Student worksheet 3: synthesising and investigating antibacterial PVC

The polymer polyvinyl chloride (PVC) is a cheap and durable plastic used in pipes, signs and clothing. Plasticisers are often added to it to make it more flexible and easier to manipulate. In this activity, you will make a membrane of PVC both with and without a plasticiser, then compare their physical and chemical properties.

Antimicrobial membranes are used in many medical technologies, and are produced by incorporating nanoparticles or microparticles of silver or other metals into polymers. In the presence of oxygen (in air) and water, the elemental silver particles react to form silver ions (Ag^{2+}), which can break down cell walls, inhibit cell reproduction and disturb metabolism in some bacteria, viruses, algae and fungi[w3, w4].

Materials

Solvent: oxolane (tetrahydrofuran, $(CH_2)_4O$)
PVC powder
Dibutyl sebacate or other plasticiser
Silver nitrate ($AgNO_3$)
Tri-sodium citrate ($Na_3C_6H_5O_7$)
Nutrient agar
Bacterial culture (e.g. *E. coli* in a nutrient broth)
A hotplate
A magnetic stirrer
75 ml beakers
A glass substrate (e.g. beaker, watch glass or glass slide)
A graduated cylinder
A Pasteur pipette
A spatula
Petri dishes
Inoculation loops

Procedure

Safety note: All steps should be carried out under the fume hood. Tetrahydrofuran is a highly flammable liquid and vapour that can cause serious eye irritation. Handle with care under the fume hood only and wear gloves when using it.

1) Making PVC without a plasticiser

1. Using the hotplate and a magnetic stirrer, warm 20 ml solvent.
2. Slowly add 1.5g PVC powder, while stirring.
3. After about 10 min, the solution should become more viscous. Remove the beaker from the heat.
4. Remove the magnetic stirrer and pour a few millilitres of the PVC solution thinly and as evenly as possible over the glass substrate (inside or outside the beaker, or on the glass slide or watch glass). To ensure a thin layer, rotate the glass substrate carefully while the solution is still hot.
5. Leave the substrate and PVC under the fume hood to allow the solvent to evaporate; this takes about 15 min. The PVC membrane can then be easily removed from the glass substrate.

2) Making PVC with a plasticiser

Repeat the steps above to create four more membranes of PVC, each with a different amount of plasticiser added to the heated solvent (see Table 4).

1. Compare your five samples of PVC membrane. What effect does the plasticiser have on the plastic?
2. What do you think happens to the plastic when more plasticiser is added?
3. Referring to the scanning electron microscopy (SEM) images on page 34, was your answer to Question 2 correct?
4. These membranes can be used in the previous activity ('Membranes with invisible holes') to investigate the relative size of the 'holes'.

3) Making antibacterial PVC

The preparation of PVC containing silver particles requires the membrane to have large holes, which is why we use a plasticiser. The silver itself is added in the form of silver nitrate, which is then reduced using sodium citrate.

1. Using the hotplate and a magnetic stirrer, warm 20 ml solvent.
2. Add 2.5 ml plasticiser, then slowly add 1.5 g PVC powder.
3. Add 2.5 ml 10 mM silver nitrate and stir for 1-2 min.
4. Divide the solution between two 75 ml beakers. Quickly rotate each beaker so that the inside is coated with solution, forming a membrane in the shape of the beaker. Ensure that there are no gaps, as the membrane must be capable of holding water.
5. Leave the beakers under the fume hood to allow the solvent to evaporate, then carefully remove the membranes. (This is quite difficult; by making two, you increase your chances of success.)
6. Make a 5 mM solution of sodium citrate and pour this carefully into one of the beaker-shaped membranes. It should pass through the membrane (hold it over a beaker), reacting with the silver nitrate, giving silver nano- or microparticles.
   Note the colour change to the membrane.
7. Allow the membrane to dry under the fume hood. Typical

Silver micro- and nanoparticles in a PVC membrane

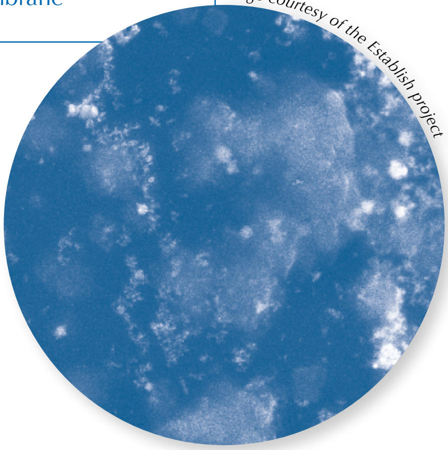
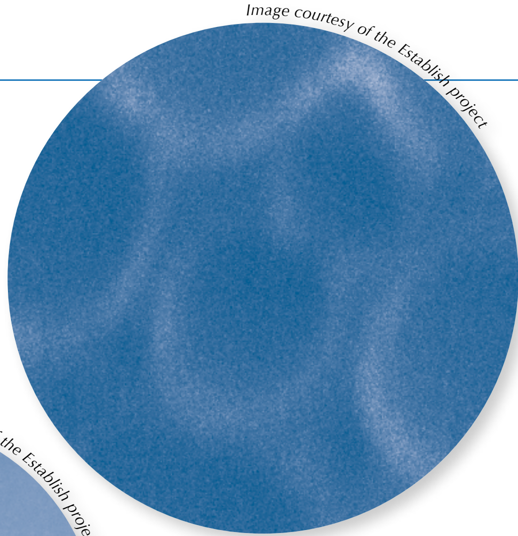
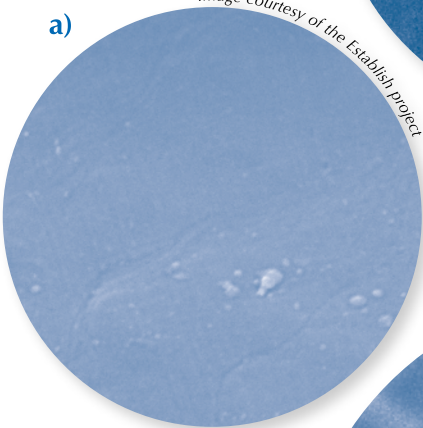


Image courtesy of the Establish project



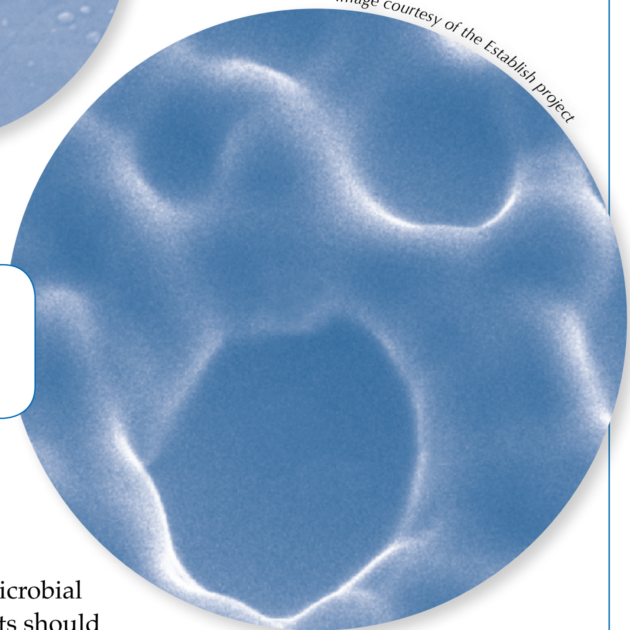
b)

Image courtesy of the Establish project



a)

Image courtesy of the Establish project



c)

Image courtesy of the Establish project

SEM images of PVC: a) unplasticised, b) with 0.5 ml plasticiser and c) with 2 ml plasticiser

SEM images (page 34) show the presence of elemental silver dispersed in a PVC membrane.

Next, you can investigate the anti-bacterial properties of the prepared membranes.

1. Prepare an agar plate with a bacterial colony: on a Petri dish containing nutrient agar, deposit about 100 μl of your bacterial culture (e.g. *E. coli* in a nutrient broth) and use an inoculation loop to spread it evenly across the plate.
2. Place approximately 1 cm^2 of your silver-impregnated PVC membrane on the plate.

   Alternatively, to provide a comparison, place three pieces of PVC membrane on the plate, one of which is untreated with silver.
3. Incubate the plate overnight at 37 °C, then measure the zone of inhibition around each piece of membrane.

Safety note: As with all microbial studies, sterilised implements should be used at all times (either sterilised in an autoclave or pressure cooker, or dipped in ethanol and then flamed). This includes the scissors that you use to cut the membrane. To prevent cross-contamination, wash the inoculation loops with antibacterial wash before use.

The antibacterial property of these membranes makes them useful for treating wounds and burns, as well as infections with bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *E. coli*.

1. Why are antibacterial PVC membranes particularly useful in the treatment of MRSA infections?
2. What other applications of antibacterial PVC membranes can you find?

Sample no.	PVC (g)	Solvent (ml)	Dibutyl sebacate (ml)
1	1.5	20	0.5
2	1.5	20	1
3	1.5	20	2
4	1.5	20	3

Table 4: Creating membranes of PVC with different amounts of plasticiser