Biochemistry Lab

Station 1: Ionic versus Covalent Bonds / Electrolytes

The salt and sugar on your kitchen table are both white crystals that dissolve easily in water, but the solutions they form have an important difference. Table salt which has the chemical name sodium chloride (NaCl) is an **ionic compound**, and when it dissolves, it **dissociates**, or breaks up into ions. The ions are free to move in the solution, and that solution, therefore, conducts electricity. The more ions in solution, the better it conducts electricity. If something produces a large amount of ions it is called a strong **electrolyte**. If something produces a small amount of ions, it is called a weak electrolyte.

The table sugar which is called glucose ($C_{12}H_{22}O_{11}$) is a **molecular compound**, and its molecules remain whole when they dissolve and do not form ions in the water. With no ions, the sugar water solution does not conduct electricity. If something produces no ions, it is called a **nonelectrolyte**.

Directions

1. Make sure the three glass beakers are clean and completely dry. Fill each beaker about half full with water.

2. Add four teaspoons of salt to the first beaker, four teaspoons of sugar to the second beaker, and four teaspoons of vinegar to the third beaker. Be sure to use a clean measuring tool for each substance to prevent cross contamination.

3. Stir each substance with a clean stirring rod until the color is uniform and you can no longer see any of the crystals. Be sure to use a clean stirring rod for each substance to prevent cross contamination.

4. Use the conductivity meter to test the electrical conductivity of each substance by placing each beaker under the meter with the end inserted into the solution. Be sure to gently clean the meter between solutions.

5. Make sure you clean your workstation before you move to the next station.
    - Wash and thoroughly dry each beaker and stirring rod.
    - Clean up any spilled substances and wipe down the work surface.
    - Gently clean the conductivity meter.
    - Notify the teacher if any of the test substances are close to running out and need to be refilled.

Biochemistry Lab

Station 2: Acids, Bases and pH

Acids and **bases** combine to create water and **salts**. Sodium hydroxide (NaOH) and hydrochloric acid (HCl) can combine to form water (H_2O) and sodium chloride (NaCl). Sodium chloride is the salt. Salts are ionic compounds that form **electrolytes** when dissolved in water but not hydrogen ions (H^+) or hydroxide ions (OH^-).

Example: $NaOH + HCl \rightarrow NaCl + H_2O \rightarrow Na^+ + Cl^- + H_2O$

Acids, bases and salts dissolve in water to create **ions** (**electrolytes**).

When an acid is dissolved in water, more H^+ ions are created than OH^- ions.

Example: CH_3COOH (vinegar) $\rightarrow CH_3COO^- + H^+$ (in water)

When a base is dissolved in water, more OH^- ions are created than H^+ ions.

Example: $NaOH + H_2O \rightarrow Na^+ + OH^-$ (in water)

Substances that create an equal number of H^+ and OH^- ions when they dissolve (**dissociate**) in water are neutral. Water itself is an example:

$H2O \rightarrow H^+ + OH^-$

The concentration of **hydrogen ions** (H^+) in water compared to **hydroxide ions** (OH^-) is measured using the pH scale. The pH scale is **logarithmic**. Each decreasing value represents a 10 fold increase in H^+ ions. So a substance with a pH of 4 has a concentration of H^+ ions 100 times greater than a substance with a pH of 2.

A pH meter or pH paper can be used to determine the pH of a solution.

Directions

CAUTION: DO NOT MIX ANY OF THE TEST SUBSTANCES!

1. Select one of the test substances. Place one drop of it on a separate, clean square of pH paper. Determine the pH of the substance by comparing the resulting color of the pH paper to the pH chart. Fill in the data table on your handout with your observations.

2. Repeat step 1for each of the test substances and fill in your observations on the data table on your handout. Be sure you do not contaminate any of your samples by using a pipette in multiple solutions unless you clean it thoroughly between uses. Also make sure you use a new square of pH paper and a clean work surface for each sample.

3. Fill a clean test tube half full with water. Use a clean drinking straw to continuously blow in the water for 2 minutes and then test the pH. Record your observations on your handout.

4. Place one drop of vinegar in a test well and add one drop of baking soda solution. Test the pH and record your observations on your handout.

5. Make sure you clean your workstation before you move to the next station.
   - Clean and dry the test tube.
   - Wipe down the table and throw away the paper towels, used pH paper, and used straw. Be carefully not to mix together any of the acids and bases.
   - Notify the teacher if any of the test substances are close to running out and need to be refilled.

Biochemistry Lab

Station 3: Buffers

The chemical reactions that occur within living cells are required for life, but often result in creating a hazardous work place for the cell. The internal pH of most living cells is close to 7, and even a small change in pH can be harmful. Many of the reactions of the cell cause changes in pH that can place the life of the cell in jeopardy. Thankfully, cells can produce **buffers** which help to maintain a stable pH inside of living cells.

The technical definition of a buffer is "a substance that consists of acid and base in a solution that minimizes changes in pH when extraneous acids or bases are added to the solution." A great example of buffers in living systems is seen in human blood. The pH of human blood is close to 7.4. A person cannot survive for more than a few minutes if the blood pH drops to 7 or rises to 7.8. It is critical that the pH of 7.4 is maintained. The presence of buffers in the blood allows for a relatively constant pH despite the addition of acids and bases. Buffers minimize changes in the concentrations of H^+ and OH^- ions. They do so by accepting hydrogen ions when they are in excess and by donating hydrogen ions to the solution when they have been depleted (there is excess OH^- ions).

Directions

CAUTION: DO NOT MIX THE ACID AND THE BASE! AVOID GETTING THE BLEACH SOLUTION ON YOU AS IT CAN BURN YOUR SKIN AND STAIN YOUR CLOTHES.

1. Fill a clean beaker with 80 ml of water. Measure the pH using a pH meter or pH paper. Record the results of each measurement for this step and each of the next steps on your handout.

2. Add one drop of vinegar (an acid) to the water, stir it for a few seconds, and then measure the change in pH (how much did the pH change from the original measure you had for water?).

3. Add a second drop of vinegar to the water, stir it for a few seconds and then measure the change in pH. Repeat this step again and again until you have added 10 drops of vinegar to the water.

4. Thoroughly wash the beaker or cup and fill it with 80 ml the liver solution. Measure the pH using a pH meter or pH paper and record it on your handout.

5. Add vinegar, drop-by-drop, to the liver solution according to the directions in step 2 and 3 and measure and record the change in pH after each addition.

6. Thoroughly wash the beaker or cup and fill it with 80 ml of the potato solution. Measure the pH using a pH meter or pH paper and record it on your handout.

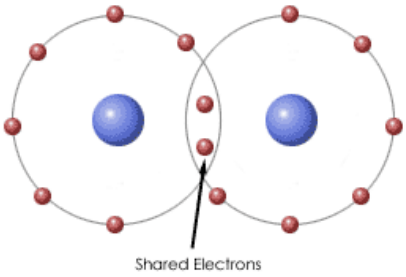
7. Add vinegar, drop-by-drop, to the potato solution according to the directions in step 2 and 3 and measure and record the change in pH after each addition.

8. Make sure you clean your workstation before you move to the next station.
   - Clean and dry all of the beakers/cups. DO NOT mix the acid solution with the base solution. Pour these into the correct disposal containers provided by your teacher and then wash each beaker/cup. Avoid getting the bleach solution on you as it can burn your skin and stain your clothes.
   - Wipe down the table and throw away the paper towels and used pH paper. Be carefully not to mix together any of the acids and bases.
   - Notify the teacher if any of the test substances are close to running out and need to be refilled.

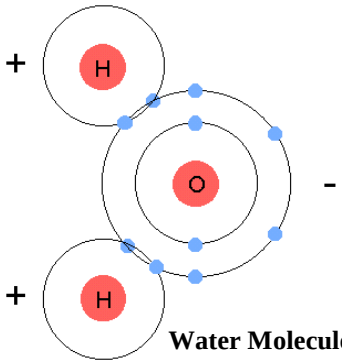
From: http://sciencestuffbyamy.blogspot.com/2012/09/how-do-living-cells-deal-with-changes.html

Biochemistry Lab

Station 4: Polarity and Surface Tension



When two or more atoms share electrons, it is called a **covalent bond**. If the electrons are shared unequally, the side of the molecule that "hogs" the electrons becomes slightly negative (since electrons have a negative charge) while the side of the molecule that does not get the electrons as often takes on a slightly positive charge. This is called a **polar molecule** and the bond in which they unevenly share the electrons is called a **polar bond**.

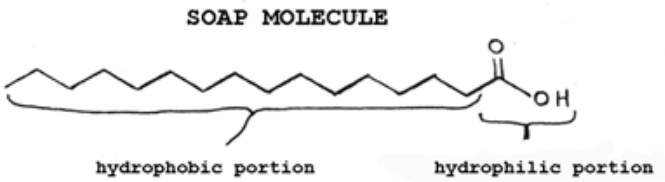


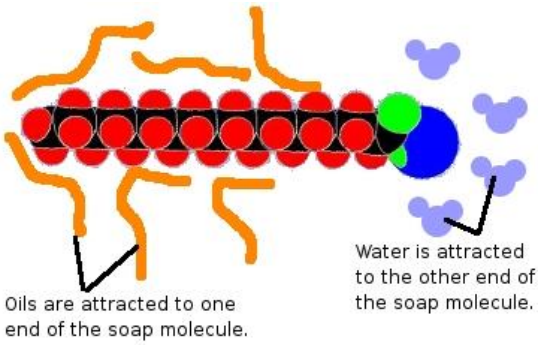
Water Molecule

Water is a very polar molecule. Water molecules are each composed of one oxygen atom and two hydrogen atoms. The oxygen atom is very **electronegative** meaning it really hogs the electrons, taking on a negative charge. The hydrogens take on a positive charge. Since opposites charges attract, the positive hydrogen regions of each water molecule are attracted (form hydrogen bonds with) the negative oxygen regions of each molecule. These hydrogen bonds are strong enough to create enough surface tension to hold very light objects up, to hold together water droplets, etc.

Polar substances will mix with other polar substances because their positive and negative regions will be attracted to one another and form weak bonds like hydrogen bonds. Polar substances are said to be hydrophilic because they are attracted to water (hydro = water, philic = lover). However, polar substances will not mix with non-polar substances which lack charged regions. Non-polar substances will mix with other non-polar substances though. Non-polar substances are said to be hydrophobic because they will not mix with water (hydro = water, phobic = fear).

Some complex molecules have both polar regions and non-polar regions. The polar regions will form weak bonds with other polar substances and the non-polar regions will be attracted to other non-polar substances. Soap is an example of one such molecule. The reason that soap helps get us clean is because the non-polar region of soap will attach to non-polar substances on our hands when we wash them. The polar region of the soap is attracted to the water we rinse our hands with. Therefore, when you lather your hands well with soap, you pick up all of the non-polar substances on them and when you rinse them off, the polar water carries off any polar substances by attracting them and also any non-polar substances by attracting the polar side of the soap molecules and carrying away the soap molecules with the non-polar substances lifted off of your hands attached to the non-polar side of the soap molecules.





Because soap has both hydrophobic and hydrophilic regions, it will mix with water but will also disrupt the hydrogen bonds between water molecules thereby affecting the surface tension.

Directions

Part 1

1. Make sure both pennies are clean and completely dry. Use the pipette to add one drop of plain water, one drop at a time, on top of one of the pennies. Count how many drops fit on the penny before it flows over the edge.

2. Repeat step one above with the other penny using the soap and water mixture.

Part 2

3. Make sure the glass bowl and paper clip are clean and dry before you begin. Fill up the glass bowl to the line with plain tap water. Rub the paperclip on your forehead to get it a little bit oily and then use the fork to slowly lower it into the glass bowl. You are trying to get it to stay on top of the water.

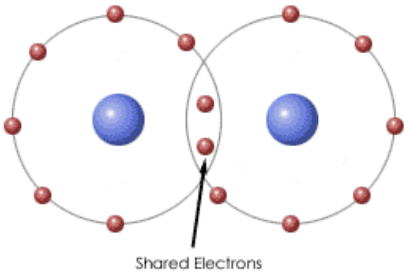
4. Use the pipette in the water cup to add ten drops of water to the water near the outer edge of the paper clip. Do not drop the water directly onto the paper clip. Does the paperclip sink? If so, after how many drops?

5. Use the pipette in the soapy water cup to add ten drops of soapy water to the water near the outer edge of the paper clip. Do not drop the soapy water directly onto the paper clip. Does the paperclip sink? If so, after how many drops?

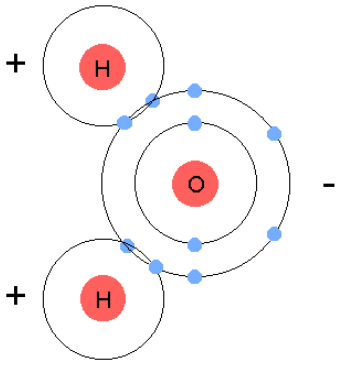
6. Make sure you clean your workstation before you move to the next station.
    - Wash and thoroughly dry the pennies, the glass bowl, and the paper clip.
    - Clean up any spilled substances and wipe down the work surface.
    - Notify the teacher if any of the test substances are close to running out and need to be refilled.

Biochemistry Lab

Station 5: Polarity and Chromatography



When two or more atoms share electrons, it is called a **covalent bond**. If the electrons are shared unequally, the side of the molecule that "hogs" the electrons becomes slightly negative (since electrons have a negative charge) while the side of the molecule that does not get the electrons as often takes on a slightly positive charge. This is called a **polar molecule** and the bond in which they unevenly share the electrons is called a **polar bond**.



Water is a very polar molecule. Water molecules are each composed of one oxygen atom and two hydrogen atoms. The oxygen atom is very **electronegative** meaning it really hogs the electrons, taking on a negative charge. The hydrogens take on a positive charge. Since opposites charges attract, the positive hydrogen regions of each water molecule are attracted (form hydrogen bonds with) the negative oxygen regions of each molecule. These hydrogen bonds are strong enough to create enough surface tension to hold very light objects up, to hold together water droplets, etc.

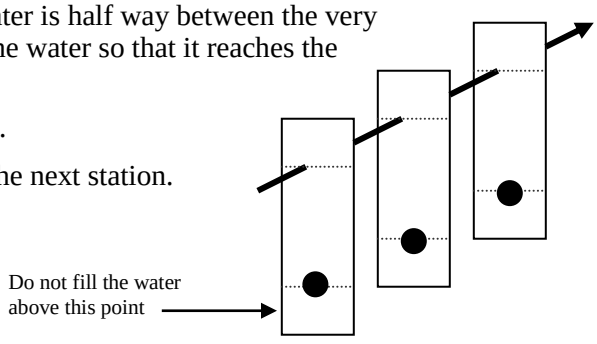
Chromatography is used to separate mixtures of substances into their components. All forms of chromatography work on the same principle. They all have a **stationary phase** and a **mobile phase**. In paper chromatography, the stationary phase is a very uniform absorbent paper. The mobile phase is a suitable liquid **solvent** or mixture of solvents. The mobile phase flows through the stationary phase and carries the components of the mixture with it. Different components travel at different rates depending on their size, polarity, and other attributes.

In this lab, you are going to use water as your solvent to separate the polar **pigments** in three different black pens. As the water soaks into the paper and travels up it via **capillary action** (water sticks to other water and pulls it like a chain up through the paper as it soaks into the dry regions), the pigments are separated from one another because of their different sizes and polarity. Molecules with greater polarity will travel faster because water is very polar and so it pulls on other very polar molecules with greater force. Also, smaller molecules will travel faster because they are better able to get around and through the fibers and molecules that make up the paper since they are smaller.

For the purposes of this lab, we are going to ignore size and just pretend that all of the movement (or lack of movement) is due to differences in polarity.

Directions

1. Select three strips of filter paper that are the same length and about the same width.
2. Use a pencil to draw a line one inch from each end of the filter paper. On one end, we'll call it the top, number the papers 1 through 3 using the pencil.
3. At the bottom end of the paper (the end that is not numbered), use one of the pens to draw a dot in the middle of the pencil line. Repeat this step with the other two pens drawing a dot on each of the other two pieces of filter paper. Record which numbered paper you used for each pen.
4. Push the wooden skewer through the middle of the pencil line in all three strips of filter paper so that they hang down with the pen dots on the bottom.
5. Lay the wooden skewer on top of the beaker and spread out the papers as far as possible so that the do not touch one another or the walls of the beaker. Slowly and carefully pour water into the beaker without splashing it on any of the papers. Fill the beaker until the water is half way between the very bottom edge of the paper and the pencil line. DO NOT fill the water so that it reaches the ink dots.
6. Observe for 5 minutes what happens and record your results.
7. Make sure you clean your workstation before you move to the next station.
   - Wash and thoroughly dry the beaker.
   - Throw away the paper slips and wipe down the work surface.



Biochemistry Lab

Station 6: Polarity; Hydrophobic and Hydrophilic Substances; Emulsions & Emulsifiers

DO NOT DO STATION 6 UNTIL YOU HAVE DONE STATION 4 AND/OR STATION 5.

Directions

Part 1a

Set up this experiment now and then proceed to the last part (Part 1b) when you are finished with Part 2 and Part 3.

1. Prepare seven test tubes each with 3 ml of water, 3 ml of oil and 1 drop of blue food coloring.
2. Use the following list to determine what to add to each test tube:

   Do not add any additional substances to test tube 1.
   Add 1.5 ml of egg white to test tube 2.
   Add 1.5 ml of egg yolk to test tube 3.
   Add 1.5 ml of lemon juice to test tube 4.
   Add 1.5 ml of corn syrup to test tube 5.
   Add 1.5 ml of honey to test tube 6.
   Add 1.5 ml of dish soap solution to test tube 7.
3. Place the caps on all 6 test tubes and shake them vigorously for 10 seconds. Set the test tube cap-side-down so it stands itself up and make your initial observations for Part 3 on the handout.

Move on to Part 2. We will finish the rest of Part 1 later.

Part 2

NOTE: During wait time in part 1 of the lab you can work on part 2 of the lab.

1. Add 3 ml of water to a clean, dry test tube. Use the measurements on the side of the pipette.
2. Add 3 ml of oil to the test tube. What happens? Record your observations on the handout and answer the questions.
3. Put the cap on and shake the test tube vigorously for 5 seconds. Set the test tube cap-side-down so it stands itself up and observe it for 3 minutes. What happens?
4. Add 1 drop of blue food coloring. What happens?
5. Put the cap on and shake it vigorously for 5 seconds. Set the test tube cap-side-down so it stands itself up and observe it for 3 minutes. What happens?
6. During the first minute you are waiting to observe step 5 above, do this step and the next step: Add 3 ml of alcohol to a clean test tube and 1 drop of the yellow food coloring. What happens?
7. Put the cap on and shake it vigorously for 5 seconds. Set the test tube cap-side-down so it stands itself up and observe it for 3 minutes. What happens?
8. Add 5 ml of the solution from the test tube with alcohol to the test tube with water and oil. Put the cap on and shake it vigorously for 5 seconds. Set the test tube cap-side-down so it stands itself up and observe it for 3 minutes. What happens?
9. Clean the test tubes and caps by washing them thoroughly with soap and water and then drying them inside and out with a paper towel. Be sure to leave the lab supplies organized as you found them. Be sure the alcohol and food coloring have their lids on.

Part 3

Water molecules are very polar. The strong attractions between water molecules affect water's surface tension, boiling point, and rate of evaporation. In this lab you will compare the evaporation rate of water and isopropyl alcohol (C_3H_8O).

Place a small sheet of brown paper towel down on your work surface and drop 1 drop of water and 1 drop of alcohol onto the paper towel about 6 inches apart at the same time. Observe what happens. Which drop evaporates first as indicated by the disappearance of the brown spot?

Part 1b

In this lab you mixed oil and water with test substances to see which are the best **emulsifiers**.

When water and oil are mixed together and vigorously shaken, a dispersion of oil droplets in water is formed. When shaking stops, the phases start to separate. However, when an emulsifier is added to the system, the droplets remain dispersed, and a stable **emulsion** is obtained.

An emulsifier is a molecule that consists of a polar, hydrophilic head and a non-polar, hydrophobic tail. The hydrophilic head is attracted to the water in the solution and the hydrophobic tail attracted to the oil in the solution. The emulsifier acts as a bridge between polar and non-polar substances enabling them to be mixed together by stabilizing them so that they separate more slowly.

Soap is an example of an emulsifier. You will learn more about how soap works at Station 4.

After you have completed Part 2 and Part 3 of the lab, and at least 10 minutes have elapsed since you set up Part 1a, examine your mixtures and write down your observations on your handout under "Part 1b".

Biochemistry Lab

Station 7: Chemical Reactions, Catalysts & Buffers

Once a reaction gets started, it may either consume or release energy. If the bonds that hold together the reactants (the chemicals you start with) require more energy than the bonds that hold together the products (the chemicals you end up with after the reaction) then the leftover energy will be released from the reaction (**exergonic**). Energy is often released in the form of heat so these types of endergonic reactions are said to be **exothermic** and they feel warm or even hot.

If the bonds that hold together the reactants require less energy than the bonds that hold together the products (that is, it requires an overall input of energy to make the products) then the extra energy needed will be absorbed from the surrounding environment, usually as heat (**endergonic**). If you touch this reaction, energy will be absorbed you're your hand and so it will feel cold. These types of endergonic reactions are said to be **endothermic**.

Photosynthesis is an example of an endothermic chemical reaction. In this process, plants use the energy from the sun to convert carbon dioxide and water into glucose and oxygen. This reaction requires 15MJ of energy (sunlight) for every kilogram of glucose that is produced:

$$\text{sunlight} + 6CO_2 + H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

Even if a reaction releases energy, it must still receive a certain amount of activation energy to get it started. **Activation energy** is the energy needed to start a chemical reaction (to break the bonds of the reactants so that the atoms can rearrange and form new bonds to form the products. Some reactions occur spontaneously because the energy of the molecules colliding at room temperature is enough to get them started. Some reactions require greater activation energy to get started – this is why heating up the products helps reactions go faster.

A **catalyst** is a substance that increases the rate of a chemical reaction, but is not consumed or changed by the reaction. A catalyst works by reducing the activation energy needed to initiate and sustain the reaction. For example, two molecules of hydrogen peroxide can react to form two molecules of water and one molecule of molecular oxygen gas by the following reaction:

$$2H_2O_2 \rightarrow 2H_2O + O_2\,(\text{gas})$$

At room temperature, this reaction occurs very slowly because few of the collisions between hydrogen peroxide molecules have sufficient energy to activate the reaction. Furthermore, commercial hydrogen peroxide solutions, such as the 3% hydrogen peroxide solution sold in drugstores and the 6% solution sold by beautician supply stores, are treated with stabilizers (sometimes called negative catalysts) that increase the activation energy for the reaction, further inhibiting it from occurring.

If you add a catalyst to a solution of hydrogen peroxide, the effect is immediately evident. The solution begins bubbling, as oxygen gas is evolved. Numerous substances can catalyze the reaction of hydrogen peroxide to water and oxygen gas. One of the most efficient catalysts for hydrogen peroxide is the enzyme **catalase**, which is contained in blood. Catalase functions in the body as a peroxide scavenger, destroying peroxide molecules that would otherwise damage cells.

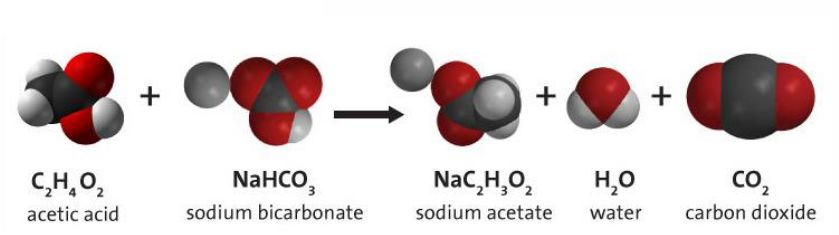
One catalase molecule can catalyze the reaction of millions of hydrogen peroxide molecules per second. Immediately after each pair of hydrogen peroxide molecules reacts, catalyzed by the catalase molecule, that catalase molecule is released unchanged and becomes available to catalyze the reaction of another pair of hydrogen peroxide molecules. When all of the hydrogen peroxide has reacted to form water and oxygen gas, you end up with as many catalase molecules remaining as you started with.

In this lab we will set up endothermic and exothermic reactions and compare the effectiveness of various catalysts at improving the reaction rate for the decomposition of hydrogen peroxide.

Directions

Part 1

Vinegar is an acid and baking soda is a base. When you mix them a chemical reaction occurs that forms sodium acetate, water, and carbon dioxide gas.



Pour 20 ml of vinegar into a plastic baggie. Add 1 teaspoon of baking soda (sodium bicarbonate). Use the temperature probe to find the temperature of the reaction. How does it compare to the room temperature? Write your observations on your handout.

Part 2

1. Fill six clean, dry test tubes each with 20 ml of hydrogen peroxide.
2. Add 1.5 ml of soap solution to each test tube. The soap solution does not participate in the reaction but it does help us see the gas released more easily so that we can observe which reactions are occurring fastest.
3. Add the following to each test tube at the same time, if possible:

    Test Tube 1: Nothing

    Test Tube 2: 1 ml of 25% diluted animal blood

    Test Tube 3: 1 ml of 10% diluted animal blood

    Test Tube 4: 1 small scoop of yeast

    Test Tube 5: 1 ml of 25% diluted animal blood that has been heated

    Test Tube 6: 1 ml of 25% diluted animal blood that has been diluted with an acid

    Observe what happens in each test tube and record your results on your handout.