Student Guide
Make a Microfluidic Device to Investigate

Properties of Solutions

Safety
Gloves and goggles should be worn when working with chemicals

DAY 1: Solutions and Guided Dialog/Vocabulary

Solutions: As a class you will review your knowledge on solutions and watch a video to help you recall your knowledge.

You make a solution when you brew coffee, make Kool-Aide, go to the bathroom, or almost anything else that has to do with life. To successfully complete this lesson on microfluidics you will need to know the following terms:

Solvation	Molarity	Mixing
Solubility	Mass/mass	PDMS
Solution	Monomer	Nanotechnology
Soluble	Polymer	Microfluidics
Insoluble	Hybridization	Diffusion
Miscible	Activation energy	Flow/laminar flow
Immiscible	Master mold	Nanoscale
Concentration	Device fabrication	Nanometer

Use your computers as a group to look up the definitions for each term. Write a one-sentence definition and draw a picture-example of what each term represents. Create a table in your lab notebooks as shown below. **If you don't finish in class, it's homework.**

Term	Definition	Picture

If time permits, have a class discussion on some of the terms you found difficult.

DAY 2: Polymers, Microfluidics and Nanotechnology

Nanoscale devices are used in many scientific disciplines and can routinely be found in many clinical, industrial, commercial, and consumer applications. You might want to check out the list of consumer products on the Project on Emerging Nanotechnologies inventory at: (https://www.nanotechproject.org/cpi/). Microfluidics, a type of nanoscale product, are devices developed to more efficiently perform what many humans and large machines have been used to accomplish in the past.

Microfluidics is the science of manipulating and controlling fluids, at nanoliter volumes in networks of micro-channels with dimensions from tens to hundreds of micrometers. This discipline takes its origins in the early 1990's and has grown exponentially. It is viewed as an essential tool for life science research or in a larger way in biotechnologies. Microfluidics emerged in the beginning of the 1980s and is used in the development of inkjet printheads, DNA chips, lab-on-a-chip technology, micro-propulsion, and micro-thermal technologies[1]

1. Wikipedia Microfluidics https://en.wikipedia.org/wiki/Microfluidics

Have you ever wondered where some of those incredible machines on CSI-type shows come from and how they work? Ever wondered how your automatic dryer knows when your clothes are dry enough? Ever wondered how a lab can run over 300 different tests on your blood for under $250? All of these are accomplished by what is now routine: microchips, the same electronic assemblies that are in your computers, your smart phones, your gaming systems, etc.

BUT, we're only getting started!!! Remember what the prefix micro means! It means 1 X 10^{-6} of whatever your measuring, in our case meters. Look at your rulers on your desk and see what the distance of a millimeter is. Now imagine that you have an object in which most of the sizes are a thousand times SMALLER than that millimeter. That's the microscale routinely in use in all microchips today.

Many of the most advanced electronics are starting to use nanoscale (1 x 10^{-9} meters) chips, chips in which the sizes are a thousand times SMALLER than your microchips. In other words, nanoscale chips are now working on the size of larger molecules and small atomic polymers. That's small!!!!

The structure of matter is due to the arrangement of atoms, molecules or ions. Atoms are less than 1 nm in diameter, typically 0.1 – 0.5 nm. Every type of atom (just over 100) has a unique electron shell configuration, energy signature and ability to bond with other atoms. Because of these unique properties, molecules are produced which have their own distinct molecular alignments or shapes, polarities, and charges. In fact, when we start looking at properties of individual molecules or smaller groups of molecules (at the nanoscale), we find that many of the properties we expect and rely on in the macroscopic world (our "normal" world) don't hold true.

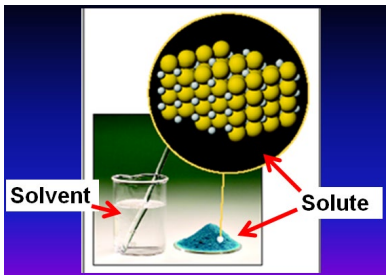
For example, in quantum mechanics we know that every orbital an electron can occupy in an atom has its own discrete energy signature at the atomic or molecular (nano) level. But, when we observe the macroscopic properties of something, we often only give it properties such as color, texture, malleability, etc. We already have some idea that the color we see is

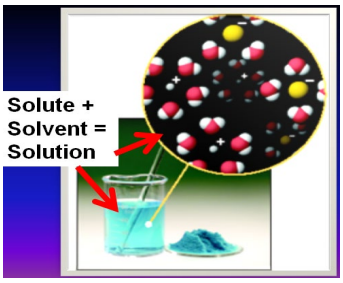
an average of the wavelengths of photons released by several different electrons returning to ground state. We also know that the texture of a substance will change based on the magnification level we are viewing it at and malleability of a metal can subjectively change with its thickness.

But why do these properties of matter seem to change? Well, first off, at the nanoscale of molecules, the force of gravity doesn't really matter compared to the electromagnetic force of electrons. Think about it; gravity will pull you in until you are stopped by the electromagnetic repulsion of a solid (you fall until you hit the ground). Secondly, quantum mechanics defines the energy and movement of these nanoparticles rather than classical mechanics (high school physics). Furthermore, in very small particles you have a greater surface to volume ratio than in large particles. For instance, the surface to volume ratio of the earth is roughly 4.7×10^{-13} whereas the surface to volume ratio of a molecule of methane is roughly 2×10^{-11}, almost 43 times greater. This means that the surface reactions of the particle with the environment become much more important than either its volume or diameter. Finally, because surface area is so much relatively greater on these small particles, their motion and collision with other particles becomes highly important.

Just how do nanoscale differences in matter end up being useful to us? For example, copper atoms move as small clusters of about 50 nm and this allows copper metal to appear malleable (bendable). Something similar happens with electrons (metallic sea of electrons) when groups of electrons of pure metal can move a certain amount, thus letting electricity flow AND promoting magnetism. In fact, aluminum, a metal not known for being magnetic, CAN become magnetic when the diameter of the particle is less than 0.8 nm. Zinc oxide, a common component of sun screen, is suspended in its solvent at a particle size of around 20 nm. This size allows the molecule to keep many of the same properties of the "bulk" material EXCEPT now the particles are transparent to most visible light but are large enough to scatter harmful UV rays. That is why nano zinc oxide sunscreen in translucent but non-nano zinc oxide is white.

Enter Microfluidics. Remember we said that microfluidics represented a type of nanoscale device that can more efficiently accomplish what larger machines perform for today. Just as the name implies, we will be working with liquids at a very small scale, in our case solutions. Remember, solutions are a homogenous mixture of a solvent (usually water or some organic liquid) and a solute (the material dissolved into the solvent).





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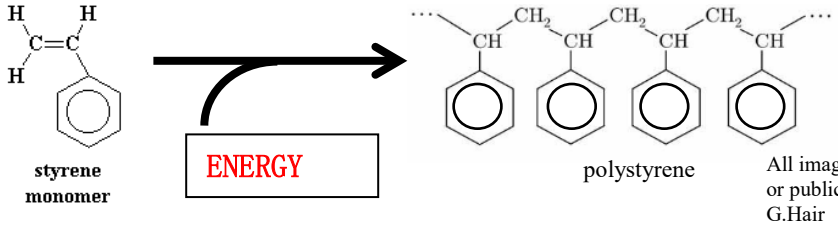
You will be designing, building, and testing a device that can accomplish a certain task working with different solutions. Finally, since we can't really make chips that are on the nanoscale in our high school chemistry labs, you will be designing chips at the millimeter

scale and shrinking them to the micrometer scale using polymer chemistry and heat energy, two more concepts to wrap our heads around!

Polymer Chemistry:

Polymer chemistry deals with the structure and properties of macromolecules, really large molecules like DNA and RNA and polymers which are long chains of repeating molecules called monomers. Polymer chemistry finds new ways to synthesize or use these molecules. You will be using sheets of a polymer called polystyrene to make your microfluidic devices. You most likely know this material as Ezee-Shrinks or Shrinky Dinks.



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Even more cool is that you can *manipulate* your sheets of polystyrene into making your microfluidics devices. A common manipulation is to cross-link or hybridize the chains of polystyrene. This will "shrink" the distances between those large aromatic rings between polystyrene chains. ***How do you think you might cross-link the polystyrene chains?***

When you shrink the distances between bonds, you bend the polystyrene sheets. You now have a rounded area on your polystyrene sheet. How do you think you could add more heat to one part of your polystyrene sheet than another?

Each group should create a list of polymeric substances. These will be shared with the entire class. Which groups can name the most?

DAY 3: Designing your Devices

What can we do with our devices? You will be designing your device to do **ONE** of the following:

1. Automatically mix two or more dyes (diffusion).
2. Automatically neutralize an acid and a base and let you know it (diffusion & titration).
3. Separate a suspension from a liquid (sedimentation).
4. Separate non-miscible liquids from each other.
5. Keep two solutions separated while flowing *TOGETHER*(This is hard but can be done by YOU)!

Please have one member of your group come to the front and pull an assignment card from the deck.

Discuss your assignment with your group. What exactly does your device have to accomplish? Look at the notes on your Appendix A. In your lab notebook list at least three ideas you will need to answer or develop to solve your assignment. Use your computer to find ideas that might answer your questions or develop your design ideas. **Sketch** your designs in you lab notebook. **Outline** in your notebook an experiment to test your prediction. **Show** your design and outline to the teacher who will approve it before your

create it on the computer using PowerPoint. Save your computer designs as they will be used tomorrow for printing. Your teacher may share possible designs with you.

DAY 4: Making the Master Mold:

You will print your computer-designed device using a laser printer onto a sheet of polystyrene. Print at least four of the designs on the sheet. Next, your polystyrene sheet will shrink by 60% and at the same time become design three-dimensional!

Collect your Materials:

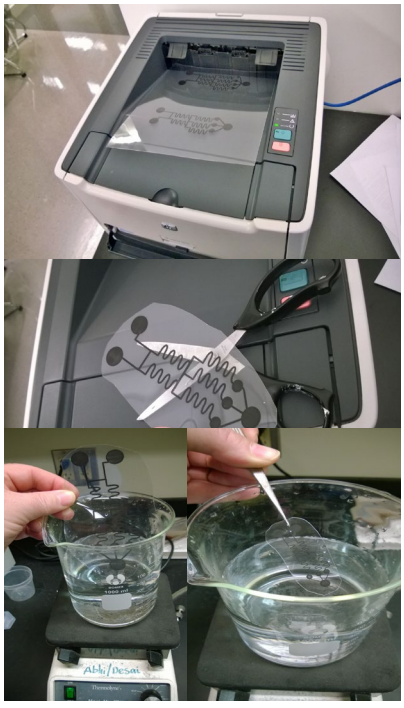
1 sheet of polystyrene (8"x 10")
1 pair scissors/group
1 600 mL beaker
1 hot plate
400 mL vegetable oil
1 thermometer
2 clean glass plates
1 petri dish
1 roll scotch tape
1 pair tongs or tweezers
1 sharpie
1 timer

This is to make one master mold. Your teacher may ask you to make 3 or 4 so that you can choose the best one for your device testing. Adjust your procedures accordingly.

Procedure:

1. Printing your design.
   a. One member of the group will pour 400 mL of vegetable oil into the beaker, place the beaker on the hot plate and begin heating the oil to 150°C. Use the thermometer to measure the temperature. (**Careful** this is really hot & can burn your skin).
   b. While the oil is warming, on your computer bring up PowerPoint and print out your design from yesterday onto the polystyrene sheet. Your design should be not be larger than 10 x 10 cm. Check with your teacher on size limit. Test it on a regular sheet of paper first!

2. Preparing your design
   a. Cut out your design. Try to leave about 1 cm (~1/2 inch) of space between your design and the edge of your cut.
   b. Continue heating and checking the oil until it reaches 150°C.



3. Shrinking your design
   a. Using the tong or tweezers, place your cut design into the hot oil.
   b. **IMMEDIATELY** start your timer set at 60 secs. When it buzzes, carefully remove your design from the oil. What has happened to your sheet & design?

4. Finalizing your master mold
   a. Lay your sheet onto one of the glass plates and **carefully** lay the second glass plate over it. Keep your polystyrene sheet flattened like this until it cools (3 – 5 minutes).
   b. Lift off the top glass plate and, using your tweezers or tongs, grab the polystyrene sheet and place it into a petri dish. **Very gently** blot your sheet dry with paper towels. Carefully tape the edge of your polystyrene sheet to the bottom of your dish with 3 small pieces of tape.

5. Using one of your phones, take a picture of your "device master mold" on the petri dish.

6. Record all observations in your lab notebook. Print and place a picture of your device master mold in the notebook.

Clean up:

Pour the used oil into the waste oil container.
Wash your beaker and glass plates in soapy water. Place them on paper towels to dry.
Wash all tweezers and thermometers in soapy water and rinse.
Dry everything.
Make sure your hot plates are turned off.
Make sure your tools & equipment are returned to the proper lab space.
Log off/shut down your computers.

Homework:

1. Why did your polystyrene shrink?
2. Why did your designs raise up to form a third dimension?
3. What do you think the logical next step will be with your master mold tomorrow?
4. When you perform your experiment later this week with your samples what do you think your results will be?

Answer questions in your lab notebook or separate paper for handing in to teacher.

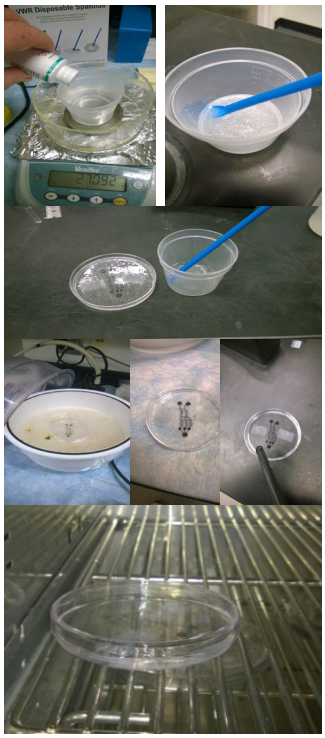
DAY 5: Making the Actual Device

You will use polymer chemistry to make the actual device from the molds you prepared yesterday. The polymer you will use is poly-dimethyl-siloxane (PDMS) and it will make the actual device from your polystyrene mold. PDMS is softer than polystyrene and is relatively safe for using around biological or water-based samples.

Collect your Materials:

1 mixing cup
1 electronic balance/group
1 plastic spatula/ wooden stirring stick
Your petri plate w/taped master or masters
Access to vacuum pump
Access to desiccator jar
1 butane lighter or sharp probe/stick
1 electronic balance
1 baking oven set at 60°C
PDMS monomer
PDMS curing agent

Procedure:



1. Make the PDMS
   a. One member of the group measures out 30 g of PDMS monomer and 3g of curing agent into the mixing cup.
   b. Use the plastic spatula to mix the two liquids completely. Will turn milky because it's full of air bubbles.

2. Label your petri dish with a sharpie and pour in the PDMS mixture over your device(s).
   a. Place your petri dish into the desiccator.
   b. Turn on the desiccator pump and leave it on for 20 minutes.
   c. Turn off the desiccator pump and gently let the air back in.
   d. Remove any remaining bubbles by either poking bubbles with a sharp probe/pointed stick or flaming with butane lighter (instructor will demonstrate).
3. Place your poured plates into the baking oven at 60°C overnight.

Record your Observations and Formative Conclusions: data collection, observation and extrapolation, and analysis of interim results. Answer the questions.

1. Why did gas bubbles form when you mixed your monomer and curing agent? If no gas bubbles formed, why not?
2. Why did most of the gas bubbles disappear in the desiccator jar? If you still had many gas bubbles, why?

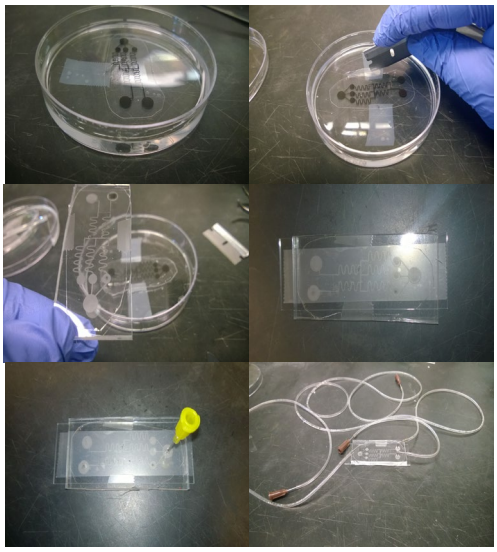
3. Why do you think you are heating your poured petri plates at a high temperature?

DAY 6: Cutting Out, Mounting and Using the Device
You will cut out your device, mount it, and then conduct your experiment with it.

Collect your Materials:

1 Scalpel
1 glass microscope slide
1 pair scissors
Biopsy punch
1" cut double-sided sticky tape
2 or more x 1" sections metal tubing
2 or more x 6" rubber tubing
Your experimental materials (vary according to your experiment)
1 Timer
Pipets and syringes

Procedure: Use gloves when handling the device.



1. One member of the group obtains the petri plate from the oven and cuts the device(s) out from the mold. Using tweezers, carefully remove the PDMS mold from the polystyrene. Choose the best mold to use in the next steps. Or your teacher may ask you to use additional devices to have controls.
    a. Use the biopsy punch to create your inlets and outlets per your design. Use caution when handling the biopsy punch.
    b. Lay a microscope slide flat on your lab bench.
    c. Carefully mount a piece of double-sided sticky tape to the slide large enough to secure the device.
    d. Mount your device to the sticky tape so that the hollow tubes face the glass slide (imprint side down). Press **gently** to make sure there are no bubbles between the tape and the device(s).
2. Attach the rubber tubing to the metal tubing sections. Attach the metal tubing sections to your device at the inlet(s).
3. Set up and prepare the chemicals for your experiment.
4. Using a syringe, place the chemicals into your device to run your experiment. This is an experiment you designed so fill in your protocol in your lab notebook.

Questions & Conclusions:

1. Did you observe what you predicted? If not, how did your observation differ from your prediction?

2. Did you have a control group? Why was it important to have a control group?

3. Do your observations leave you with any more questions? Do they enable you to make more predictions? If so, what are they?

4. What other issues or concerns were raised during your experiment and what might you do to address them?

5. How did the fluid move through your device (mix, separate, laminar flow, turbulent, etc.)? If you were to scale up this device so that you were in the meter rather than the nanometer/micrometer scale, what would happen to your separation or mixing that you observed? Why?

Draw Conclusions

Example: Based on your results, do you feel that _____? Explain your answer.