

# Optically Transparent and Electrically Conductive Polymers for the Manufacture of Western Blot Cassettes

This section was prepared by



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## 1. Introduction

Karma Biotechnologies was tasked by Woodham Biotechnology Holdings to develop an optically transparent, electrically conductive cassette suitable for western blotting.

About Karma Biotechnologies:

*“Karma is a Los Angeles biotechnology startup that uses a unique multidisciplinary approach to innovatively solve problems using biomaterials. Our team of biologists, chemists, and materials scientists collectively has decades of experience in developing novel polymer-based nanotechnologies in multiple industries. Karma was founded specifically to commercialize our polymer nanoparticle technologies to allow in vivo tissue engineering on demand. In addition to developing our own core technologies, we offer consulting services to companies in need of polymer design, optimization, and characterization. We recently developed a novel polymer for a life sciences company, and we are developing two different products for use in the aerospace and defense industries. Karma thrives on collaboration and is always happy to explore partnerships with other innovators.”*

For Woodham Biotechnology Holdings, Karma has synthesized and tested multiple conductive polymers in polystyrene matrices, and has fabricated prototype western blot cassettes for further assessment by Woodham.

## 2. Synthesis of Electrically Conductive Polymer Matrix Formulations

To produce the electrically conductive and optically transparent polymer matrices required by Woodham Biotechnology, Karma has researched and developed methods to improve the electrical properties of a conductive polymer, specifically polyaniline (PANI). In addition, Karma has also researched and developed two methods for dispersing the PANI in a polystyrene matrix which confers the desired optical and mechanical properties.

### 2.1 Chemical doping of PANI with 0.5M HBr to increase electrical conductivity

In Phase I, Karma determined that the unmodified emeraldine base of PANI was insufficiently electrically conductive to be useful in producing the electrically conductive polymers required by Woodham Biotechnology. Karma then tested a number of chemical doping protocols aimed at increasing PANI's electrical conductivity. It was determined that doping PANI with 0.5M HBr yielded the greatest improvement in PANI electrical conductivity. The protocol for this doping process is below. PANI doped with 0.5M HBr was used for all experiments requiring PANI shown in the remainder of this report.

#### 2.1.1 Materials

1. Polyaniline, emeraldine base – CAS #: 25233-30-1
2. Distilled water
3. Hydrobromic acid – CAS #: 10035-10-6
4. Grade 2 filter Paper, Whatman 1002 (or equivalent)

### ***2.1.2 Standard Operating Procedure: HBr doping of PANI***

1. Prepare a 10 g/L suspension of polyaniline, emeraldine base in 0.5 M HBr.
2. Stir at room temperature for 24 hours.
3. Using a vacuum funnel, filter the suspension through Grade 2 filter paper.
4. Rinse the filtered polyaniline with three volumes of distilled water.
5. Dry at 400°C for 24 hours.
6. Store at room temperature in a dark bottle.

### ***2.2 Dispersion of PANI in polystyrene matrix via dichloromethane method***

The first method that Karma developed to disperse PANI in a polystyrene matrix was via the use of dichloromethane (DCM,  $\text{MeCl}_2$ ). This method achieved adequate dispersion of PANI particles throughout the matrix. It also resulted in a matrix that was optically clear (other than the dispersed PANI particles) and had the requisite stiffness. Despite the initial promise of this method, Karma discovered a significant limitation: Due to the large volume loss as  $\text{MeCl}_2$  evaporates during manufacture, the matrix tends to shrink as it cures. This does not pose significant problems when making large plates of  $\text{MeCl}_2$ -PS-PANI from which to cut specific shapes. However, when poured into an acrylate frame to make a cassette suitable for western blotting (Section 6), this contraction either results in the polystyrene matrix pulling away from the edges of the frame (breaking the cassette) or causes the cassette to warp (making it unusable for its intended purpose). Given these limitations, Karma developed an improved method of dispersing PANI in a liquid polystyrene matrix (see Section 2.3).

#### ***2.2.1 Materials***

1. Dichloromethane ( $\text{MeCl}_2$ ) – CAS #: 75-09-2.
2. Poly(styrene), average MW 250,000 – CAS #:9003-53-6
3. Polyaniline (PANI) (prepared according to the polyaniline doping protocol in Section 2.1.2)

#### ***2.2.2 Standard Operating Procedure: Dispersion of PANI in polystyrene matrix via dichloromethane method***

1. Working in a fume hood, dissolve polystyrene in  $\text{MeCl}_2$  at a ratio of 1 g polystyrene to 10 mL  $\text{MeCl}_2$ . Stir until fully dissolved.
2. Add the desired mass of PANI to the solution and stir until fully dispersed.
3. Gently pour the mixture into the desired mold, taking care to spread it evenly.
4. Store the sample in a well-ventilated location and allow it to dry until the  $\text{MeCl}_2$  is fully evaporated (approximately 48 hours).

### ***2.3 Dispersion of PANI in polystyrene matrix via liquid polystyrene method***

The second method that Karma developed to disperse PANI in a polystyrene matrix was via the use of so-called “liquid polystyrene” (LiqPS). This method achieved superior dispersion of PANI particles throughout the matrix compared to the  $\text{MeCl}_2$  method. It also resulted in a matrix that was optically clear (other than the dispersed PANI particles) and had the requisite stiffness. There is significantly less loss of volume via evaporation using this method, alleviating the warping and cracking problems associated with the  $\text{MeCl}_2$  method.

### 2.3.1 Materials

1. Styrene – CAS #: 100-42-5
2. Poly(styrene), average MW 250,000 – CAS #:9003-53-6
3. Phenylbis(2,4,6-trimethylbenzoyl)phosphine oxide (PPO) – CAS #: 162881-26-7
4. Polyaniline (PANI) (prepared according to the polyaniline doping protocol in Section 2.1.2)
5. UV Lamp

### 2.3.2 Standard Operating Procedure: Dispersion of PANI in polystyrene matrix via liquid polystyrene method

1. Working in a fume hood, prepare a 25% (w/w) solution of polystyrene in styrene by gradually adding solid polystyrene to liquid styrene, while stirring constantly. When not adding polystyrene to the solution, keep the solution covered. Stir until the polystyrene is completely dissolved.
2. Add the desired mass of PANI to the solution and stir until fully dispersed. Due to viscosity, it should be stirred by hand for small batches.
3. Weigh out 5 milli-equivalents of PPO (relative to styrene monomer) and stir it thoroughly into the suspension.
4. Gently pour the mixture into a desired mold, taking care to spread it evenly.
5. Expose the poured sample to 10 minutes of direct UV light at 370 nm wavelength.
6. Allow the sample to cure fully in a fume hood (approximately 48 hours).

## 3. PANI Polymer Electrical Conductivity Analysis

### 3.1 Overview

Woodham requires a polymer matrix that is electrically conductive within a range of  $10^{-3}$  to  $10^{-8}$  S/cm (optimal range of  $10^{-3}$  to  $10^{-6}$ ). Karma tested multiple polymer/matrix combinations at a variety of voltages to determine which of these meets the requirements of this project.

### 3.2 Materials and Methods

#### 3.2.1 Standard Operating Procedure: Measurement of electrical resistance of a conductive polymer plate at 100 V

1. Set up video camera to record data from resistance tester in real time.
2. Make plate (10 cm by 10 cm by 1 mm) of polymer to be tested for conductivity.
3. Cut plate into four equal test samples of approximately 4 cm by 4 cm by 1 mm.
4. Measure thickness of test sample using Vernier calipers, and record in the Karma Final Experiments Logbook Excel spreadsheet (available on request).
5. Wipe surface of test sample with 70% ethanol using a Kimwipe and allow to air dry.
6. Attach Red Dot monitoring electrode (3M, manufacturer number 2560-1) to surface of test sample.
7. Wipe opposite surface of test sample with 70% ethanol and allow to air dry.
8. Attach Red Dot monitoring electrode to opposite surface of test sample, taking care to line up the protruding electrodes.
9. Label Red Dot monitoring electrode with sample type, number, and date of manufacture
10. Repeat steps 4-9 for a total of three test samples from each plate.
11. Label a Ziploc bag with the type and date of manufacture of the plate.

12. Place the fourth test sample from the plate in the labelled Ziploc.
13. Plug two cables (one red, one black) with alligator clips into the “L” (red) and “E” (black) ports of the resistance tester (DOUYI model number DY5106A).
14. Gently clamp the first test sample onto stand.
  - A. The clamp should ONLY make contact with the foam of the Red Dot monitoring electrode.
  - B. The clamp should NOT make contact with the metal electrodes of the Red Dots.
  - C. Take care NOT to close the clamps with too much force; this may crack the sample.
15. Attach one lead to each Red Dot monitoring electrode of the test sample using the alligator clips.
16. Begin recording with video camera.
17. Turn dial of resistance tester to the 100 V setting.
18. Press red “Test” button.
19. Run test for approximately 40 seconds.
20. End test by pressing red “Test” button again.
21. Turn dial of resistance tester to the “OFF” position.
22. Stop recording with the video camera.
23. Remove alligator clips from sample, and sample from clamp stand.
24. Place the test sample tested into the labelled Ziploc bag.
25. Repeat steps 14-24 for each of the three test samples cut from the plate being measured.
26. Review video recordings, and for each sample record the resistance measurement at 10 s, 20 s, and 30 s of the test in the appropriate cell of the Karma Final Experiments Logbook Excel spreadsheet (available on request).
  - A. Take care to note the SI unit that the resistance meter is reading at – it will be GOhm or MOhm.
  - B. A reading of “1” means that the resistance is too high for the device to measure (i.e., the sample is an excellent electrical insulator; assume electrical conductivity is zero).
  - C. A reading of less than 0.01 MOhm means that the material is an excellent electrical conductor, repeat the test using the resistance test function of a standard voltmeter.
27. Save data.

### *3.2.2 Standard Operating Procedure: Measurement of electrical resistance of a conductive polymer plate from 100 V to 5000 V*

1. Set up video camera to record data from resistance tester in real time.
2. Make plate (10 cm by 10 cm by 1 mm) of polymer to be tested for conductivity.
3. Cut plate into four equal test samples of approximately 4 cm by 4 cm by 1 mm.
4. Measure thickness of each sample using Vernier calipers, and record in the Karma Final Experiments Logbook Excel spreadsheet (available on request).
5. Wipe surface of test sample with 70% ethanol using a Kimwipe and allow to air dry.
6. Attach Red Dot monitoring electrode (3M, manufacturer number 2560-1) to surface of test sample.
7. Wipe opposite surface of test sample with 70% ethanol and allow to air dry.
8. Attach Red Dot monitoring electrode to opposite surface of test sample, taking care to line up the protruding electrodes.
9. Label Red Dot monitoring electrode with sample type, number, and date of manufacture.
10. Repeat steps 5-9 for a total of three test samples from each plate.
11. Label a Ziploc bag with the type and date of manufacture of the plate.
12. Place the fourth test sample from the plate in the labelled Ziploc.

13. Plug two cables (one red, one black) with alligator clips into the “L” (red) and “E” (black) ports of the resistance tester (DOUYI model number DY5106A).
14. Gently clamp the first test sample onto stand.
  - A. The clamp should ONLY make contact with the foam of the Red Dot monitoring electrode.
  - B. The clamp should NOT make contact with the metal electrodes of the Red Dots.
  - C. Take care NOT to close the clamps with too much force; this may crack the sample.
15. Attach one lead to each Red Dot monitoring electrode of the test sample using the alligator clips.
16. Begin recording with video camera.
17. Turn dial of resistance tester to the 100 V setting.
18. Press red “Test” button.
19. Run test for approximately 10 seconds.
20. Turn dial of resistance meter to the 250 V setting.
21. Run test for approximately 10 seconds.
22. Repeat steps 20 and 21 for each remaining voltage setting on the resistance meter (500 V, 1000 V, 2500 V, 5000V).
23. End test by pressing red “Test” button again.
24. Turn dial of resistance tester to the “OFF” position.
25. Stop recording with the video camera.
26. Remove alligator clips from sample, and sample from clamp stand.
27. Place the test sample tested into the labelled Ziploc bag.
28. Repeat steps 13-27 for each of the three test samples cut from the plate being measured.
29. Review video recordings, and for each sample record the resistance measurement at each voltage tested (100 V, 250 V, 500 V, 1000 V, 2500 V, 5000 V) in the appropriate cell of the Karma Final Experiments Logbook Excel spreadsheet (available on request).
  - A. Take care to note the SI unit that the resistance meter is reading at – it will be GOhm or MOhm.
  - B. A reading of “1” means that the resistance is too high for the device to measure (i.e., the sample is an excellent electrical insulator; assume electrical conductivity is zero).
30. Save data into the Karma Final Experiments Logbook Excel spreadsheet (available on request).

### 3.2.3 Calculation of sample resistivity and conductivity

The resistivity ( $\rho$ ) of each sample was calculated using the formula:  $\rho = R \frac{A}{l}$  where  $R$  is the electrical resistance of a uniform specimen of the material (as recorded using the SOPs described above).

$l$  is the length of the piece of material (this was the measured thickness of each sample).

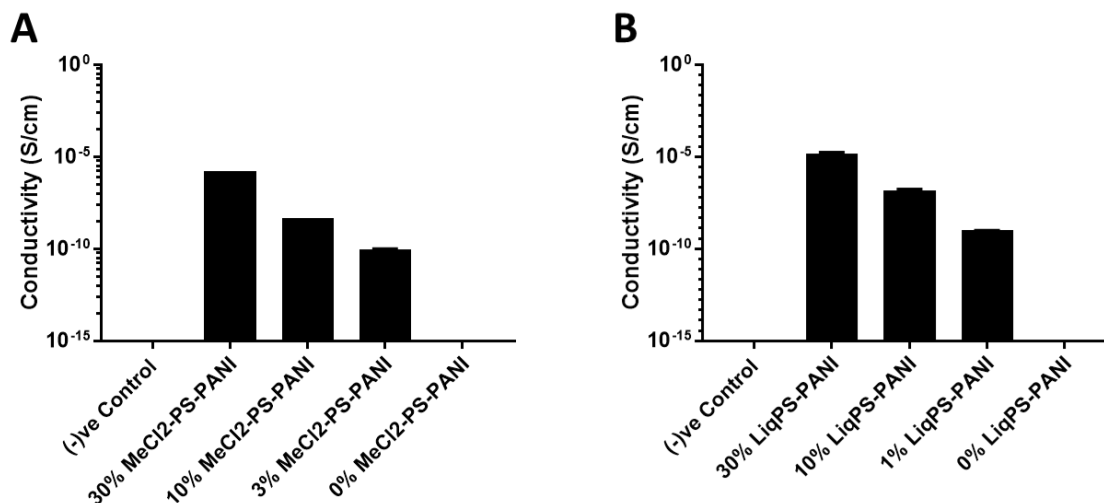
$A$  is the cross-sectional area of the specimen (this was uniformly 2.827 cm<sup>2</sup>, the gel contact surface area of 3M Red Dot electrodes used for resistivity/conductivity testing).

Conductivity was calculated as the inverse of the resistivity of each sample.

### 3.3 Results and Discussion

#### 3.3.1 Measurement of polymers conductivity at 100 V

Samples of polystyrene matrix prepared using the  $\text{MeCl}_2$  method and by the LiqPS method were prepared containing 30%, 10%, 3% (for  $\text{MeCl}_2$ ), 1% (for LiqPS), and 0% of HBr-doped PANI. LiqPS 1% PANI samples were tested rather than 3% as they had been more recently developed and had the requisite optical properties (3% PANI samples did not). The electrical conductivity of each sample was tested at 100 V in triplicate as described in Section 3.2.1. Conductivity was measured at 100 V because the target customer demographic for Woodham Biotechnology is expected to have equipment capable of running an electrophoresis blot transfer at approximately this voltage. For both PS matrices, conductivity decreased with decreasing amount of embedded PANI (Figure 3-1). The recorded conductivity for a given amount of embedded PANI was higher when embedded in LiqPS matrix compared to  $\text{MeCl}_2$ -PS matrix. Of all the samples measured, only 30% PANI in LiqPS matrix demonstrated the highest conductivity at 100 V ( $1.6 \times 10^{-5} \text{ S/cm}$ ) with 10% and 1% PANI in LiqPS coming in at  $1.4 \times 10^{-7} \text{ S/cm}$  and  $1 \times 10^{-9} \text{ S/cm}$ , respectively (Figure 3-1B).



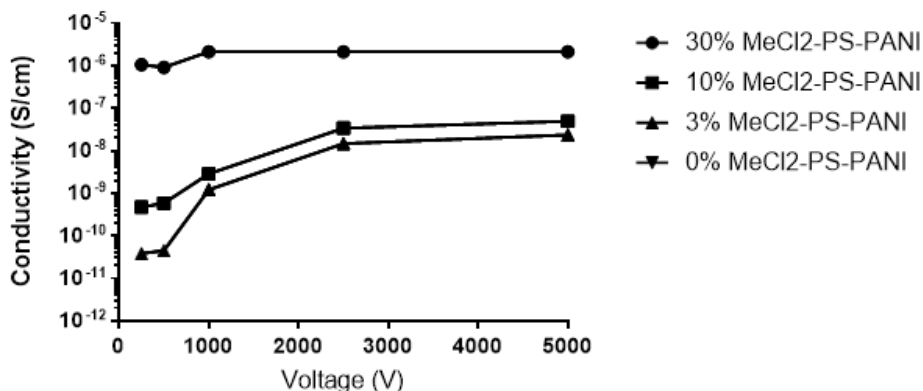
**Figure 3-1: Conductivity of HBr-doped PANI embedded in polystyrene matrices at 100 V.** Sheets of polystyrene containing varying quantities of HBr-doped PANI were made via the  $\text{MeCl}_2$  method (A) or the LiqPS method (B). As a negative control, 2 mm sheets of acrylate were used as a known electrical insulator. The recorded electrical resistances of the negative control acrylate and 0%  $\text{MeCl}_2$ -PS-PANI samples were off the scale, so their electrical conductivity is shown as zero above. As a positive control, 3M Red Dot electrodes were stuck to each other without a sample between them. These recorded an electrical resistance of zero (data not shown).

#### 3.3.2 Measurement of polymers conductivity at voltages up to 5000 V

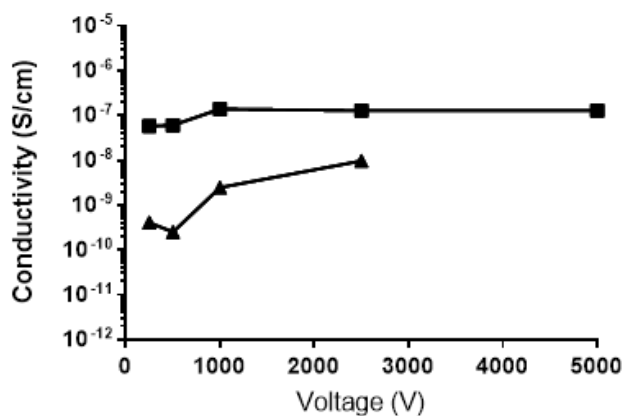
Given that most polymers tested fell outside the optimal conductivity range ( $10^{-3} \text{ S/cm}$  to  $10^{-6} \text{ S/cm}$ ) at 100 V, Karma next investigated whether some or all of the polymers were more conductive at higher voltages. The samples tested in Section 3.3.1 were retested at increasing



voltages using the SOP described in Section 3.2.2. In most cases, higher voltage resulted in increased sample conductivity, as expected. The largest increase in conductivity typically occurred between 500 V and 1000 V. Most samples failed at either 2500 V or 5000 V (the highest voltages resulted in an electrical arc across the samples, burning holes in them). Taken together, these data indicate that the highest practical voltage for using the PANI/PS polymer matrices created by Karma during this project is 1000 V.



**Figure 3-2: Conductivity of HBr-doped PANI embedded in MeCl<sub>2</sub>-PS matrix at increasing voltages.** Sheets of polystyrene containing varying quantities of HBr-doped PANI were made via the MeCl<sub>2</sub> method. As a negative control, 2 mm sheets of acrylate were used as a known electrical insulator. The recorded electrical resistances of the negative control acrylate and 0% MeCl<sub>2</sub>-PS-PANI samples were off the scale, so their electrical conductivity is shown as zero above. As a positive control, 3M Red Dot electrodes were stuck to each other without a sample between them. These recorded an electrical resistance of zero (data not shown).



**Figure 3-3: Conductivity of HBr-doped PANI embedded in LiqPS matrix at increasing voltages.** Sheets of polystyrene containing varying quantities of HBr-doped PANI were made via the LiqPS method. As a negative control, 2 mm sheets of acrylate were used as a known electrical insulator. The recorded electrical resistances of the negative control acrylate and 0% MeCl<sub>2</sub>-PS-PANI samples were off the scale, so their electrical conductivity is shown as zero above. As a positive control, 3M Red Dot electrodes were stuck to each other without a sample between them. These recorded an electrical resistance of zero (data not shown).

These recorded an electrical resistance of zero (data not shown). Similarly, for this formulation, 30% LiqPS PANI recorded an electrical resistance of zero at all voltages (data not shown).

## **4. PANI Polymer Stiffness Analysis**

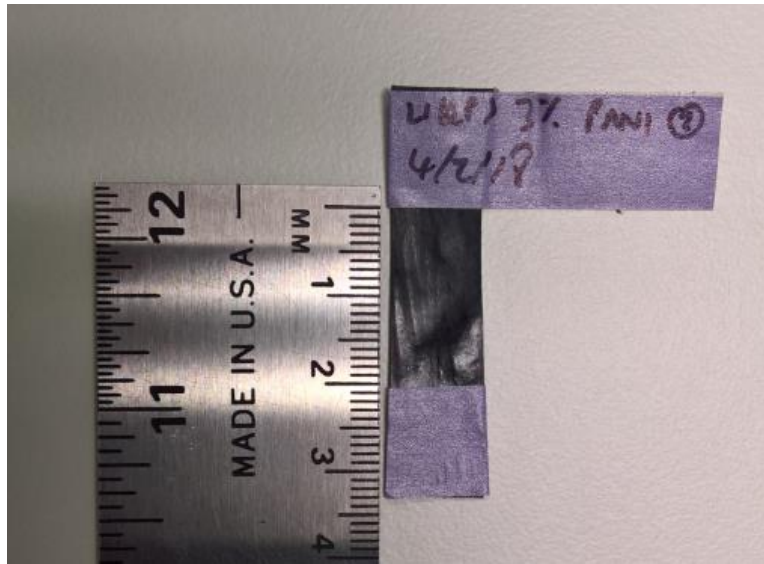
### **4.1 Overview**

Woodham requires an electrically conductive polymer matrix with a stiffness of over 1 kN/mm<sup>2</sup>. Karma tested the stiffness of multiple polymer/matrix combinations to determine which of these meets the requirements of this project.

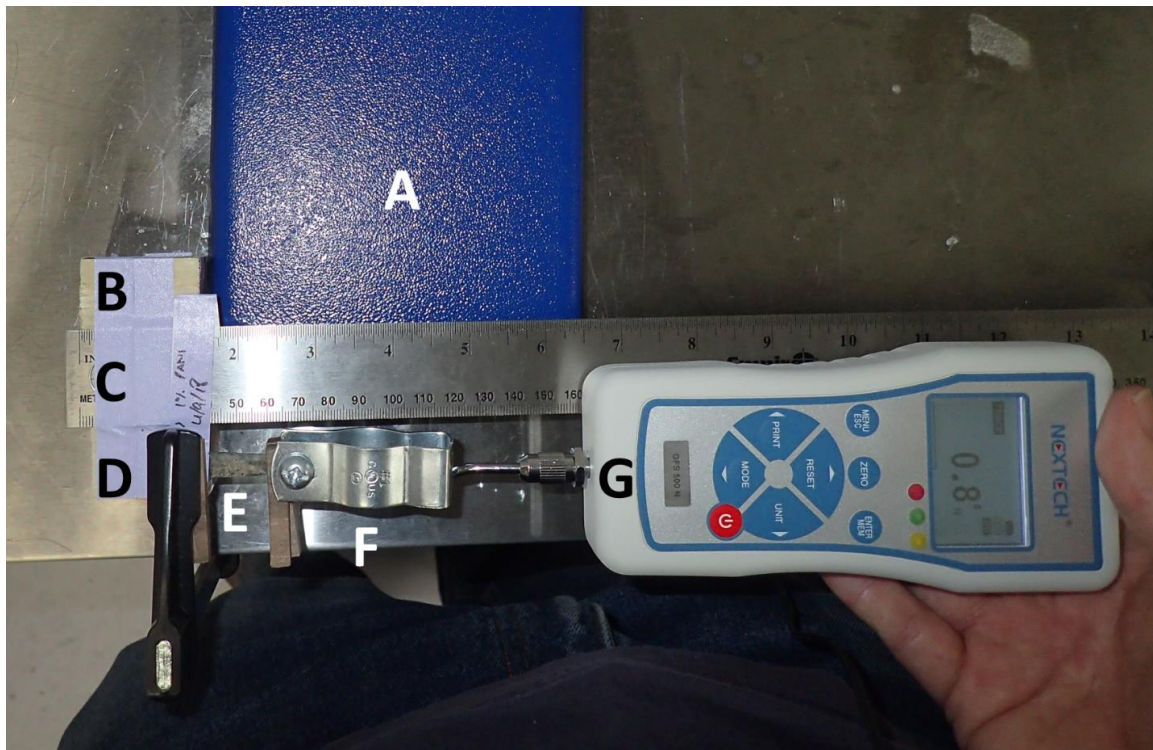
### **4.2 Materials and Methods**

#### *4.2.1 Standard Operating Procedure: Measurement of stiffness of a conductive polymer plate*

1. Set up video camera to record data from force gauge in real time.
  - a. Ensure that the LCD screen of the force gauge, the sample, and the ruler are all in shot (Figure 4.1).
2. Make plate (10 cm by 10 cm by 1 mm) of polymer to be tested for conductivity.
3. Cut from plate a test sample of approximately 4 cm by 4 cm by 1 mm.
  - a. Note that this is typically the fourth test sample leftover from electrical conductivity analysis conducted on the same plate.
4. Cut test sample into four equal strips of approximately 4 cm by 1 cm by 1 mm.
5. Label a Ziploc bag with the type and date of manufacture of the plate.
6. Place the fourth test strip from the plate in the labelled Ziploc.
7. Measure thickness and width of test strip using Vernier calipers, and record in the Karma Final Experiments Logbook Excel spreadsheet (available on request).
8. Attach a tape label to each end of each test strip (Figure 4-1) exactly 20 mm apart. Write the sample type, date and number on the longer label.
9. Clamp force gauge to end of test strip with short label.
10. Clamp end of test strip with long label to table via wooden block (Figure 4-2).
11. Begin recording with video camera.
12. Keeping test strip parallel to bench, pull horizontally on force gauge directly away from where it is clamped to the bench.
13. Increase pulling force as uniformly as possible until sample fails (snaps) or deforms 5 mm (total length of test strip between the two labels is 25 mm).
14. Stop recording with video camera.
15. Remove the test strip and place it into the labelled Ziploc bag.
16. Repeat steps 7-15 for each of the three test strips cut from the plate being measured.
17. Review video recording, and for each record the force measurement at 1 mm, 2 mm, 3 mm, 4 mm, and 5 mm of test strip deformation in the appropriate cell of the Karma Final Experiments Logbook Excel spreadsheet (available on request).
  - A. If the strip fails (snaps) without any apparent deformation, record the deformation as 0.1 mm.
18. Save data.



**Figure 4-1: Labelling of sample for stiffness testing.**



**Figure 4-2: Stiffness test rig setup.** The video camera is mounted to a clamp stand (A) directly above the rest of the test rig. A pair of wooden blocks (B) of approximately 1.5"x1.5"x1.5" is used to secure a ruler (C) to the bench via a clamp (D), which also secures one end of the sample (E)

to the rig. The other end of the sample is secured via a clamp (F) to the force meter (G). Force is applied to the sample by pulling directly away from the wooden block to which the sample is secured (i.e., the force meter is pulled towards the right edge of this photograph).

#### 4.2.2 Calculation of sample stiffness

Sample stiffness was calculated as follows:

1. Sample *stress* was calculated at each recorded deformation point (1 mm, 2 mm, etc.) using the following formula:

$$stress = \frac{F}{A}$$

where:

$F$  = force in kN measured at that deformation point

$A$  = cross sectional area of test strip in mm<sup>2</sup>

2. Sample *strain* was calculated at each recorded deformation point (1 mm, 2 mm, etc.) using the following formula:

$$strain = \frac{l}{L}$$

where:

$l$  = length of deformation at that point (1 mm, 2 mm, etc.)

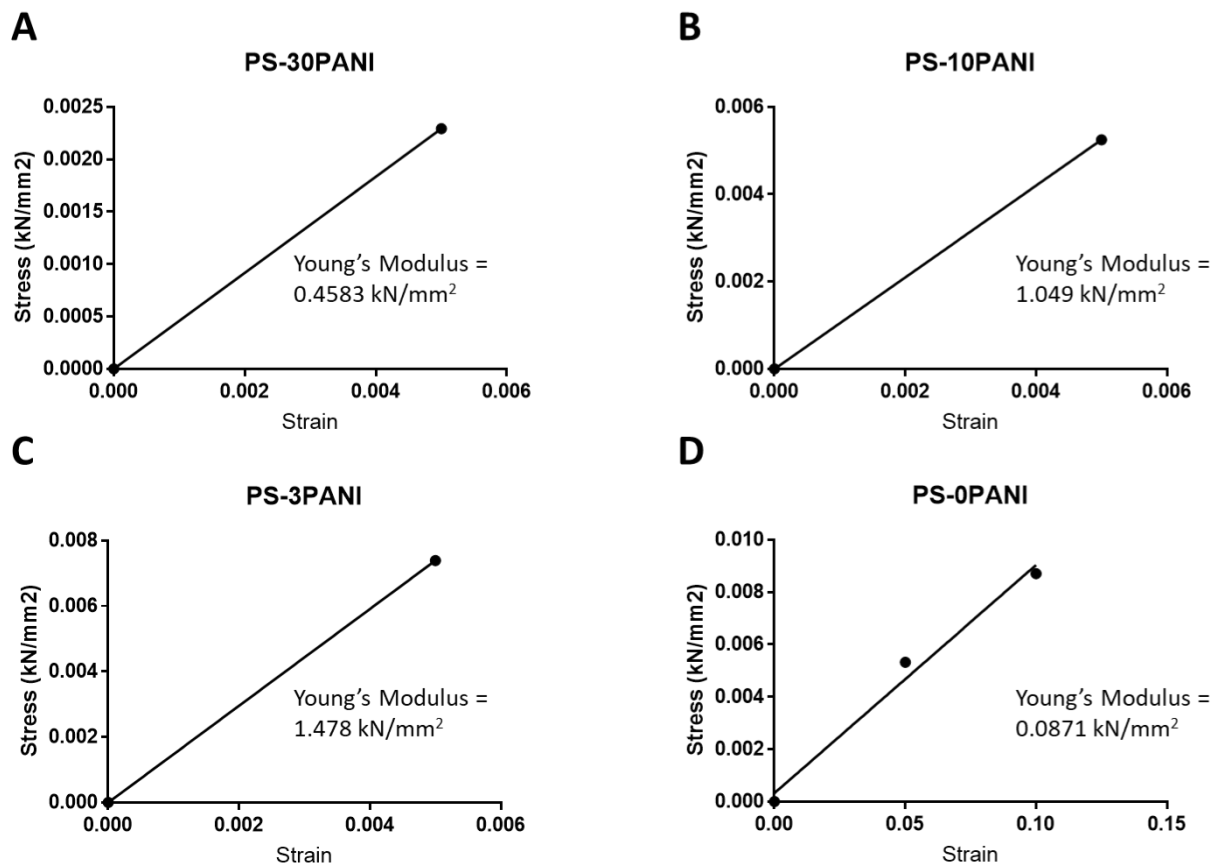
$L$  = starting length of test strip (20 mm, per the setup of this experiment)

3. Plot stress (y axis) against strain (x axis).
4. Calculate the slope of the graph to obtain the value for the Young's modulus for that test strip.

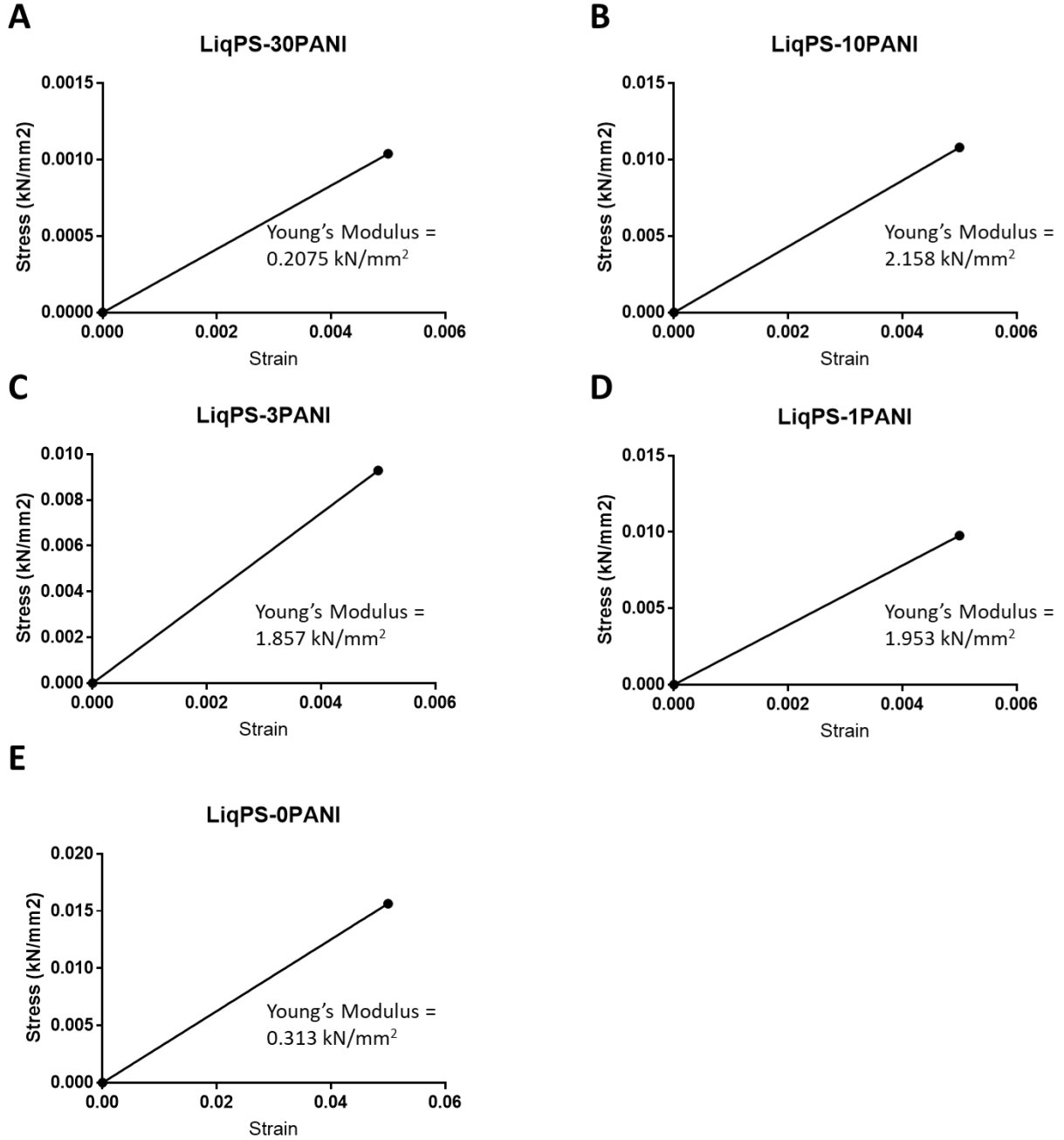
### 4.3 Results and Discussion

#### 4.3.1 Measurement of polymer stiffness

Samples of polystyrene matrix prepared using the MeCl<sub>2</sub> method and by the LiqPS method were prepared containing 30%, 10%, 3%, and 0% of HBr-doped PANI. In addition, a sample of 1% PANI in the LiqPS formulation was prepared. The stiffness of each sample was tested in triplicate as described in Section 4.2.1. For both PS matrices, stiffness increased with increasing amounts of embedded PANI (Figures 4-3 and 4-4). Both 30% PANI formulations were stiff to the point of brittleness. This made it impossible to accurately measure the stiffness of the samples using this method (they typically snapped with zero measurable deformation with virtually no lateral force being applied). With this in mind, the recorded stiffnesses shown for both 30% PANI samples are likely to be underestimates (Young's modulus for 30% MeCl<sub>2</sub>-PS-PANI was 0.4583 kN/mm<sup>2</sup>, and for 30% LiqPS-PANI was 0.2075 kN/mm<sup>2</sup>). This is because the assumed deformation before failure used in the calculations herein (0.1 mm) is likely to be an overestimate of the actual deformation of the fragile 30% PANI samples that occurred prior to failure. The Young's moduli calculated for 10%, 3%, and 1% PANI embedded in both formulations were all in the desired range (>1 kN/mm<sup>2</sup>) (Figures 4-3 and 4-4). Note that in most cases the samples failed (snapped) without measurable deformation. In these cases, it was assumed that the total deformation was 0.1 mm to allow calculation of Young's modulus for each.



**Figure 4-3: Stiffness of HBr-doped PANI embedded in MeCl<sub>2</sub>-PS matrix.** Stiffness (Young's modulus) of each sample (A: 30% MeCl<sub>2</sub>-PS-PANI, B: 10% MeCl<sub>2</sub>-PS-PANI, C: 3% MeCl<sub>2</sub>-PS-PANI, and D: 0% MeCl<sub>2</sub>-PS-PANI) calculated as the slope of the *stress/strain* graph plotted for each sample. Each data point represents the mean *stress/strain* result of three strips of each sample tested.



**Figure 4-4: Stiffness of HBr-doped PANI embedded in LiqPS matrix.** Stiffness (Young's modulus) of each sample (A: 30% LiqPS-PANI, B: 10% LiqPS-PANI, C: 3% LiqPS-PANI, D: 1% LiqPS-PANI, and E: 0% LiqPS-PANI) calculated as the slope of the *stress/strain* graph plotted for each sample. Each data point represents the mean *stress/strain* result of three strips of each sample tested.

## 5. PANI Polymer Optical Transparency Analysis

### 5.1 Overview

Woodham requires an electrically conductive polymer matrix that transmits at least 50% visible light. Karma tested multiple polymer/matrix combinations at a variety of wavelengths of visible light (representing violet, blue, green, yellow, orange and red light) to determine which of these meets the requirements of this project.

### 5.2 Materials and Methods

#### 5.2.1 Required Materials

1. Optical plate reader such as the Biotek Synergy H1 Hybrid Multi-mode Plate Reader
2. 96-well plates with optically clear well bottoms (Thermo Scientific Nunc MicroWell 96-Well Optical-Bottom Plates with Polymer Base, manufacturer number 265301)
3. Super glue
4. Scissors/scalpel

#### 5.2.2 Standard Operating Procedure: Measurement of optical transparency of a conductive polymer plate

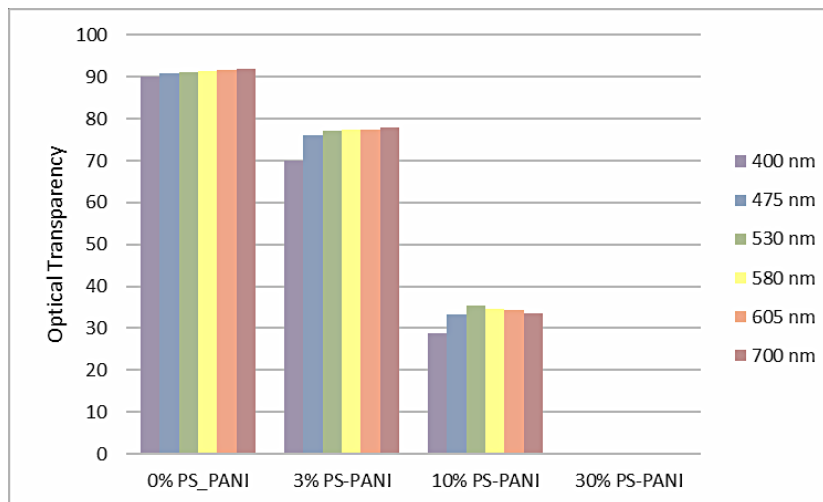
1. Make plate (10 cm by 10 cm by 1 mm) of polymer to be tested for conductivity.
2. Cut from plate a test strip of approximately 4 cm by 1 cm by 1 mm. Note that this is typically the fourth test strip leftover from stiffness analysis conducted on the same plate.
3. Cut test sample into three equal squares of approximately 0.9 cm by 0.9 cm by 1 mm.
4. Using superglue, stick each square onto the underside of a 96 well optical plate such that each square completely covers the bottom of one well. Take care not to allow any superglue to stick to the optically clear plastic at the bottom of the well, as this will interfere with absorption readings.
5. Using an optical plate reader, read the absorption of each well at 400, 475, 530, 580, 605 and 700 nm.
6. Save data.

### 5.3 Results

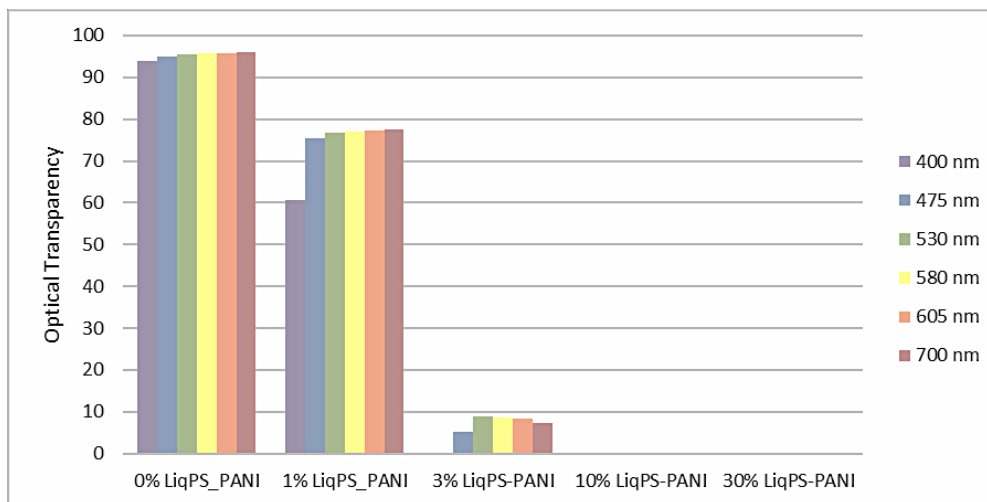
#### 5.3.1 Measurement of polymer optical transparency

Samples of polystyrene matrices prepared using the  $\text{MeCl}_2$  method and by the LiqPS method were prepared containing 30%, 10%, 3%, and 0% of HBr-doped PANI. For the LiqPS matrix, a preparation containing 1% HBr-doped PANI was also prepared. The absorbance of each sample was measured in triplicate at six wavelengths of visible light (400, 475, 530, 585, 605 and 700 nm, approximating violet, blue, green, yellow, orange, and red light) as described in Section 5.2.1. From these data, the optical transparency of each sample for each wavelength of light was calculated (Figures 5-1 and 5-2). For both types of PS matrices, optical transparency at all measured wavelengths increased with decreasing amount of embedded PANI. Both 30% PANI formulations were black and absorbed all visible light (optical transparency is zero at all wavelengths). The optical transparency of 3%  $\text{MeCl}_2$ -PS-PANI and 1% LiqPS-PANI formulations were within the desired range of over 50% (approximately 77% transparency in both cases for all wavelengths except 400 nm, which was more highly absorbed). Interestingly, the LiqPS

formulations were generally darker (less transparent) than their MeCl<sub>2</sub>-PS-PANI equivalents. This is likely due to the superior dispersion of PANI in the LiqPS formulation. This results in a more uniform coloration of LiqPS formulations, as opposed to the patchier dispersion of PANI in MeCl<sub>2</sub>-PS formulations where visible light can pass through the clearer parts of the matrix without interruption.



**Figure 5-1: Optical transparency of HBr-doped PANI embedded in MeCl<sub>2</sub>-PS matrix.** Optical light absorption at six wavelengths of visible light (400, 475, 530, 580, 605 and 700 nm) of each sample was measured using an optical plate reader. The percentage of light at each wavelength of each sample was calculated as a percentage of the absorption of the control (a piece of completely black plastic), and this number subtracted from 100% to calculate the optical transparency of the sample at each wavelength.



**Figure 5-2: Optical transparency of HBr-doped PANI embedded in LiqPS matrix.** Optical light absorption at six wavelengths of visible light (400, 475, 530, 580, 605 and 700 nm) of each sample was measured using an optical plate reader. The percentage of light at each wavelength of each sample was calculated as a percentage of the absorption of the control (a piece of completely black plastic), and this number subtracted from 100% to calculate the optical transparency of the sample at each wavelength.



## 6. LiqPS-PANI Polymer Cassettes for Western Blotting

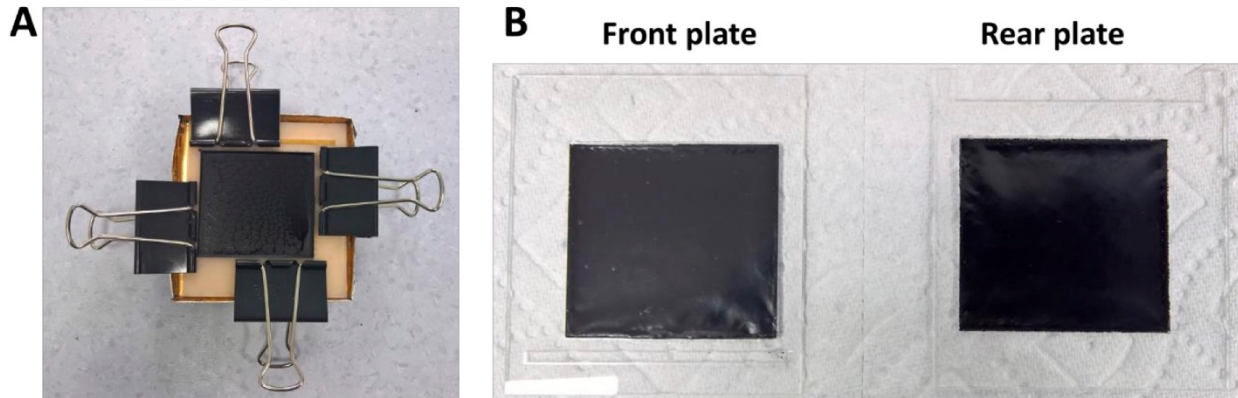
### 6.1 Overview

Woodham requires prototype western blot cassettes made from one or more of the electrically conductive polymer matrices developed above. Karma produced multiple polymer/matrix cassettes to be further evaluated by Woodham. Particularly, Karma developed a hybrid cassette consisting of a clear plastic frame with an integral PANI polymer window which allows protein gels to be run and transferred to an internal blotting membrane without opening the cassette.

### 6.2 Materials and Methods

#### 6.2.1 *Manufacture of acrylate frame/PANI polymer hybrid western blot cassette (Woodham cassette).*

1. Laser cut outer frame of Woodham cassette from 2 mm clear acrylate sheet (Uxcell 2 mm clear Perspex Acrylic Cut Plexiglass Sheet A4 Size 210 mm x 297 mm) using the template files (available on request).
2. Make an aluminum mold with dimensions of 10 cm by 10 cm by 0.5 cm.
3. Fill aluminum mold with Envirotex Pour-On Lite to a depth of 2 mm and allow to cure on a level surface in a well-ventilated area for 72 hours.
4. Place acrylate frame in aluminum mold and clamp around the edges of its central window (Figure 6-1).
5. Make polystyrene matrix containing the desired concentration of PANI using the LiqPS method (Section 2.3). Approximately 8 mL is required to fill the central window of each frame; starting with 7.5 g of polystyrene per frame is recommended.
6. Place mold containing clamped frame on a stand on a level surface.
  - A. A wooden block or piece of Styrofoam with approximate dimensions of 3 cm by 3 cm by 3 cm works well for this.
  - B. Ensure that the top surface of the block is level.
7. Pour LiqPS-PANI into central window of frame, taking care not to overspill onto the frame itself.
  - A. Depending on the viscosity of the LiqPS-PANI (which depends on the concentration of PANI used), it may need to be spread manually to all four corners of the window. A glass pipette works well for this.
  - B. LiqPS will dissolve the acrylate frame. This is necessary to fuse the LiqPS-PANI to the frame around the edges of the window. However, if any LiqPS spills over the edges, the frame will be damaged irreparably.
8. Expose the poured sample to 10 minutes of direct UV light at 370 nm wavelength.
9. Allow the LiqPS to cure for approximately 48 hours in a well-ventilated area.
10. Carefully remove clamps from around frame.
11. Refrigerate molds for 30 minutes. This makes it easier to remove the Woodham cassette frame from the mold.
12. Carefully separate the Woodham cassette frame from the mold using a large metal spatula. A typical gel knife used for cutting PAGE gels is ideal for this.
13. Remove the grey protective film from the acrylate frame.

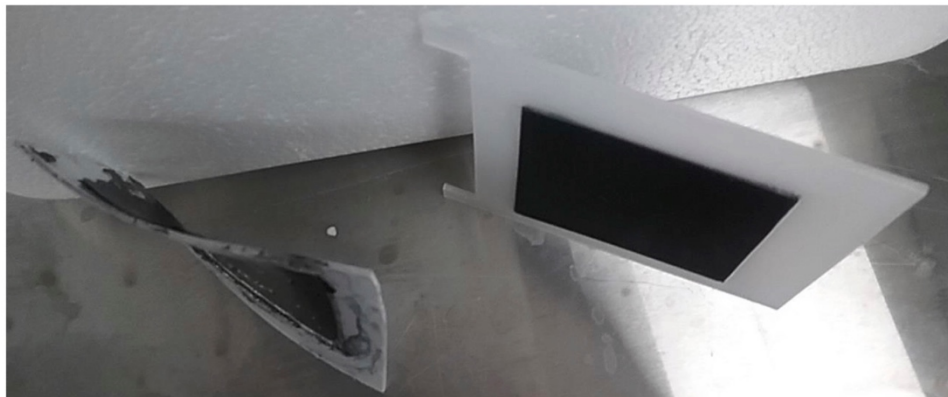


**Figure 6-1: Woodham cassette manufacture.** (A) Woodham cassette frame clamped in aluminum mold. 10% LiqPS-PANI has been poured into the central window. Note that care has been taken to avoid overspill of the LiqPS-PANI onto the acrylate frame. (B) Front and rear Woodham cassette plates removed from mold after curing. Note that the protective film supplied with the clear acrylate has been peeled off the final product.

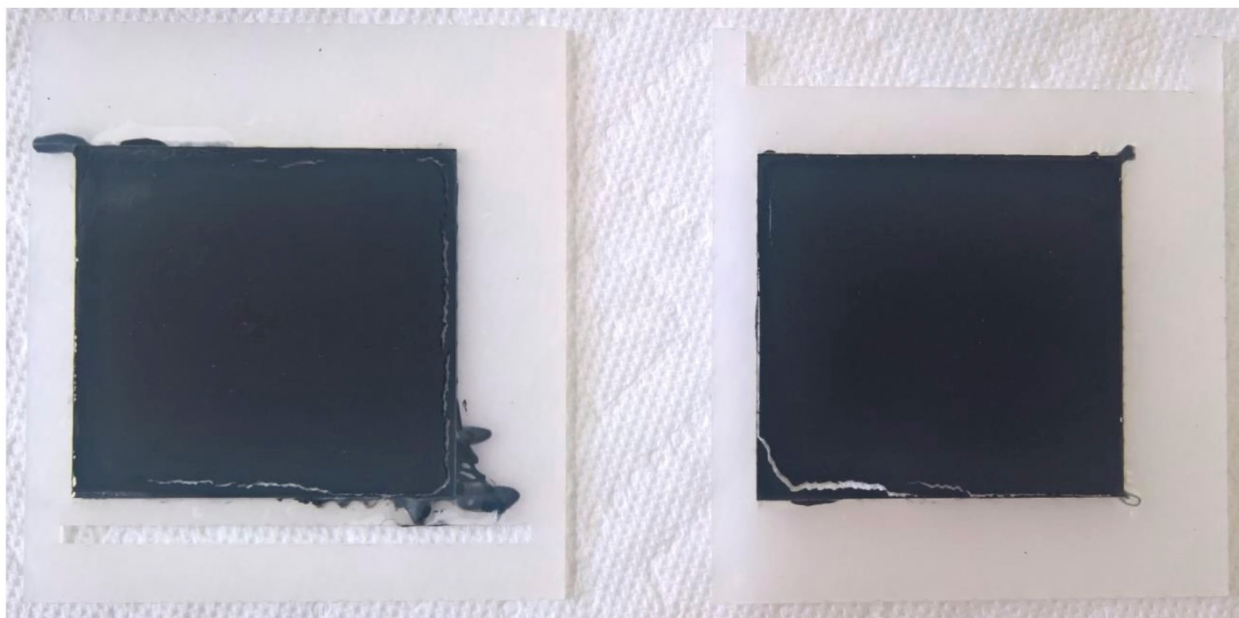
### 6.3 Results and Discussion

Karma's first efforts in making a hybrid Woodham cassette involved dispersing PANI in a polystyrene matrix via the  $\text{MeCl}_2$  method, then pouring this polymer into the windows of the Woodham cassette frames. This was designated the Mark IV Woodham cassette. Karma found that this method was unworkable. The very low viscosity of polystyrene dissolved in  $\text{MeCl}_2$  resulted in leaks out of the window, damaging the frame (acrylate is soluble in  $\text{MeCl}_2$ ). Additionally, the large volume loss as  $\text{MeCl}_2$  evaporates results in significant shrinkage of the PANI polymer as it cures. This resulted in significant force being applied to the frame, causing it to warp (Figure 6-2). The PANI polymer would also frequently crack in these instances. In either case, the resulting Mark IV Woodham cassettes were not fit for purpose. This led directly to Karma developing the LiqPS method for dispersing PANI in polystyrene matrices to alleviate these problems. The resulting hybrid acrylate/LiqPS-PANI prototypes were designated Mark V Woodham cassettes.

Karma manufactured several Woodham cassette prototypes with varying concentrations of LiqPS-PANI polymer (30%, 20%, 10%, and 1%). In all cases, the reduced volume loss during curing alleviated the frame warping issue. However, cassettes containing 30% and 20% PANI were rejected because these LiqPS-PANI polymers were too brittle to be used. The PANI windows always cracked in these instances (Figure 6-3). In contrast, the cassettes containing 10% and 1% PANI were robust enough for potential repeated use in experiments running PAGE gels and transferring them to nitrocellulose membranes.



**Figure 6-2: Comparison of Mark IV and Mark V Woodham cassettes.** The PANI polymer in the central window of the Mark IV Woodham cassette was manufactured using the  $\text{MeCl}_2$  method (left). This resulted in significant problems with leakage, warping, and cracking. The PANI polymer in the central window of the Mark V Woodham cassette was manufactured using the LiqPS method (right). This completely alleviated warping, and practically eliminated leaks during pouring of the PANI polymer. Furthermore, at lower concentrations of PANI (10% and 1%), instances of cracking in the central window were dramatically reduced.



**Figure 6-3: Woodham cassette prototype with 20% LiqPS PANI window.** The increased brittleness of high PANI concentration LiqPS formulations results in cracking around the edges of the window.

## 7. Final Comments and Recommendations

Karma has produced several prototypes of the Woodham cassette for further testing by Woodham. We have provided the following versions of the prototype cassettes:

1. 3X Mark V hybrid Woodham cassettes consisting of acrylate frame and 10% LiqPS-PANI transfer window
2. 2X Mark V hybrid Woodham cassettes consisting of acrylate frame and 1% LiqPS-PANI transfer window
3. 3X Mark VI Woodham cassettes consisting completely of 1% LiqPS-PANI

Most of the polymer matrix combinations tested did not have all of the properties required by Woodham (electrical conductivity of between  $10^{-3}$  and  $10^{-8}$  S/cm, stiffness of  $>1$  kN/mm<sup>2</sup>, and optical transparency of  $>50\%$ ). LiqPS formulations containing 30% PANI have the requisite conductivity, but are too brittle to be practical and are completely black (i.e. the optical transparency is zero). LiqPS formulations containing 1% PANI have the requisite optical transparency, but to be electrically conductive must be no greater than 0.2 mm thick and thus cassettes made solely out of the 1% PANI will not likely have the structural properties necessary for western blotting. Even at that thickness, the electrical conductivity is outside the range initially requested by Woodham though still may be suitable for transfer under the right conditions. To circumvent some of these problems, it was decided to create hybrid cassettes for western blotting in which a clear plastic frame contained a rectangle of electrically conductive polymer matrix (Figure 6-1). This has the advantage that a protein size ladder can be run within a part of the gel contained within the clear plastic frame, allowing the user to track the progress of the gel as it runs. Structural integrity is conferred by the stiff plastic frame, so the cassette can be moved in its entirety into a protein transfer rig. The integral conductive polymer window can be made with a variety of PANI concentrations. Karma manufactured a series of these hybrid cassettes containing various concentrations of PANI.

The hybrid acrylate 10% LiqPS-PANI Mark V Woodham prototype cassette has the structural characteristics to be practical, has the conductivity in the range originally requested by Woodham ( $2.7 \times 10^{-6}$  S/cm at 250 V), but is completely black (non-transparent). Though outside the optimal range, this conductivity may actually be an advantage if highly conductive cassettes interfere with protein separation during PAGE. The hybrid acrylate 1% LiqPS-PANI cassette also has the structural characteristics requested by Woodham, and achieves the requested transparency ( $>50\%$  of visible light). However, it is significantly less conductive at low voltages, though conductivity is increased with increasing voltage ( $4.1 \times 10^{-10}$  S/cm at 250 V to  $2.5 \times 10^{-9}$  S/cm at 1000 V). Thus, it is advised that Woodham further tests these prototypes using protein transfer equipment that can run at high voltages, as this may be sufficient to suite Woodham's purposes.