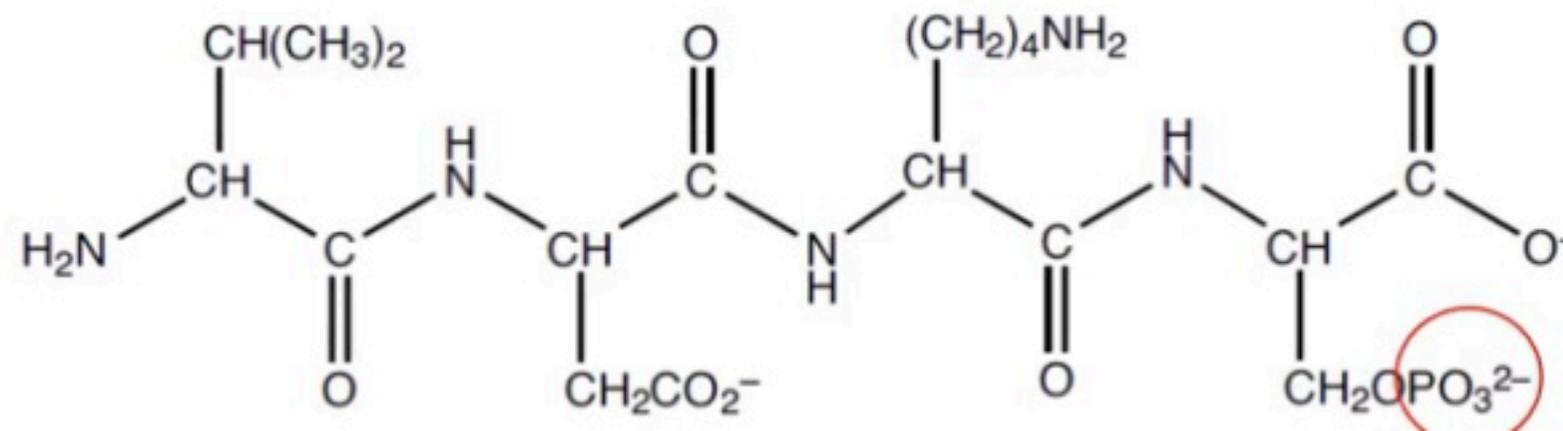


# Casein protein

- Casein is a group of phosphoproteins that are commonly found in the mammalian milk.
- It contains a large number of phosphate groups attached to the amino acid side chains in its polypeptide structure.
- The negatively charged phosphate groups are balanced by positive calcium ions and are responsible for the high nutritional calcium content in milk.
- Casein is relatively hydrophobic and making it poorly soluble in water.



فوسفيت جروب بالاضافة لستركشرز ال amino acid مرتبطين مع بعض ب peptide linkage

\* الحليب يحتوي على عدة عناصر مهمة مثل المعادن ويحتوي على نسبة كبيرة من الماء بالإضافة انه يحتوي على نسبة من الدهون والبروتينات

\* من أهم البروتينات في الحليب هو **casein protein** بشكل ما نسبة 80% من البروتينات الموجودة بالحليب

\* بالنسبة لتجربة اليوم الهدف منها استخلاص ال **casein** من الحليب الموجود عنا

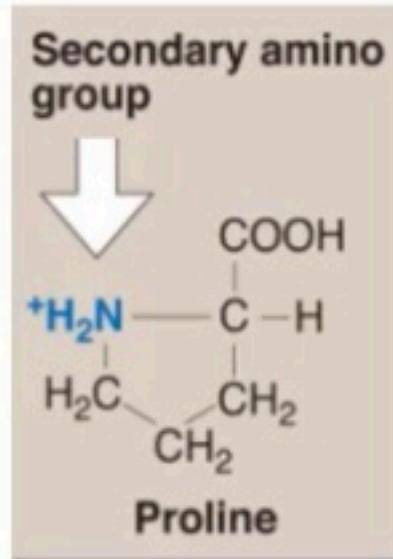
\* ال **casein** هو عبارة عن جروب من **phosphoproteins** يلي بالعادة بنلاقيه بحليب الثدييات (**mammalian milk**)

يتم موازنة مجموعات الفوسفات سالبة الشحنة بأيونات الكالسيوم الموجبة وهي مسؤولة عن محتوى الكالسيوم الغذائي العالي في الحليب

وطبعا ال **casein** هو **hydrophobic** نسبيا وهاد الاشئ سبب بانه يكون ضعيف الذوبان في الماء

# Casein protein

- Almost all essential amino acids are found in casein protein in high proportion.
- Casein, like other proteins, contains amino groups and carboxyl groups on its terminal amino acids as well as in non-terminal amino acids.
- It contains a fairly high number of proline residues, which do not interact. There are also no disulfide bridges. As a result, it has relatively little tertiary structure.



طبعا بنعرف ال amino acids بنقسموا لقسمين:

**essential and nonessential**

تقريبا كل ال essential amino acids موجودين بال

casein بنسبة عالية

يحتوي ال casein مثله مثل البروتينات الثانية على amino group

+ carboxyl group على ال amino acids الطرفية وغير

الطرفية

بتحتوي كمان على عدد كبير من proline residues (بقايا البرولين)

يلي ما بتتفاعل اصلا

كمان ما في عنا ( disulfide bridge ) ومشان هيك بكون في عنا

هيكل ثلاثي الابعاد بنسبة قليلة نسبيا

# Casein protein

- It is found in milk as a suspension of particles called "casein micelles" which show only limited resemblance with surfactant-type micellae in a sense that the hydrophilic parts reside at the surface and they are spherical.
- However, in sharp contrast to surfactant micelles, the interior of a casein micelle is highly hydrated.
- The caseins in the micelles are held together by calcium ions and hydrophobic interactions.

بكون موجود بالحليب على شكل جزيئات معلقة اسمها (casein micelles)

ال hydrophilic parts بتكون موجودة على السطح وبشكل كروي

مع هيك في تناقض حاد مع ال micelles الفاعل بالسطح  
بكون الجزء الداخلي ال casein micelles رطب كثير

بنجمع ال casein في ال micelles عن طريق ايونات الكالسيوم وتفاعلات ال hydrophobic

# Casein micelles

Any of several molecular models could account for the special conformation of casein in the micelles.

- 1) One of them proposes the micellar nucleus is formed by several submicelles, the periphery consisting of microvellosities of  $\kappa$ -casein.
- 2) Another model suggests the nucleus is formed by casein-interlinked fibrils.
- 3) The most recent model proposes a double link among the caseins for gelling to take place.

اي من النماذج الجزئية العديدة ممكن تفسر  
تشكل ال casein في ال micelles

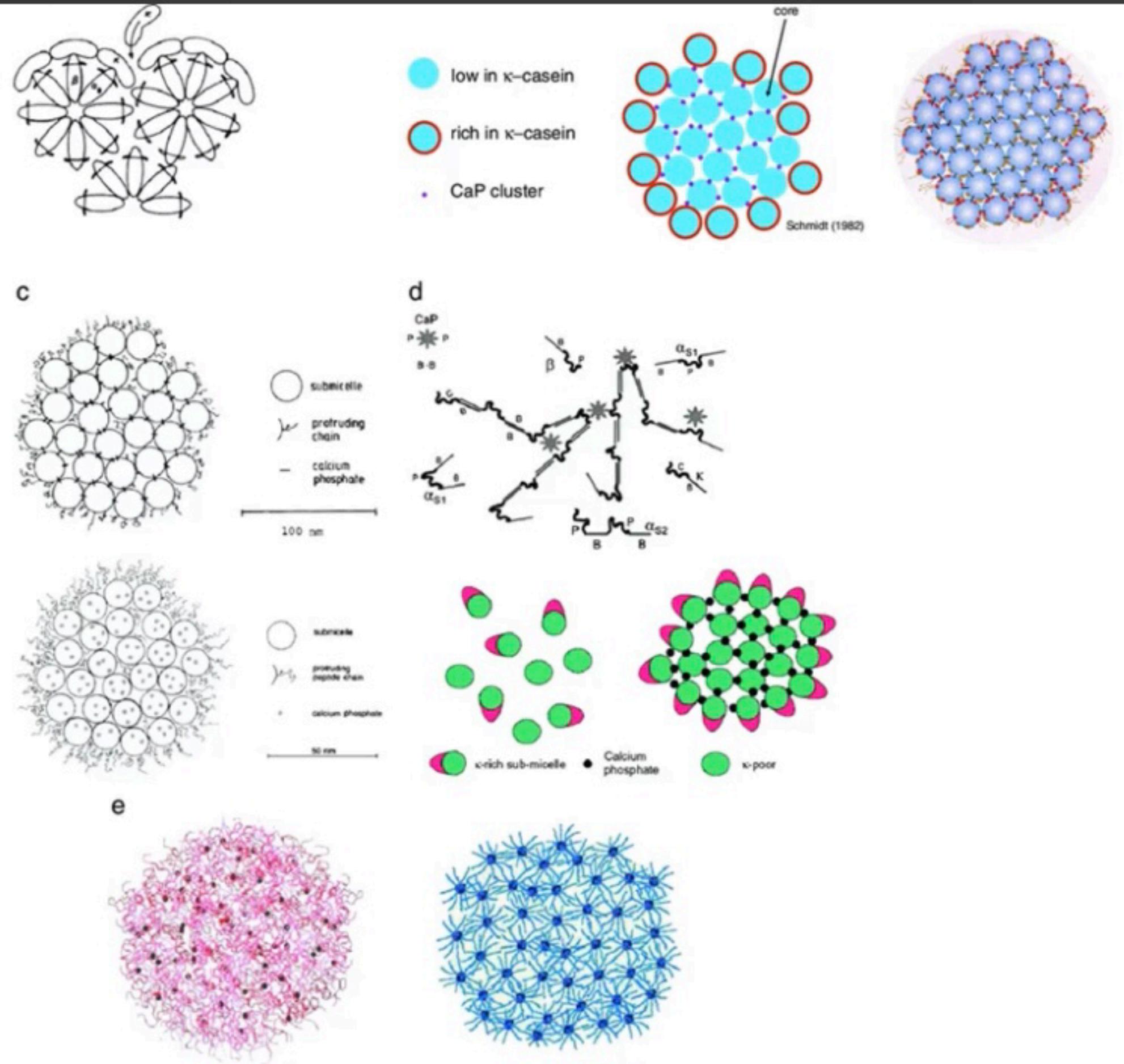
١- واحد من ال models انه نواة ال micellar بتكون  
من عدة اجزاء فرعية والمحيط بتكون من  
microvellosities of *k-casein*

٢- ثاني model يقترح انه النواة بتتكون من الياف متشابكة من ال  
casein

٣- احدث model يقترح ارتباط مزدوج بين ال casein  
مشان يصير gelling

➤ All three models consider casein micelles as **Colloidal particles** formed by casein aggregates wrapped up in soluble  $\kappa$ -casein molecules.

➤ The outer surface of the micelle are rich with  $\kappa$ -casein, has protruding hairs of negative charges which induce a steric repulsion between micelles, thus preventing them to coagulate and hence stabilizes the milk.

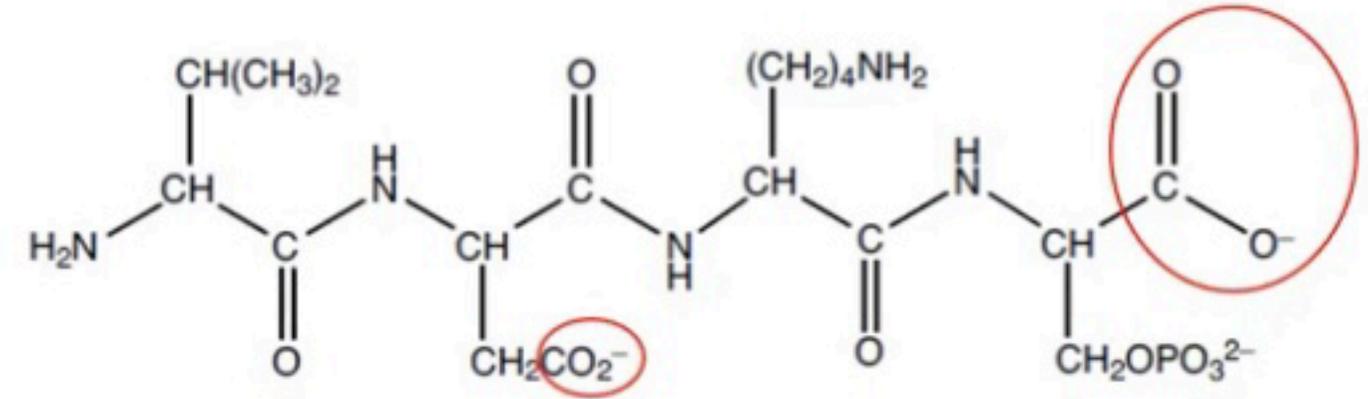


تعتبر ال three models لل casein micelles انها  
colloidal particles تكونت من تكتلات ال casein  
وملفوفة في *k-casein molecules* قابلة للذوبان

السطح الخارجي لل *micelle* غني بال *k-casein*  
واله شعيرات بارزة من الشحنة السالبة يلي بتحفز  
التنافر بين ال *micelles* وبالتالي بتمنعها من التخثر  
وبالتالي استقرار الحليب

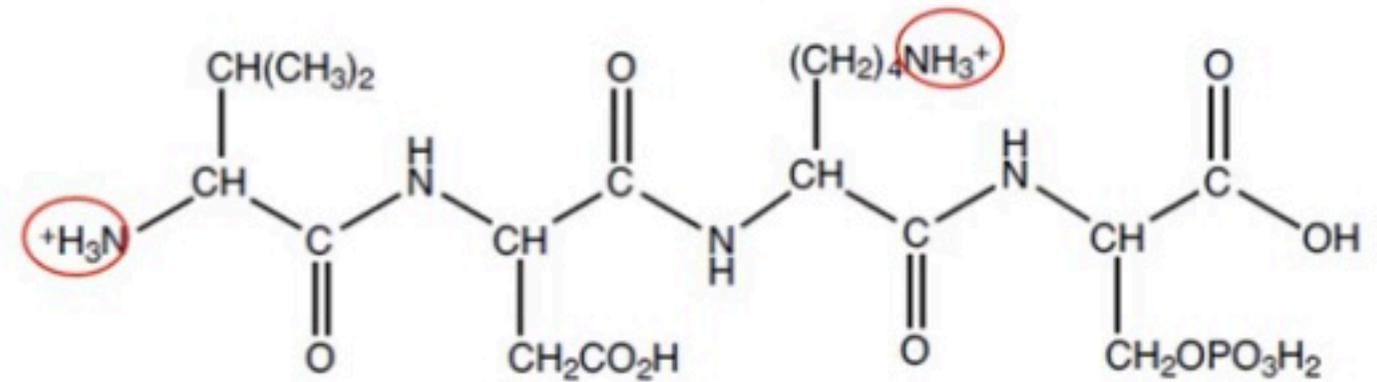
# Casein and pH

- At high pH, casein will have a net negative charge due to ionization of its acidic side chains ( $-\text{COO}^-$ ). Because casein is ionized at high pH values, it is soluble in dilute sodium hydroxide solution.



\* Casein is readily dispersible in dilute alkalis and in salt solutions such as sodium oxalate and sodium acetate

- At low pH, casein will have a net positive charge due to protonation of its basic side chains ( $-\text{NH}_3^+$ ). Because casein is ionized at low pH values, casein is also soluble in strongly acidic solutions.



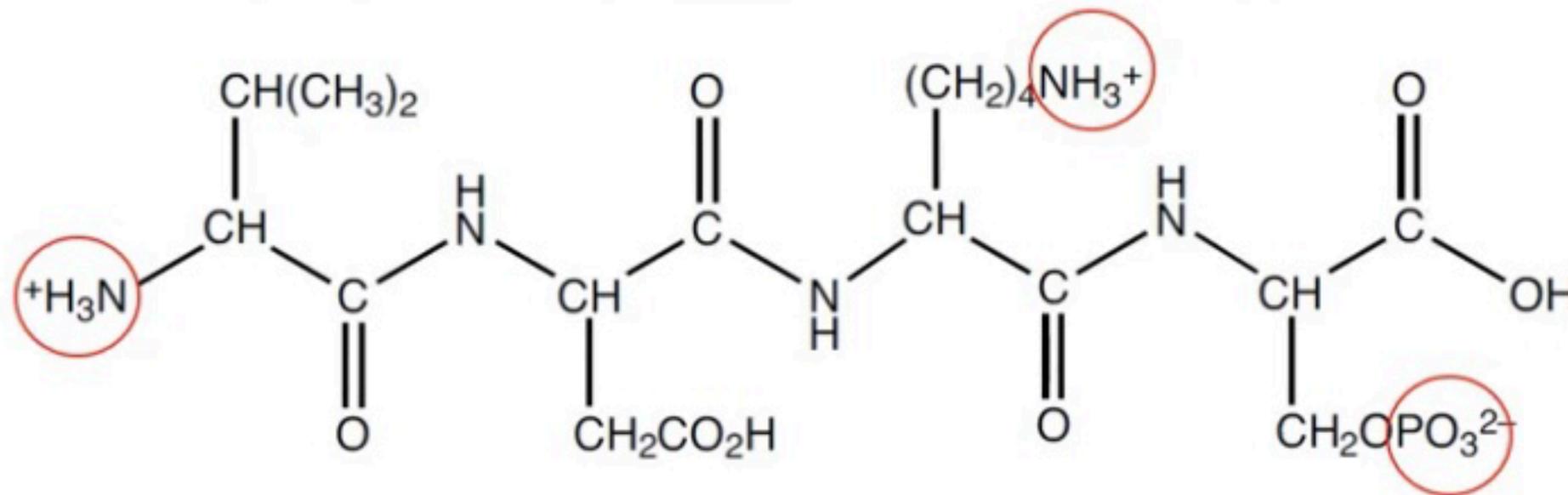
عند ال ph المرتفع ال casein رح يكون ال **net negative charge** بسبب انه بصير **ionization** لسلاسه الجانبية الحمضية .  
ال casein يكون **soluble** في محلول هيدروكسيد الصوديوم المخفف لانه ال  
casein بصيرله **ionization** عند قيم ال ph العالية

ال casein قابل للتشتت بسهولة (**dispersible**) في القلويات المخففة (**dilute alkalis**) ومحاليل  
ملحية مثل ال **sodium oxalate + sodium acetate**

عند ال ph المنخفض ال casein رح يكون ال **net positive charge** بسبب انه بصير **protonation** لسلاسه الجانبية  
القاعدية (**+NH<sub>3</sub>-**)  
وال casein soluble في المحاليل الحمضية بشدة بسبب انه  
بصيرله **ionized at low PH**

# Casein and pH

- At intermediate pH values, casein will contain an equal number of positively and negatively charged groups and the protein will have a net charge of zero. Casein is insoluble in neutral solutions because it is not charged under these conditions.



- The solubility of a protein is usually at a minimum at its isoelectric point (IP). The isoelectric point is defined as the pH at which a protein has a net charge of zero. For casein, due to the attached phosphate groups, the isoelectric point is close to  $\text{pH} = 4$  (ranges from 4.6-4.8).

عند قيم ال PH المتوسطة ال casein رح يحتوي على مجموعة من الشحنات الموجبة والسالبة متساوية وبتكون صافي شحنة البروتين صفر

ال casein يكون insoluble في المحاليل المحايدة (neutral solutions) لانه ما يكون مشحون بهاي الحالة

ال isoelectric point : نقطة تساوي الكهرباء عند ال ph اما يكون البروتين عنده net charge of zero

نوبان البروتين عادة يكون عند الحد الادنى عند ال isoelectric point (IP)

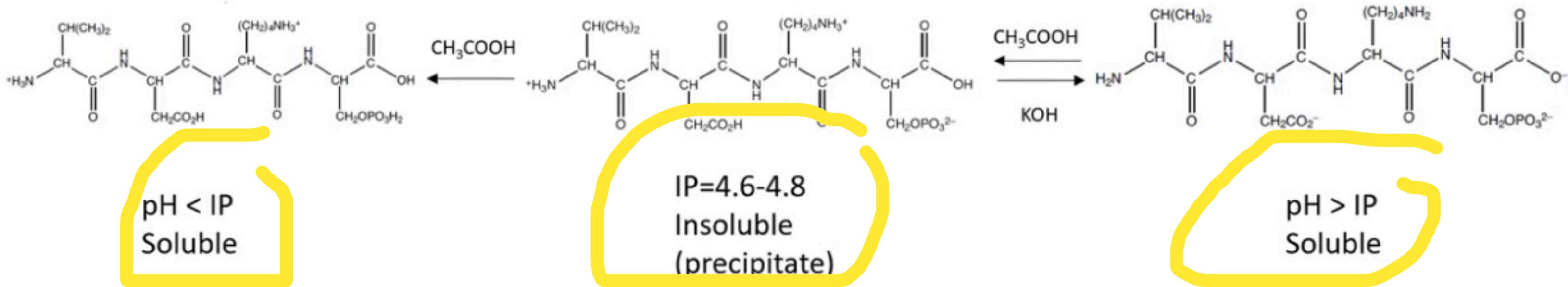
ال isoelectric point لل casein قريبة لل  $ph=4$  بتتراوح ما بين (4.8-4.6) بسبب وجود مجموعات فوسفات مرفقة

# Casein isolation:

Since milk's pH is 6.6 (which is higher than the isoelectric point of casein), so casein has a negative charge in milk; which induce a steric repulsion between micelles, thus preventing them from coagulation and hence stabilizes the milk.

To precipitate the casein → acetic acid is added dropwise until the isoelectric point is reached.

**Note:** if excess  $\text{CH}_3\text{COOH}$  is added the precipitate redissolves



بما انه ال ph للحليب =6.6 ( وهو اعلى من ال  
isoelectric point of casein ) ف ال casein  
اله شحنة سالبة بالحليب ويلى بتحت على التنافر  
الفراغي بين ال **Micelles** وبالتالي منعها من  
التخثر وبالتالي استقرار الحليب

# Casein isolation:

- Casein is insoluble in ethanol which is a property used to isolate it from the unwanted fat
- Ether is also used in casein isolation to remove impurities and to remove any traces amounts of remaining water (drying).
- Unlike many proteins, casein is not coagulated by heat

واحنا بنغلي الحليب ما ممكن  
يصير عنا denaturation  
للحليب

# *Casein has many uses including:*

## *1. Paint*

- Casein paint is a fast-drying, water-soluble medium used by artists since ancient Egyptian times as a form of tempera paint, and was widely used by commercial illustrators as the material of choice until the late 1960s when, with the advent of acrylic paint, casein became less popular.

## *2. Glue*

- Casein-based glues, formulated from casein, water, hydrated lime and sodium hydroxide were popular for woodworking, including for aircraft.

# *Casein uses:*

## *3. Cheese making:*

Cheese consists of proteins and fat from milk, usually the milk of cows, buffalo, goats, or sheep. It is produced by coagulation of casein. Typically, the milk is acidified and then coagulated by the addition of rennet, containing a proteolytic enzyme, typically obtained from the stomachs of calves. The solids are separated and pressed into final form.



### 3. *Cheese making:*

اهم طريقة بينهم



- Calf rennet, which consists of over 90% chymosin, is commonly used in cheese industries for the curdling of milk.
- During the process of clotting, milk-clotting; **proteases** act on the soluble portion of the caseins,  $\kappa$ -casein, thus resulting in an unstable micellar state that causes clot formation.
- **Chymosin** is an aspartic protease that specifically hydrolyzes the peptide bond in Phe105-Met106 of  $\kappa$ -casein, and is considered to be the most efficient protease for the cheese-making industry.
- When coagulated with chymosin, casein is sometimes called paracasein.
- British terminology, on the other hand, uses the term caseinogen for the uncoagulated protein and casein for the coagulated protein. As it exists in milk, it is a salt of calcium.

# *Casein uses:*

## *4. Protein supplement*

- An attractive property of the casein molecule is its ability to form a gel or clot in the stomach, which makes it very efficient in nutrient supply. The clot is able to provide a sustained slow release of amino acids into the blood stream, sometimes lasting for several hours.
- Often casein is available as hydrolyzed casein, whereby it is hydrolysed by a protease such as trypsin. Hydrolysed forms are noted to taste bitter and such supplements are often refused by infants and lab animals in favour of intact casein



# Procedure

## Materials:

1. Milk
2. Acetic acid ( $\text{CH}_3\text{COOH}$ ) (10% v/v)
3. Ethanol (95%v/v) (flammable)
4. Diethyl ether (highly flammable)
5. Thermometer  $100^\circ\text{C}$
6. Muslin
7. Filter paper
8. Watch glass

# Procedure

## Method:

1. In 250ml-beaker, place 100 ml of milk and warm to 40°C on a hot plate.
2. Once temperature is reached, remove the beaker containing milk, and start adding acetic acid slowly (drop by drop) to the milk while stirring with a stirring rod.

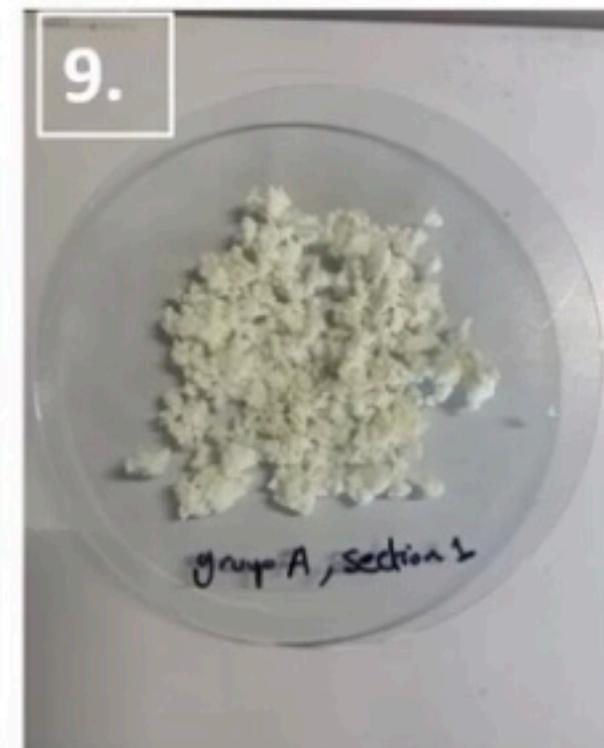
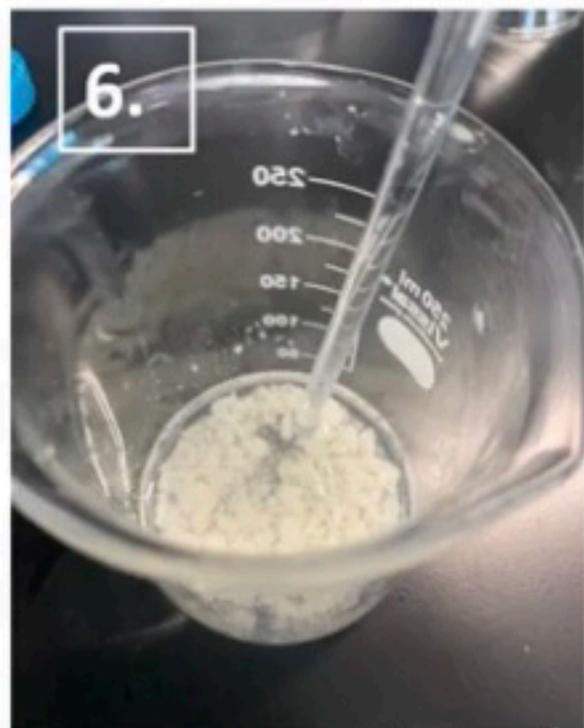
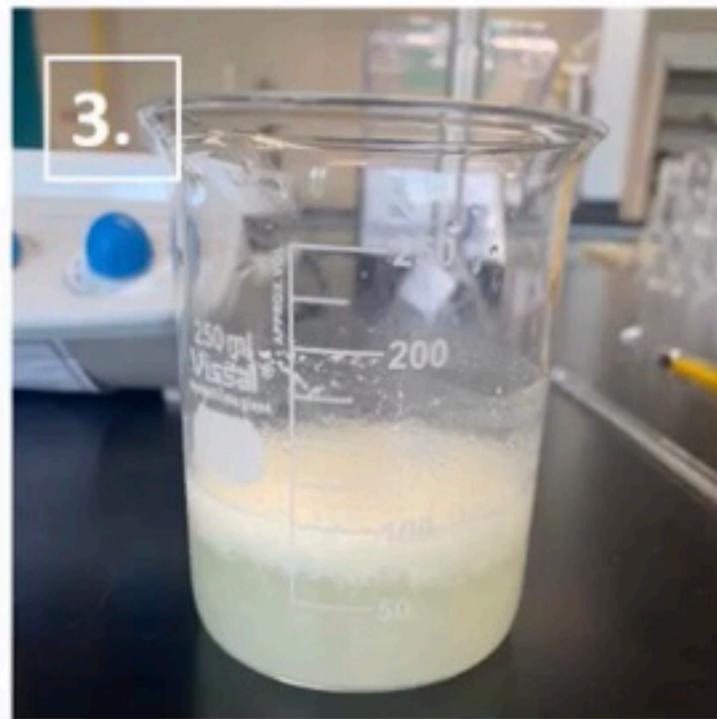
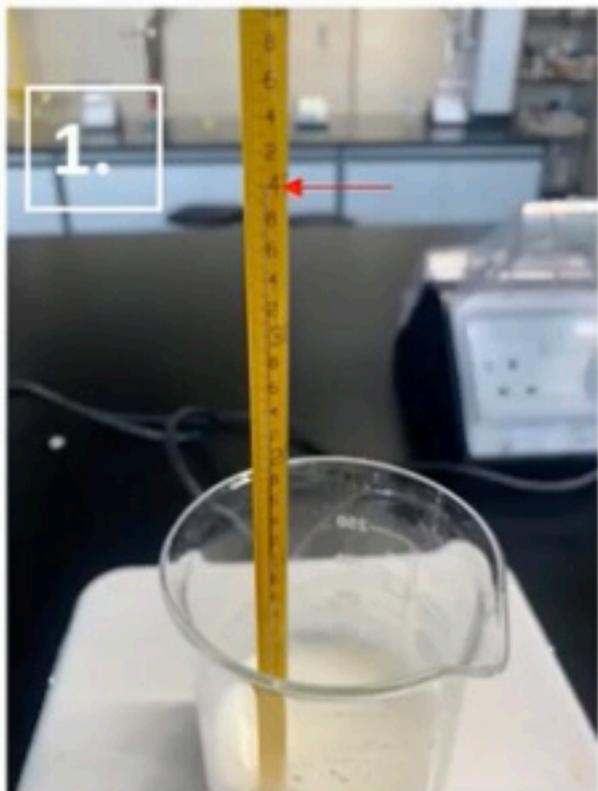
**Note:** never transfer the acetic acid all at once.

3. Keep adding acetic acid dropwise until milk clots starts to form and the casein is precipitated (~1 ml  $\text{CH}_3\text{COOH}$ ). This point means that you have reached the a pH of 4.6-4.8

# Procedure

4. Leave to stand for 5 min and filter through **muslin cloth** (you may need to wait for 10-15 minutes for the filtration to be completely finished).
5. Collect the casein precipitate and transfer into a beaker to suspend it in 25 ml mixture of equal volume of **ethanol and ether** (1:1) and mix by glass rod so that the extra fat may come down to the solvent system.
6. Filter the precipitate again using filter paper. Wait until the solution is totally filtered.
7. Once all the solvent has been removed from casein. Remove the powder and spread out on a watch glass (or petri dish) until it is completely dry (usually overnight).





# Report

- Weigh your sample and calculate the percent yield you obtained given that milk contains 35g casein/L of milk

$$\%Yield = \frac{\textit{Experimental weight}}{\textit{Theoretical weight}} \times 100$$