Name: Section: Date:

Lab #33:
Calcium as Essential Mineral—A Simulation of Blood Anti-Coagulation

Pre-Lab Activities

1. Read through the first section of the **Introduction,** *Calcium Structure and Behavior,* and complete the tasks.

2. Complete the *Reactivity Series of Metals Challenge*.

3. Read from *The Role of Calcium in the Human Body* up to *Intake and Absorption of Calcium*. Be sure answer prompts along the way and to ***"talk to the text"*** as you read.

4. Read *Intake and Absorption of Calcium*. Be sure answer prompts along the way and to ***"talk to the text"*** as you read.

5. **Interactive Lecture**: Why is the calcium ion so important to the human body?

6. Read through the **Materials and Methods** section, completing the given tasks along the way, and continuing to ***"talk to the text."*** Circle the materials you will be using in Lab #33.

7. For each chemical you will be using, record the required 3 pieces of information from the MSDS sheets: Ratings, Handling, and Disposal.

8. Set up your lab notebook for Lab #33. Write the purpose of this lab in your lab notebook.

9. Complete *Properties of Milk.*

10. Read through the entire **Materials and Methods** sections *a second time*. Circle the variables you will be expected to use in Lab #33. As a group, discuss these variables and list them in your lab notebook.

11. **Interactive Lecture**: What is the purpose of a scientific control?

12. As a lab group, diagram your **Experimental Set-up**, construct an initial **Materials List,** and write out a **Procedure** for Lab #33. Include a one paragraph **Justification** of why you chose to set up the experiment the way you did. This should be done on a separate sheet of paper (one sheet per group).

13. Have your group's **Materials List, Experimental Set-up, Procedure, and Justification** approved by your teacher. ***Note:** This approval simply means that your experimental set-up and procedure appear to pose no danger, if handled properly, to you and your lab partners.

14. Transfer your approved **Materials List, Apparatus Diagram, Procedure, and Justification** to your lab notebooks—every group member needs his or her own. ***Note:** If there were problems with your initial set-up, be sure to revise and seek teacher approval before transferring it to your lab notebooks.

15. In your lab notebook, identify the experimental group, the control group, and the controlled conditions under which this lab will be conducted.

16. Review your **Procedure** and construct the necessary data tables in your lab notebook.

17. Propose a hypothesis for Lab #33 and record it in your lab notebook .

18. Select one of the body systems and explain what you think would happen in the absence of calcium ions. Start at the molecular level; then do cellular or tissue level and end with system level. Be very specific and use diagrams and/or equations to make your case.

Introduction

➢ ***Calcium Structure & Behavior***
Calcium is the 20th element on the Periodic Table. Sir Humphrey Davy discovered calcium in 1808.

Use your **NYSPS Chemistry Reference Tables** to complete the information about Calcium.

Calcium

average atomic mass:	_____	**number of electrons in a neutral atom:**	_____
group number:	_____	**period number:**	_____
group name:	_____	**usual ion formed:**	_____
atomic radius:	_____	**ionic radius, GREAT THAN or LESSER THAN atomic radius? (circle one)**	
electronegativity:	_____	**ionization energy:**	_____

Figure 2 represents the valence electrons of calcium. Circle the valence electrons in **Figure 1**.

What do the rings in **Figure 1** represent?

Explain why calcium will always form a +2 ion.

Figure 1: Calcium Atom

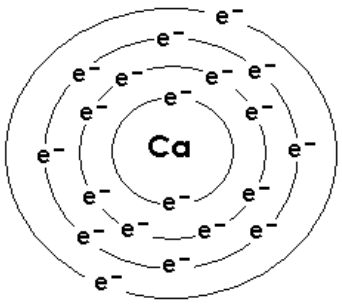
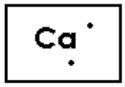


Figure 2: Calcium Lewis Dot Model



Comment on whether or not all Group 2 elements will behave in the same way as calcium during ion formation.

Reactivity is a property that depends on how easily a given element undergoes a chemical change. Group 18 elements, also known as the *noble gases*, are considered non-reactive or inert because they do not undergo chemical changes under standard conditions. Their behavior is defined by their electron configuration. Draw and create **Figure 3a:** Orbital diagram of Neon and **Figure 3b**: Lewis dot diagram of Neon.

Figure 3a: Orbital Diagram of Neon

Figure 3b: Lewis dot diagram of Neon

Review the diagrams for Calcium and Neon in **Figures 1-3**. Compare calcium's reactivity vs. neon's reactivity.

Calcium, as a Group II element, is considered a reactive element. The Group II elements are slightly less reactive than Group I elements. This means that calcium requires very little *activation energy* to undergo chemical changes at standard conditions. For example, if you were to place calcium metal into water it would react immediately to produce calcium hydroxide and hydrogen gas.

Write a balanced chemical equation that represents the chemical reaction described above.

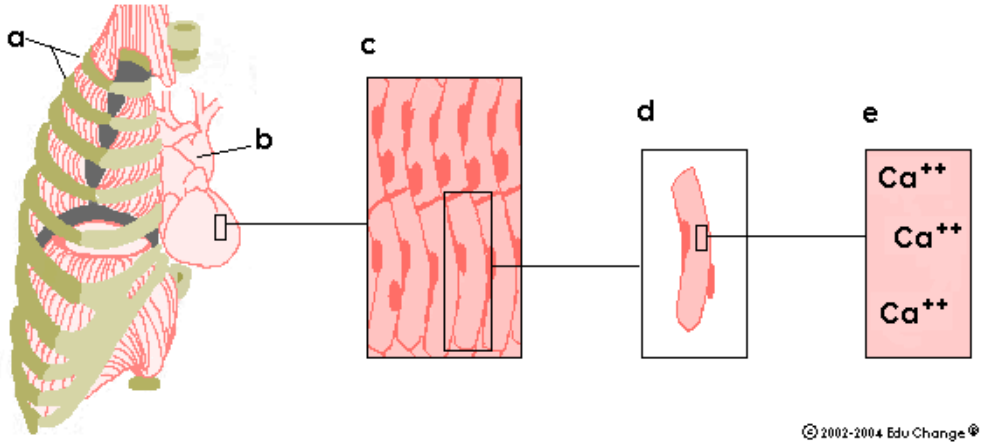
The reaction of calcium and water can be viewed at: http://www.angelo.edu/faculty/kboudrea/demos/calcium_H2O/calcium_H2O.htm
***Safety Note:** It is recommended that this demonstration NOT be recreated, based on the volatile nature of the reactants.

➢ *The Role of Calcium in The Human Body*

Calcium is the most abundant mineral in the human body and plays an important role at all levels of biological organization. About 99% of the calcium in our bodies is found in the bones and teeth where it plays a structural role. The remaining 1% is present in body tissues and fluids where it is essential for cell metabolism, muscle contraction and nerve impulse transmission. At the molecular level, calcium ions play a key role in the functioning of muscle cells and tissues. (See **Figure 5**).

Figure 5: Calcium At All Levels of Organization

Calcium is found primarily in our bones, such as our ribs (a), and teeth, but plays a critical role in organs such as the heart (b). The heart pumps as the muscle tissue (c) contracts and relaxes. Each individual muscle cell (d) contracts in response to changing concentrations of calcium in the cytoplasm (e).



➢ *Calcium and the Skeletal System*

The primary function of calcium in the human body is structural. The skeleton of a young adult male contains about 1.2 kg of calcium. The calcium is bound in a compound called *calcium hydroxyapatite.* This molecule is composed of three ions: calcium, phosphate and hydroxide.

Use your ***NYSPS Reference Tables*** to write the molecular formula for the *ions* that compose calcium hydroxyapatite:

The *molecular formula* for calcium hydroxyapatite is $Ca_{10}(PO_4)_6(OH)_2$. Often it will appear in text as an *empirical formula*, the smallest whole number ratio of atoms in a compound. The molecular formula can be coverted to the empirical formula of a compound by dividing all of the subscripts by a common denominator.
NOTE: You should *not* reduce the subscripts within the polyatomic ion.

Write the *molecular formula* and *empirical formula* for calcium hydroxyapatite. Molecular formula : Empirical formula:

As long as there is adequate calcium from dietary sources in the bloodstream, the amount of calcium in bones can remain fairly consistent. In a feedback loop directed by hormones, a dynamic equilibrium between blood and bone calcium is maintained. Calcium can be reabsorbed from bone into the blood and deposited into bone from the blood at similar rates. However, when blood calcium levels are continuously low, the body reabsorbs calcium into the blood from the bone faster than it can be deposited back, resulting in a net loss of bone mass.

As children, we depend on calcium to grow a healthy skeleton. By age 20 in men and age 16 in women, bones typically stop elongating and bone mineral density (BMD) increases more slowly from this point until our 30's. Our bone density as young adults is largely dependent upon our calcium intake as children and teenagers. The greater this peak bone mass, the less likely our bones are to become porous and fragile later in life (See **Figure 6,** source: http://www-ermm.cbcu.cam.ac.uk/nfig001mbo.gif).

Figure 6: Bone Density

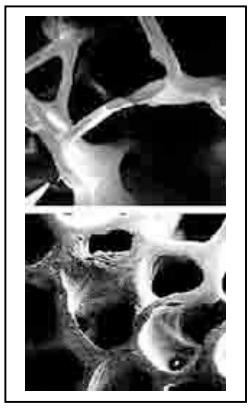
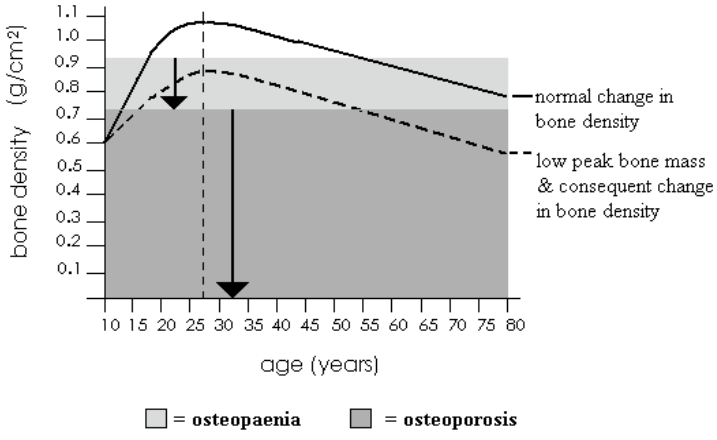


Figure 7: Onset of Osteopaenia and Osteoporosis Due to Change in Bone Density *



*Adapted from Variation in the bone density of women at different ages, Expert Review in Molecular Medicine ©1999 Cambridge University Press ©2002-2004 EduChange®

Osteoporosis generally refers to the severe condition of low BMD accompanied by fractures **(see Figure 7**). At-risk are smokers, excessive drinkers, older women, people with high caffeine intake, and people with a hereditary predisposition.

In 2001 Ethel S. Siris, MD, and her colleagues at Columbia University College of Physicians and Surgeons in New York City reported the initial findings from the National Osteoporosis Risk Assessment (NORA), the largest study of osteoporosis conducted to date in the US. The study was presented in the *Journal of the American Medical Association* (JAMA), and indicated that nearly 1 in 2 women had undetected low BMD. Read the abstract to learn more: http://jama.ama-assn.org/cgi/content/short/286/22/2815

➢ ***Calcium and the Muscular System***

The movement of calcium ions allows for the contraction of muscle tissue. In the heart, a group of muscle tissues functioning as an organ, calcium is essential for the proper contraction of heart muscle. If the heart were to function irregularly due to calcium deficits, there would be far reaching effects on the distribution of oxygen and glucose throughout the body.

Ultimately, muscle contraction is regulated by the level of available calcium ions in the cytoplasm. If there is not enough free calcium ions in the blood the body will make up the deficiency by taking calcium from the bone tissue.

➢ ***Calcium and the Circulatory System***

Calcium indirectly effects the movement of materials through the circulatory system via its roles in the muscle system. Calcium also activates clotting *factors* (another term for proteins) in our blood. The circulatory system has to be self-sealing, otherwise continued blood loss from even the smallest injury would be life-threatening.

Blood clots ultimately contain platelets, fibrin (a protein), and usually trapped red blood cells that give the clot a red color. Fibrin is essential for creating the semi-permanent clot and calcium ions are crucial for the formation of fibrin. Therefore a deficiency in available blood calcium can lead to an impaired clotting function.

Individuals with cardiovascular issues such as atheroschloerosis, the hypercoagulation of blood, are at risk for clot formation and are often prescribed blood-thinning treatments known as *anticoagulants*. These treatments generally do one of two things: 1) thin the blood by increasing the volume of water or decreasing the volume of platelets, or 2) reduce the naturally occurring clotting factors.

Sodium citrate, $Na_3C_6H_5O_7$, is an *anticoagulant*. When an individual is taking sodium citrate, the sodium ion *dissociates* readily, leaving the citrate ion with a stronger attraction to calcium ions in the blood. When citrate binds with a calcium ion, this ion is now unavailable for clotting activities. The citrate has effectually "occupied" calcium. If the blood does not clot as readily, the likelihood of having a clot form in an artery is reduced. <u>The effects of sodium citrate may be readily simulated, and that is what we will be doing in Lab #33!</u>

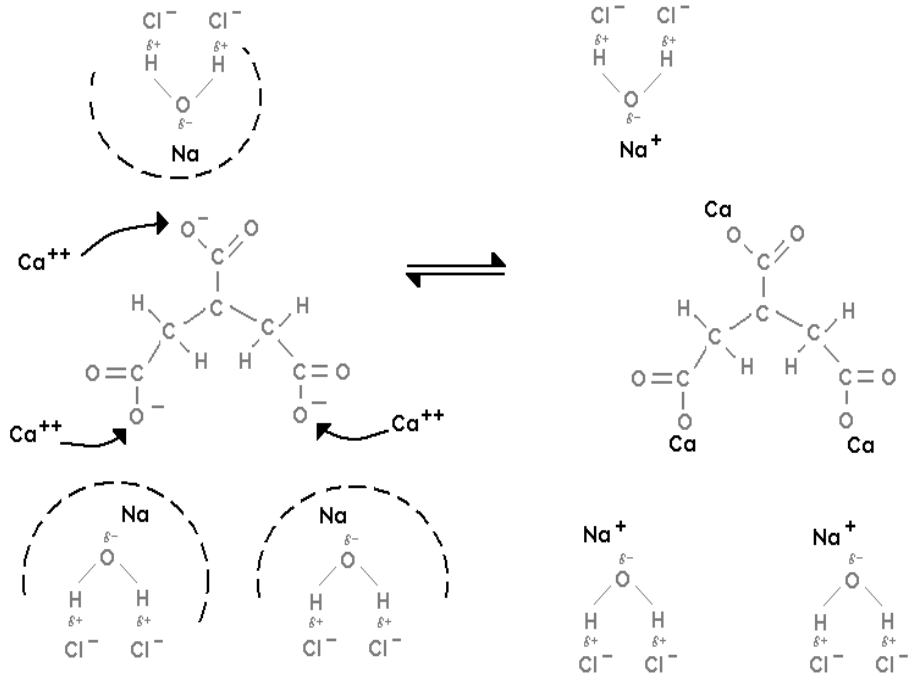
Of course, there are risks associated with using *anticoagulants*. If a wound does not clot, it leads to massive blood loss called *hemorrhaging*. Calcium chloride may be administered to patients who are taking sodium citrate blood thinners, to create an excess of calcium ions. In this situation, additional calcium ions from the dissociation of $CaCl_2$ will be available in the blood to create the necessary clots. Administering sodium citrate and $CaCl_2$ acts like a human-created feedback loop, and simulates a homeostasis mechanism (see **Figure 9)**.

Figure 9: Sodium Citrate and Calcium Binding

Chemical equation, symbols:

$$3CaCl_2 + 2Na_3C_6H_5O_7 \rightleftharpoons Ca_3(C_6H_5O_7)_2 + 6NaCl$$

Chemical structures:



➢ ***Intake and Absorption of Calcium***

Are you getting enough calcium? The body has a carefully regulated system to ensure that a good supply is always and immediately available:

- It takes calcium from our bones if there is not enough available in our blood. When this happens, the bones become less dense and more fragile.
- It absorbs calcium directly from the food we eat.

- It slows down the amount of calcium that leaves the body in the urine by returning some to the blood stream where it remains available to organs and cells.

The main goal of good calcium nutrition is to keep an adequate supply available to the body. In other words, you must try to maintain a situation in which your calcium loss (some of which is normal) does not exceed your dietary intake. This balance is important so that your body does not have to dip into your only calcium reservoir — your bones.

A person's need for calcium varies according to age and certain risk factors. The National Institutes of Health (NIH); http://consensus.nih.gov/cons/097/097_statement.htm#CDC97T1; recommends the following total calcium intakes in all forms--dietary and supplemental:

- Children one to five years old: 800 mg a day
- Children six to ten years old: 800 mg a 1200 mg a day
- Adolescents: 1200 mg to 1500 mg a day
- Adults between the ages of twenty-five and fifty: 1000 mg a day
- Pregnant or lactating women: 1500 mg a day
- Women over fifty: 1500 mg a day

Calcium can be obtained from a variety of sources including:

- Dairy products such as low fat or nonfat milk, cheese and yogurt;
- Powdered dry milk can also be added to everything from soups to muffins.
- Dark green leafy vegetables such as broccoli or bok choy
- Calcium fortified foods including orange juice, cereal, bread and soy products
- Nuts

Crucial to the body's absorption of calcium is vitamin D. Much of our vitamin D supply is made in the skin, in a process that occurs when we are exposed to sunlight (a total of just 15 minutes each day is all that is needed). Additional sources of Vitamin D include:

- Egg yolks
- Saltwater fish,
- Liver
- Vitamin D-fortified dairy products.

Using the list above, identify the primary source(s) of calcium & Vitamin D in your diet.

In addition to dietary sources of calcium, there exists a wide range of calcium supplements. Different types of calcium supplements contain different amounts of *elemental calcium*, which is the form of calcium available to your body. Calcium in its ionic form is not stable enough to form a solid, therefore it is most often found in complexes such as calcium carbonate, calcium citrate, calcium lactate, and calcium gluconate. **Table 2** summarizes the usable calcium in three different calcium compounds used in supplements. Fill in the formula mass for each compound and then calculate the % by mass of calcium in each compound.

Table 2: Calcium Supplements

Calcium Compound	Formula	Formula Mass	% Calcium by mass	% Usable Calcium	Brand Examples of Supplements
Calcium Carbonate	$CaCO_3$			40 %	Caltrate, Oscal, Tums
Calcium Citrate	$Ca_3(C_6H_5O_7)_2$			21 %	Citrical
Tri-Basic Calcium Phosphate	$Ca_3(PO_4)_2$			39 %	Posture, Posture-D

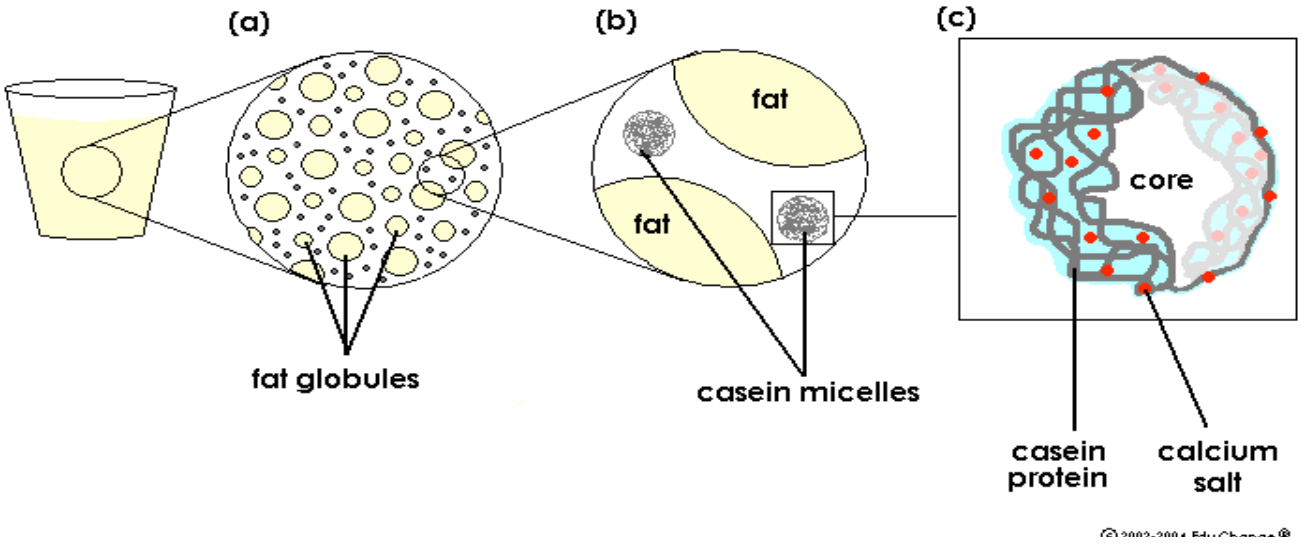
Materials and Methods

➢ ***Got Milk? Simulating Coagulation with Calcium and Casein***

In Lab #33 we will be investigating the effects of calcium on milk coagulation to create a model of human blood clotting processes. What will the milk simulate in our model?

Figure 10: Composition of Milk

Diagram (a) illustrates the heterogeneous mixture of milk. Diagram (b) is a magnification of diagram (a): notice that the casein micelles are significantly smaller in size than the fat globules. Diagram (c) is a schematic drawing of what scientists currently believe to be a general structure for casein micelles. This schematic is a cross-section of a micelle to illustrate how the casein proteins provide the micelle's structure, maintaining a hydrophillic surface for holding calcium salts.



Cow's milk is a heterogeneous mixture of about 5% lactose (milk sugar), 4% proteins, 5.5% fats and 85% water (See **Figure 10).** These substances form a colloid that has the familiar white creamy appearance. The two main proteins in milk are whey and casein. About 80% of the protein in milk is *casein*. Casein is composed of several similar proteins that form a multi-molecular, granular structure called a *casein micelle*. In addition to casein, the casein micelle contains water and salts (mainly calcium and phosphorous).

Casein *coagulates* in the presence of a milk enzyme called *rennin*. Coagulation is just another was of saying denatures or changes shape. All proteins, not just enzymes, can denature thereby changing their structure and function. The denaturing of casein that plays a key role in the cheese making process.

Just as the formation of fibrin in human blood clotting requires the presence of calcium, enzymatic rennin can only clot milk in the presence of calcium. The calcium is functioning as a co-factor to the enzyme. If calcium is removed from the milk, the rennin will not be able to facilitate coagulation and the milk will remain fluid. Based on this information what are the casein and the rennin simulating in our model?

In this lab, you will design a **Procedure** to test the effects of rennin and sodium citrate on milk coagulation. Think about Lab #32 and other experiments in which scientific controls were used. <u>One of the first things that we must consider is, on what substance are we conducting the experiments?</u> This is the material that we must

ensure we have a control for. In this way, we will be able to see how the material behaves at standard environmental conditions, in addition to its behavior when materials are added or the environment changed.

In our model, what materials/ substance do we need a control for?

The environment in which we will be working is a 37ºC water bath. Why is 37ºC our standard environmental condition?

The additional materials we can add to our system include:

- Rennin
- Sodium citrate

Rennin, as we have already discussed, serves to speed up the clotting of milk under the correct conditions. We will be determining the effect of sodium citrate, the same molecule that acts as human anti-coagulant, on the action of rennin in milk.

What do you think would happen to a sample of milk that has rennin AND sodium citrate in it?

Good scientists always question their peers: "How do you know that the results correlate to your variable, and not to some other condition that went awry?" We need to have control groups in order to make our case to other scientists. Remember, every variable you have adds possible sources of error and your properly constructed controls can help reduce this error and make your case stronger when presenting it to others.

Materials

goggles	Test tube stoppers	Sodium citrate
thermometers	10 mL graduated cylinders	stop watches, watch, or clock
400 ml beakers	5 mL pipettes	25 mL Volumetric Flasks
hot plate	Whole milk	100 mL Beakers
test tube rack	Filter paper	Stirring Rod
20 mm test tubes	Rennin	

Procedure --To be constructed by lab group.

<u>*Safety Note: All procedures must be approved by the teacher before used.</u>

Post-Lab Activities

1. Based on your results, do you think that the calcium ion inactivation by sodium citrate is a reversible reaction? Explain your answer.

2. Based on your response to #1, write a balanced equation for the reaction of Sodium citrate with calcium ions. (You will have free ions on each side of the equation.)