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Structural Properties of Casein Micelles in Milk, the effect of salt, temperature, and pH

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Abstract:

Milk is a complex liquid, which contains many different species, for example proteins, fat, minerals etc. It is the primary source of nutrition for young mammals before they are able to digest other types of food. The proteins can be divided into two groups: caseins and whey. Whey proteins are about 20 wt % of the total protein amount in milk, whereas the caseins corresponds 80 wt % of the total protein content in milk. The largest structures in the fluid portion of the milk are “casein micelles” which are aggregates of several thousands of protein molecules. The micelle is considered to be spherical and the diameter is in the micrometer size.

The caseins can be divided in four types: αs1- casein, αs2 - casein, β - casein, and k - casein. ß - casein is one of the most abundant caseins and it also self - assembles to larger aggregates. In this thesis we have used a simple model to try to capture how electrostatic interactions affects the structure of β - casein micelles in milk. The micelles have been modeled as hard spheres, with a central net charge of ---140e, and a radius of 75 Å. These parameters have been taken from experimental data published in the literature. The structure of the solution has been studied by comparing the radial distribution functions for different solution conditions, such as the salt concentration and valency, pH, and the temperature.

Popular scientific description

Milk is a complex liquid, which contains many different species, for example proteins, fat, minerals etc. It is the primary source of nutrition for young mammals before they are able to digest other types of food. The proteins can be divided into two groups: caseins and whey. Whey proteins are about 20 wt % of the total protein amount in milk, whereas the caseins corresponds 80 wt % of the total protein content in milk. The largest structures in the fluid portion of the milk are “casein micelles” which are aggregates of several thousands of protein molecules. The micelle is considered to be spherical and the diameter is in the micrometer size.

In this thesis we have used a simple model and computer simulations to try to capture how electrostatic interactions affects the structure of β - casein micelles in milk. The micelles have been modeled as hard spheres, with a central net charge of -140e, and a radius of 75 Å. The volume fraction of was set to 5%, which is the actual volume fraction in the real product. The structure of the solution has been studied by comparing the radial distribution functions for different solution conditions, such as the salt concentration and valency, pH, and the temperature.

It was noticed that due to the fact that the micellar charge is very large, the electrostatic repulsive interaction dominates, and the mean distance between the micelles are almost always obtained. Moreover, an increase of the temperature does not affect the structure at all i.e. the entropic contribution due to increased temperature can be neglected in comparison with electrostatic repulsion between the micelles. Also, there might also be an influence of the electric permittivity since it was kept constant during the simulations,

When the salt concentration was increased to 80 mM, which corresponds to the ionic strength in milk, the structure of the β - casein micelles resembles the structure of an ideal gas i.e. the electrostatic repulsive interactions are screened

Introduction:

Milk is as ancient as human beings themselves. Historians believe that humans started to drink milk over 10,000 years ago, along with the start of the domestication of animals. Milk is the normal product of the mammary glands of female mammals. Its purpose is primarily to meet the nutritional requirements of the neonate. It contains more than 100 substances, and it is the most complete and complex known natural food. Milk is perishable because it is a very good environment for the growth of the microorganisms that caused the FDA to warn people from drinking raw milk in March 2007. They wrote: Pasteurized milk does safe live! [41] The pasteurization of milk is heating the milk in a certain way to save the nutritional value of the milk while destroying the harmful bacteria.

The tropical countries are not considered consumers of milk, because of the high temperature and the lack of refrigeration, which makes the storage of milk difficult. The north part of the world, especially northern Europe and North America, is considered a big consumer of milk and milk products. Milk has traditionally been preserved by converting it to more stable products like making cheese and yogurt by milk fermentation. [33]

Milk processing:

Pasteurization:

Because of the perishability of milk, milk storage has been an important process. The most widely used process, for preserving fresh milk, is pasteurization, which was named after the French microbiologist Louis Pasteur in 1864.

The most popular methods of pasteurization are:

1)HTST: high temperature short time pasteurization, which requires heating milk to 720 C for 15 seconds, and can extend the shelf life to a week.

2)UHT: ultra high temperature pasteurization, which requires heating the liquid to 1380 C for 2 seconds, and can extend the shelf life for weeks. It causes some changes of the structure of the milk protein.[2]

Fermentation

Other processes, that are popular for preserving milk, involve preserving milk components like fat and protein. These processes are modifying the environment of the milk and extend the lifetime of the products. Adding lactic acid bacteria to the milk converts lactose (milk sugar) to lactic acid, which leads to the

dissociation of the casein micelles. After that, the viscous gel solution has the sour taste of yogurt. After adding the lactic acid bacteria to the milk, the lactose is converted to lactic acid, which decreases the pH of the milk. Rennet is added to convert the casein micelle into a stable protein that does not dissociate in water.

The content of the milk differs according the mammals it comes from as shown in table 1. The components also depend on the nutrition and the season. Cow's milk for example contains about 87% water. [34]

Species	Total solid [%]	Fat [%]	Protein [%]	Lactose [%]	Ash [%]
Human	12.2	3.8	1.0	7.0	0.2
Cow	12.7	4.5	2.9	4.1	0.8
Sheep	19.3	7.4	4.5	4.8	1.0
Pig	18.8	6.8	4.8	5.5	-
Horse	11.2	1.9	2.5	6.2	0.5
Donkey	11.7	1.4	2.0	7.4	0.5
Reindeer	33.1	16.9	11.5	2.8	-
Domestic rabbit	32.8	18.3	11.9	2.1	1.8
Bison	14.6	3.5	4.5	5.1	0.8
Indian elephant	31.9	11.6	4.9	4.7	0.7
Polar bear	47.6	33.1	10.9	0.3	1.4
Grey seal	67.7	53.1	11.2	0.7	-

Table (1) The composition (%) of milk of different species. [2]

Milk components:

Milk fat:

Fat represents about 3.5-□6.5 % of milk, and it is the most variable component. It is secreted as a fat globule surrounded by a lipid bilayer membrane. When raw milk is centrifuged, the fat moves to the top and forms a cream layer, because the lipid is lower in buoyant density, see Figure 1.

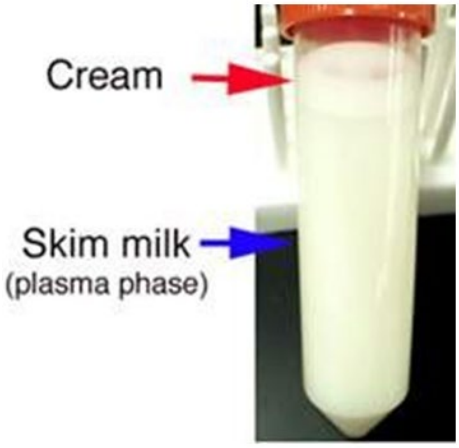

Figure 1 shows the formation of two layers upon centrifugation of the milk. The upper layer is the lipid and it forms a creamy layer, and the layer at the bottom is formed from skim milk (milk without fat). This is because the lipid layer has a low buoyant density.

The cream contains some proteins (Mucin, Xanthine oxidase and butyrophilin), which are carried by the fat globules. It has been suggested that the fat globules are accumulated between the two halves of the milk lipid globule membrane (MLGM), which is part bilayer and part protein (see figure 2). Milk fat globules (MFG), whose diameters range from 0.2 □m to 20 □m, with a mean diameter of 4□m (Mulder and Walstra, 1974), are essentially from triglycerides, which are esters derived from glycerol combined with three fatty acids. It has been found that the triglyceride forms 98% of the fat content, and the phospholipid forms 1% of the fat content.

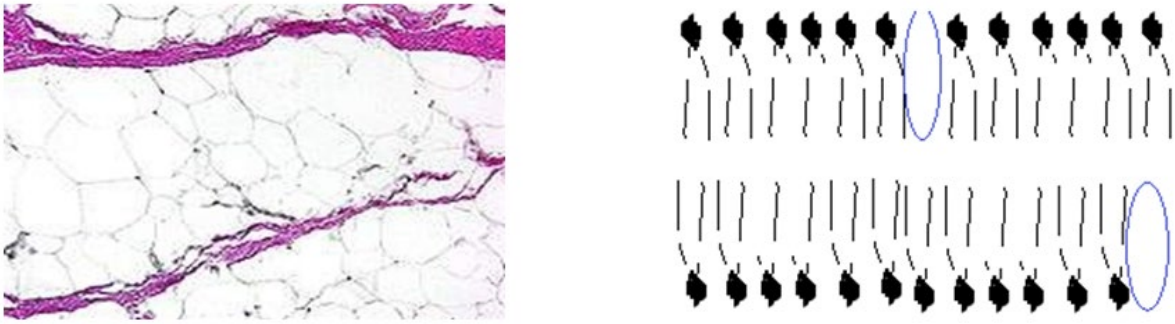

Figure 2. The left image of the adipocytes ''lipid cells' shows the fat globules between the membrane, and the right image shows the membrane, which is composed of a combination of a phospholipid bilayer and the protein (blue ovals).

Over 400 fatty acids have been identified in milk fat. The most common type of fatty acids is triglycerol or triglyceride, see figure 3, which is composed of three fatty acids.

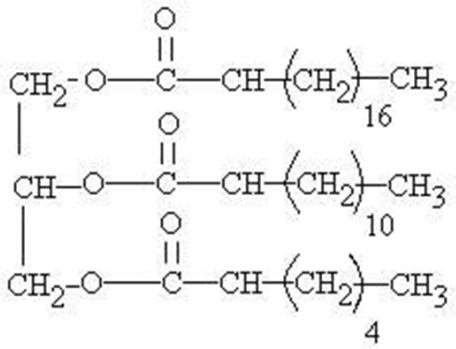

Figure 3 Three fatty acids form a triglyceride.

Milk fat melts over a wide range of temperatures from -□400C to 400C, and it can be degraded by excess heat or light, and actions of some enzymes cause it to be inactivated.

Lactose

Lactose is the major carbohydrate in milk, and it is a disaccharide derived from galactose and glucose as seen in Figure 4. It is found dissolved in the milk serum in two forms: alpha-□anomer and beta-□anomer. The ratio between the two forms changes with temperature. The beta-□anomer is sweeter and more soluble than the alpha-□anomer. Short pasteurization time does not have a significant effect on the glucose, but (UHT pasteurization) motivates the Brownian reaction between the Lactose and the protein, leading to undesirable flavor and color.

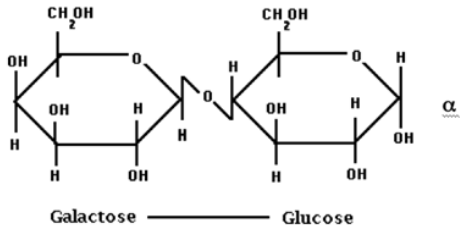

Figure 4 shows the Lactose structure.

Minerals:

The majority of minerals found in milk are Ca, P, Mg, K and Zn. Calcium and phosphorus combine together to form Calcium Phosphate salt and it is called Colloidal Calcium Phosphate (CCP). It was suggested to play an important rule in binding the casein micelle by immigrating in or out the micelle with changing temperature.

Vitamins

Milk is not considering a major source of vitamins in a diet. There are two groups of vitamins that exist in milk according to their solubility:

•The water-□soluble vitamins group, including the vitamins (B1, B2, B3, B5, B6, B12, C).

•The fat-□soluble vitamins group, including the vitamins (A, D, E, K).

HTST does not affect the vitamins, but UHT decreases the water-□soluble vitamins. Exposure to light affects the vitamin A content.

Milk proteins

The proteins in milk are unique, and make milk an important source of human nutrition. Milk proteins are digestible by almost everyone in the intestine. Generally, there are many types of proteins according to the sequences of the amino acids. There are 20 amino acids, which form the different protein types where 9 of them are essential for diet and all of which are present in milk. The amino acids are bonded to each other by polypeptide bonds, see Figure 5. The N-□ terminal (terminus) is the amine part and the C-□terminal (terminus) is the carboxylic acid part. They carry negative charge (the N terminal) and positive charge (C-□terminal), and they neutralize each other. If the hydrocarbon side chain can be titrated, the charge of the protein depends on the pH, which will be discussed in detail later.

A denatured protein is the protein unfolded from its native state. The denaturation can be useful in the industry or digestion as it gives the enzymes access to the protein chains.

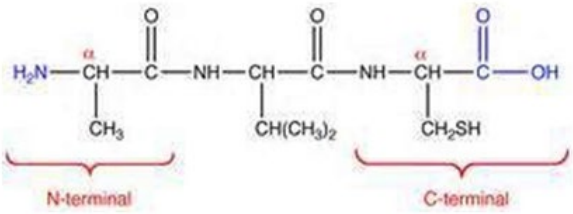

Figure 5 shows how the peptide bond binds the amino group to the carboxylic acid to connect the amino acids.

Bovine milk proteins are the most common proteins. Caseins resemble about 80% of all the protein and it is present in milk of all species. All other proteins are grouped together in a group called whey proteins. The major whey protein in cow milk is alpha-□lactalbumin and beta-□lactoglobulin. The only known milk allergy is caused by beta-□lactoglobulin, because of its poor digestibility. The whey protein has also an important use in the industry as it is used in binding meat and water in meat and sausage products, and it will not form a gel upon denaturation and acidification.

There are four types of caseins in milk, each having appropriate amino acids that make the milk essential for growth and development of the young. They they are also phosphorylated to various degrees, which plays an important role in the stabilization of the casein itself.

A casein micelle is composed of several similar proteins,

forming a multi-□ molecular granular structure. The casein micelle contains water plus salts (mainly from Calcium and Phosphorus). The casein micelle plays an important role in many milk product industries such as in cheese making. The casein can be separated from whey by centrifugation where the casein is pelleting and the whey is in supernatant, or it can be precipitated by adding acid.

The average diameter of a casein micelle is around 120 nm to 180 nm, and it is about 1/50 of the fat globule size. The micelle is composed of proteins with salt and the salt is primarily nano-□clusters of Calcium and Phosphorus. Researchers think that the Calcium and Phosphorus play a role in connecting the proteins because by removing the salts, the micelles dissociate into small parts called sub-□ micelles. The micelles were found to have essentially 4 proteins; □ -□s1, □-□s2, □ and χ-□casein in the ratio 4:1:4:1. [2] The isoelectric point of the casein is at pH

4.6 i.e. the pH at which the net charge is zero. At pH 6.6, the nano-□clusters, which are mainly Calcium and Phosphorus salts, are dispersed as small particles with a radius of 1.72 nm-□ 2.27 nm. There are many articles about the internal structure, and all of them agree that there is a micelle formed by the proteins and the colloidal calcium phosphate (CCP). [27]–[30]

Generally, the studies that use light and x-□ray and neutron scattering show that the micelle is formed from sub-□units (called sub-□micelles) with the help of CCP. CCP acts like cement, as it connects the sub-□micelles. The studies that use electron microscopes show that there are no sub-□micelles, but there is a protein matrix and channels in the inside of the micelle. However, some recent studies, using high-□resolution microscopes and carefully made samples show that the sub-□micelle model probably exists. Horne (2006) has given an explanation, to the sub-□micelle model, as is shown in Figure 6, and illustrates that the whole micelle consists of small spherical sub-□units (sub-□ micelles) connected with the colloidal calcium phosphate. The sub-□micelles on the outer surface of the micelle are rich with k-□casein, having protruding hairs of negative charges which induce a steric

repulsion between micelles, thus preventing them to coagulate and hence stabilizes the milk.

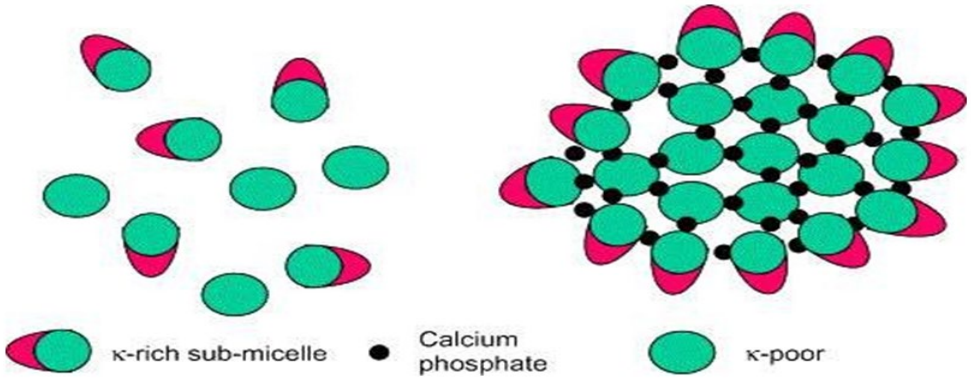

Figure 6 shows a schematic drawing of the casein micelle according to Horne model.

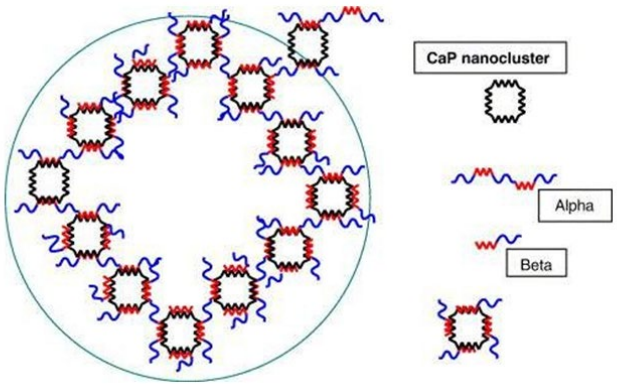

Figure 7 illustrates the network between nanoclusters in the Holt model, and how the alpha and beta casein bind to Calcium Phosphate to form a bridge and complete the chain. [20]

The Holt model explains how the nano-□cluster of the CCP acts like cement, and connect the proteins to form the big micelle as is shown in Figure 7. Both alpha
and beta casein are attached to the cluster. The difference between the proteins enables them to make a bridge with the cluster.
Old electron microscope studies say that there is no inhomogeneity in the micelle more than a few nanometers, which means that all the components form one part, and that there are no small molecules looking like the big micelle. However, the previous electron microscope photos contain artifacts, since the samples were supposed to be fixed by drying or freezing, with a possible loss of protein flexibility.
Recent studies by more advanced electron microscopes, with carefully prepared samples, declare that the sub-□micelle particles might exist, see Figure 8.

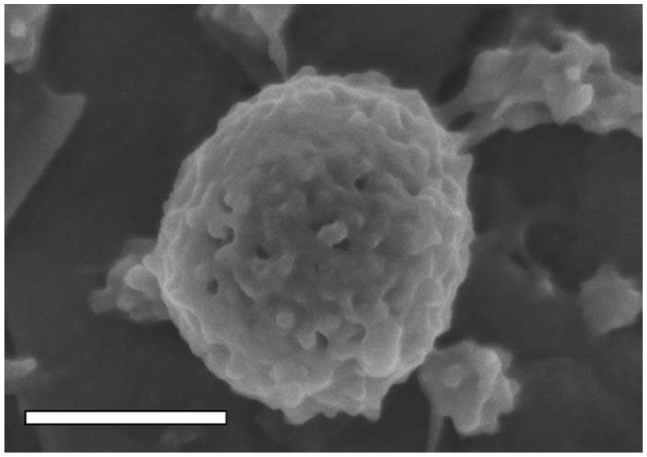

Figure 8 shows an electron microscope photo of a casein micelle. The photo shows that there are small aggregates around the big micelle, which probably resemble the sub-□ micelle. The bar length is 200 nm. [27] Figure 8 shows an electron microscope photo of a casein micelle. The photo shows that there are small aggregates around the big micelle, which probably resemble the sub-□ micelle. The bar length is 200 nm. [27]

In general all studies confirm that there is a casein micelle, composed of four types of protein, namely alpha s1, alpha s2, beta and kappa casein. The protein micelle is similar to the surfactant micelle with both a hydrophobic part and a hydrophilic one, motivating the micelle formation by their behavior towards the water phase. The polar part, which is presented by the N and C terminals, tends to face the water. The hydrophobic part is presented by two portions, the first of which consisting of non polar side chains and the second is the repeated sequence of P, F (Phenylalanine and Proline). It has been found that P, F sequences have less polarity than other sequences.
Milk can be considered as a colloidal solution that contains:
Solution from milk sugar in water.
Fat emulsions (oil in water).
Proteins suspended in water.
Because of the great role played by the casein micelle, a simple model has been used to simulate the role of electrostatic interactions between ß-□casein micellar structures in milk with emphasize on the following:
1)The effect of the electrostatic environment on the micelle-□micelle interaction.
2)The effect of temperature of the micelle-□micelle interaction.
3)The effect of changing pH on the protein charge on the micelle-□micelle interaction.

Theoretical Background

Electrostatic interactions:

Milk contains approximately 87% percent water. It is extremely time consuming to consider the effects of all the individual water molecules in computer simulations, and therefore we will only consider the global effect of water, by letting the electrostatic interaction energy (uel) between charges, be screened by the dielectric permittivity of water, denoted □r.

$$u \propto \frac{!}{!" \quad \varepsilon.}$$

The electrostatic energy (□!") depends on the electrostatic potential (Φ!"). The electrostatic potential depends on the charges found in the system. The electrostatic potential in vacuum at a point, separated by a distance rj from a point charge j, is given by:

Φ □ = !!!
!” ! !!□!!!

2.3

where □! is the dielectric permittivity of vacuum. The electrostatic interaction energy between two particles i and j (in vacuum) can be obtained by:

□ □ = !!!!!
!” !” !!□!!!”

2.4

This equation represents Coulomb’s law. Coulomb’s equation for electrostatic interaction in water is given by:

□ □ = !!!!!
!!□ □ !
2.5

! ! !”
As mentioned above, the introduction of the dielectric permittivity coefficient of water □!, accounts for the screening of the electrostatic interaction between charges when treating water molecules implicitly as a structureless continuum.

The effect of the concentration, the valence of the ions, and the temperature on the system, can be studied using the model (explained above). The Debye length can be considered as a resisting factor of the electrostatic force and is defined as
length . The Debye length can be depicted as the radius of the circle with a
point charge oriented in its origin, and the circumference of this circle is the farthest position that can be influenced by this point charge.
□!! = E!E!!”
!” !!
!
!
The Debye length affects the electrostatic potential, and the electrostatic potential from particle j, which affect particle i, becomes,

(□) = 2.7

which is the Debye Hückel expression for the electrostatic potential. Here Rj is the radius of the particle, and rij is the distance between i and j. It is obvious that the Debye screening length decrease by the increasing of the concentration of salt and the charge, but also while increasing temperature.
In our model, the radial distribution function between the micelles has been calculated numerically to show the electrostatic behavior of the model when changing the environment.

2.2 Protein charge
The N and C terminus, and several side chains of a peptide group can be charged. The protein charge can be estimated by calculating the sum of the amino acids charges. The terminals of the amino acids, which are an amine and a carboxylic acid group, compensate each other, so they can be considered as neutral. The side chain can be charged and it can be dissociated at a suitable pH. The acidic groups (AH) dissociate into a proton (H+) and the conjugate negative ion (A-□):

□□ ↔ □! + □! 2.8

The basic groups (BoH) dissociate into a hydroxyl group (OH-□) and a positive ion
(B+):

□□□ ↔ □! + □□! 2.9

The equations (1.1) and (1.2) can be combined as

□□! ↔ □! + □! 2.10
This equation can be generalized for any titration process as B is the titrand with the charge ne against a titrant with a charge me, resulting in ABP where p= n+ m, where (e) is the elementary charge.
For this reaction the dissociation constant is:

□! =

!!! !”#!$#%&’%(“# !” !!! !”#$%&’(□!!!”# !”#$%$#&
!”#$$%!%#&’(2.11
!”#!$#%&’%(“# !” !!! !”#$%#&%’□!!!”# !” !”#”!”
!”#$$%!%#&’(
which is equivalent to:
□! =

!!!□!!!
!!!!

□ !!!□!!!
!!!!

≈ !!!□!!!
!!!!
2.12
By assuming that the activity coefficient is unity, if An is a proton and Kd is referred to acid dissociation constant Ka, pA = pH
□□ = − log!” □!! 2.13
□□! = − log!”(□!) 2.14

= − log!”

!!!!!!
!!!!

= □□ − log!”
2.15
The equation (2.15) is the expression for Henderson-□Hassel-balch expression.[35] The ratio can be expressed as the number of moles (C=n/V)B

−log!” = □□! − 2.16
= 10!”!!!! 2.17

□!! = !

!!!
!!!!!®!

, □□□□ □□□ □□□□□ □□ □!

. 2.18

□!! =

!”!”!!!!
!!!”!”!!!!2.19

□!!! is obtained by the same steps.
!
!!! !!!”!”!!!!

When pH is equal to pKd, the exponents in the mole fractions equations will be zero, which leads to be ½ for both the titrant and the titrand. When pH <pKd, there are an increase of the number of titrant sites. For pH >pKd the number of titrant sites decreases.

It is possible afterward to express the total charge by mole fractions at constant pH by summing □!! over all the basic groups, substracting them from the sum of
□!”! over all the acidic groups.
< □ > =

! □!”! −

! □!!

2.21

!
! !!!”!”!!!!,!

!”!”!!!!,!
− ! !”!!!!,®

2.22

Here < z > is the net average charge of the protein.

3. Model and Method

3:1 Model

A simple model system has been designed to study the micelle-□ micelle interactions in milk. The charge of the proteins which build up the micelle, can be estimated from the following steps:

1. Finding the sequence of amino acids of the protein. [36]
2. Picking up the polar chains and knowing if they are positive or negative according the pH. [37]
3. Substituting in equation (2.22)

At a temperature of 250C and pH=6.7 (conditions of fresh milk) the net charge of a micelle protein is about -□7 elementary charges.

In our model, we treated the micelles as hard spheres with a radius of 75 Å, and we neglected any titration effects during the coarse of the simulations. Practical experiments have been done to calculate the micellization number of the ß casein.[38] It is found to be around 20, i.e., there are 20 proteins/micelle, and the total charge would thus be around (-□140) e/micelle.

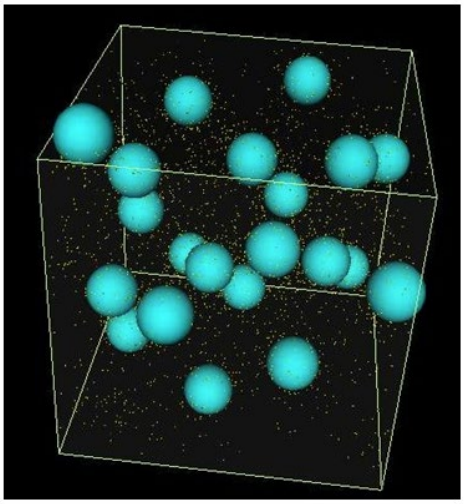

Figure 9: Schematic draw of the reference model consisting of 21 micelles and 2940 counterions. All species are modeled as hard spheres, and the water is treated by applying a dielectric continuum.

In order to make the system overall electroneutral, 140 counterions/micelle were added to the system. The ions were modeled in the same way as the micelles, i.e. as point charges, enclosed in impenetrable spheres, but with a diameter of 4 Å. The smallest investigated system consisted of 21 micelles and 2940 counterions, enclosed in a cubic simulation box with side lengths equal to 906 Å. The dimensions of the box were chosen as to give a micelle volume fraction of about 5 %, a value corresponding to the conditions in milk. The volume fraction was simply calculated as the total volume of the spherical model micelles, divided by the box volume. A snapshot of the model system is provided in Figure 8.

Method:

Fortunately, most of the computer simulations are based on the assumption that classical mechanics can be used to describe the motion of atoms and molecules. The fact that the quantum system can be found in different states is needed. Some important definitions with respect to computer simulations are:

Ergodic system: when the time average of the sequence of events is equal to the ensemble average.

The difference between analytical and numerical calculations: the analytical calculations are exact and can be done by using pen and paper and the solutions are exact. The numerical calculations are more complicated and can be done by using computer simulations, accompanied with an inevitable statistical noise.

Markov chain: a system is said to undergo Markov chains when it undergoes a chain of transitions among finite possible states and the next state only depends on the current state and not the sequence of the state.

Importance sampling: A technique used for estimating a particular distribution for a sample generated by random distribution. For example, two distributions as shown in Figure 10 where f(x) is the random distribution, and h(x) is the particular one ''certain one''.

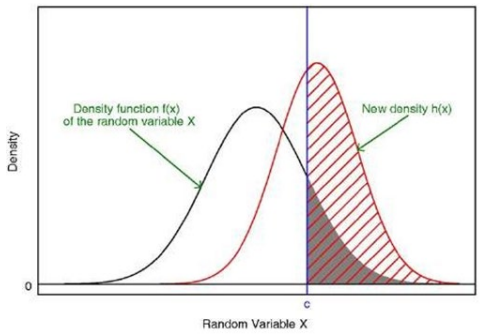

Figure 10 the f(x) is a random distribution and h(x) is a particular one.

Importance weights: It is a function used to measure the error between the two distributions,

= 3.1

hence the rejection rate depends on the importance weights. We need a new distribution related to h(x) only. By using the importance weight technique, approximation of the new distribution can be obtained.

□ = □□ □ = !
!

!
!!!
[□ □

□ (□)] 3.2

where E is called the Monte Carlo estimator. The variance can be calculated.

□!(□) = ! □![□ □ □(□)]3.3
!

3.2.1 Metropolis method

Assume that we have two states (o) and (n), and these states are obeying Markov chain, where:

- the N function denotes the probability density,
- acc(initial state→final state) denotes the accepted move from the initial state to final one,
- □ (Initial state→final state) denotes the transition matrix from the initial state to the final one and □ is the underlying matrix of the Markov matrix, chosen to be symmetric.

In the equilibrium condition to move between the states is equal so that the system does not change so:

□□□ □ → □ = □□□ □ → □ . 3.4

The probability density of the certain state is the probability to find this state inside the system,
!!" !

□(□) = !
!

3.5

where Z is configurational integral.
□ □ =!

!!" !

!

3.6

The choice of Metropolis:

□□□ □ → □ =
□□ □ □ < □ □

□□ = 1 □ ≥ □(□) 3.7

or □ □ < □(□), the accepted moves will be !(!) from the total moves
!(!)

!(!)=□!!(! ! !! ! 3.8
!(!)

3.2.2 Cluster moves technique

The cluster moves technique is a sampling technique in order to speed up the simulation. It is an artificial move and instead of moving one particle from the cluster in an unfavorable environment, one moves the particle with surrounding ions and thereby one can speed up the simulation and so it increases the acceptance rate. From the previous explanation of Metropolis method, the acceptance of moving the cluster from the position (o) to a new position (n),

□(□ → □) = □□□ [1, □□□(−ßΔ□)] 3.9

But this equation doesn't care about the equilibrium conditions for the micelle with the counterions (the cluster in our model). In order to modify the equation to serve the equilibrium conditions of the cluster, the equation should not accept moving more than one particle to or from the cluster. This term has been introduced p(k,l), where p is the probability of moving the particle, which is a part from the cluster between two positions k, l : p(k,l)= 1 when rk,l< rc and = 0 when rk,l> rc
where rc is the radius of the cluster. The acceptance equation will be

!"#
acc(n→o)= min [1,exp(-□ßΔU) ! !,!] 3.10
!" !!"#(!,!)

This equation guarantees the acceptance moves will obey the equilibrium of the cluster.

3.2.3 Ensembles

One special class of ensemble is the ones that do not evolve over time. These ensembles are known as equilibrium ensembles and their condition is known as statistical equilibrium. Statistical equilibrium occurs if, for each state in the ensemble, the ensemble also contains all of its future and past states with probabilities equal to that state. The study of equilibrium ensembles of isolated systems is the focus of statistical thermodynamics. In this study the NVT ensemble has been used i.e. the number of molecules, volume and temperature are being constant.

3.3 Structural analysis

A protein is a polymer formed naturally from a specific sequence of amino acids (monomers), and the amino acids are bonded together by peptide bonds, as shown in Figure 11. To calculate the size of the proteins precisely, the radius of gyration is recommended because it considers the distance between all the mono-

mers, not the average distance as in the hydrodynamic radius. The radius will be discussed later in this section.

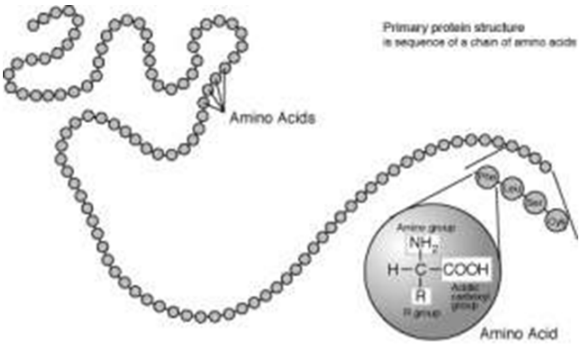

Figure 11 shows the structure of the protein.

The radial distribution function:
The radial distribution function (r.d.f or g(r)) is the variation of the number
density (= !, the unit of is number/volume) of particle as a function of the
!
distance from a certain particle, see schematic picture in Figure 12 (left). Or
more simply spoken: it is the probability of finding a particle at a distance r far from a reference particle.
The g(r) is calculated by using a Histogram technique, and it is a graphical representation of data distribution to estimate the probability distribution of a contentious variable. The radial distribution function is determined by calculating the distances between all the particles pairs, and put them all together in a histogram.

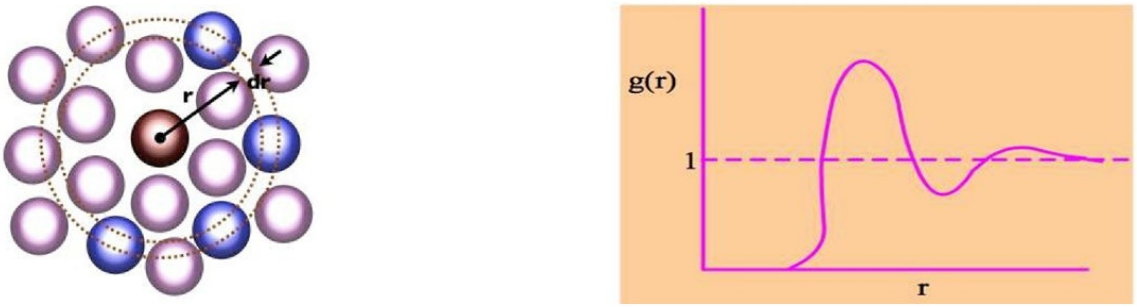

Figure 12 shows the radial distribution function g(r).[42] It is a schematic picture to the left and the histogram in a graphical representation to the right

The graph of the radial distribution function can be understood from the following: The beginning of moving from zero in g(r) axis is the start of appearing a particles after the center particle, and it is equal to twice of the radius, if it is a pure hard sphere potential.
By assuming that (rmax) is the distance (r) at the maximum g(r), means that at the (rmax) distance from the center particle is the most crowded position. Also the height of the peak refers to the number of the configuration. The g(r) gives a link between the microscopic determined from the intermolecular forces, like in this thesis ¨electrostatic interactions¨, and the other microscopic thermodynamic properties like energy (E) and entropy (S).

End to end distance of the polymeric chain
The distance from one end to another. By assuming that the polymeric chain has N monomers, and bonds with length req bond the polymers to each other. The end-to-end distance Ree corresponds to the displacement Ld of a random walk with number of steps Nr – 1 and step length req. Then
! ! !
! !
< !! >!= < ! >!= ! − 1 !!” 3.11

3.3.3 The hydrodynamic radius
It is also related to the number of monomers and the distance between monomers.
!
!!” = !! <

!!! □!"

3.12

where N is number of monomers and rij the distance between i and j monomers.

3.3.4 The radius of gyration

Is the root mean square of the distances between the monomers and their centers of mass, and it can be expressed by the formula:
! !

3.13

□! = !!!

!,!

□! − □!

the ratio between the mean square of the end to end distance(Ree), and the radius of gyration (Rg), is related to the factor, and the shape factor is related to the polymer conformation.

Results and discussion:

The charges of the micelles have been calculated according to equation (2.22) at the pH of the normal fresh milk (6.7) and it has been found to be (-□140e), which for 21 micelles gives rise to 2940 counterions. In the simulations, all the counterions have been explicitly taken into account, hence the systems are electroneutral. The existence of charged species in the solution gives rise to Columbic forces, i.e. attractive electrostatic interactions between particles of the opposite sign, and repulsive electrostatic interactions between particles of the same sign of charge. Electrostatic interactions have an effect on the distribution of micelles, and since the micelles repel each other, and attract the counter ions, a cluster move technique has been applied i.e. it has been possible to move a micelles with condensed counterions in one MC step.

The effect of the electrostatic forces:

The radial distribution function between the micelles is shown in Figure 13 (blue curve), and the corresponding g(r) for non-□ charged micelles are shown in the red curve. The blue curve shows the effect of the electrostatic interactions on the micellar structure. The g(r) is zero for r less than one molecular diameter due to hard sphere repulsive forces. The electrostatic interactions induce repulsion between the micelles and there is a prominent peak at around r = 328 Å, which is more than the double of the radius of the micelles (150 Å). The height of the peak reaches two, which means that it is two times more likely to find two micelles of this distance than in an ideal gas.

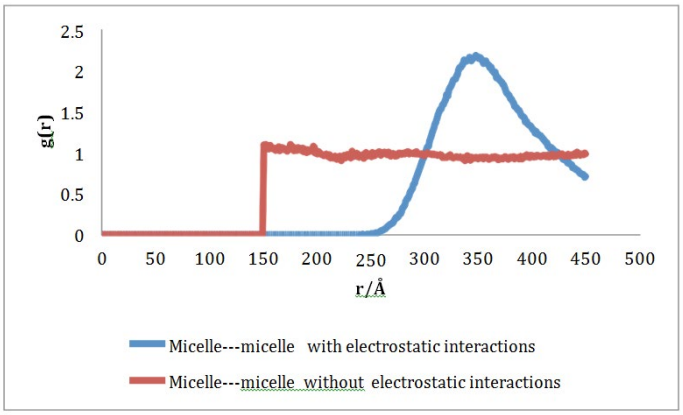

Figure 13 shows the radial distribution between charged micelles (blue curve), and neutral micelles (red curve).

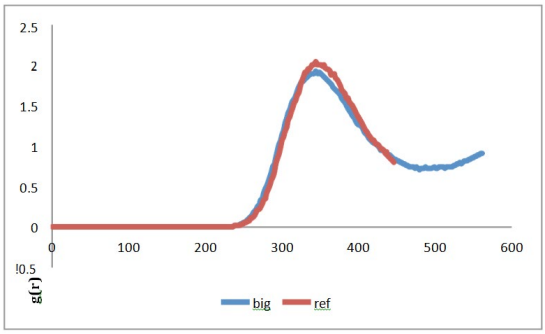

Figure 14 shows the slight system size dependence.

The ideal system and the effect of counterion volume

In Figure 15, the blue curve shows the "ideal system¨ with neutral micelles, and as expected, the closest approach distance between the micelles is twice the micelle radius due to hard sphere interactions. The red curve gives the structure for a system with again 21 neutral micelles, but now including 2940 neutral hard sphere "ions". From this simple test, we conclude that the excluded volume effect from the small ionic species is negligible.

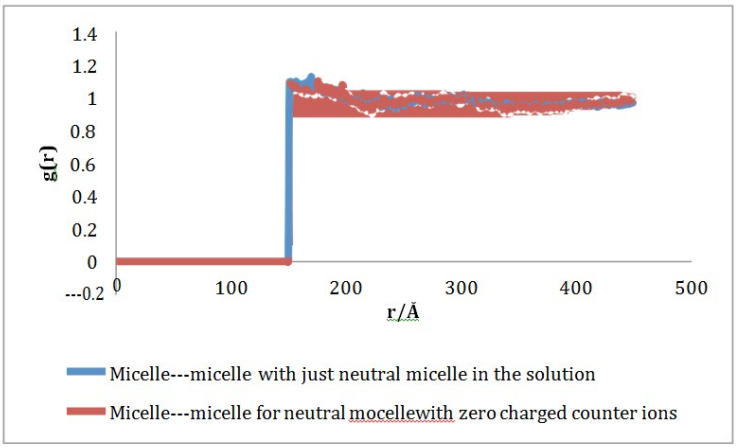

Figure 15: The radial distribution function versus the distance between the micelles for two different systems. The blue curve corresponds to a system with 21 neutral micelles, while the red curve corresponds to a system with 21 neutral micelles together with 2940 neutral "counterions".

The effect of salt concentration:

The monovalent salt concentration of 80 mM is interesting, since it corresponds to the real ionic strength in milk. The salt solution screens the electrostatic interactions and to be able to study the effect of salt, a screened electrostatic potential has been used. The g(r) in Figure 16 shows that when the salt concentration is increased, the electrostatic interaction is not as strong as in the reference system, and that it becomes more like the ideal system c.f. green curve (ideal system) with the red curve (80 mM).

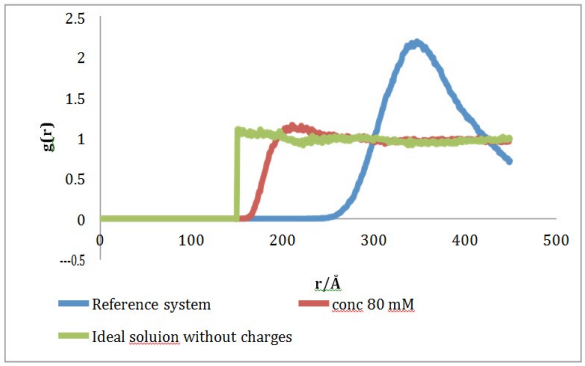

Figure 16 shows the response in the micelle-micelle structure with respect to the addition of 80 mM of monovalent salt.

The blue curve is for the reference system (explained above) and it is clear that the micelles are more separated than in the red curve, which is for the system with 80mM concentration (more screened electrostatic interaction). The third system is the ideal solution without any charges, where no long-ranged order is visible. Notice that the red and green curve deviate from each other at shorter interparticle distances, hence at these distances the micelles "feel" each other and repulsion is obtained. The screening length for 80 mM salt is ≈ 11 Å.

The effect of salt valence on milk:

The monovalent counterions in the reference system have been replaced by divalent and tetravalent ions where the aim was to study how the valency of the ions affects the structure, and if correlation effects can be obtained. Correlation effects means that two highly charged objects of the same sign can attract each other due to charge fluctuations, see schematic mechanism in Figure 17. This contra intuitively effect is only visible for higher ordered salts and most probably important in the aggregation process of proteins.

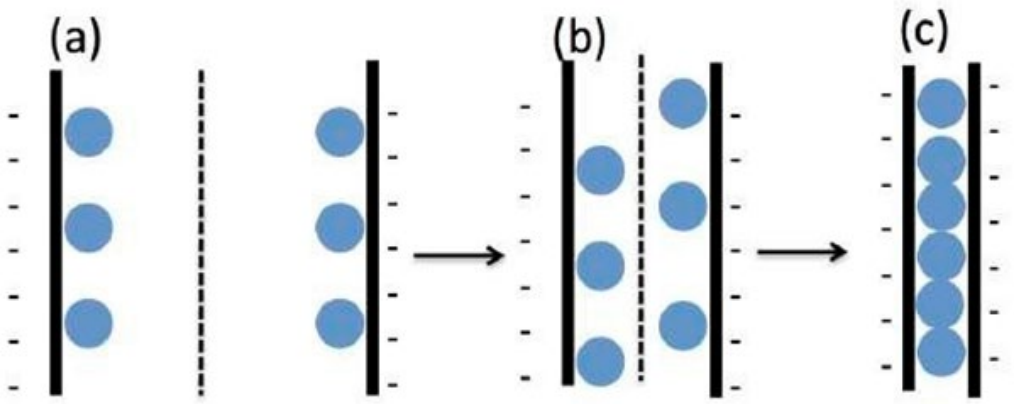

Figure 17. Correlations effects. Each half of the system is electroneutral, and the blue spheres correspond to salt ions of higher valency, and the surfaces correspond to negatively charged colloids. In (a) the two colloids are well separated and do not feel each other, (b) they feel each other and charge fluctuations are induced, which results (c) in the aggregation of the colloids.

The blue curve in Figure 18 corresponds to the reference system with monovalent counterions, the red curve corresponds to a system where the monovalent ions have been replaced by divalent ions, and the green curve corresponds to a system where the monovalent ions have been replaced by tetravalent ions. As clearly shown, when the valency is increasing the interparticle distance between the micelles is decreasing and the largest effect is visible for system with tetravalent ions. Here the micelles are more or less in molecular contact with each other, only separated by the counterions. Moreover, in the tetravalent system g(r) ≈ 13 at the peak position, which indicates that at r ≈ 160 Å, it is 13 times higher probability to find neighboring micelles in comparison with an ideal gas.

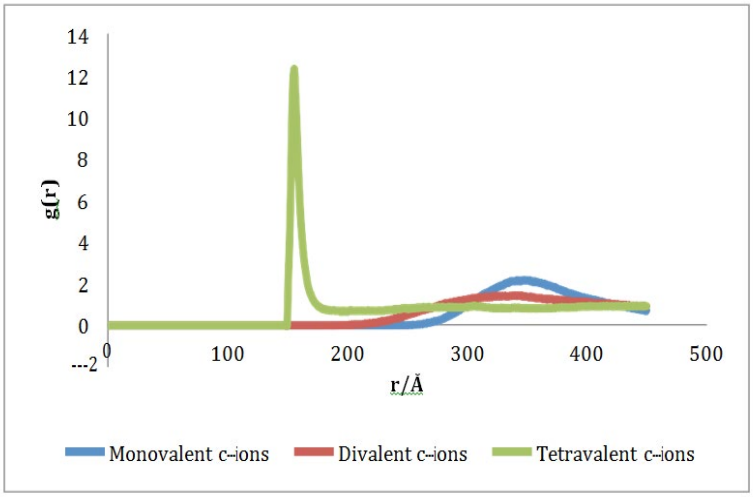

Figure 18 shows the difference in the distribution of micelles with the variation of the valency of the counterions. In the green curve, the micelles look freer than in the red and the blue curve, and this behavior occurred because of the screening of the electrostatic force, which increases sharply when increasing the valence. The maxima of the r.d.f:s are varying as well, which indicate the variations of structures

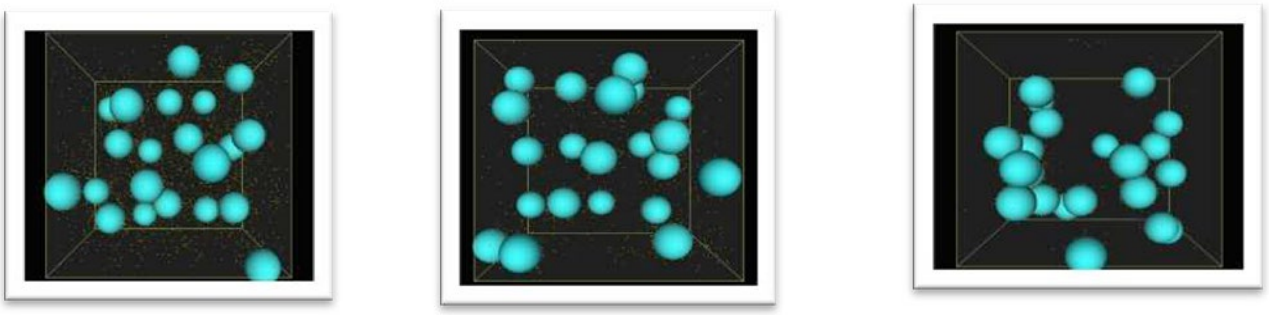

Figure 19 shows micelles in monovalent and divalent and tetravalent respectively from right to left.

The effect of pasteurization:

Increasing the temperature is of special interest, because that is used in the pasteurization processes. Even without adding the values of the right permittivity coefficient, the graph still shows some variations of the behaviors of the micelles. These variations occur because of the effect of temperature on the Debye screening length, which is directly proportional to the root mean square of the □!!.

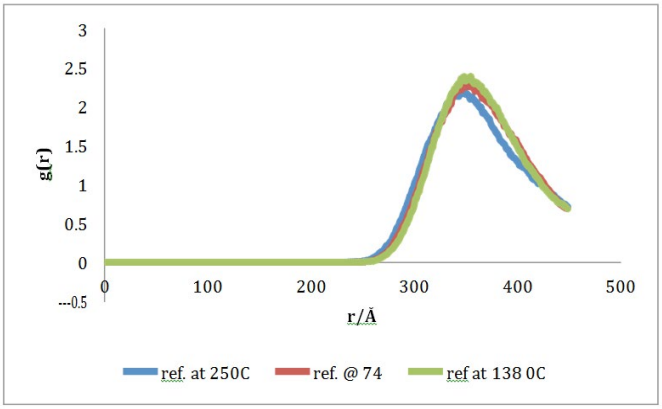

Figure 20. The micellar structure has been studied as an effect of temperature to mimic the pasteurization process

The radial distribution functions for the three different temperatures are given in Figure 20. As shown, they coincide and one reason might be because we kept the permittivity coefficient constant in the simulation. At 740C, the permittivity coefficient of the water falls to 63:61, and at 1380C it falls to 48:46.[40] The entropy increases by increasing the temperature, but since the inter-□ micellar electrostatic repulsion is very strong, it does not have any influence on the structure.

The effect of fermentation

It is well-□known that the charge of the protein depends on the pH as explained above. The protein charges have been calculated by the equation (1.15) for different pH as shown in Figure 21

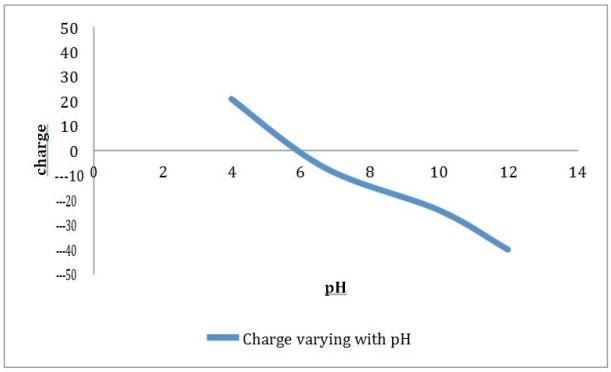

Figure 21 shows how the protein charge is varying with the pH

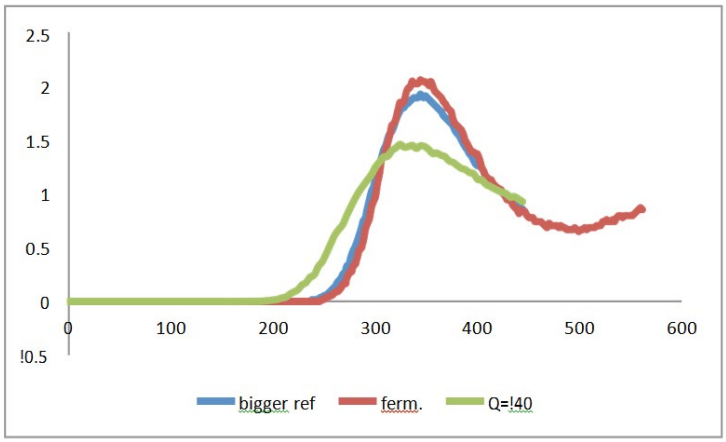

Figure 22 shows the g(r) for micelles in ordinary milk (blue curve) and in fermented milk (red curve) as well as test micelle where the charge is 1/3 of the micellar charge in milk.

As visible there are no traceable effects on micellar structure between milk and fermented milk system (in which we increased the micelle charge to 420 e.) This is due to the fact that the screening of the counter decreases the Debye length to 21.5Å from 37.3Å for the reference system.

Summary

Milk is indeed a complex liquid, which contains many different components. In this study, we have used a simple model of hard spheres to mimic □-□casein micelles in milk. The aim was to investigate how electrostatic interactions affect the micellar structure by varying the solution conditions.

The structure of the solution has been analyzed by comparing the radial distribution function as a function of pH, salt concentration and valency, and temperature. It was noticed that due to the fact that the micellar charge is very large, the electrostatic repulsive interaction dominates, and the mean distance between the micelles are almost always obtained. The mean distance was calculated to be approximately 330 Å for a micellar volume fraction of five percent.

Moreover, an increase of the temperature does not affect the structure at all i.e. the entropic contribution due to increased temperature can be neglected in comparison with electrostatic repulsion between the micelles.

When the salt concentration was increased to 80 mM, which corresponds to the ionic strength in milk, the structure of the □-□casein micelles resembles the structure of an ideal gas i.e. the electrostatic repulsive interactions are screened. 80 mM salt corresponds to a screening length of approximately 11 Å.

6.Suggestions for future work

I.Verifying the model by simulation larger systems.

II.Investigate how a temperature affects the dielectric constant, in our study; it was kept constant to 78.4.

III.Investigated the effect of mixed micelles.

IV.Investigate how the protein volume fraction affects the structure.

V. It is of interest to get a general understanding of the micellar formation, and which theory that is applicable.

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