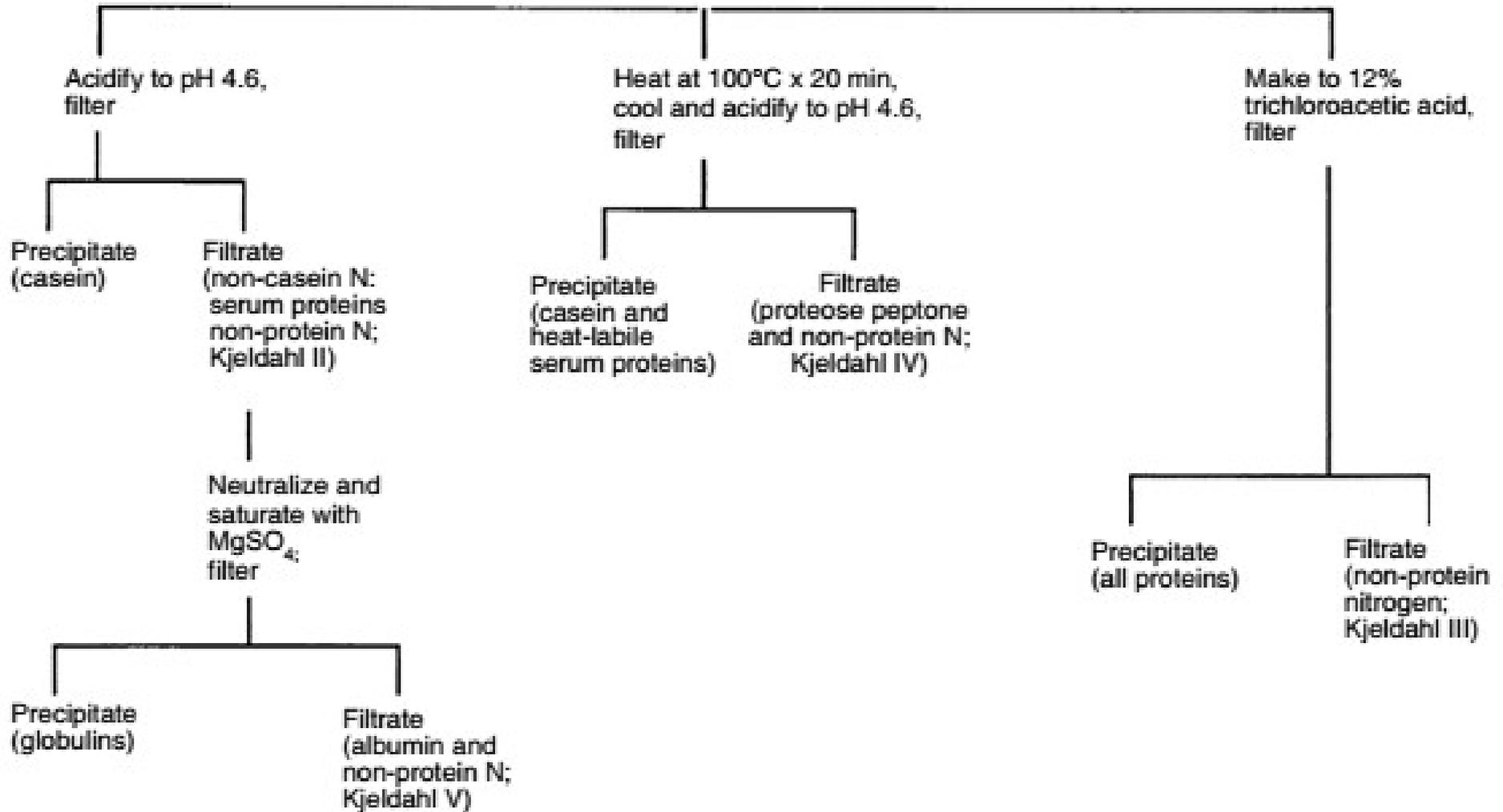


**Milk Fractionation:** The behaviour of individual milk proteins and their concentration will be known when they are fractionated from milk.

### **Rowland method of fractionation**

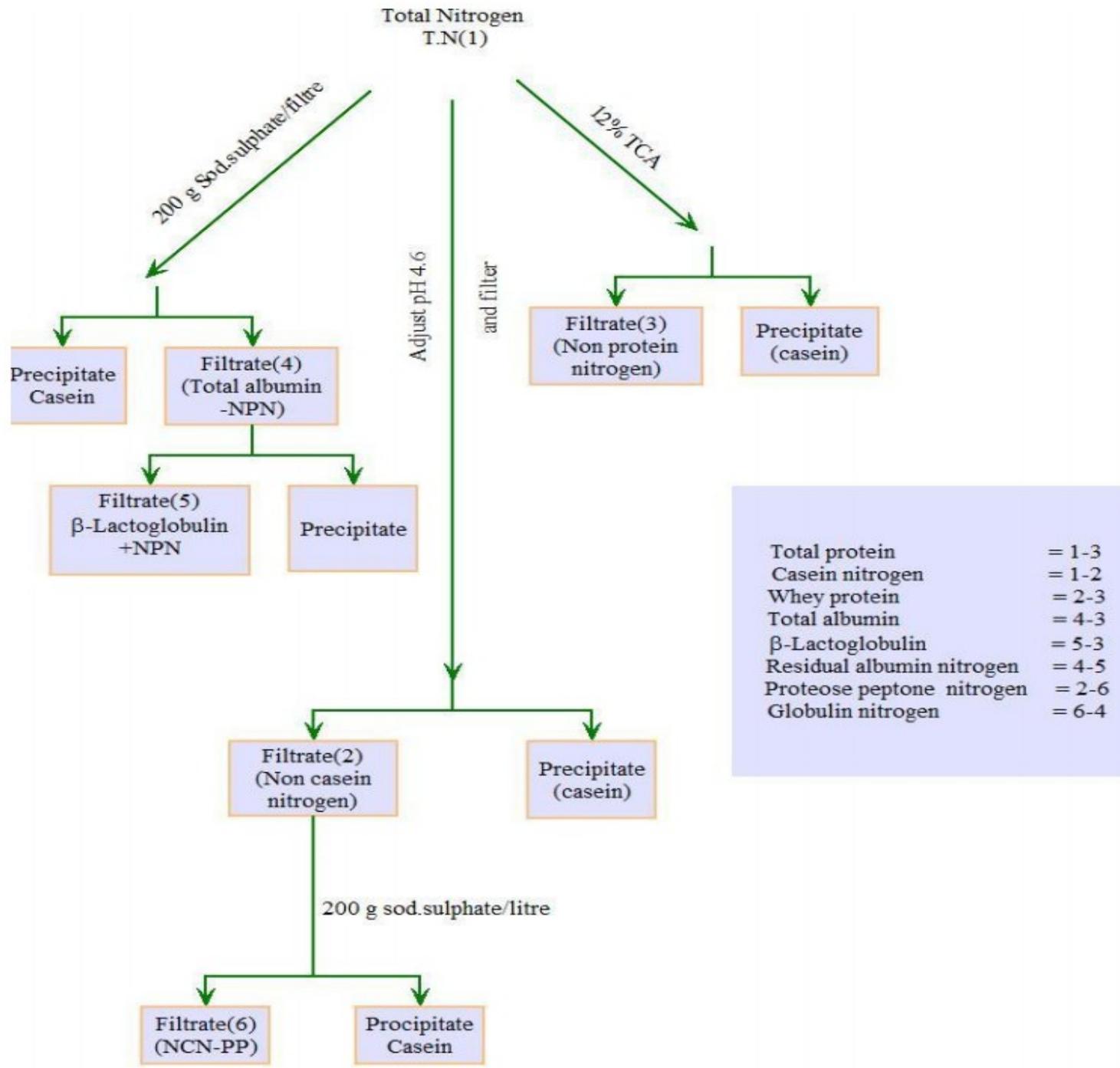
- 1. Rowland observed that when milk was heated to 95 C for 10 min, 80% of the nitrogenous compounds in whey were denatured and co-precipitated with the casein when the pH of the heated milk was adjusted subsequently to 4.6.**
- 2. He considered that the heat-denaturable whey proteins represented the lactoglobulin and lactalbumin fractions and designated the remaining 20% 'proteose-peptone'.**
- 3. The proteose peptone fraction, which is quite heterogenous is precipitated by 12% trichloroacetic acid (TCA) but some nitrogenous compounds remain soluble in 12% TCA and are designated as non-protein nitrogen.**

Skimmilk (Kjeldahl I)



**Aschaffenburg and Drewry method (1959):** In the modified method, the solubility of some fractions of the proteins in sodium sulphate solutions is utilized where the total albumin along with NPN (Non-protein nitrogen) compounds will be soluble. While in saturated sodium sulphate the albumins get precipitated.

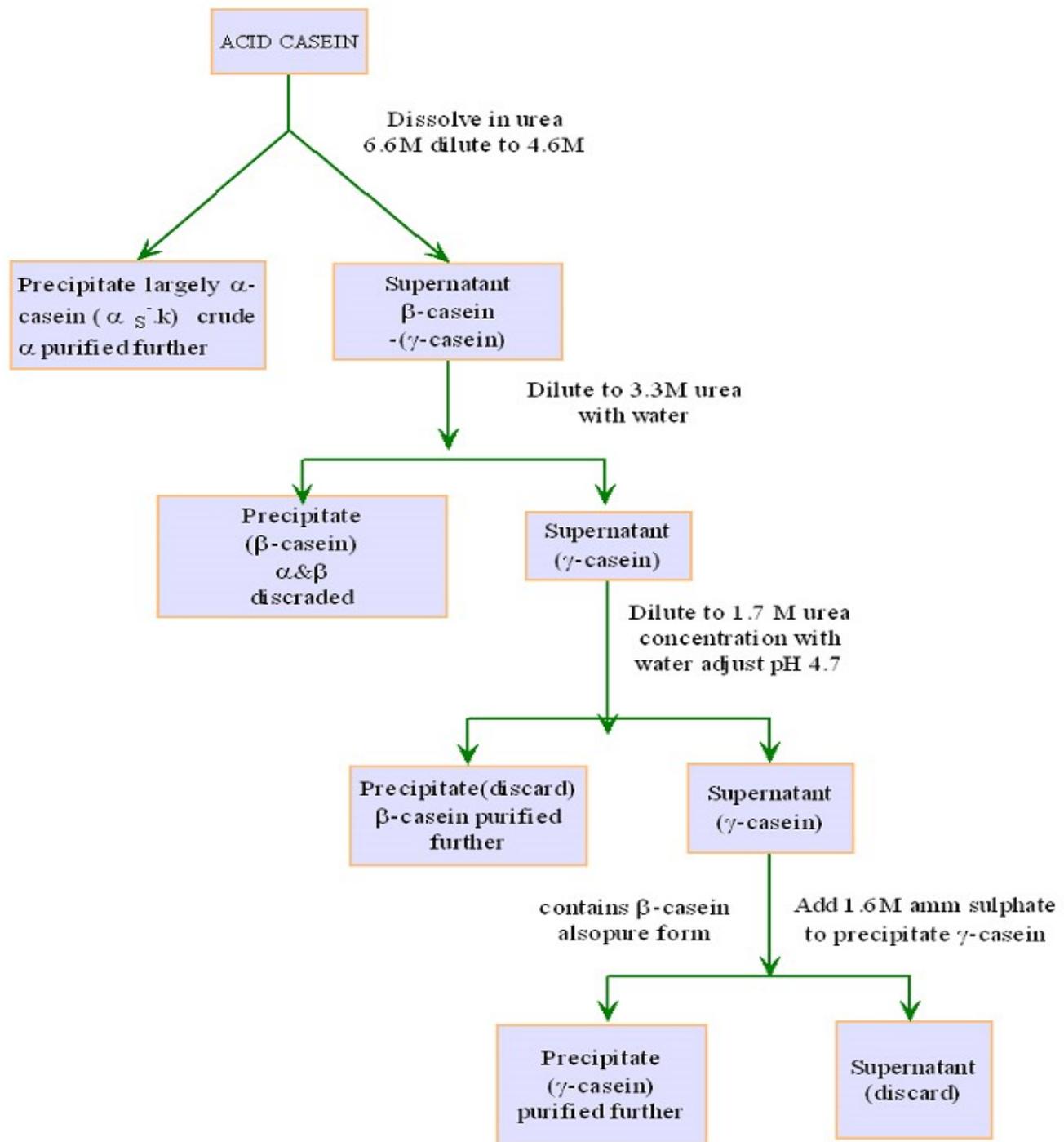
# Protein fractionation by Aschaffenburg and Drewry (1959)



1. Several methods have been developed to obtain one or more of the various caseins from whole casein or directly from skim milk based on their differential solubility.
2. Hipp et al (1952) developed two procedures which have been used extensively or partly incorporated into other methods.
  - The first one is based on the differential solubility of casein in 50% alcohol in the presence of ammonium acetate by carrying the pH, temperature and ionic strength.
  - The second procedure involves the dispersion of whole casein in 6.6 M urea and separation of casein fractions by dilution, pH adjustment and finally addition of  $(\text{NH}_4)_2\text{SO}_4$ .
3. The order of precipitation of the casein in both methods is  $\alpha$ -casein,  $\beta$ -casein and then  $\gamma$ -casein.
4. Warner's casein fractions are now identified as follows,  $\alpha$ -casein is a mixture of  $\alpha_s$  and  $\kappa$ -caseins.

5. The  $\alpha$ s caseins and  $\kappa$ -caseins have been prepared largely from either whole casein or the  $\alpha$ -casein fraction of Hipp and co workers.
6. Crude  $\alpha$ s-casein is prepared from casein obtained by  $\text{CaCl}_2$  precipitation at 37 C by removing calcium with oxalate to solubilize the casein and reprecipitation with 0.25M  $\text{CaCl}_2$  at 37 C and pH 7.0.
7. The  $\kappa$ -caseins are removed from the supernatant by precipitation with  $\text{Na}_2\text{SO}_4$ , followed by re-precipitation from 50% ethanol with ammonium acetate or by using calcium oxalate as a precipitate to enhance the removal of the other caseins.
8. Adjustment of the pH of whole casein dispersions in urea also has been used to precipitate the  $\alpha$ s-casein either by adjusting 6.6 M urea dispersion to pH 1.3-1.5 with  $\text{H}_2\text{SO}_4$  or by adjusting a 3.3 M urea to pH 4.5.
9. The crude  $\kappa$ -caseins can be obtained from supernatant of the  $\text{H}_2\text{SO}_4$  method, by precipitating with  $(\text{NH}_4)_2\text{SO}_4$  and purified by reprecipitation from aqueous ethanol.
10. More than 90% of  $\alpha$ s1-caseins have been recovered from acid casein by precipitation with 75mM  $\text{CaCl}_2$  at 5 C.

**Fractionation of casein by Hipp et al (1952) base on urea solubility**



**Physicochemical characteristics of caseins:** Number of these parameters is derived from the composition based on determination of the primary structure.

Protein	Charge at pH 6.6	Isotonic pH	Partial specific volume	Absorptivity (cm <sup>2</sup> /g) 280nm	Molecular weight
$\alpha_{s1}$ -casein					
A	-20.0	4.97	0.722	1.13	22066
B	-20.9	4.96	0.725	1.05	23612
C	-20.0	5.00	0.725	1.06	23540
D	-22.6	4.91	0.723	1.05	23722
$\alpha_{s2}$ - casein A					
P <sub>10</sub> ( $\alpha_{s6}$ )	-13.2	5.39	0.722	1.11	25148
P <sub>11</sub> ( $\alpha_{s4}$ )	-14.8	5.32	0.721	1.10	25228
P <sub>12</sub> ( $\alpha_{s3}$ )	-16.4	5.25	0.720	1.10	25308
P <sub>13</sub> ( $\alpha_{s2}$ )	-18.0	5.19	0.718	1.10	25388
$\beta$ -casein					
A <sup>3</sup>	-12.8	5.11	0.742	0.46	23.971
A <sup>2</sup>	-12.3	5.19	0.742	0.46	23980
A <sup>1</sup>	-11.8	5.27	0.742	0.46	24020
B	-10.8	5.35	0.742	0.46	24089
C	-8.2	5.53	0.742	0.46	24939
$\kappa$ -casein					
A	-3.9	5.43	0.734	0.95	19037
B	-3.0	5.64	0.734	0.95	19005

## **Structure of Casein Micelle:**

- 1. Casein in cow milk forms intricate particles which are recognized as casein micelles.**
- 2. Highly insoluble material (calcium phosphate) has to be carried without disturbing either stability or increase in its size.**
- 3. The whole casein will form aggregate when it is in solution at a concentration, pH, and ionic strength as in milk and low  $\text{Ca}^{2+}$  activity.**
- 4. The micelles contain about 10-100 casein molecules.**
- 5. The aggregates like globular proteins will have a dense hydrophobic core in which most hydrophobic parts of the casein molecules are buried and a more loosely packed, hydrophilic outer layer containing most of the acidic (carboxylic and phosphoric) and some of the basic groups.**

6. Each of these small aggregates of the whole casein, usually called submicelles contains different casein molecules. The relative proportion of  $\alpha s1$ :  $\alpha s2$ :  $\beta$  and  $\kappa$  in casein micelle is 3: 0.8: 3:1 respectively.
7. Moreover,  $\kappa$ -casein probably exists in milk as an oligomer containing several molecules and held together by covalent bonds (S-S linkages).
8. Consequently there may be essentially two types of submicelles with and without  $\kappa$ -casein.
9. Earlier casein micelle models described were:
  - 1) Core-coat model
  - 2) Internal structure model
  - 3) Sub-unit model

# Dual Binding model for micelle assembly and structure:

1. According to this model, micellar assembly and growth take place by a polymerization process involving two distinct forms of bonding: cross linking through hydrophobic regions of the casein or bridging across calcium phosphate nanoclusters.
2. Micellar integrity and stability is maintained by a localised excess of hydrophobic attractions over electrostatic repulsion.
3. Each casein micelle effectively functions as a block co polymer, with each block possessing different and possibly multiple functionality for the cross linking paths.
4.  $\kappa$ -casein is the most important of the caseins in this model of micellar assembly and structure. It is linked to the growing chain through its hydrophobic N-terminal block but its C-terminal block has no phosphoserine cluster and therefore cannot extend the polymer chain through an nanocluster link.
5. Final is a chain and network growth of casein micelle with a surface layer of  $\kappa$  -casein molecules.

6. There will be considerable increase in the association of the casein micelle with increase in the  $\text{Ca}^{2+}$  activity.
7. Calcium phosphate is present submicelles aggregate in to micelles. The calcium phosphate acts as cementing agent. The aggregation will go on until a gel or a precipitate was formed if there were no  $\kappa$ -casein, which thus acts as a “protective colloid”.
8. The C-terminal part of  $\kappa$ -casein is very hydrophilic particularly those molecules that contain carbohydrate and it also has a considerable negative charge. This part of the molecule sticks partly out in to the surrounding medium as a flexible “hair” more or less behaving as a random coil polymer chain.
9. Some areas have steric repulsion causing hindrance for aggregation of the submicelles. In this way aggregation of submicelles would go on until the surface of the micelle was more or less covered with  $\kappa$ -casein. The  $\kappa$ -casein will be interspaced with other caseins, instead of covering the entire surface of the micelles.

- 10. Most of the  $\kappa$ -casein is at the outside and the protruding chains of its C-terminal end give the micelles a hairy surface which are flexible and show perpetual Brownian motion. The effective thickness of the hairy layer is at least 5 nm. A small part of  $\kappa$ -casein is in the interior.**
- 11. About half of the voluminosity of casein micelle is due to the submicelles while remaining volume is serum between sub micelles.**
- 12. Taking the hairy outer layer in to consideration, micelles voluminosity is higher on average about 4 ml per gram of casein.**
- 13. The hydrodynamic voluminosity increases with the decreasing micelles size.**

- 15. The casein micelles are not static and have three dynamic equilibrium with its surroundings. The equilibrium is between the free casein molecules and sub micelles (a tiny part of the casein is in true solution) the equilibrium strongly depends on temperature, between the dissolved and colloidal Ca and phosphate, between free sub-micelles and micelles.**
- 16. The casein micelles are rather variable particularly in milk of individual cows.**
- 17. The proportion of  $\kappa$ -casein varies by about 9-15% which will be reflected in the average casein micelle size.**
- 18. The voluminosity varies by a factor of 2 variations in the size and voluminosity usually from 6-9 g per 100 g dry casein.**

19. The quantity of calcium phosphate in the micelles increases on heating and decrease when lowering the pH.
20. The casein micelles will be dispersed into smaller units by any treatment that dissolves a considerable proportion of the calcium phosphate at constant pH such as adding NaCl.
21. Micelles disperse in to submicelles when milk is subjected to high pressure (100 MPa for 1 h) this disintegration is irreversibly and it may be caused by the formation of hydroxyl apatite from the calcium phosphate.
22. Casein micelles also dissolve when submicelles dissociate.
23. In the sub micelles the molecules are held together mostly by hydrophobic interaction and by H bonds in the hydrophobic interior of the submicelles.
24. Consequently addition of large quantities of urea or guanidinium chloride and sodium dodecyl sulfate in small quantities will dissolves the micelles.
25. Electrostatic interactions also participate in keeping the submicelles together, but they cannot be broken without dissolving the calcium phosphate.
26. Reagents that break S-S linkage do not disintegrate the micelles fully.
27. Lowering the temperature (5 °C)considerably effects the casein micelles.
28. Hydrophobic interactions become much weaker and part of the casein particularly of the beta casein dissociate from the micelles.
29. A small part of the calcium phosphate dissolves. These changes may be the cause of the slight disintegration of the micelles despite the increase in

## Properties:

1. Almost all casein in un-cooled milk is present in roughly spherical particles mostly 0.02-0.30  $\mu\text{m}$  in diameter, comprised of some 20-150000 casein molecules.
2. These micelles also contain inorganic matter mainly calcium phosphate, about 8 g per 100 g casein.
3. The casein micelles are voluminous and hold more water than pure casein. They also contain small quantities of other proteins such as milk enzymes lipase and plasmin and part of proteose-peptone.

## **Importance:**

- 1. The stability of milk products during heating, concentrating, and holding is largely determined by these micelles.**
- 2. The rheological properties of sour milk and concentrated milk are largely determined by the changes occurring in these micelles. The interaction of these micelles with air, water and oil-water interfaces is important in homogenization.**
- 3. The casein micelles also influence some of the properties of the homogenized products.**