Teacher Guide

EXPERIMENT A - NATURAL NANOMATERIALS

In this experiment students will study the relations between nanostructures and properties of natural nanomaterials. In this worksheet we provide you with some background information as well as instructions for preparing the experiments.

Aims

- Be aware of the existence of natural nanomaterials (for instance, gelatine and milk)
- Have an indirect evidence of the presence of nanoparticles in natural nanomaterials through light interaction with colloids
- Understand the link between nanoscale structure and function in the observable world,
- Understand how the reorganisation of molecules of a material, like milk, leads to obtaining different products (cheese, yogurt, etc) with different macroscopic properties (taste, smell, etc.)

This experiment has two parts:

Part A: Test natural nanomaterials to have indirect evidence of their nanostructure

Part B: Test natural nanomaterials to understand the relation between nanostructure and properties.

Disclaimer: *Please note that the experiments described in the following training kit use chemicals which need to be used according to MSDS specifications and 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.*

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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

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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**. The particles in a colloid can be so well dispersed that they have the appearance of a solution (e.g. transparent). Therefore the laser test provides indirect evidence of nanoparticles in the colloid.

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.

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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.

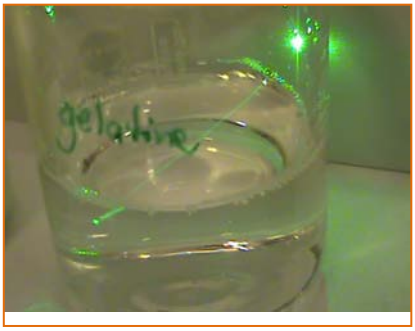
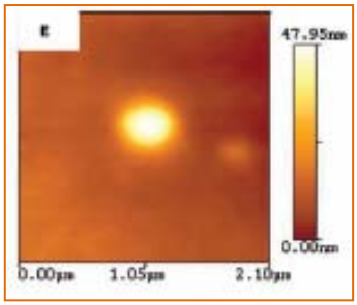


Figure 2. Testing a gelatin sample with a laser pen. (Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Attribution ShareAlike 3.0.)

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**.

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**.



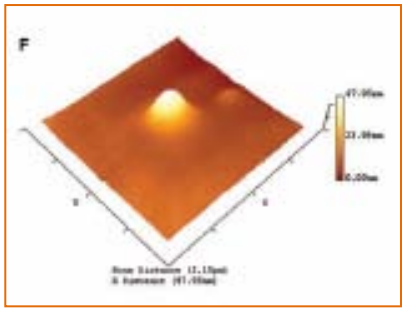


Figure 3. AFM images of a gelatine extracted from catfish revealing the presence of spherical nanostructures. (Image credit: reprinted by permission of Wiley-Blackwell Publishing Ltd from Yang et al., Journal of Food science (2006), 72(8), pp c430-c440, copyright (2006) Wiley-Blackwell Publishing Ltd.

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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!

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

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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.

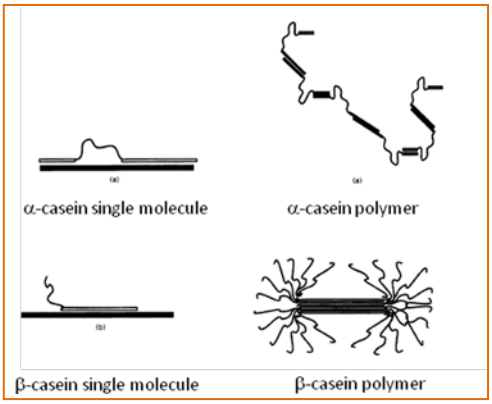

Figure 4. Schematic structures of caseins and their polymers. Rectangles in the images represent hydrophobic regions. Reprinted from: Horne D.S., Inter. Dairy Journal (1998), 8 (3), 171-177, with permission from Elsevier.

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

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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.

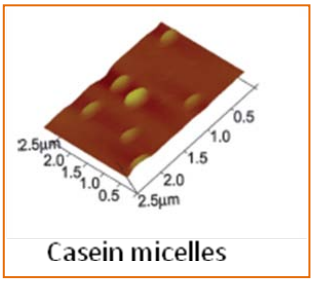

Figure 5. AFM image of milk casein micelles. (Reprinted with permission from: Shekar et al., PNAS (May 23, 2006), vol. 103, no. 21, pp 8000-8005. Copyright 2006 National Academy of Sciences, U.S.A.)

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.

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

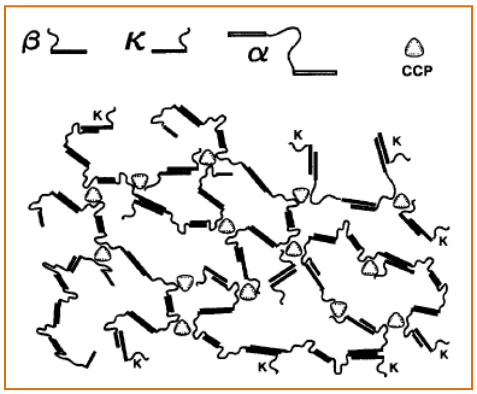

Figure 6. Dual bonding model in casein micelles, with α, β and k-casein depicted as indicated. Reprinted from: Horne D.S., Inter. Dairy Journal (1998), 8 (3), 171-177, with permission from Elsevier.

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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.

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 not entirely connected to electrostatic interaction, but also to hydrophobic interactions. The latter are extremely temperature-dependent: hydrophobic interactions are stronger at higher temperatures.

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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

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(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. 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.

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

Suggested strategies for teaching

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!

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MATERIALS 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
- 4 pieces of pH paper
- 1 tablespoon
- 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.

Part A: Test natural nanomaterials to have indirect evidence of their nanostructure

To perform the experiment, each pair of students should have:

A cool sample of gelatine,

A beaker of plain water

A beaker of milk

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A beaker of diluted milk: Take 150 mL of distilled water in a beaker or glass, and add 1-2 droplets of milk (using a pipette). Mix well and let stand for a couple of minutes (you don't want air bubbles). The liquid will look pale-grey.

How to prepare gelatine: Prepare 10 mg/mL gelatine by mixing, for each pair, 0.5 mg of gelatine powder with 50 mL of cold water. Place on the hotplate and heat the water + gelatine powder. Stir gently with the spatula (so no bubbles will be formed) as the mixture heats up. Bring close to boil (check temperature with thermometer), then turn off the hotplate and let the gelatine cool down. You can either distribute the gel to the small beakers while it is still liquid (pour onto the glass sides, to avoid bubbles), or you can wait for the gel to solidify, and then cut it into square samples for the students.

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.

Q1. Can you see any small particles by looking at the different samples? Ideally – No.
Can you see any small particles by looking at the different samples with a magnifying glass?
Ideally - No (some bubbles may be seen if bubbles were formed during the preparation process)

Q2. Look from above and describe what you can see. Record how the laser beam behaves as it penetrates each sample.
Gelatine: The laser can be seen as a red line
Water: The laser can only be seen on the glass, not in the water
Milk: The laser forms a large spot of light in the milk
Diluted milk: The laser can be seen as a red line

Q3. Did all the samples behave the same? Can you guess what disrupted the laser's path (notice that if you can see the laser's path, then something disrupted its path, otherwise its light wouldn't reach your eyes).
No, the samples did not behave the same way. There was something small (nanoparticles) that disrupted its path in all samples except for the water.

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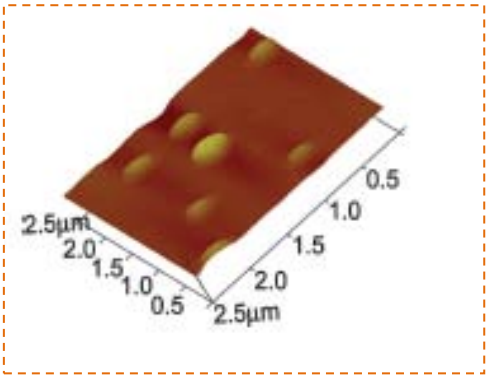
Q4. So what happened here? Please fill in the missing words

Nanoscale particles are suspended in the sample, and when the laser light hits them, it changes its direction. This is seen as a path of light in the sample, as can be seen in the _gel___ sample and the diluted milk_sample. The laser forms a spot of scattered light in the sample if the particles are so dense that the light can't pass through, as seen in the _milk_ sample. In the _water_, the light passes through without hitting anything, and so we cannot see the laser light.

Q5. Milk owes its properties to the existence of casein micelles, which are spherical nanostructures with a diameter of about 50-300 nm. Select from the images provided at the end of this worksheet the AFM image that you think corresponds to casein micelles (**take into account that 1000 nm = 1 µm**). Paste the image here.

Image C (the particles are spherical, and smaller than 0.5 microns)



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Part B: Test natural nanomaterials to understand the relation between nanostructure and properties.

pH acid indicators are used to determine the pH level of common materials such as vinegar, lemon juice, battery acid and even yeast infections.

- A pH of 7 indicates neutral solution.
- A pH above 7 indicates alkaline solution.
- A pH below 7 indicates acidic solution.

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To perform this experiment you need:

1. A beaker of cold milk

2. A beaker of warm milk (60°C)

Heating milk

Place the beaker containing milk 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 to heat the milk to about 60°C when using that specific microwave.

STEP 1

- Use the same beaker containing milk that you used in part 1. With the use of a pH paper (which checks for the level of acidity) record the acidity (pH) of skim milk (fill table provided in next page).

STEP 2

- Take a clean beaker and add another sample of 400 mL of cold milk. Place the beaker containing milk on a hotplate, turn the hotplate on and warm the milk to about 60°C. If a hotplate is not available, the milk can be heated using water that has been boiled separately and poured into a water bath container. Your teacher will instruct you on how to warm up the milk. When the milk is warm, check the acidity with a new piece of pH paper, and record the result in the table below. Observe the milk and stir it with a spoon, and record the way it looks, smells, and feels in the table below.

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STEP 3

- To the cool milk add 2 tablespoons of white vinegar (an acidic solution) and stir well as you do so. What happens (look, smell, feel)? Record your observations in the table provided. Record the **pH of the liquid** (in the table provided). **Safety note**: You should not taste aggregated acid-milk!

STEP 4

- Repeat the test, adding the same amount of vinegar (2 tablespoons) but to the **warm milk**. Stir, and let stand for a minute or two. Remember that the beaker will be hot! What happens? (Remember, do not taste!) Record your observations in the table provided. Record the **pH of the liquid** (fill the table provided below):

Step:	**Sample**	**Temperature**	**pH**	**state of the milk (appearance, colour, odour, viscosity)**
TEST 1	**Milk**	cold	6.7	*Smooth white liquid, with no macroscopic aggregates*
TEST 2	Milk	60°C	6.7	*Smooth white liquid, with no macroscopic aggregates, aromatic*
Test 3	Milk + **2 tablespoons vinegar**	Room temperature	Lower pH than in test 1,2	*Thick white liquid, with no macroscopic aggregates*
Test 4	Milk + **2 tablespoons Vinegar**	60 ^{0} C	Same as test 3	*Yellowish liquid with macroscopic aggregates (lumps) sinking to the bottom.*

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Q6. Was there a clear difference in the milk state and pH ***that took place just by heating the milk, without adding vinegar*** (Test 1 & 2)? If yes, describe the difference.

The milk became more aromatic, no pH change was observed; no other change is observed.

Q7. Was there a clear difference in the milk state and Ph ***when adding*** **vinegar** to warm milk or cold milk (Test 3 & 4)? If yes, describe the difference.

Yes, there was a clear difference between test 3 and 4. Solids were separated from the liquid in test 4, while the liquid only got thicker in test 3.

Q8. Based on the results of the test of adding vinegar to warm milk or to cold milk, do you think the reaction that takes place is based only on the acidity (pH)? Explain.

Since the pH was the same in test 3 and 4, but the results were different, the reaction couldn't be based only on the acidity

TIP FOR THE TEACHER

Use the following table to show the students that in order to get the full effect (separation of solid from liquid), both parameters (pH and temperature) is needed to be changed.

	Room temperature	60^{0} C
Milk	*Smooth white liquid, with no macroscopic aggregates* pH= 6.7	*Smooth white liquid, with no macroscopic aggregate* s pH= 6.7
Milk + 2 tablespoons Vinegar	*Thick white liquid, with no visible particles* pH= ?	*Yellowish liquid with macroscopic aggregate (solid lumps) sinking to the bottom.* pH= ?

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Q9 Which of the processes explained above did you perform on the milk in this experiment?

We decreased pH by adding an acid.

Q10. Use the information you have just read to explain the difference between the result of the cold milk experiment and the warm milk experiment.

When the milk is cold, the addition of acid disrupts the micelles to some extent, but the hydrophobic interactions maintain some stability of the casein micelles, so they don't precipitate, and we get a thick liquid. When the milk is warm, there are two factors that together disrupt the stability of the casein micelles, the acidity (which leads to the release of calcium phosphate) and the high temperature, which make hydrophobic interactions stronger. Those two effects together disrupt the stability of the casein micelles to the point that they precipitate.

Q11. Did the protein molecules change to new ones in the experiment? If not, what did change?

The molecules did not change, the way they are linked changed.

Q12. Using the information you have just read, can you describe in your own words what happens to the micelles in the process that results in your favourite dairy product?

Cheese: attack by chymosin; yogurt: controlled addition of lactic acid bacteria.

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