BioPharmaceutical Technology Center
I N S T I T U T E

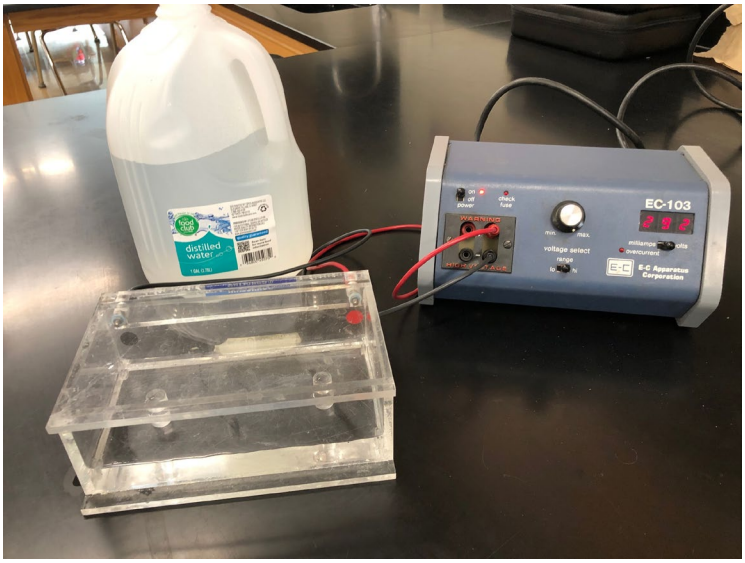
A SIMPLE DEMO FOR ANSWERING A GREAT QUESTION: Solving the TBE Mystery

I was reminded last week of a lab demonstration that I used to do with my Biotechnology students. We were preparing agarose gels for electrophoresis and a student asked "What is the purpose of TBE buffer when running gels and what is 'TBE' anyway?" Those are great questions and there is a very simple demonstration which addresses them.

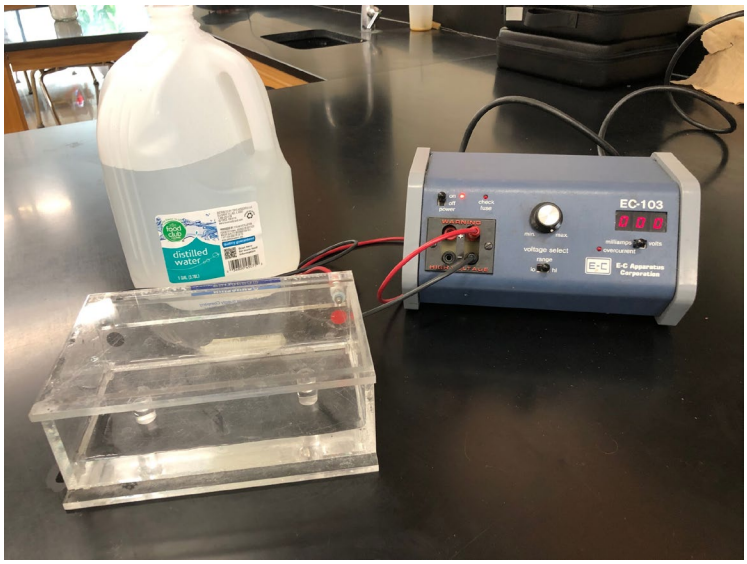
You will need the following materials:

- an electrophoresis gel box
- a power supply which shows current and voltage
- Distilled water
- A salt solution (about 1 gram in 100 mL of distilled water works fine)
- Phenolphthalein (check with your Chemistry teachers - you will only need 1 - 2 milliliters)
- A concentrated solution of TBE buffer (10X works fine)

Start by adding distilled water to the gel box and connect it to the power supply. When you turn on the power supply, you will see that it measures a voltage, but that the current is 0 mAmps.

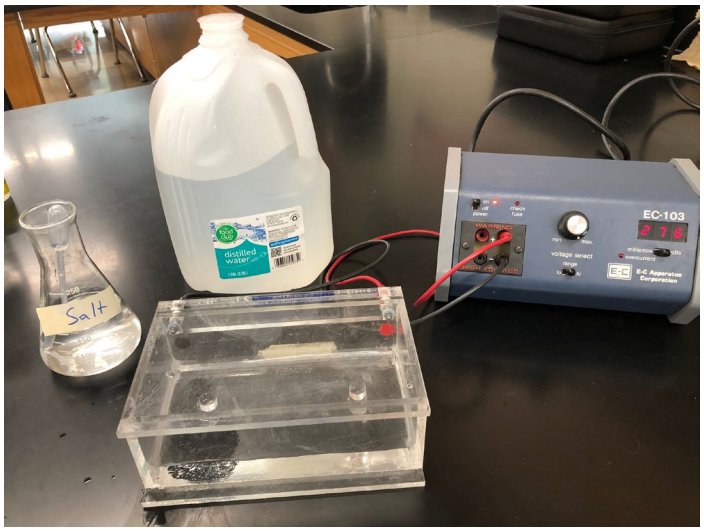


VOLTAGE - Distilled Water 292 volts

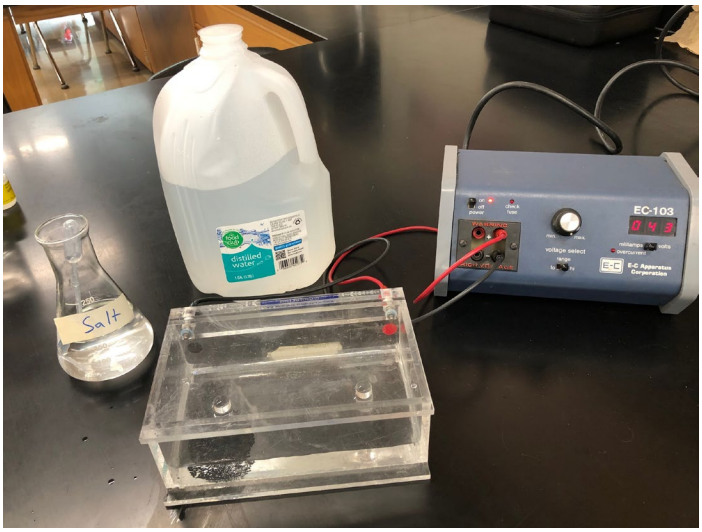


CURRENT - Distilled Water 0 mA

This is because voltage is a measure of potential "push" the power supply is giving the electrons. However, current is a measure of how much the electrons are flowing from one side of the gel box to the other. Distilled water has no salt ions so no conductivity. This also means that DNA will not be moving through the gel either. Turn off the power supply and add a few milliliters of the salt solution to the gel box. When you reconnect the power supply, you will find that the voltage has not changed appreciably, but there is now a current.



VOLTAGE - Salt 276 Volts



CURRENT - Salt 43 mA

Salt provides the ions necessary to allow the electrons to flow which can be measured in this case as 43 mAmps. If electrons are moving, DNA (which has a negative charge) would be moving towards the positive end.

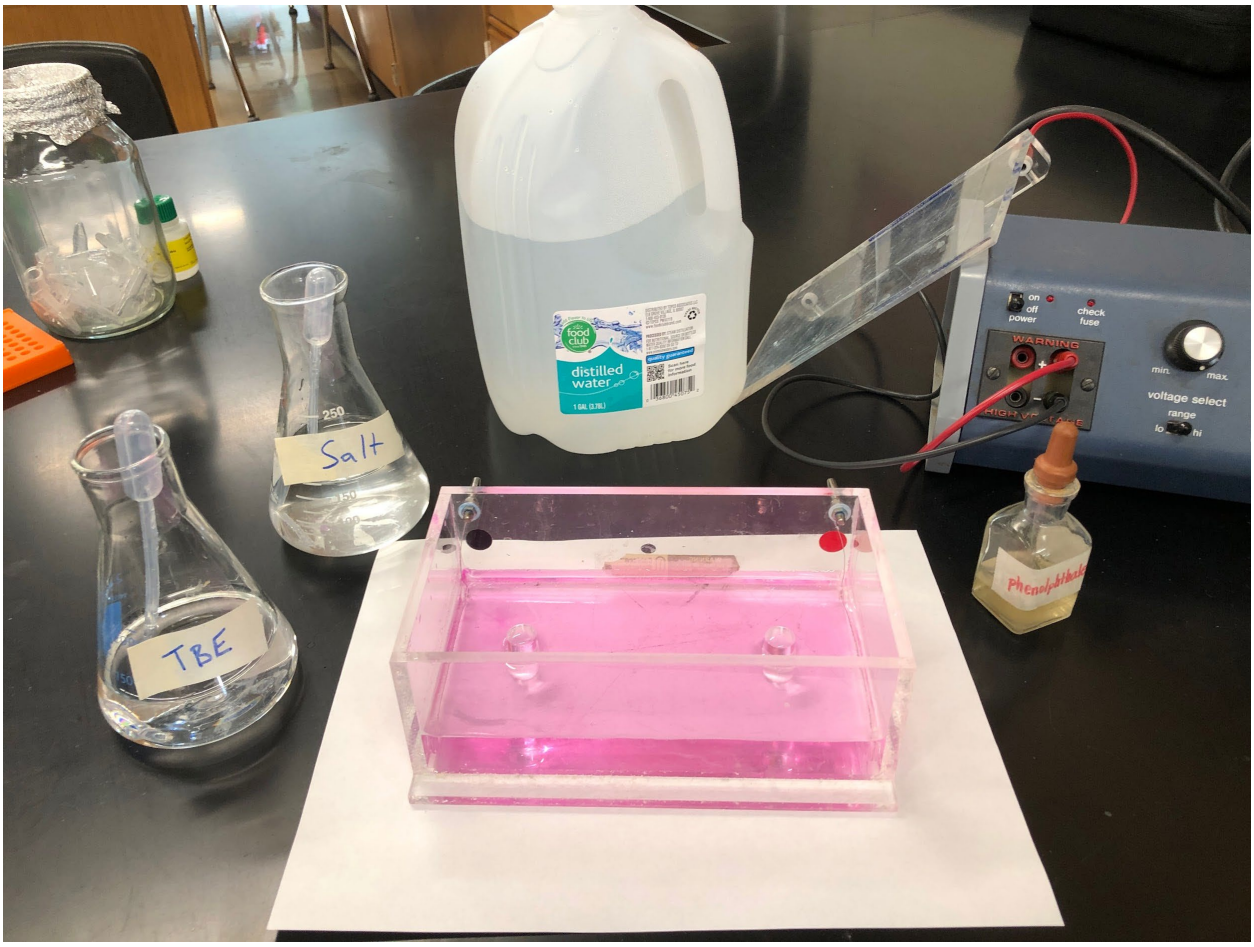
The next step is to add a few drops of phenolphthalein - an indicator which turns pink in the presence of basic solutions and clear in the presence of acidic solutions. Turn on the power supply and let it run for approximately one minute. During this time, the color of the negative end (black wire) will turn pink and the positive end (red wire) will become clear. This is due to the buildup of OH^- at the negative end and H^+ ions at the positive end.

PHENOLPHTHALEIN - Power Supply OFF

PHENOLPHTHALEIN - Power Supply ON

The problem is that the DNA would cease to move in this case or, worse, would start to move back to the negative end where the positively-charged H^+ ions are gathering. This would defeat the whole purpose of electrophoresis which is to separate DNA based on length as it gets pulled towards the positive end. (NOTE: The same effect could be achieved with phenolphthalein. This indicator turns pink in a base and stays clear in an acid.)

The final step is to add a few milliliters of 10X TBE. Turn the power supply on for approximately one minute. This time, the solution in the gel box stays a uniform color. This is because TBE is a buffer and prevents the buildup of H^+ and OH^- ions. The DNA will be consistently pulled towards the positive end.



GEL BOX WITH TBE BUFFER AFTER 1 MINUTE WITH POWER SUPPLY ON

So, why do we need TBE Buffer in a gel box? It provides the salt ions for the electrons and DNA to move and the buffer to keep the pH constant. The full name of this buffer is Tris-Borate-EDTA. The Tris and Borate (or Boric Acid) act as the salt and the buffer thereby allowing the current to flow and maintain a constant pH. The EDTA acts as a preservative. It chelates or binds to divalent cations like Ca^{2+} and Mg^{2+}. These ions are necessary for many different enzymes including many which would degrade DNA. We would not want our DNA to be destroyed before it even had a chance to run in the gel, right?
Now, there are other buffers used in gel boxes. You may have encountered other gel electrophoresis buffers including TAE (Tris-Acetate-EDTA) and even simply Sodium Borate. They each have their own specific application, but all provide the necessary ingredients for the salt and the buffer.

Anyway, I just wanted to share the memories that this student's question generated. I used to go over this information and started to filter it out in favor of covering other information later in the semester. However, I may bring it back next year. It's a simple way to show why TBE buffer is used in our gel boxes.

Until next time.