NANOYOU Teachers Training Kit in Nanoscience and Nanotechnologies

Experiment A – Natural Nanomaterials

Experiment Module

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MATERIAL INCLUDED IN THIS EXPERIMENT PACKAGE:

For teacher:

TEACHER RESOURCES FOR EXPERIMENT A

For students:

EXPERIMENT A-STUDENT BACKGROUND READING

EXPERIMENT A-STUDENT LABORATORY WORKSHEET

LEVEL OF EXPERIMENT: Simple

DISCLAIMER: The experiments described in the following training kit use chemicals which need to be used according to MSDS specifications and according to specific school safety rules. Personal protection must be taken as indicated. As with all chemicals, use precautions. Solids should not be inhaled and contact with skin, eyes or clothing should be avoided. Wash hands thoroughly after handling. Dispose as indicated. All experiments must be conducted in the presence of an educator trained for science teaching. All experiments will be carried out at your own risk. Aarhus University (iNANO) and the entire NANOYOU consortium assume no liability for damage or consequential losses sustained as a result of the carrying out of the experiments described.

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 233433

TEACHER RESOURCES EXPERIMENT A: NATURAL NANOMATERIALS

AIM: We have numerous **natural nanomaterials around us** and in this experiment students will learn that two very common materials, gelatine and milk, are indeed two of these. Milk as we know it is white and its appearance is connected to the presence of numerous proteins which are self-assembled in specific nano-structures, called *casein micelles*, 50-300 nm in size. When milk is heated and an acid such as vinegar is added, this molecular organisation is disrupted and milk agglomerates and a "curd" precipitate forms. Interestingly, if the same is done using cold milk, only thickening occurs but no precipitate is formed. Students will learn that this is due to the fact that the stability of casein micelles in milk is due to both electrostatic and hydrophobic interactions; therefore depending on the degree of disruption of these interactions different effects are obtained. Overall the experiment will show how appearance (colour, odour) and **function of a material such as milk is profoundly connected to its molecular supra-organisation (nanostructures).** By altering this organisation, new materials are obtained (in the example of milk processing, these are cheese, yogurt, etc). This is a fundamental concept of nanoscience.

FIELD OF NANOTECHNOLOGY APPLICATION: Fundamental concepts in Nanoscience

EXTRA TEACHERS' READING: Chapter 2 "Natural nanomaterials" and Chapter 4 "Fundamental nano-effects" in Module 1 of "NANOYOU Teachers Training Kit in Nanotechnologies".

REQUIRED STUDENT PRE-KNOWLEDGE:
- Light absorbance and scattering
- Protein structure (primary, secondary, etc.) and relationship between protein structure and function

STUDENT READING:
- NANOYOU Students' background document for Experiment A

EXPECTED OUTCOME:
- Existence of natural nanomaterials: gelatine and milk as examples of natural colloids
- Light interaction with colloids
- Protein self-assembly into nanostructures
- Relationship between milk's "macro" properties (colour, smell, taste, consistency) and its molecular structure and how these can be manipulated to obtain different products (cheese, yogurt, etc).

STUDENT ASSESSMENT:
-NANOYOU Student laboratory worksheet Experiment A

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BACKGROUND INFORMATION

Natural nanomaterials

By natural nanomaterials we mean here materials that **belong to the natural world** (animal and mineral), without human modification or processing, and that have remarkable properties because of their **inherent nanostructure**.

The chemical identity and properties of a substance depend upon its molecular structure. The nanostructure of a biological material is due to its supramolecular organisation – the arrangement of tens to hundreds of molecules into shapes and forms in the nanoscale range. The interaction of light, water and other materials with these nanostructures gives the natural materials remarkable properties that can be appreciated at the macro scale.

We have **hundreds of examples of nanoscience under our eyes daily**, from geckos that walk upside down on a ceiling, apparently against gravity, to butterflies with iridescent colours, to fireflies that glow at night. In Nature we encounter some outstanding solutions to complex problems in the form of fine nanostructures with which precise functions are associated.

Figure 1. Examples of natural nanomaterials. From top left corner clockwise: a butterfly, the foot of a gecko, nasturtium leaves, milk. (Image credits: Top left, Wiki commons, Creative Commons Attribute ShareAlike 3; top right: A. Dhinojwala, University of Akron, NISE network, reprinted under NISE network terms and conditions; bottom, left: Wiki commons, Creative Commons Attribute ShareAlike 3; bottom right: iNANO, University of Aarhus, Creative Commons Attribution ShareAlike 3.0.)

Natural nanomaterials provide an **inspiring way of bringing nanoscience into the classroom**. Many natural materials that students will be very familiar with **owe their properties to nanostructures in their composition**. In this experiment the natural nanomaterials that will be analysed are **gelatine and milk**. Both are types of **colloids**. A *colloid* is another type of chemical mixture where one substance is dispersed evenly throughout another but **the particles of the dispersed substance are only suspended in the mixture**, they are not completely dissolved in it (unlike a *solution*). This occurs because the particles in a colloid are larger than in a solution. Generally speaking, a **colloid is composed of particles in the range of 10-300nm**. They are small enough to be dispersed evenly and maintain a homogenous appearance, but large enough to **scatter light**.

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The particles in a colloid can be so well dispersed that they have the appearance of a solution (e.g. transparent).

A simple way to test if a mixture is a solution or a colloid is to **shine a laser beam though the mixture**: the light will be scattered only by the colloid. **WARNING**: never shine a laser beam near the eyes nor look straight into the beam!

In this experiment student will realise that **without these nanostructures common materials like milk lose their appearance and function**.

In this experiment student will:

1. Prepare gelatine and test it with a laser pen to confirm its colloidal nature.
2. Confirm that milk is a colloid and treat it with acid to induce its aggregation. This experiment will give students practical evidence of the link between structure and function, and how manipulation of the molecular organisation of a material, like milk, leads to materials with different colour, odour and taste!

Gelatine

Gelatine is a tasteless solid substance, derived from the collagen inside animals' skin and bones. It is used as a gelling agent in food products (cakes, etc.), in pharmaceuticals (e.g. gelatine capsules), in cosmetic products and photography.

Gelatine is a **protein** produced by **partial hydrolysis of collagen** found in the bones, connective tissues, organs and some intestines of **mammalian animals** such as pigs. However gelatine from **fish** is also becoming a common source.

During the partial hydrolysis of collagen some molecular bonds between individual collagen strands are broken down into a form that rearranges more easily (gelatine). For this reason gelatine chemical composition is, in many respects, very similar to that of its parent collagen.

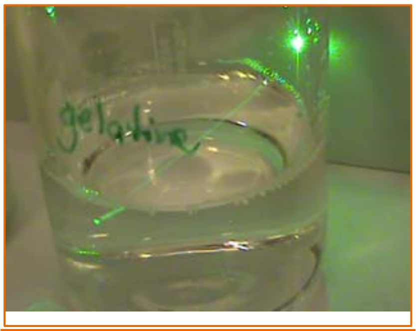

Figure 2. Testing a gelatin sample with a laser pen. (Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Attribution ShareAlike 3.0.)

Gelatine is often found in the form of powder. When mixed with water it forms a solution of high viscosity, which sets to a gel on cooling, forming a **colloid gel**. Gelatine gel melts to a liquid when heated and solidifies when cooled again. Therefore its existence as a gel is limited to a **specific temperature window**.

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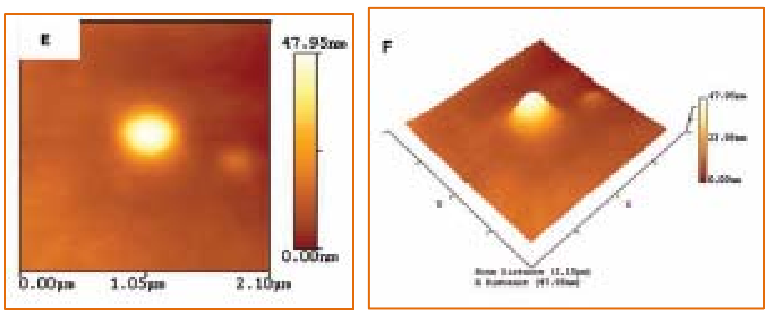
The fact that gelatine is a colloid rather than a solution can be easily seen by using a **laser pen and shining light through the gel**. A path of scattered light is clearly visible (Tyndall effect). This effect is due to the scattering of light by the nanoparticles inside the colloid Students will test this effect. **WARNING**: never shine a laser beam near the eyes nor look straight into the beam!

HOW IS IT "NANO"? Recent studies with the **Atomic Force Microscope (AFM)** have shown that gelatine is indeed formed by numerous **nanostructures** which have various shapes depending on the type of gelatine analysed. For instance AFM analysis of gelatine extracted from catfish (*Ictalurus punctatus*) skin has revealed the presence of **annular pores** with diameters averaging 118 nm and **spherical nano-aggregates** with diameter around 260 nm. It is hypothesised that these structures are formed during the penetration of water inside the collagen molecules during hydrolysis. **The presence of these nanostructures proves that gelatine is a colloid and explains its light scattering behaviour**.

Figure 3. AFM images of a gelatine extracted from catfish revealing the presence of spherical nanostructures. (Image credit: reprinted by permission of Wiley-Blackwell Publishing Ltd from Yang et al., Journal of Food science (2006), 72(8), pp c430-c440, copyright (2006) Wiley-Blackwell Publishing Ltd.



Milk

Bovine milk contains a number of biomolecules, such as lipids and proteins, which are dispersed in water. The amount of proteins is between 2.5 and 3.5% depending on the animal breed, of which about 80% are **caseins** (the rest being whey or serum proteins). Four proteins comprise the casein group and are: α_{s1}-casein, α_{s2}-casein, β-casein and k-casein. The caseins are characterised by the fact that they are **phosphoroproteins** that precipitate at pH 4.6 (isoelectric point, I.P.), at which pH whey proteins remain soluble. Another property of caseins is their existence as **casein micelles** which are in the **range 50-300 nm in dimension**. Micelles contain the caseins combined with calcium, phosphate and small amount of citrate. As such, **milk is a colloid** (a mixture of nanoparticles evenly dispersed but only suspended in a liquid medium). The presence of these micelles (together with other biomolecules like lipids) determines the **white colour of milk** due to their light scattering.

Although milk is a colloid, it is not transparent, therefore if you shine milk in a glass, the Tyndall effect is not visible. However, if milk is diluted (1% milk in water) the effect is seen. Students will see this effect. **WARNING**: never shine a laser beam near the eyes nor look straight into the beam!

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FROM STRUCTURE TO FUNCTION

The fine molecular self-organisation of proteins and minerals in milk is fundamental for realising its natural function of transporting calcium from the mother to the offspring. Numerous studies have revealed that this organisation results in nanostructures which have precise functions (**casein micelles**). In the next section we will describe how this organisation is determined by electrostatic interactions but also hydrophobic interactions between the proteins that constitute milk and some minerals that are associated with the proteins. Without this fine organisation calcium would not be "trapped" inside the milk micelles and the biological function of milk would not be realised.

MILK PROCESSING

Processing of milk with various treatments is widely used in the dairy industry. For instance, yogurt is a fermented milk product obtained by the controlled growth of specific microorganisms, mainly bacteria that convert lactose (milk sugar) into lactic acid. By lowering the pH of milk, its consistency and taste change. In cheese making, enzymes are used to induce the aggregation and precipitation of caseins. As will be discussed in the next section, in all milk processing methods **the molecular organisation of caseins is altered**, which leads to thickening, precipitation and other effects. The appearance, taste and other "macro" properties of milk are deeply connected to its supra-molecular (nano) structure. **In this experiment students will use vinegar (a source of acid) and heat to alter the properties of milk.**

CASEINS

Caseins are one of the types of proteins found in milk. Casein in milk (which has a pH near to neutrality, about 6.7) are negatively charged (I.P. is 4.6). All caseins, except k-casein, possess the ability to **bind to Ca^{2+} which occurs mainly through their phosphate residues**. The binding of Ca^{2+} is fundamental for milk to fulfil its function, that is, to transport calcium (and other nutrients) from the mother to the offspring. Each casein is composed of a different peptide sequence and therefore has a different secondary and tertiary structure.

The precise **structure** of casein is still a matter of debate and study within the scientific community. This arises from the fact that **caseins cannot be crystallised** (as opposed to other types of proteins) and NMR structural studies have so far extended to peptide analysis. The current model of casein tertiary structure is based on numerous studies performed using circular dichroism, Raman spectroscopy and FTIR analysis.

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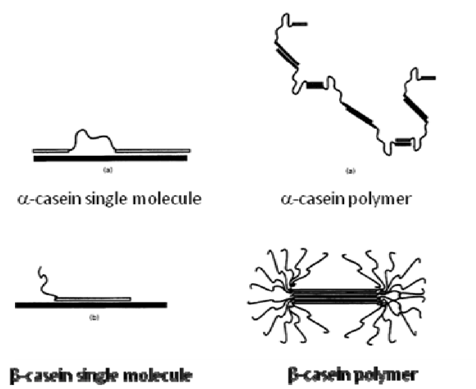

Figure 4. Schematic structures of caseins and their polymers. Rectangles in the images represent hydrophobic regions. Reprinted from: Horne D.S., Inter. Dairy Journal (1998), 8 (3), 171-177, with permission from Elsevier.

The most current model of casein tertiary structure is based on considering **caseins as block co-polymers**. In the case of α-casein the protein has **two hydrophobic regions separated by a hydrophilic core**. It is predicted that this protein self-assembles into a "train-loop-train structure" as illustrated in **Figure 4**. It is believed that this protein links inter-molecularly to form a worm-like polymeric chain. On the other hand **β-casein** has a **highly charged N-terminal region and a hydrophobic C-terminal region** and it is believed it assumes a tail-train structure. Self-association of these molecules is believed to lead to a "micellar polymer" with a hydrophobic core and a hydrophilic "hairy" outside. **K-caseins** have a structure which is a mirror image of β-casein, and thus have a **hydrophobic neutral N-terminal region and a highly charged C-terminal** peptide. K-caseins do not have the ability to bind Ca^{2+} but have a stabilising function.

CASEIN MICELLES: STRUCTURE AND FUNCTION

Caseins in milk are believed to exist as **casein micelles** in the range **50-300 nm in dimension**. Micelles contain the **caseins combined with calcium, phosphate and small amount of citrate.** The structure of casein micelles (like that of caseins itself) is still a matter of debate and intense research. Since all caseins posses a hydrophobic region and a polar region, it is believed that hydrophobic interactions as well as electrostatic interactions play a role in the self-association of caseins to form casein micelles. Casein micelles differ from the polymers of the individual caseins in one crucial aspect: they contain inorganic calcium phosphate, which exists in the form of small microcrystalline inclusions termed **calcium nanoclusters**. The fact that the stability of casein micelles is not due only to electrostatic interaction has been demonstrated by the fact that **casein micelles can be dissociated using urea**, which is an agent that does not rupture the calcium phosphate linkages.

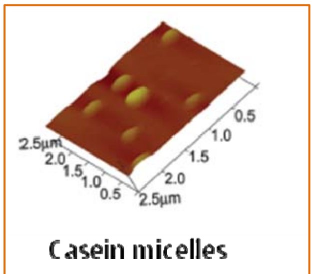

Figure 5. AFM image of milk casein micelles. (Reprinted with permission from: Shekar et al., PNAS (May 23, 2006), vol. 103, no. 21, pp 8000-8005. Copyright 2006 National Academy of Sciences, U.S.A.)

Two types of linkages between casein in the casein micelles have been postulated:

- The first linkage is **hydrophobic**, where two or more hydrophobic regions from different molecules (α-caseins and β-caseins) form a bonded cluster. These are indicated as a **rectangular bar** in **Figure 6**.

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- The second linkage of **hydrophilic charged regions** containing phosphoserine clusters which bind to colloidal calcium phosphate nanoclusters (indicated as CCP in **Figure 6**).

The k-caseins do not have the phospheroserine group to link with the calcium nanocluster, therefore their association is only possible through hydrophobic interactions. Also, the micelle cannot grow further beyond the k-caseins, which therefore act as an outer layer in the micelle. The **role of k-caseins is to stabilise the casein micelle**, preventing excessive growth and micellar aggregation which would otherwise lead to precipitation.

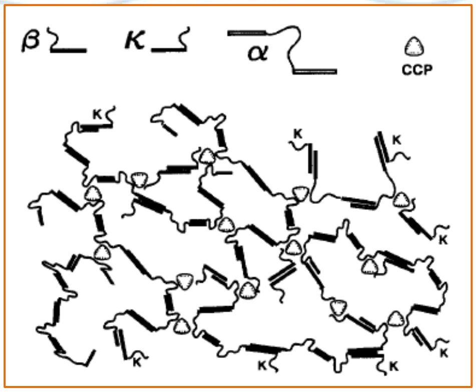

Figure 6. Dual bonding model in casein micelles, with α, β and k-casein depicted as indicated. Reprinted from: Horne D.S., Inter. Dairy Journal (1998), 8 (3), 171-177, with permission from Elsevier.

CASEIN MICELLES DISSOCIATION AND AGGREGATION

As outlined above, casein micelles are believed to have an intricate structure which is an interplay of hydrophobic and electrostatic interactions. Maintenance of micellar integrity is a balancing act and numerous methods exist to disrupt this balance. These methods are widely used in the diary industry to make cheese and fermented products like yogurt. Below we will quickly review these methods; the aim here is not to detail the production of milk products, but the interested teacher can find more information at the end of this document in the “Further reading” session.

- **increasing pH (to about 8)** leads to casein **micelle dissociation**, and the effect is that heated milk becomes more translucent. The reason for this is that increasing the pH from the natural neutrality converts the phosphoseryl groups from singly to doubly charged units which are no longer capable of linking the calcium phosphate nanoclusters. The increased negative charge of the micelle induces electrostatic repulsion, and the micelles dissociate.

- **decreasing pH to the isoelectric point (4.6)** induces dissociation of the casein micelles. The reason for this is that calcium micelles only exist because of the presence of calcium phosphate; therefore its dissolution necessarily causes changes in the stability of the micelle. The consequence of lowering the pH is the titration of the phosophoseryl and carboxyl groups in the proteins. Without their negative charge these groups cannot link to the colloidal calcium phosphate nanoclusters, so these are released from the micelle. It should be noted that this does not necessarily cause the caseins to dissociate from the micelles and lead to precipitation. At temperatures below 25°C, increasing dissociation occurs, but otherwise the caseins remain in the micelles. This effect will be tested in this experiment by adding vinegar (a source of acid) to cold milk. The reason lies in the fact that the stability of casein micelles is

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not entirely connected to electrostatic interaction, but also to hydrophobic interactions. The latter are extremely temperature-dependent: hydrophobic interactions are stronger at higher temperatures. **Therefore hydrophobic interactions maintain the stability of casein micelles in cold milk even when its pH has been lowered to the isoelectric point.** On the other hand, **if acidification occurs after milk has been warmed (about 60°C), micelles are dissociated (calcium phosphate is released from the micelle) and will aggregate due to increased electrostatic forces *and* increased hydrophobic interaction.** This will be tested in this exercise by adding vinegar to warm or cold milk.

- **Attack by chymosin leads to micelle precipitation and formation of a curd**. This process is employed in **cheese manufacturing**. Chymosin is a proteolytic enzyme which is the active principle in rennet, the extract of calf's stomach used in cheese making. Chymosin specifically attacks a single bond in the k-casein, breaking the molecule into two peptides: one remains attached to the micelle, while the other one diffuses in solution. As mentioned above, the presence of k-caseins is fundamental for the overall stability of the casein micelle; therefore its disruption leads the micelle to lose stability, aggregate and eventually form a curd.

- The controlled addition of **lactic acid bacteria** (bacteria that produce lactic acid such as Lactobacillus, Lactococcus, and Leuconostoc) under specific processing conditions leads to fermented milk products such as yogurt. This process differs from simple acidification as milk is heat-treated and whey proteins are also incorporated. The coagulation is induced by the acidification but does not lead to the formation of a curd but to a product which is more viscous than plain milk.

TIP FOR TEACHER

The phenomenon of aggregation of milk can easily be seen in milk that is old and has long passed its sell-by date. In this case, it is lactic acid bacteria that are responsible for the acidification of milk and consequent aggregation to form acid-smelling lumps. **In this experiment students will use vinegar (a source of acid) and heat to alter the properties of milk.**

WHAT CAN THIS EXPERIMENT TEACH ABOUT NANOTECHNOLOGY?

Through this exercise students will learn two fundamental concepts:

- **Structure means appearance**: materials in the "real" natural world, like milk, appear as they do because of fine nanostructures they possess. Milk is white because it contains colloidal nanoparticles (micelles). If we alter the structure of these micelles, we alter some "macro" properties of milk like **colour** and **odour.**

-**Structure means function:** natural materials have very specific functions which are dictated by the fine supra-organisation of their molecules (nanostructures). If we alter these, we can obtain a material with a new function. In cheese production, altering the casein micelles through specific processes (e.g.

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chymosin treatment or lactic acid bacteria fermentation) leads to different products (cheese, yogurt, etc.). **This is exactly the concept of nanotechnologies**: to engineer new materials with new functions from the manipulation of their molecular organisation.

THIS EXPERIMENT IN CLASS

1. Start with a discussion on natural nanomaterials. What are they? Let the students think of materials they know already and/or discuss examples such as gecko, butterflies, bones, or biological nanostructures such as DNA, ferritin, chlorophyll, etc.

2. Discuss the relationship between structure and function. This can start from the macro-level (e.g. structure of a building to serve its function to resist an earthquake) and move to the nanoscale.

3. Discuss with the students what they know about gelatine and milk. What happens when you heat them? Or cool them? What happens if milk is left in a fridge way past its sell-by date?

4. Proceed with the experiment as outlined in the next section.

5. Conclude with a discussion on other natural colloids such as blood, custard, smoke. Nano is all around us!

MATERIAL NEEDED

The material below is indicated assuming students will work in pairs.

Materials for the entire class (to be shared)

- Gelatine from pig skin (Sigma-Aldrich product number G1890, 100 gr about 34 Euros)
- 1 L white vinegar
- 1 laser pen (to be shared by the class; ideally more than one should be available)
- a water kettle if hotplates are not available for students to use

Materials for each student pair:

- Hotplate
- 2x beaker 50 mL
-1x beaker 200 mL
- 2x beaker 500 mL
- 0.5 gr gelatine powder
- 1 tablespoon

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- 800 mL of skim milk
- 4 tablespoons of white vinegar
- thermometer
- 1 spatula
- latex gloves
- safety glasses

SAFETY NOTE: This experiment doesn't use chemicals but only common liquids and solids. Nevertheless staining is possible so wash hands and surfaces thoroughly after handling. Use appropriate clothing protection, gloves and eyes protection. Collect all liquids and washing water is glass/plastic containers and dispose of in sink. All experiments will be carried out at your own risk. Aarhus University (iNANO) and the entire NANOYOU consortium assume no liability for damage or consequential losses sustained as a result of the carrying out of the experiments described.

PROCEDURE

1. Is gelatine a colloid?

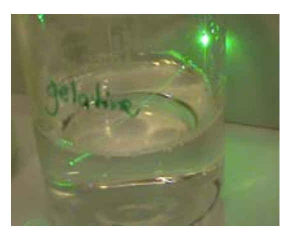
In this part of the experiment students prepare gelatine and test it with a laser pen to confirm its colloidal nature.

STEP 1

Prepare a 10 mg/mL gelatine sample by mixing 0.5 mg of gelatine powder with 50 mL of cold water. Place on the hotplate and heat the water + gelatine powder. Stir with the spatula as the mixture heats up. Bring close to boil (check temperature with thermometer), then turn off the hotplate and let the gelatine cool down. **SAFETY NOTE!** Do not touch the beaker immediately as it will be very hot. After it has cooled down, remove it from the hotplate and place on the bench safely. Otherwise use safety gloves.

STEP 2

Once the gel is formed, test it with a laser pen. Place a piece of white paper on the other side of the beaker. Shine the laser beam through the gelatine sample and ask students to record their observations. **WARNING**: never shine a laser beam near the eyes nor look straight into the beam! Students should wear **safety glasses** when doing this test.



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STEP 3

Repeat the laser test but testing a beaker with plain water. Does plain water scatter light?

Students should complete questions Q1 and Q2 in the Student Worksheet.

2. Milk and its properties

Milk is a natural colloid and students will confirm this as they did with gelatine, using a laser pen (using diluted milk). They will then treat milk with acid to disrupt its molecular nanostructure and induce aggregation.

STEP 1

Milk is a natural colloid but unlike gelatine it is not transparent. Pour 400 mL of milk in a beaker. Try to test it with a laser pen as you did with gelatine, can you see a path of scattered light? No, because the light is reflected back (back scattering).

WARNING: never shine a laser beam near the eyes nor look straight into the beam! Students should wear **safety glasses** when doing this test.

Now ask the students to dilute milk. Take 150 mL of distilled water in a beaker or glass, and add 1-2 droplets of milk (using a pipette). Mix and let the solution stand for a couple of minutes (you don't want air bubbles). The solution will look pale-grey. Now ask the students to test it again using the laser pen. They will see a path of scattered light.

Students should complete questions Q3 and Q4 in the Student Worksheet.

STEP 2

With the use of a pH paper, record the pH of skim milk.

Now place the same beaker containing milk used in STEP 1 on a hot plate, turn the hot plate on and warm the milk up to about 60 C. If a hot plate is not available the milk can be heated using water that has been boiled separately and added to the water bath container (as shown in figure).

TIP TO TEACHER: A **microwave** can be used as an alternative but the milk should **not boil nor become too hot**, therefore a test should be done beforehand to assess the time required for heating the milk to about 60°C when using that specific microwave.

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STEP 3

- To the hot milk (about 60°C) add 2 tablespoons of white vinegar and stir well as you do so. You will notice that the milk will immediately aggregate and form a "milk ball". Make sure students wear gloves as they do this, since the beaker will be hot.

- Ask the students to record the pH of the liquid

- Students should write down their observations.

SAFETY NOTE: Aggregated acid-milk should not be tasted by the students!

STEP 4

- Repeat the test but without heating the milk. Give each pair of students another 400 mL of cold milk, and ask them to add 2 tablespoons of white vinegar. The milk will not agglomerate but only become a bit thicker.

- Ask the students to record the pH of the liquid

- Ask the students to record their observations.

The reason why the milk only becomes a bit thicker but does not agglomerate should be discussed.

Students should complete questions Q5 and Q6 in the Student Worksheet.

TIP TO TEACHER: This part of the experiment can be used to connect it with the process of making yogurt.

Why does this happen? In normal milk (pH close to neutral) the casein micelles are formed of caseins (charged negatively, I.P. is 4.6) which are neutralised by calcium clusters, resulting in a stable micelle. In addition to electrostatic interaction hydrophobic interactions among the caseins stabilise the micelle. By adding vinegar the pH of milk is lowered enough to induce dissociation of the casein micelles because calcium phosphate is released from the milk micelles. The reason for this is that lowering the pH to the isoelectric point leads to the titration of the phosophoseryl and carboxylgroups in the proteins. Without their negative charge these groups cannot link to the colloidal calcium phosphate nanoclusters, so these are released from the micelle.

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When vinegar is added to warm milk a curd is formed because the proteins that make up the micelles are no longer charged and aggregate due to hydrophobic interactions. This aggregation leads to precipitation because the milk is warm. In fact, if the vinegar is added to cold milk, an increase in viscosity is noted (sign of a coagulation of the micelles) but a curd is not formed. This is because at temperatures below 25°C, acidification of milk leads to an increase in micelle dissociation but otherwise the caseins remain in the micelles. The reason lies in the fact that the stability of casein micelles is not entirely connected to electrostatic interactions but also to hydrophobic interactions. The latter are extremely temperature-dependent: hydrophobic interactions are stronger at higher temperatures. **Therefore hydrophobic interactions maintain the stability of casein micelles in cold milk even when its pH has been lowered to the isoelectric point.** On the other hand, **if acidification occurs after milk has been warmed (about 60°C), micelles are dissociated (calcium phosphate is released from the micelle)** ***and*** **will aggregate due to increased electrostatic forces** ***and*** **increased hydrophobic interaction.**

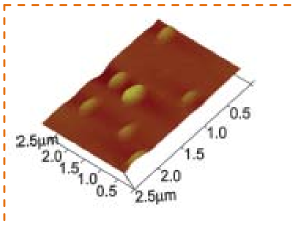
ANSWERS TO QUESTIONS

Q1. Yes, gelatine is a colloid since a path of scattered light is visible through it.

Q2. No, water is not a colloid since no path of scattered light is visible.

Q3. Yes, milk is a colloid since a path of scattered light is visible through it.

Q4. Image should be the one represented here.[1]



Q5. When vinegar is added to warm milk, a curd is immediately formed. When vinegar is added to cold milk, no curd is formed; it only becomes a bit thicker. The difference is very evident.

Q6. The fact that the reaction is temperature-dependent is an indication that the stability of casein micelles is not only due to electrostatic interactions (which can be altered through addition of an acid) but also to some other form of physical interactions among the molecules that form the micelles. In this case it is hydrophobic interactions, which are very temperature-dependent.

FURTHER READING

- D. G. Dalgleish, "Bovine milk protein properties and the manufacturing quality of milk", Livestock Production Science (1992), 35: 75-93

- D. S. Horne, "Casein interactions: casting light on the black boxes, the structure of dairy products", Int. Diary Journal (1998), 8: 171-7

D. S. Horne, "Casein structure, self-assembly and gelation", Current Opinion in Colloid and Interface Science (2002), 7: 456-61

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