JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS Vol. 19, No. 7 - 8, July – August 2017, p. 528 - 537

Statistical analysis of the effects of the variation of mediator concentration, spin rate, and solvent type on spin coated $[Ru(NH_3)_6]^{3+}$/Nafion/ITO modified electrodes

S. T. PALISOC[a,c], K. A. Y. KAW[a], F. F. CO[b,c], M. T. NATIVIDAD[a,c*]
[a]*Condensed Matter Laboratory, Physics Department, De La Salle University, 2401 Taft Avenue, Manila 0922, Philippines*
[b]*Mathematics Department, De La Salle University, 2401 Taft Avenue, Manila 0922, Philippines*
[c]*Condensed Matter Research Unit, Center for Natural Science and Environmental Research, College of Science, De La Salle University, 2401 Taft Avenue, Manila 0922, Philippines*

Hexaammineruthenium-Nafion films were spin coated on indium tin oxide coated glass substrates to protect them against fouling and to enhance their transport properties. The mediator concentration (hexaamineruthenium or RuHex), spin rate, and solvent type (ethanol, methanol, and isopropanol) were varied to determine the optimal composition of $[Ru(NH_3)_6]^{3+}$/Nafion/ITO modified electrodes that will yield the best electrode as defined by their transport properties. Cyclic voltammetry results show that spin rate provides minimal peak current change. RuHex concentration had the highest influence on peak height. Isopropanol diluted solutions had higher anodic peaks compared to other alcohols. Ethanol diluted solutions had the highest reversibility ratio, albeit by a small margin. Methanol diluted solutions were the most stable in terms of relative standard deviation. Statistical analyses with 3-way ANOVA reveal that the 3 parameters do not have an interaction effect. The 2-way ANOVA test, however, identified that there is an interaction effect between solvent type and RuHex concentration.

(Received July 18, 2016; accepted August 9, 2017)

Keywords: Nafion, hexaamineruthenium, RuHex, cyclic voltammetry, spin coating, 3-way ANOVA, 2-way ANOVA, Tukey-Kramer post-hoc test

---

1. Introduction

Research on the development of modified Nafion-coated indium tin oxide (ITO) electrodes have surged in recent years due to the need for cost-effective techniques in the determination of heavy metals present in the environment [1–20]. In this study, the electrodes are modified by coating them with Nafion® using RuHex as mediator in different solvent types.

Nafion®, by DuPont, is a perfluorosulfonated cation exchange polymer with the following characteristics: antifouling capacity, chemical inertness, and high permeability to cations. It has been frequently used as an electrode modifier for organic molecules [21]. However, it is an ionomer and does not conduct electrons. Redox mediators were used to remedy this situation since mediators facilitate electron transport between the analyte and the electrode.

Hexaammineruthenium or RuHex redox couple is a widely used redox couple that presents a reversible redox behavior in aqueous solution [22, 23]. It has been mainly used as an electrochemical indicator or redox probe for biosensing applications such as DNA-based sensors [24-29].

Given Nafion's expensive nature, it is natural to dilute it with alcohols to reduce cost. A number of studies reported to have diluted their Nafion solution with ethanol [30-34] whereas some diluted Nafion with methanol [35] and isopropanol [36-38]. There has been no study that identifies the effects of different solvent types on the electrochemical properties of RuHex-Nafion modified electrodes.

In this study, RuHex incorporated Nafion films were spin coated on indium tin oxide coated glass substrates. The RuHex-Nafion coating solutions were diluted with ethanol, methanol, or isopropanol. The modified electrodes were tested via cylic voltammetry to identify the effects of various parameters such as mediator concentration, solvent type, and spin rate to the overall performance of the modified electrode. Statistical analyses were performed to identify the relevance of the discrepancies i.e. to identify interaction effects.

2. Methodology

2.1. Reagents and materials

Nafion® solution (5 wt%) was procured from Fuel Cell Earth (Woburn, MA, USA). Hexaamineruthenium(III) chloride (98%) and Indium tin oxdie coated rectangular glass slide with surface resistivity of 15-25 Ω/sq, were purchased from Aldrich (Sigma-Aldrich Pte. Ltd., Singapore). Isopropyl alcohol, GR grade, was purchased from Duksan Pure Chemicals Co., Ltd. (Seonggok-dong, Ansan-si, Gyeonggi-do, South

Korea). Ethanol and Methanol, 99.9% was purchased from RCI Labscan (RCI Labscan Limited, Bangkok, Thailand).

2.2. Preparation of substrates

The indium tin oxide coated glass slides were cut into 2.5 × 1.0 cm segments using a diamond tip glass cutter. The cut segments were to be used as substrates. They were first washed under running tap water to remove glass fragments. The substrates were then placed in a Petri dish which was filled with enough solution (deionized water, acetone, and then isopropyl alcohol) to completely submerge the substrates. The petri-dish was then set afloat on a Ultrasonic bath (Bandelin Sonorex RK-100, Bandelin electronic GmbH & Co. KG, Heinrichstraße, Berlin, Germany) and sonicated for 5minutes each (in sequence) to remove surface contaminants. The substrates were air dried before spin coating.

2.3. Preparation of casting solution

For the preparation of the solutions used to coat the substrates, an appropriate amount of hexaammineruthenium (III) chloride (RuHex, 25mg, 50mg, and 75mg) was weighed and placed in a 1% Nafion-alcohol solution. The 1% Nafion-alcohol solution was prepared by adding 24mL of alcohol (methanol, ethanol, or isopropanol) to 6mL of 5% Nafion. The mixture was then partially immersed in an ultrasonic bath and sonicated for 5 minutes, six times with swirling and cooling intervals in between to aid dissolution and to prevent evaporation.

2.4. Fabrication of modified electrodes

Spin coating was done with a Spin Coat G3P-8 (Specialty Coating Systems, Indianapolis, USA). The coating solution was placed in the spin coater canister, sealed and pressurized to 10psi with compressed air. The substrate was placed on the spin coater chuck and centered. A vacuum pump, connected to the spin coater, was used to hold the substrate while spin coating.

The substrates were first spun at 750rpm for 10s and were then spun to 1500, 2000, or 2500rpm for 30s. The initial spin rate was where the coating solution was sprayed onto the substrate. The final spin rate was where the deposited solution was thinned out.

The coated substrates are the fabricated substrates. They were placed in a Thermolyne 4800 furnace (Barnstead Thermolyne Corporation, Iowa, USA) where the temperature was raised from room temperature to 70°C at a rate of 2.2°C/min. The temperature was kept constant at 70°C for 30 minutes; afterwhich, the furnace was slowly cooled to room temperature. The annealing process was monitored with a Fluke 53 II B thermometer (Fluke Corporation, Washington, USA).

2.5. Cyclic voltammetry

Cyclic voltammetry (CV) was performed with a BST8-stat potentiostat/galvanostat (MTI Corporation, Richmond, California USA) which was interfaced to a computer with BST80stat software (V.50.2.12.730). The spin coated electrode was used as the working electrode and a KCl-saturated calomel electrode was sued as the reference electrode. A platinum coil was used as the counter electrode. For all measurements, 100mL of 0.1M NaCl was used as the supporting electrolyte. Furthermore, to ensure a constant exposure area, the electrodes were wrapped with polytetrafluoroethylene tape to expose 1.0cm^2 on one end. The electrodes were connected to the potentiostat via an alligator clip.

Two CV tests were performed: a 10-cycle test and a multiple scan rate test. The 10-cycle test was a potential sweep -0.8V to +0.2V and vice versa at 0.1V/s and 0.001V increments for 10 consecutive times (**Table 1**). For the multiple scan rate test, the scan rate was varied from 0.03V/s to 0.3V/s. The potential step to scan rate ratio was maintained at 0.1. Detailed parameters for the multiple scan rate test are presented in **Table 2**.

Table 1. Parameters for 10 cycle CV test

# of loops	Potential (V) Initial	Potential (V) Final	Scan rate (V/s)	Potential step (V)
10	- 0.8	+ 0.2	0.1	0.001

Table 2. Parameters for Multiple Scan Rate CV Test

#	Potential (V) Initial	Potential (V) Final	Scan rate (V/s)	Potential step (V)
1	- 0.8	+ 0.2	0.3	0.03
2	- 0.8	+ 0.2	0.25	0.025
3	- 0.8	+ 0.2	0.2	0.02
4	- 0.8	+ 0.2	0.15	0.015
5	- 0.8	+ 0.2	0.1	0.01
6	- 0.8	+ 0.2	0.09	0.009
7	- 0.8	+ 0.2	0.08	0.008
8	- 0.8	+ 0.2	0.07	0.007
9	- 0.8	+ 0.2	0.06	0.006
10	- 0.8	+ 0.2	0.05	0.005
11	- 0.8	+ 0.2	0.04	0.004
12	- 0.8	+ 0.2	0.03	0.003

Three tests were derived from the 10-cycle scans, namely: Peak Current, Reversibility ratio, and %RSD. The peak current test is a comparison of different anodic peak heights. The reversibility ratio test is a comparison of ratios between the anodic peak heights and the anodic peak heights. And the %RSD test is a comparison of the %RSD of the 10-cycle test of different modified electrodes.

2.6. Statistical analyses

The results from CV tests were subjected to statistical analyses using Statistical Analysis System (SAS) version 9.3 software. A 3-way factorial analysis of variance (ANOVA) was used to determine if there were interaction effects between the parameters of the study (RuHex concentration, Solvent type, and Spin rate). Results from the 3-way ANOVA did not yield significant 3-factor interaction effects and thus a 2-way factorial ANOVA test was performed. Finally, the Tukey-Kramer post-hoc test was used to perform the appropriate pairwise treatment comparisons.

3. Results and discussion

3.1. Cyclic voltammetry

An averaged CV plot comparing isopropanol-diluted 25 mg RuHex, 50 mg RuHex, and 75 mg RuHex electrodes is shown in Fig. 1.

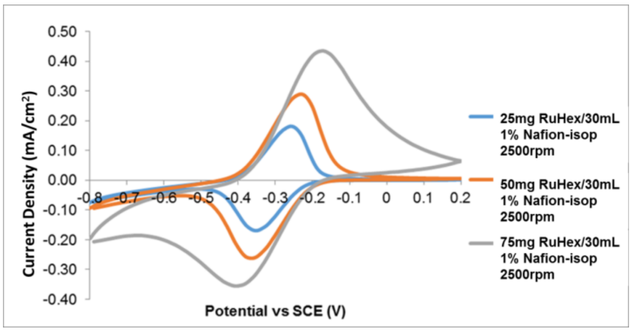
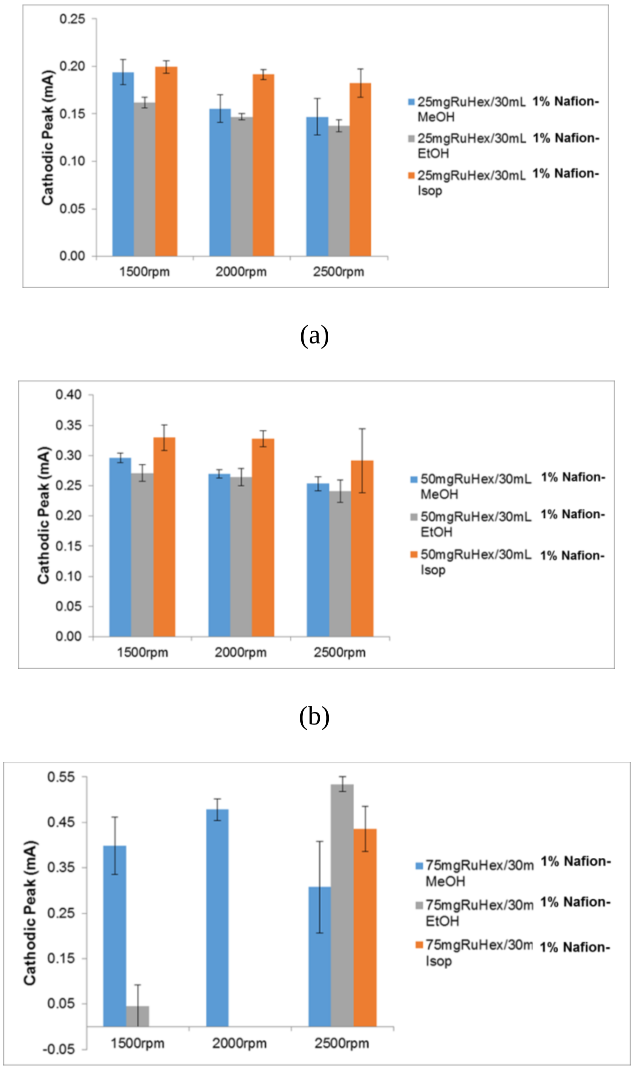


Fig. 1. Comparison of isopropanol diluted 25 mg RuHex, 50 mg RuHex, and 75 mg RuHex electrodes

3.1.1. Peak current

A comparison of different anodic peak heights is shown in **Fig.2**. The plots were grouped according to the RuHex content of the electrodes and compared with different solvent types. For 25 mg and 50 mg RuHex, isopropanol peaks were highest followed by methanol, with ethanol having the lowest. The 75 mg RuHex electrodes had very inconsistent results because they had poor reversibility as shown in Fig. 3. Higher RuHex concentration resulted to higher peaks.



(a)

(b)

(c)

Fig. 2. Comparison of anodic peaks for 25mg RuHex (a), 50mg RuHex (b), and 75mg RuHex (c) electrodes

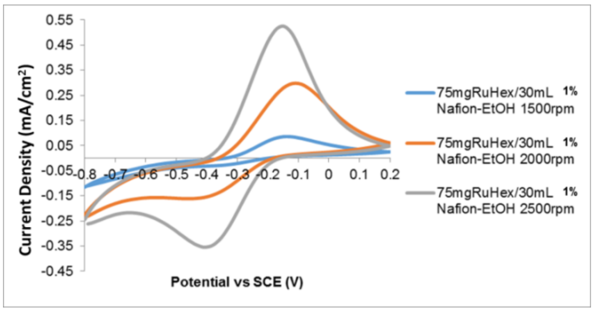
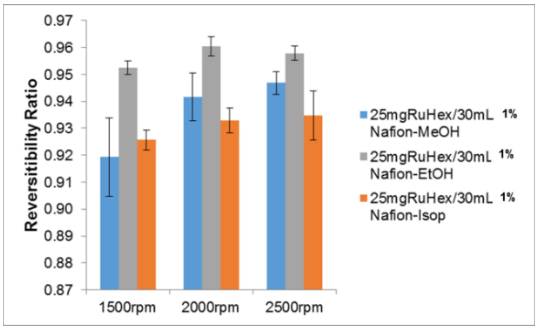


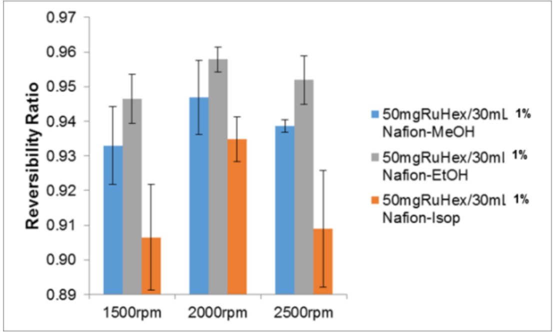
Fig. 3. Cyclic voltammogram of 75 mg RuHex – ethanol diluted electrodes

3.1.2. Reversibility ratio

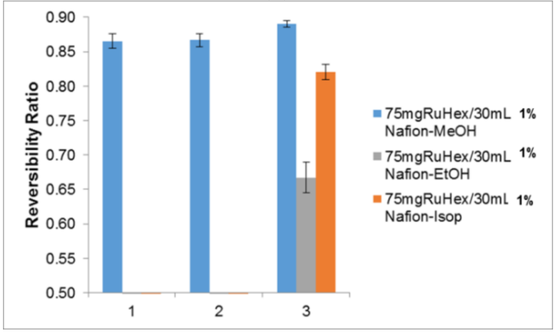
Plots of reversibility ratios, grouped by RuHex concentration, are shown in Fig. 4. Results show that reversibility ratios were highly reversible (above 0.9) except for the 75mg RuHex electrodes. In particular, the solvent type with the highest reversibility was ethanol, followed by methanol, and then isopropanol. Results for 75 mg RuHex electrodes were blank as its CV scans did not reveal any anodic peaks. The low reversibility ratio of the 75mg RuHex electrodes can be explained by the inconsistency in the homogeneity of the coating. The solutions were so saturated with RuHex that it was difficult to dissolve and homogenize the solution. They had a high chance of transitioning from a liquid phase to a semi-solid jelly-like phase. The increase in viscosity can be attributed to the intermolecular interactions between the Nafion’s sulfonate group [39-42]. The viscosity of the solutions shoot up after reaching a critical micelle concentration which is the point at which hydrophobic molecules aggregate and form micelles [43-45].



(a)



(b)

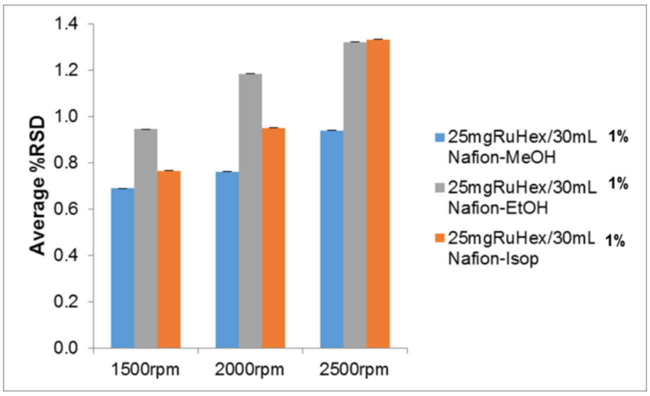


(c)

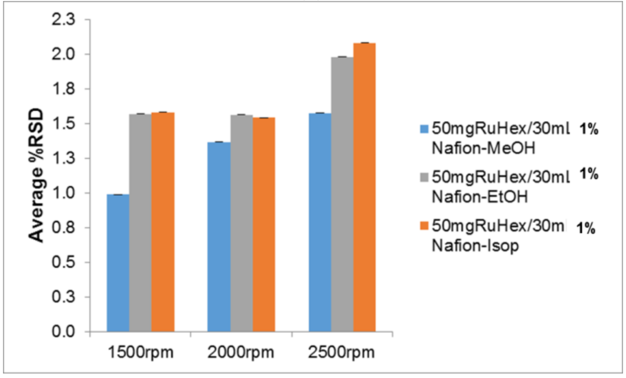
Fig. 4. Comparison of reversibility ratios for 25mg RuHex (a), 50mg RuHex (b), and 75mg RuHex (c) modified electrodes

3.1.3. Relative standard deviation

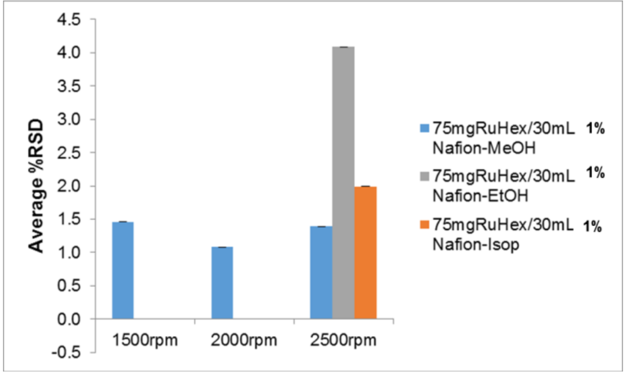
The %RSD plots are shown in Fig. 5. Overall 25mg RuHex electrodes have a lower %RSD compared to 50mg RuHex. In general, majority of results have a %RSD of less than 2% with methanol consistently having the lowest %RSD. A low %RSD simply means accuracy in the measurements.



(a)



(b)



(c)

Fig. 5. Comparison of %RSDs of 25mgRuHex (a), 50mg RuHex (b), and 75mg RuHex(c)

3.1.4. Multiple scan rate

The multiple scan rate plot in Fig. 6 shows voltammograms from scan rates of 0.03V/s to 0.3V/s. The plots display perfect reversibility in all scans. The peaks were plotted against the square root of the scan rate and shown in Fig. 7. A linear relationship was observed which signifies that the redox mediator immediately underwent redox reaction and did not require any charging-up period.

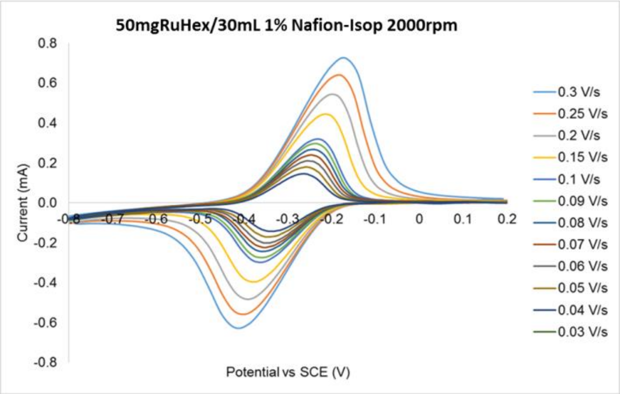


Fig. 6. Multiple scan rate voltammogram for isopropanol-diluted 50mg RuHex electrode

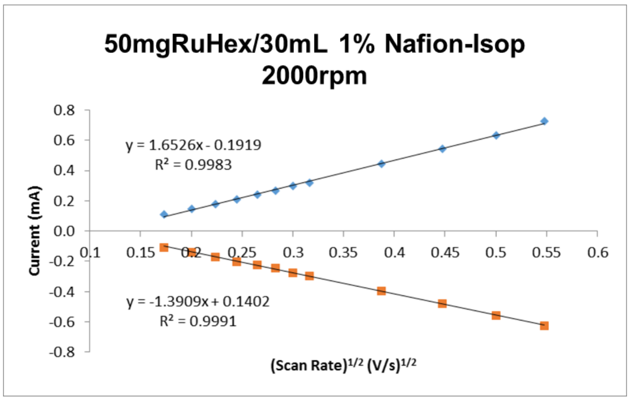


Fig. 7. Peak height vs the square root of the scan rate of isopropanol diluted 50mg RuHex electrode

3.2. Statistical Analysis

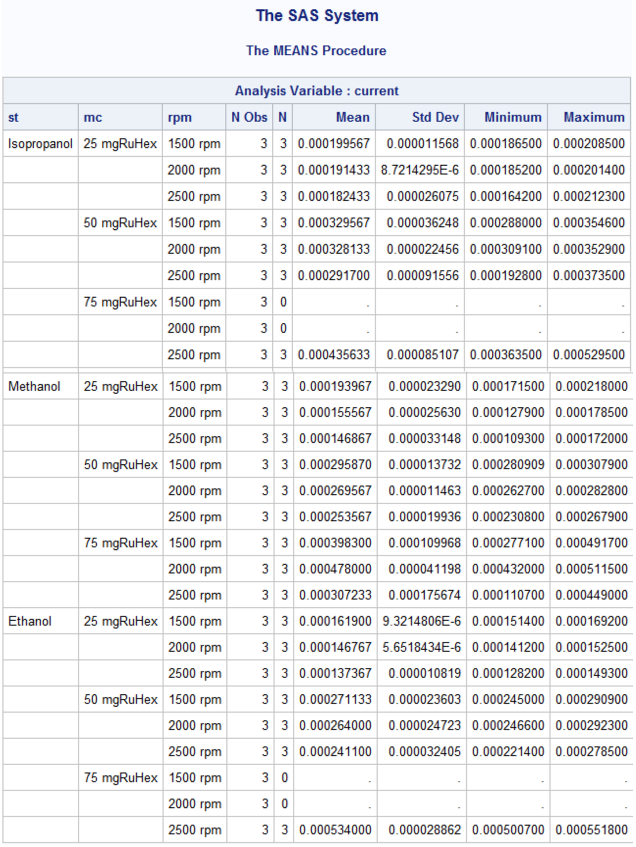
3.2.1. Peak current

The previous section showed a descriptive analysis of the behavior of the anodic current in response to solvent type (st), mediator (RuHex) concentration (mc), and spin rate (rpm). This section discusses the statistical analysis of the empirical data in the study. It is more inferential since it establishes the trend; hence, it gives a generalization beyond the scope of the data collected. The general protocol for factorial designs is that if there are more than one (1) factors, start with the highest order interaction effect. In this study, it is a three – way interaction, hence the 3-way factorial ANOVA was used initially. The type IV sums of squares option was used in the 3-way factorial ANOVA due to the presence of missing or blank cells.

Table 3 shows the descriptive statistics for the dependent variable CURRENT across the 27 treatment combinations of the factors SOLVENT TYPE, ST (Isopropanol, Methanol, Ethanol), MEDIATOR CONCENTRATION, MC (25mgRuHex/30mL, 50mgRuHex/30mL, 75mgRuHex/30mL), and SPIN COATING ANGULAR SPEED (1500rpm, 2000rpm, 2500rpm).

Results from a three-factor factorial analysis of variance (3-WAY ANOVA) using a 5% significance level indicate that there is no significant interaction effect among the three factors in the analysis ($p = 0.9989$). On the other hand, the two-way interaction effects between MC and RPM ($p = 0.0825$) and ST and RPM ($p = 0.9779$) are not significant while that between ST and MC is significant ($p = 0.0026$).

Table 3. Descriptive statistics for current across 27 treatment combinations



The SAS System

The MEANS Procedure

Analysis Variable : current

st	mc	rpm	N Obs	N	Mean	Std Dev	Minimum	Maximum
Isopropanol	25 mgRuHex	1500 rpm	3	3	0.000199567	0.000011568	0.000186500	0.000208500
		2000 rpm	3	3	0.000191433	8.7214295E-6	0.000185200	0.000201400
		2500 rpm	3	3	0.000182433	0.000026075	0.000164200	0.000212300
	50 mgRuHex	1500 rpm	3	3	0.000329567	0.000036248	0.000288000	0.000354600
		2000 rpm	3	3	0.000328133	0.000022456	0.000309100	0.000352900
		2500 rpm	3	3	0.000291700	0.000091556	0.000192800	0.000373500
	75 mgRuHex	1500 rpm	3	0	.	.	.	.
		2000 rpm	3	0	.	.	.	.
		2500 rpm	3	3	0.000435633	0.000085107	0.000363500	0.000529500
Methanol	25 mgRuHex	1500 rpm	3	3	0.000193967	0.000023290	0.000171500	0.000218000
		2000 rpm	3	3	0.000155567	0.000025630	0.000127900	0.000178500
		2500 rpm	3	3	0.000146867	0.000033148	0.000109300	0.000172000
	50 mgRuHex	1500 rpm	3	3	0.000295870	0.000013732	0.000280909	0.000307900
		2000 rpm	3	3	0.000269567	0.000011463	0.000262700	0.000282800
		2500 rpm	3	3	0.000253567	0.000019936	0.000230800	0.000267900
	75 mgRuHex	1500 rpm	3	3	0.000398300	0.000109968	0.000277100	0.000491700
		2000 rpm	3	3	0.000478000	0.000041198	0.000432000	0.000511500
		2500 rpm	3	3	0.000307233	0.000175674	0.000110700	0.000449000
Ethanol	25 mgRuHex	1500 rpm	3	3	0.000161900	9.3214806E-6	0.000151400	0.000169200
		2000 rpm	3	3	0.000146767	5.6518434E-6	0.000141200	0.000152500
		2500 rpm	3	3	0.000137367	0.000010819	0.000128200	0.000149300
	50 mgRuHex	1500 rpm	3	3	0.000271133	0.000023603	0.000245000	0.000290900
		2000 rpm	3	3	0.000264000	0.000024723	0.000246600	0.000292300
		2500 rpm	3	3	0.000241100	0.000032405	0.000221400	0.000278500
	75 mgRuHex	1500 rpm	3	0	.	.	.	.
		2000 rpm	3	0	.	.	.	.
		2500 rpm	3	3	0.000534000	0.000028862	0.000500700	0.000551800

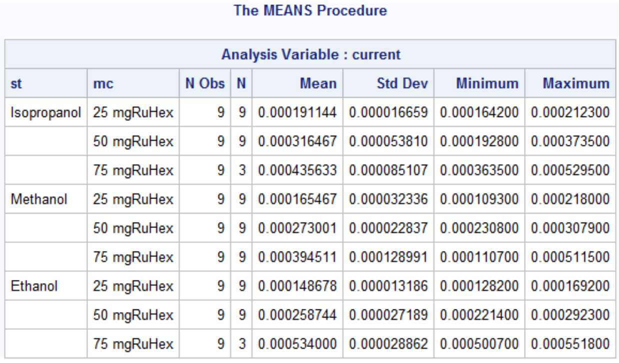
The significance of the combined effect of mediator concentration and solvent on the anodic current proves that the main mechanism in the measurement of the transport property of the electrode is the RuHex concentration in which the RuHex ions provide the vehicle for electron hopping. More electrons reach the working electrode from the analyte because of the RuHex ions on which they hop.

The greater the RuHex concentration, the higher the anodic current.

However, we cannot take the mediator concentration alone since a two-way interaction effect is established by solvent type and mediator concentration (st*mc) with a p-value of 0.0026.

Table. 4 shows the descriptive statistics for the dependent variable CURRENT across the 9 treatment combinations of the factors SOLVENT TYPE (Isopropanol, Methanol, Ethanol) and MEDIATOR CONCENTRATION (25mgRuHex/30mL, 50mgRuHex/30mL, 75mgRuHex/30mL).

Table 4. Descriptive statistics for current across 9 treatment combinations



The MEANS Procedure

Analysis Variable : current							
st	mc	N Obs	N	Mean	Std Dev	Minimum	Maximum
Isopropanol	25 mgRuHex	9	9	0.000191144	0.000016659	0.000164200	0.000212300
	50 mgRuHex	9	9	0.000316467	0.000053810	0.000192800	0.000373500
	75 mgRuHex	9	3	0.000435633	0.000085107	0.000363500	0.000529500
Methanol	25 mgRuHex	9	9	0.000165467	0.000032336	0.000109300	0.000218000
	50 mgRuHex	9	9	0.000273001	0.000022837	0.000230800	0.000307900
	75 mgRuHex	9	9	0.000394511	0.000128991	0.000110700	0.000511500
Ethanol	25 mgRuHex	9	9	0.000148678	0.000013186	0.000128200	0.000169200
	50 mgRuHex	9	9	0.000258744	0.000027189	0.000221400	0.000292300
	75 mgRuHex	9	3	0.000534000	0.000028862	0.000500700	0.000551800

Results from a two-factor factorial analysis of variance (2-WAY ANOVA) using a 5% significance level indicate that there is a significant interaction effect between ST and MC, ignoring RPM, on the response variable CURRENT (p = 0.0101).

Table 5 summarizes the results of the Tukey-Kramer post-hoc test at the 5% significance level. The Tukey-Kramer groupings in the last column indicate the ST-MC treatment combinations belonging to a homogeneous subgroup (same letter code). To illustrate, the mean current under the ST-MC treatment combination Ethanol-75mgRuHex and the mean current under the ST-MC treatment combination Isopropanol-75mgRuHex do not differ significantly (code a). While the mean current of these two ST-MC treatment combinations (code a) differs significantly from the mean current of the other ST-MC treatment combinations (codes b to e). The optimized electrodes showing the greatest anodic current are those of greatest RuHex concentration with either ethanol or isopropanol as solvents.

The results in Table 5 are also shown in the ST-MC interaction plot that follows (please refer to the ST-MC code* in the table).

Fig. 8 shows that the 75 mg RuHex concentration yields consistently the highest anodic currents. The difference in the slopes of the 3 plots are indicative of a statistical interaction effect between mediator concentration (MC) and solvent type (ST). This signifies a combined effect of the mediator concentration and solvent on the anodic current. This is validated by the p-value in the ANOVA table. The same slope means that there is no significant interaction between the factors in the interaction plot

Table 5. Tukey-Kramer post-hoc test for current

Solvent Type	Mediator Concentration	ST-MC CODE*	Mean Current (in descending order)	95% Confidence Limits	Tukey-Kramer Grouping
Ethanol	75mg RuHex	3,3	0.000534000	(0.000468, 0.000600)	a
Isopropanol	75mg RuHex	1,3	0.000435633	(0.000370, 0.000501)	a, b
Methanol	75mg RuHex	2,3	0.000394511	(0.000357, 0.000432)	b
Isopropanol	50mg RuHex	1,2	0.000316467	(0.000279, 0.000354)	b, c
Methanol	50mg RuHex	2,2	0.000273001	(0.000235, 0.000311)	c, d
Ethanol	50mg RuHex	3,2	0.000258744	(0.000221, 0.000297)	c, d
Isopropanol	25mg RuHex	1,1	0.000191144	(0.000153, 0.000229)	d, e
Methanol	25mg RuHex	2,1	0.000165467	(0.000128, 0.000203)	e
Ethanol	25mg RuHex	3,1	0.000148678	(0.000111, 0.000187)	e

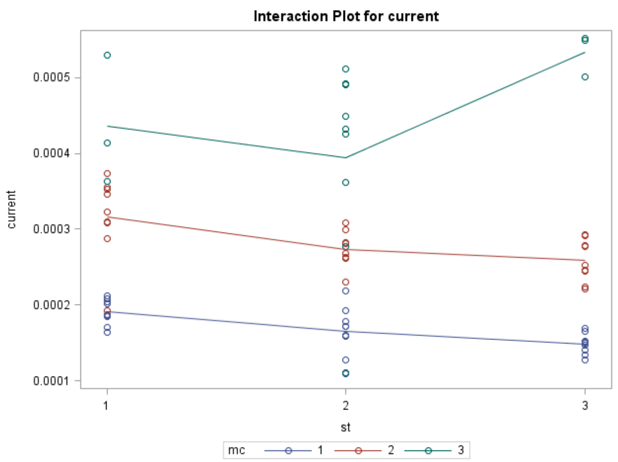


Fig. 8. Interaction plot for current

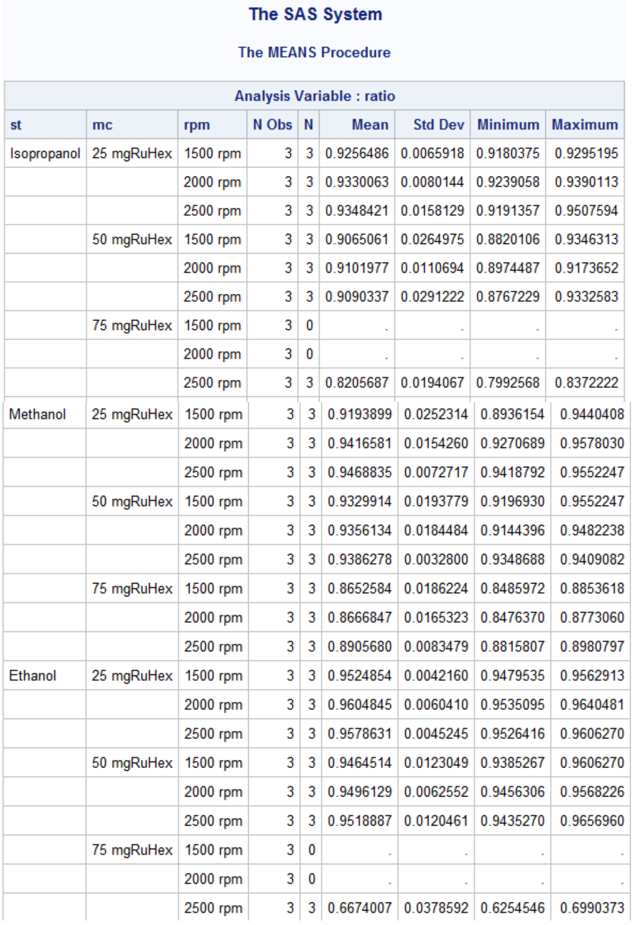
3.2.2. Reversibility Ratio

Table 6 shows the descriptive statistics for the dependent variable Reversibility Ratio (RR) across the 27 treatment combinations of the factors SOLVENT TYPE (Isopropanol, Methanol, Ethanol), MEDIATOR CONCENTRATION (25mgRuHex, 50mgRuHex, 75mgRuHex), and SPIN COATING ANGULAR SPEED (1500rpm, 2000rpm, 2500rpm). Note that the mean RR is lowest (below 0.9rpm) when the treatment combination of the three factors involves a mediator concentration of 75mgRuHex. Otherwise, for the other treatment combinations involving a mediator concentration of 25mgRuHex or 50mgRuHex, the mean RR is above 0.9.

Results from a three-factor factorial analysis of variance (3-WAY ANOVA) under the type IV sums of squares option using a 5% significance level indicate that there is no significant interaction effect among the three factors in the analysis ($p = 0.9395$) on the reversibility ratio. The same can be said about the two-way interaction effects between MC and RPM ($p = 0.5822$) and ST and RPM ($p = 0.9296$). On the other hand, there is a significant two-way interaction effect between ST and MC ($p < 0.0001$) on RR. The main effect of RPM on RR is not significant ($p = 0.0947$).

Table 7 shows the descriptive statistics for the dependent variable RR across the 9 treatment combinations of the factors SOLVENT TYPE (Isopropanol, Methanol, Ethanol) and MEDIATOR CONCENTRATION (25mgRuHex, 50mgRuHex, 75mgRuHex), ignoring RPM. Again, note that the mean RR is lowest (below 0.9) when the treatment combination of the two factors involves a mediator concentration of 75mgRuHex. Otherwise, for the other treatment combinations involving a mediator concentration of 25mgRuHex or 50mgRuHex, the mean RR is above 0.9.

Table 6. Descriptive statistics for reversibility ratio across 27 treatment combinations

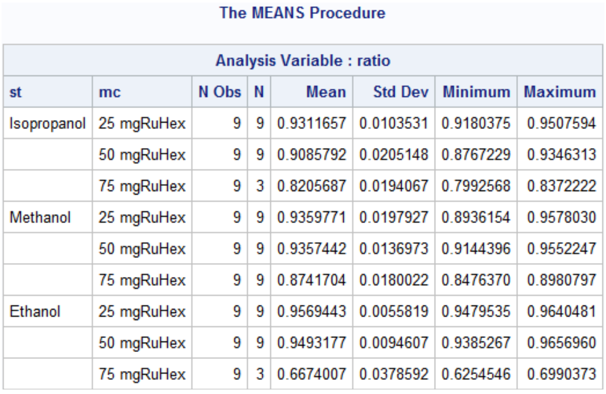


The SAS System

The MEANS Procedure

Analysis Variable : ratio								
st	**mc**	**rpm**	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**Maximum**
Isopropanol	25 mgRuHex	1500 rpm	3	3	0.9256486	0.0065918	0.9180375	0.9295195
		2000 rpm	3	3	0.9330063	0.0080144	0.9239058	0.9390113
		2500 rpm	3	3	0.9348421	0.0158129	0.9191357	0.9507594
	50 mgRuHex	1500 rpm	3	3	0.9065061	0.0264975	0.8820106	0.9346313
		2000 rpm	3	3	0.9101977	0.0110694	0.8974487	0.9173652
		2500 rpm	3	3	0.9090337	0.0291222	0.8767229	0.9332583
	75 mgRuHex	1500 rpm	3	0	.	.	.	.
		2000 rpm	3	0	.	.	.	.
		2500 rpm	3	3	0.8205687	0.0194067	0.7992568	0.8372222
Methanol	25 mgRuHex	1500 rpm	3	3	0.9193899	0.0252314	0.8936154	0.9440408
		2000 rpm	3	3	0.9416581	0.0154260	0.9270689	0.9578030
		2500 rpm	3	3	0.9468835	0.0072717	0.9418792	0.9552247
	50 mgRuHex	1500 rpm	3	3	0.9329914	0.0193779	0.9196930	0.9552247
		2000 rpm	3	3	0.9356134	0.0184484	0.9144396	0.9482238
		2500 rpm	3	3	0.9386278	0.0032800	0.9348688	0.9409082
	75 mgRuHex	1500 rpm	3	3	0.8652584	0.0186224	0.8485972	0.8853618
		2000 rpm	3	3	0.8666847	0.0165323	0.8476370	0.8773060
		2500 rpm	3	3	0.8905680	0.0083479	0.8815807	0.8980797
Ethanol	25 mgRuHex	1500 rpm	3	3	0.9524854	0.0042160	0.9479535	0.9562913
		2000 rpm	3	3	0.9604845	0.0060410	0.9535095	0.9640481
		2500 rpm	3	3	0.9578631	0.0045245	0.9526416	0.9606270
	50 mgRuHex	1500 rpm	3	3	0.9464514	0.0123049	0.9385267	0.9606270
		2000 rpm	3	3	0.9496129	0.0062552	0.9456306	0.9568226
		2500 rpm	3	3	0.9518887	0.0120461	0.9435270	0.9656960
	75 mgRuHex	1500 rpm	3	0	.	.	.	.
		2000 rpm	3	0	.	.	.	.
		2500 rpm	3	3	0.6674007	0.0378592	0.6254546	0.6990373

Table 7. Descriptive statistics for reversibility ratios across 9 treatment combinations



The MEANS Procedure

Analysis Variable : ratio							
st	**mc**	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**Maximum**
Isopropanol	25 mgRuHex	9	9	0.9311657	0.0103531	0.9180375	0.9507594
	50 mgRuHex	9	9	0.9085792	0.0205148	0.8767229	0.9346313
	75 mgRuHex	9	3	0.8205687	0.0194067	0.7992568	0.8372222
Methanol	25 mgRuHex	9	9	0.9359771	0.0197927	0.8936154	0.9578030
	50 mgRuHex	9	9	0.9357442	0.0136973	0.9144396	0.9552247
	75 mgRuHex	9	9	0.8741704	0.0180022	0.8476370	0.8980797
Ethanol	25 mgRuHex	9	9	0.9569443	0.0055819	0.9479535	0.9640481
	50 mgRuHex	9	9	0.9493177	0.0094607	0.9385267	0.9656960
	75 mgRuHex	9	3	0.6674007	0.0378592	0.6254546	0.6990373

Results from a two-factor factorial analysis of variance (2-WAY ANOVA) using a 5% significance level indicate that there is a significant interaction effect between ST and MC, ignoring RPM, on the response variable RR ($p < 0.0001$). The spin rate can be analyzed alone since it has no interaction effect with the other two factors.

Although st and mc have $p < 0.0001$, individually, they must not be evaluated alone because of their respective interaction effect with each other (st*mc with $p < 0.0001$).

Statistical interaction effect depends on the combination of two factors. This means that in statistics, solvent type alone or mediator concentration alone cannot be considered as significant because of the statistical interaction effect of one on the other. Hence, the significance of one factor, say ST, cannot be discussed without the other, say MC.

Table 8 summarizes the results of the Tukey-Kramer post-hoc test at the 5% significance level. The Tukey-Kramer groupings in the last column indicate the ST-MC treatment combinations belonging to a homogeneous subgroup (same letter code). To illustrate, the mean reversibility ratios (RR) under the ST-MC treatment combinations Ethanol-25mgRuHex, Ethanol-50mgRuHex, Methanol-25mgRuHex, and Methanol-50mgRuHex do not differ significantly (code a). While the mean RRs of these four ST-MC treatment combinations (code a) differ significantly from the mean RRs of the other five ST-MC treatment combinations (codes b to f). The results in this table are also shown in the ST-MC interaction plot for the Reversibility Ratio (RR) that follows (please refer to the ST-MC code* in the table).

Table 8. Tukey-Kramer post-hoc test for reversibility ratio

Solvent Type	**Mediator Concentration**	**ST-MC CODE***	**Reversibility Ratio (in descending order)**	**95% Confidence Limits**	**Tukey-Kramer Grouping**
Ethanol	25mg RuHex	3,1	0.9569443	(0.946043, 0.967845)	a
Ethanol	50mg RuHex	3,2	0.9493177	(0.938417, 0.960219)	a, b
Methanol	25mg RuHex	2,1	0.9359771	(0.925076, 0.946878)	a, b
Methanol	50mg RuHex	2,2	0.9357442	(0.924843, 0.946645)	a, b
Isopropanol	25mg RuHex	1,1	0.9311657	(0.920265, 0.942067)	b, c
Isopropanol	50mg RuHex	1,2	0.9085792	(0.897678, 0.919480)	c
Methanol	75mg RuHex	2,3	0.8741704	(0.863270, 0.885071)	d
Isopropanol	75mg RuHex	1,3	0.8205687	(0.801688, 0.839449)	e
Ethanol	75mg RuHex	3,3	0.6674007	(0.648520, 0.686282)	f

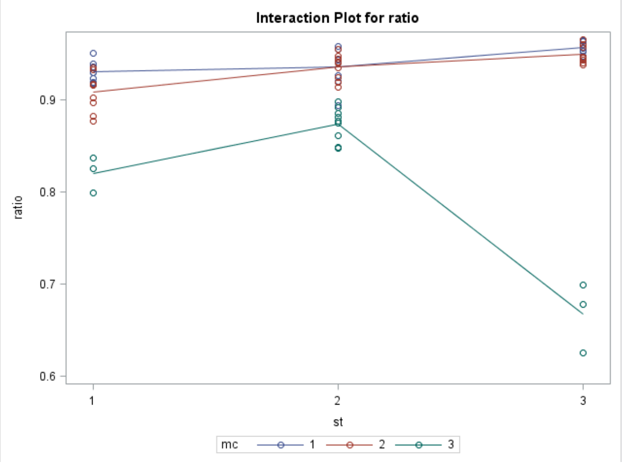


Fig. 9. Interaction plot for reversibility ratio

The different slopes for all three lines in Fig. 9 indicate the presence of interaction between solvent type and mediator concentration (st*mc). There is no significant difference between 25mg and 50mg RuHex as far as methanol and ethanol solvent types are concerned. RuHex concentrations (25mg and 50mg) for isopropanol also have no significant difference. RuHex concentrations (25mg and 50mg) for all solvent types are statistically equal. For 75mgRuHex, the solvent types are significantly different from each other. In statistics, the solvent types are significantly different from each other since they have remarkable difference in the reversibility ratios for all three solvent types. This is due to the viscosity and the formation of micelles in the 75 mg RuHex electrodes. The RuHex solution was just too viscous that the thin films were just not homogeneous.

4. Conclusions

Anodic current peak, reversibility, and stability were selected as parameters to determine the best electrode because of the following: electrodes with sharp anodic current peaks indicate the abundance of redox mediators that ensure a rapid electron transfer; high reversibility indicates that the redox mediator is stable and secured in the Nafion matrix; and even after ten scans, the modified electrode did not exhibit any degradation.

With all parameters considered, the modified electrode with 50mgRuHex/30mL, using isopropanol as solvent, and spun at 2000rpm was chosen as the best. The chosen electrode had the highest anodic current peak, very high reversibility ratio (~1), and high stability (RSD=1.5% even after 10 scans). Consequently, the electrode which was chosen as the best was used to fabricate more electrodes for stripping voltammetry tests.

Acknowledgments

This work was supported in part by the Department of Science and Technology – Science Education Institute (DOST-SEI) of the Republic of the Philippines and the University Research Coordination Office (URCO) of De La Salle University, Manila, Philippines.

References

[1] S. Palisoc, M. Natividad, P. DeVera, B. Tuason, Phil. Sci. Lett. **7**, 372 (2014).
[2] S. Palisoc, M. Natividad, P. DeVera, B. Tuason, J. N. Mat. Electr. Sys. **17**, 205 (2014).
[3] S. Palisoc, M. Natividad, N. Martinez, R. Ramos, K. Kaw, e-Polymers **16**, 117 (2016).
[4] S. Palisoc, B. Tuason, M. Natividad, Optoelectron. Adv. Mat. **9**, 1435 (2015).
[5] S. Palisoc, M. Natividad, F. Co, K. Kaw, Optoelectron. Adv. Mat. **9**, 1010 (2015).
[6] K. Kaw, S. Palisoc, M. Natividad, Phil. Sci. Lett. **1**, 171 (2014).
[7] B. Tuason, S. Palisoc, M. Natividad, IOP Conf. Series: Materials Science and Engineering **79**, 012032 (2015).
[8] S. Palisoc, M. Natividad, D. M. Calde, E. R. Rosopa, J. N. Mat. Electr. Sys. **19**, 223 (2017).
[9] S. Palisoc, N. C. Valeza, M. Natividad, Int. J. Electrochem. Sci. **12,** 3859 (2017).
[10] S. Palisoc, M. Natividad, C. E. A. Tan, J. N. Mat. Electr. Sys. **20**, 089 (2017).
[11] S. Palisoc, K. Kaw, M. Natividad, J. Robles, J. N. Mat. Electr. Sys. **20**, 077 (2017).
[12] S. Palisoc, A. M. Causing, M. Natividad, Anal. Methods (2017).
[13] S. Bouden, A. Dahi, F. Hauquier, H. Randriamahazaka, J. Ghilane, Sci. Rep. **6,** 36708 (2016).
[14] R. A. Soomro, M. M. Tunesi, S. Karakus, N. Kalwara, RSC Adv. **7**, 19353 (2017).
[15] T. Chowdhurya, A. A. Shaikh, H. Akter, M. M. Neaz, P. K. Bakshi, A. J. Saleh Ahammad, ECS Solid State Lett. **3,** 14 (2014).
[16] Y. Wang, L. Zhu, Y. Hu, Z. Deng, Z. Lou, Y. Hou, F. Teng, Opt. Express **25**, 7719 (2017).
[17] W. Deng, C. Chu, S. Ge, J. Yu, M. Yan, X. Song, Microchim. Acta **182**, 1009 (2015)
[18] W. A. El-Said, J-W. Choi, Electrochimica Acta **123**, 51 (2014).
[19] Y. Watanabe, K. Suemori, S. Hoshino, AIP Adv. **6**, 065121 (2016).
[20] J. Sempionatto, L. Recco, V. Pedros, J. Braz. Chem. Soc. **25**, 453 (2014).
[21] S. Chaiyo, E. Mehmeti, K. Zagar, W. Siangproh, O. Chailapakul, K. Kalcher, Analytica Chimica Acta. **918**, 26 (2016).
[22] Y. Wang, J. G. Limon-Petersen, R. G. Compton, Journal of Electroanalytical Chemistry **652**, 13 (2011).
[23] Strem Chemicals Inc. (n.d.). Hexaammineruthenium(III) Chloride as an Electron Mediator for Rapid Whole Blood Glucose Detection. Retrieved on 19 Nov., 2014 from http://www.strem.com/uploads/resources/documents/hexaammineruthenium_chloride_lit_sheet_copy1.pdf.
[24] M. Steichen, Th. Deneux, C. Buess-Herman Electrochimica Acta **53**, 6202-6208. (2008).
[25] G. J. Li, L. H. Liu, X. W. Qi, Y. Q. Guo, W. Sun, X. L. Li, Electrochimica Acta. **63**, 312 (2012).
[26] J. Wang, S. Zhang, Y. Zhang, Analytical Biochemistry. **396**, 304 (2010).
[27] J. Chen, J. Tang, J. Zhou, L. Zhang, G. Chen, D. Tang, Analytical Chimica Acta. **810**, 10 (2014).
[28] J. Zhang, Y. Yuan, S. B. Xie, Y. Chai, R. Yan, Biosensors and Bioelectronics **60**, 224 (2014).
[29] H. J. Kim, K. S. Yun, J. Kwak, Electrochimica Acta **50**, 205 (2004).
[30] L. Chen, Z. Li, Y. Meng, P. Zhang, Z. Su, Y. Liu, Y. Huang, Y. Zhou, Q. Xie, S. Yao, Sensors and Actuators B: Chemical. **191**, 94 (2014).
[31] I. A. Ges, F. Baudenbacher, Enzyme-coated microelectrodes to monitor lactate production in a nanoliter microfluidic cell culture device. Biosensors and Bioelectronics. **26**, 828 (2010).
[32] A. P. Soldatkin, A. V. El'skaya, A. A. Shul'ga, L. I. Netchiporouk, A. M. Nyamsi Hendji, N. Jaffrezic-Renault, C. Martelet, Anal. Chim. Acta **283**, 695 (1993).
[33] J. Murimboh, M. T. Lam, N. M. Hassan, C. L. Chakrabarti, Anal. Chim. Acta **423**, 115 (2000).
[34] J. M. Zen, F. S. Hsu, N. Y. Chi, S. Y. Huang, M. J. Chung, Anal Chim Acta **310**, 407 (1995).
[35] T. Onikubo, L. R. Jang, M. Kaneko, Journal of Electroanalytical Chemistry **361**, 143 (1993).
[36] J. Li, S. Guo, Y. Zhai, E. Wang, Anal. Chim. Acta **649**, 196 (2009).

[37] T. C. Jao, G. B. Jung, S. C. Kuo, W. J. Tzeng, A. Su, International Journal of Hydrogen Energy. **37**, 13623 (2012).

[38] T. T. Ngo, T. L. Yu, H. S. Lin, Journal of Power Sources. **225**, 293 (2013).

[39] R. N. Goyal, V. K. Gupta, M. Oyama, N. Bachheti, Electrochemistry Communications. 7(8), 803 (2005).

[40] J. W. Shie, U. Yogeswaran, S. M. Chen, Talanta **78**, 896 (2009).

[41] Q. Guo, J. Shao, T. Sun, H. Li, S. Lan, Z. Xu, Electrochimica Acta **56**, 1432 (2011).

[42] Y. Wang, S. Wang, M. Xiao, S. Song, D. Han, M. A. Hickner, Y. Meng, International Journal of Hydrogen Energy **39**, 16123 (2014).

[43] Perkin Elmer. (n.d.). Atomic Spectroscopy: A Guide to Selecting the Appropriate Technique and System. Retrieved on 30 March 2016 from https://www.perkinelmer.com/PDFs/downloads/BRO_WorldLeaderAAICPMSICPMS.pdf

[44] K. Jha, A. Bhattarai, S. J. Chatterjee, BIBECHANA **11**, 131 (2014).

[45] N. Sata, K. Tyuzyo, Colloid and Polymer Science **175**, 40-50. (1961).

---

[*] Corresponding author: michelle.natividad@dlsu.edu.ph