GENERAL LABORATORY RISK ASSESSMENT

1. General information.

Department:	Department of Nanotechnology	Date:	October 23, 2024
Procedure:	Lp-PLA2 ECL Biosensor Fabrication	Revision Number:	1
Principal Investigator:	Dr. John Saad	PI Phone Number:	519-888-4567 x39296
PI Signature: *By signing you are indicating that the tasks are planned for in such a way that the risk is tolerable.		X _____	

2. Identify if any of the following hazards or materials are present.

☒ Yes ☐ No	Nanomaterials	☐ Yes ☒ No	Cannabis
☒ Yes ☐ No	Biohazards	☐ Yes ☒ No	Nonionizing Radiation
☐ Yes ☒ No	Radioactive materials	☐ Yes ☒ No	Designated Substances (acrylonitrile, benzene, silica, isocyanates, vinyl chloride, As, Pb, Hg, etc.)
☐ Yes ☒ No	X-ray sources		
☐ Yes ☒ No	Class 3B or Class 4 Lasers		

If you checked "yes" for any of the items above, review the associated program to ensure you have managed those requirements prior to or in conjunction with completing this risk assessment.

3. Describe the project steps in point form detail. Identify equipment and materials at relevant steps.

A) CoFe PBA Fabrication

1. Solution A is made by dissolving 436.2 mg of cobalt (II) nitrate hexahydrate ($Co(NO_3)_2 \cdot 6H_2O$) and 441.2 mg of trisodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) in 50 mL of deionized water
2. Solution B is made by dissolving 329.25 mg of potassium ferricyanide (III) ($K_3[Fe(CN)_6]$) in 50 mL of deionized water
3. The two solutions were mixed with a magnetic stir plate for 5 minutes
4. The new solution was aged for 24 hours at room temperature
5. The solution was spun in a centrifuge at 8000 rpm for 5 minutes
6. The dark purple precipitates were collected and washed three times with deionized water and ethanol separately
7. The substance was dried overnight (12 hours) at 60°C

B) PEI@CoFe PBA Combination

1. The CoFe PBA (10 mg) were dispersed via sonification in 10 mL of deionized water alongside 100 µL of polyethylenimine (PEI)
2. The mixture was magnetically stirred for 4 hours at 800 rpm at room temperature
3. The mixture was spun in a centrifuge at 8000 rpm for 5 minutes to separate the precipitates
4. The precipitates were washed three times with deionized water
5. The precipitates were dispersed in about 10 mL of deionized water (subject to yield) to obtain 1 mg/mL of PEI@CoFe PBA
6. The mixture was stored at 4°C in the dark

C) AuNP@CoFe PBA Nanocomposites Adhesion

1. 1 mL of the PEI@CoFe PBA solution was added to 9 mL of a 25 nm AuNP colloid and stirred at room temperature for 2 hours
2. The solution was spun in a centrifuge at 8000 rpm for 5 minutes
3. The precipitate was collected and washed three times with deionized water
4. The particles were dispersed in about 5 mL of deionized water (subject to yield) to make 0.2 mg/mL of AuNP@CoFe PBA nanocomposites

D) ITO Electrode Preparation

1. The ITO-coated substrate was sanitized before usage

    a. For ITO-coated glass/PEN, it was sonicated in a 1:1 v/v 2% sodium hydroxide (NaOH) and ethanol for 30 minutes; acetone and deionized water were also used for sonification for 30 minutes
2. It was sonicated in a 30% ammonia solution for 30 minutes and soaked overnight to hydrolyze its surface
3. The ITO was cleaned with deionized water and dried with nitrogen gas 3 times
4. 10 µL of a 0.01% v/v APTMS and ethanol solution was dropped onto the ITO surface
5. It was placed in a water saturated environment at 55°C for 4 hours
6. 50 µL of the AuNP@CoFe PBA solution was dropped onto the ITO surface for 5 minutes
7. The excess solution was washed off with deionized water and dried with nitrogen gas
8. The electrode was stored at 4°C until next stage

E) Antibody Adherence

1. The 10 µL Lp-PLA2 antibody mixture (0.2 ng/mL) was dropped onto the electrode surface and incubated at 30°C for 2 hours
2. The excess solution was washed off with 0.01 M of phosphate buffered saline (PBS) at 7.4 pH
3. The remaining exposed active sites were closed off with 10 µL of 1% v/v bovine saline albumin (BSA) at 25°C for 1.5 hours
4. The excess solution was washed off with 0.01 M of PBS at 7.4 pH and dried with nitrogen gas
5. The completed sensor was stored at 4°C until usage

F) Sample Testing

1. A 0.2 M PBS solution at 8 pH containing 1.0×10^{-5} M of Luminol was prepared as the electrolytic solution
2. The ITO electrode was placed on the circuit containing the counter and reference electrodes
3. The electrolytic solution was dropped onto the circuit ensuring all the electrodes are covered
4. The circuit was place into the dark box containing the photodiode sensor
5. The circuit was powered by a pulse square wave with a V_{pp} of 1.7 V with a maximum of 1.3 V and minimum of -0.4 V and a period of 3 seconds
6. Its baseline light intensity was recorded, and solution was dried off with nitrogen gas
7. The sample containing the Lp-PLA2 was coated on the ITO sensor and left to incubate at 35°C for 80 minutes maximum
8. The electrolytic solution was dropped onto the circuit ensuring all the electrodes are covered
9. The circuit was place into the dark box containing the photodiode sensor
10. The circuit was powered by a pulse square wave with a V_{pp} of 1.7 V with a maximum of 1.3 V and minimum of -0.4 V and a period of 3 seconds
11. Its light intensity was recorded

4. Identify the WHIMIS hazard classes, categories, and anticipated control measures used to reduce worker exposure. (Hover mouse here to learn how to add more rows)

Name of chemical	List all WHMIS physical and health hazard classes and categories (drop-down list and free-form text)	Category / Type	Using WHMIS precautionary statements as a guide, identify what control practices are required to minimize worker exposure for handling these chemicals – consider ventilation, PPE, containment, etc.
3-Aminopropyltrimethoxysilane (APTMS)	Skin corrosion	1	• Chemical must **ALWAYS** be used in the fume hood or a well-ventilated environment • Chemical can react with water and produce methanol vapours (**BAD**) so use in fume hood • **ALWAYS** wear eye protection and gloves around substance
	Skin irritation	2	
	Serious eye damage	1	
	Eye irritation	2	
	Substances and mixtures which, in contact with water, emit flammable gases	1	

Cobalt (II) nitrate hexahydrate	Oxidizing solids	2	• Keep out of reach of flammable material and chemicals when possible
			• Chemical must **ALWAYS** be used in the fume hood or a well-ventilated environment
			• **ALWAYS** wear eye protection and gloves around substance
			  ○ **ENSURE** gloves are the following materials:
			  ▪ Natural Rubber
			  ▪ Nitrile Rubber
			  ▪ Neoprene
			  ▪ PVC

	Acute toxicity - oral	4	
	Skin sensitizer	1	
	Serious eye damage	1	
	Acute toxicity - inhalation	4	
	Respiratory sensitizer	1	
	Germ cell mutagenicity	2	
	Carcinogenicity	1B	
	Reproductive toxicity	1B	
	Skin corrosion	1B	
	Specific target organ toxicity - repeated exposure (list organs) • Cardiovascular Organs • Thyroid • Bone Marrow	1	
Potassium ferricyanide (III)	Acute toxicity - oral	4	• Will decompose into hydrogen cyanide (**BAD**) when heated, so **NEVER** heat this outside a fume hood or well-ventilated area • Will decompose into cyanide (**ALSO BAD**) when reacted with an acid, so **NEVER** bring into contact with an acid • Chemical must **ALWAYS** be used in the fume hood or a well-ventilated environment • **ALWAYS** wear eye protection and gloves around substance
	Skin irritation	2	
	Eye irritation	2	
	Specific target organ toxicity - single exposure (list organs) • Respiratory Organs	3	
	Reproductive toxicity	2	
Polyethylenimine (PEI)	Acute toxicity - oral	4	• **ALWAYS** wear eye protection and gloves around substance
	Serious eye damage	1	
Indium tin oxide	Specific target organ toxicity - repeated exposure (list organs) • Respiratory Organs	1	• Do not inhale ITO dust, so minimize chances of producing this • Wash hands thoroughly after handling material
Bovine saline albumin (BSA)	Specific target organ toxicity - single exposure (list organs) • Respiratory Organs	3	• Do not inhale fumes or vapours from substance, so handle in well-ventilated area
Luminol	Acute toxicity - oral	4	• Wear eye protection and gloves around substance • Handle in well-ventilated area
	Skin irritation	2	
	Eye irritation	2	
	Specific target organ toxicity - single exposure (list organs) • Respiratory Organs	3	

Sodium hydroxide	Corrosive to metals	1	• Produces heat when dissolved in water or neutralized with acid, so be wary when using with flammable substances • **ALWAYS** wear eye protection and gloves around substance
	Skin corrosion	1A	
	Serious eye damage	1	
	Eye irritation	2	
Ethanol	Flammable liquids	2	• Be cautious of placement near an ignition source or an oxidizer • Wear eye protection around this substance
	Eye irritation	2	
Acetone	Flammable liquids	2	• Be cautious of placement near an ignition source or an oxidizer • Work with substance in a well-ventilated environment • Wear eye protection around this substance
	Eye irritation	2	
	Specific target organ toxicity - single exposure (list organs) • Narcotic Effects	3	
Ammonia solution	Skin corrosion	1B	• **ALWAYS** wear eye protection and gloves around substance • Work with substance in a well-ventilated environment
	Specific target organ toxicity - single exposure (list organs) • Respiratory Organs	3	

5. List equipment being used.

Equipment used consists of devices that impart energy or contain reactions – examples include rotovaps, ovens, pressure devices, material test stands etc. (Hover mouse here to learn how to add more rows)

Identify equipment (Click box to enter text)	Step #	List Potential Equipment Hazards	Identify anticipated controls to control the identified risks
Magnetic Stir & Hot Plate	A3, A7, B2, C1	Vibration Source of heat - potential burns Moving parts	• Place a sign nearby the hot plate to signify its usage
Sonicator	B1, D1, D2	Vibration Noise	• Use ear protection due to high frequency noise generated • Place a sign nearby the sonicator to signify its usage
Centrifuge	A5, B3, C2	Vibration Moving parts	• **ALWAYS** ensure the inner stage is balanced with 2 or more vials • Place a sign nearby the centrifuge to signify its usage
Function Generator	F5, F10	Unprotected electrical source	• Ensure it is not set to a high voltage before touching outputs
UV-Vis	Characterization	Radiation emission - UV, IR, Visible light, microwave, radiofrequency	• Do not look at light source during operation
SEM	Characterization	Moving parts High pressure release	• Follow approved operation steps to be safe

6. Hazardous conditions.

Please identify how experimental conditions (pressure, temperature, humidity, etc.) may alter the behaviour of chemicals being used. Remember to consider reactive intermediates as well.

- APTMS will be dropped onto the ITO electrode and placed in a water saturated environment at 55°C for 4 hours, which will produce methanol as a byproduct
  - This is very dangerous, so it must be done in the fume hood and the experimenter must wear proper PPE
- Residual potassium ferricyanide (III) may be present during the overnight drying process at 60°C, which may decompose and produce hydrogen cyanide gas
  - This is very dangerous, so this step must also be left in the fume hood with a corresponding sign and the experimenter must be careful not to inhale the gas

7. Managing hazardous waste.

Please include waste disposal methods in your SOPs. Guidance can be found on the Hazardous Waste website or by emailing esf@uwaterloo.ca. (Hover mouse here to learn how to add more rows)

Contents and Anticipated Class	Waste Type	Anticipated Amount
Potassium nitrate (Aqueous liquid waste – inorganic salts)	☐ Solid ☐ Radioactive ☐ Biological ☒ Liquid ☐ Battery ☐ Chemical	<100 mL
Polyethylenimine (Aqueous liquid waste – organics)	☐ Solid ☐ Radioactive ☐ Biological ☒ Liquid ☐ Battery ☐ Chemical	<10 mL
Sodium hydroxide (Base)	☐ Solid ☐ Radioactive ☐ Biological ☒ Liquid ☐ Battery ☐ Chemical	250 mL
Ethanol (Organic solvents)	☐ Solid ☐ Radioactive ☐ Biological ☒ Liquid ☐ Battery ☐ Chemical	400 mL
Acetone (Organic solvents)	☐ Solid ☐ Radioactive ☐ Biological ☒ Liquid ☐ Battery ☐ Chemical	500 mL
Ammonia (Aqueous liquid waste – inorganic salts)	☐ Solid ☐ Radioactive ☐ Biological ☒ Liquid ☐ Battery ☐ Chemical	500 mL
Phosphate buffered saline (Aqueous liquid waste – inorganic salts)	☐ Solid ☐ Radioactive ☐ Biological ☒ Liquid ☐ Battery ☐ Chemical	300 mL
Luminol (Organic solvent)	☐ Solid ☐ Radioactive ☐ Biological ☒ Liquid ☐ Battery ☐ Chemical	50 mL
☒ Yes ☐ No	All necessary labels, containers, transportation means are available to start the research process.	
☒ Yes ☐ No	All waste generators have taken the online Chemical Waste Segregation SO2070	

8. Standard operating procedures and emergency planning.

All medium to high-risk activities require an SOP. Work with toxic, pyrophoric, or water reactive materials require emergency planning SOPs. Identify what SOPs will be created for this project in the table below and where they are located. SOP template is available from the Safety Office.

SOP Name	Procedure available	Indicate how this SOP covers anticipated risks
Overall Process	☒ Yes ☐ No	• Guides the experimenter safely • Ensures nothing is done incorrectly
Spill, Exposure, or Emergency	☒ Yes ☐ No	• In the scenario that something occurs, the experimenter can safely navigate the situation • Mainly deals with spillage, and accidental exposure

9. Personal protective equipment.

Note: Closed toed shoes and lab coat are mandatory for work with chemicals.

PPE Type	PPE Storage Location	When it is Worn
Nitrile Gloves	QNC Undergraduate Labs	Always
Goggles	Personal Item	Always

10. Supervisor commentary.

11. Worker sign-off.

By signing the sheet below, you acknowledge that you have:

1. Understood the stipulations, hazardous, and control requirements outlined in this document.
2. You have completed practical training and had the opportunity to ask questions

Name (Print)	Signature	Date
Brandon Kong	X	Click or tap to enter a date.
Magilan Varatharuban	X	Click or tap to enter a date.
Rand Dakhil	X	Click or tap to enter a date.
Andrew Wang	X	Click or tap to enter a date.