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VOLTAMMETRIC ANALYSIS OF HYDROQUINONE IN SKIN WHITENING COSMETIC USING FERROCENE MODIFIED CARBON PASTE ELECTRODE

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ABSTRACT

Hydroquinone is the most effective whitening agent in skin whitening cosmetics. It has the ability to whiten the skin in a relatively shorter time compared to other whitening agents. However, based on the regulations of the Indonesian Food and Drug Administration (BPOM) and the United States Food and Drug Administration (FDA), the use of hydroquinone in whitening cosmetics is prohibited and is given zero tolerance. Therefore, it is the aim of this study to develop an alternative method to analyze the presence of hydroquinone in cosmetics that is easier, inexpensive, selective, and accurate. The result showed that the best composition of the electrode membrane which gave the best outcome was carbon: paraffin: ferrocene with each ratio of 6 : 3: 1. The validity of the hydroquinone analysis method that uses carbon/ferrocene paste as the electrode shows good results with the working range of 0.20-10 μM , the sensitivity of 10.436 $\mu\text{A}/\mu\text{M}$, and detection limit of 0.06 μM . It is also selective in the analysis of a mixture of hydroquinone and arbutin. These electrodes have excellent accuracy in recovering hydroquinone analysis in cosmetic samples with an average recovery value of 99%. This electrode can also be used for arbutin analysis in cosmetics with an accuracy of 99%. This method is suitable to be used in skin whitening analysis, both hydroquinone and arbutin.

Keywords: Hydroquinone, Arbutin, Voltammetry, Ferrocene, Cosmetics, Whitening Agent

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INTRODUCTION

Hydroquinone is one of the most effective whitening agents compared to other whitening agents because it has the ability to whiten the skin in a relatively short time with only low concentration.¹ However, based on the regulations of the Indonesian Food and Drug Administration and the Food And Drug Administration (FDA), the use of hydroquinone in whitening cosmetics is prohibited and is given zero tolerance due to its negative impact on health.² There are many side effects of using hydroquinone in whitening cosmetics such as skin irritation, skin redness, burning sensation, and black patches on the skin. Meanwhile, the long-term side effects of hydroquinone can lead to skin cancer, kidney and liver function disorders.¹ However, in case of medical purposes such as treatment of hyperpigmentation, hydroquinone is still permitted under the strict supervision of a doctor because hydroquinone is on the list of hard drugs.³ The Indonesian Food and Drug Supervisory Agency notes that there are still many labeled whitening cosmetics containing hydroquinone. Those whitening cosmetics are also circulating illegally on the market, not to mention that the concentration level is relatively high which is more than 2%. Odumosu & Ekwe (2010) analyzed the presence of hydroquinone in 10 whitening cosmetics on the market and they

were all tested positive in containing hydroquinone ranging from 2% to 5% of concentration level.⁴ A similar test was done by Siddique et al. (2012) who found that 11 out of the 22 whitening cosmetics analyzed were tested positive in containing hydroquinone with various concentration levels ranging from 0.002% to 0.092%.⁵ Based on the result of several tests, it was proven that the use of hydroquinone in skin whitening cosmetics is still widely used and circulated in the community. Therefore, it is needed to develop a faster method that is also inexpensive and accurate to detect the use of hydroquinone in cosmetics. The method is expected to be the first step in preventing and controlling the negative effects caused by the use of these compounds.

Various methods have been developed for hydroquinone analysis, including High-Performance Liquid Chromatography (HPLC),⁶ GC-MS,⁷ Chemiluminescence Flow Injection,⁸ Capillary electrophoresis⁹ and Spectrophotometry.⁴ Some of the methods that have been developed have good sensitivity, but have complicated sample preparation stages and relatively expensive analytical costs. This is what drives the need for the development of hydroquinone analysis methods that are not only easier, cheaper, faster, and more efficient, but are also sensitive, selective and accurate. Hydroquinone is an electroactive compound that can undergo oxidation and reduction reactions so that it can be detected by electrometry. The development of hydroquinone analysis using the voltammetry method has been carried out by developing various types of working electrodes.¹⁰⁻¹⁵

In this study, the working electrode used was carbon paste electrode (CPE) which was modified by ferrocene (Fc). The application of carbon as a working electrode is based on the inert nature of carbon. A good conductive material that has a wide potential range besides carbon is also cheap and easy to obtain.¹⁶ Ferrocene functions as a mediator to increase the electrocatalytic activity of redox reactions.¹⁷ Ferrocene that is used as a mediator of the voltammetry method has been carried out in the analysis of dopamine¹⁷ and levodopa¹⁸. Based on the result of several studies, the addition of ferrocene increased the sensitivity of analyte measurements. The selectivity of the Fc / CPE electrode was studied by the detection of hydroquinone in its mixture with the skin whitening compound named arbutin (hydroquinone- β -D-glukopyranoside) since these compounds are widely used as a substitute for hydroquinone. Based on the result of the study, arbutin compounds can be hydrolyzed to hydroquinone by the skin's bacterial activity.¹⁹

EXPERIMENTAL

Chemicals and Reagents

Activated carbon was obtained from the Forest Products Research and Development Center, Bogor, Indonesia. This carbon is made from teak wood (*Tectona grandis*) which is activated at a temperature of 850 °C for 120 minutes. Ferrocene and hydroquinone were purchased from Sigma. All other used materials were in an analytical reagent grade.

Apparatus

Voltammetric experiments were performed using a potentiostat edaq e-corder 201 (model ed201) which is equipped with 3 pieces of electrodes, namely the reference electrode (Ag/AgCl), auxiliary electrode (Pt), and ferrocene modified carbon paste (Fc/CPE) as the working electrode.

Electrode Preparation

The electrodes were prepared by mixing carbon, paraffin pastilles, and ferrocene with variations in the composition of ferrocene (w/w). Electrodes with a composition of 10% ferrocene consist of carbon, paraffin, and ferrocene with a ratio of 6: 3: 1. Other compositions are regulated by changing the amount of ferrocene and carbon, while paraffin is fixed. The mixture in the watch glass was heated at 40 °C using a hotplate until the paraffin melts, then the mixture was stirred to form a paste. The warm paste than was inserted into the micropipette tip (electrode body) which has been installed with copper wire and later it was pressed until there was no empty space in it. Afterward, the electrode surface was rubbed on HVS paper until it shines which indicates that the electrode surface is flat and smooth.

Measurement Voltammetry

Ferrocene/CPE was used as working electrode, Ag / AgCl was used as reference electrode, while platinum was used for the auxiliary electrode. The three electrodes were dipped in 1 mM hydroquinone

solution using the cyclic voltammetry (CV) technique in the potential range of -0.2 V to +1.0 V in order to study the effect of pH and scan rate. Linear sweep voltammetry (LSV) in the same potential range was used for quantitative analysis and validation. A phosphate buffer solution (PBS) is used to adjust the pH of the solution.

Sample Preparation

The sample used was a cosmetic cream free of whitening agent that later spiked with hydroquinone until the concentration reached 2%. It was then diluted and immediately analyzed using the LSV technique. In the same way, a cream that is routinely spiked with hydroquinone until the concentration reached 4% was also being diluted quantitatively and analyzed by LSV to determine accuracy.

RESULTS AND DISCUSSION

Characterization

The Scanning electron microscopy (SEM) of the teak wood carbon (*Tectona grandis*) which was activated by heating at 850 °C for 120 minutes shows that the activated carbon has various pore sizes and shapes (Fig.-1) and BET surface area was 861.900 m². Cyclic voltammogram in Fig.-2 and Table 1 data show that the value of ΔE_p in hydroquinone analysis with the Ferrocene/CPE electrode is in a smaller number compared to the value of ΔE_p on CPE electrode. This indicates that the Fc/CPE electrode provides better reversibility compared to the CPE electrode.

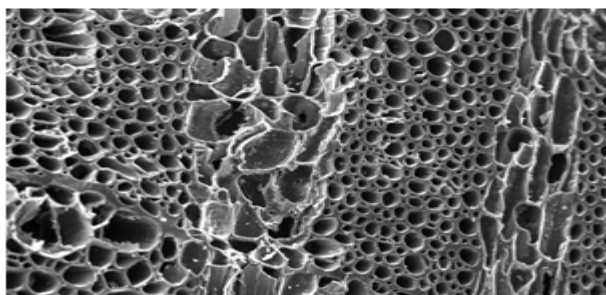


Fig.-1: SEM Image of the Activated Carbon

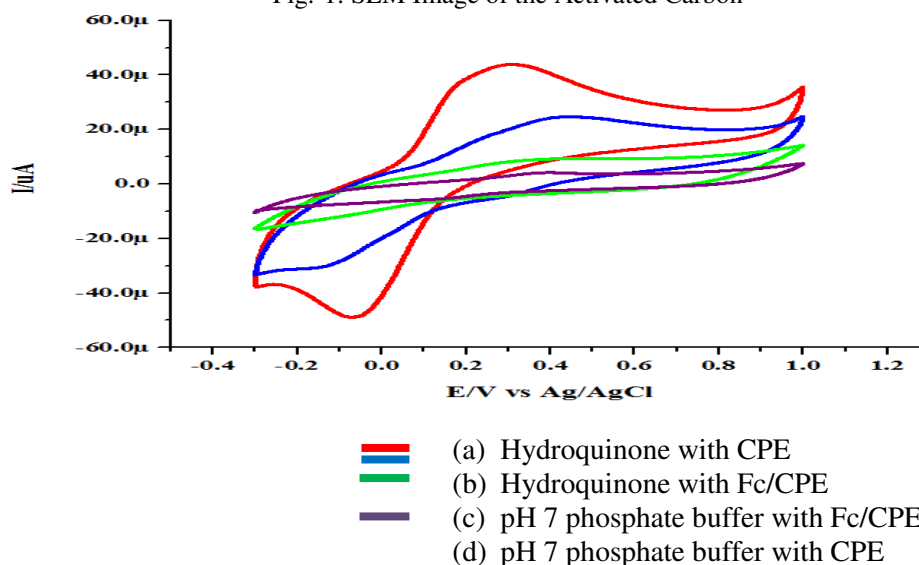


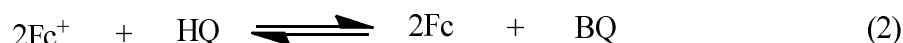
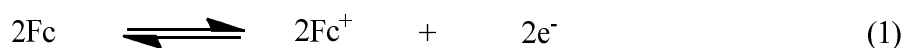
Fig.-2: Cyclic Voltammogram of 1mM Hydroquinone in pH 7 PBS with a Scan Rate of 100 mV/s by using 10 %Fc/CPE and CPE Electrodes

Ferrocene functions as a mediator for electron transfer, so that the electrons received and released in the reduction and oxidation reactions on the surface of the working electrode are faster.¹⁷The buffer solution functions as a supporting electrolyte that does not electroactive so it does not provide peak current.

Table-1: Data on E_{pa} , E_{pc} , I_{pa} , I_{pc} and ΔE_p of 1mM Hydroquinone Analysis

Electrode	E_{pa} (mV)	E_{pc} (mV)	ΔE_p (mV)	I_{pa} (μ A)	I_{pc} (μ A)
Fc/CPE	0.297	-0.060	0.357	31.880	-27.960
CPE	0.410	-0.137	0.547	12.567	-7.630

The magnitude of the separation value of anodic and cathodic peak potential (ΔE_p) can be used to determine the number of the transferred electrons during the voltammetry hydroquinone analysis process. The value of ΔE_p can also be used as a determinant of the reversibility criteria of the reaction.²⁰ In addition to showing a smaller separation of peak potential (ΔE_p), hydroquinone analysis with Fc/CPE working electrodes produces anodic and cathodic current peaks that are 2.5 times higher than using CPE. It indicates that with the addition of Ferrocene, the transfer speed of the electrons to the electrode surface becomes faster which allows greater production of electrode sensitivity and results in the higher current. The proposed mechanism for catalytic reaction on the Fc/CPE in hydroquinone analysis is as follows:



When the Fc /CPE is electrified from a negative potential to a positive potential, the Ferrocene contained in the working electrode is oxidized to form an Fc^+ ion. This ion then oxidizes hydroquinone (HQ) to form benzoquinone (BQ) by releasing 2 electrons while the Fc^+ ion is reduced by taking 2 electrons to form Fc. The occurrence of electron transfer during the oxidation process and the reduction of the analyte on the surface of the working electrode voltammetry produces a current response that is proportional to the amount of concentration of the analyte in the solution. The description of the hydroquinone reaction on the Fc/CPE surface can be seen in Fig.-3.

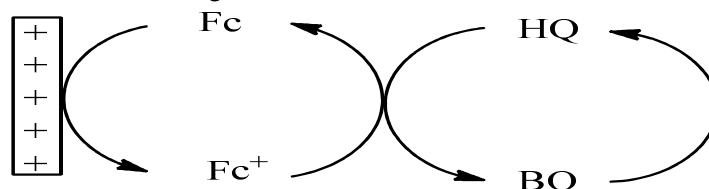


Fig.-3: Model of the Hydroquinone Reaction on Fc/CPE

In addition, to demonstrate the effectiveness of hydroquinone analysis, the Fc/CPE electrode also showed good stability in the solution. The result of 1mM hydroquinone analysis with repetitive measurements by 20 cycles can be seen in Fig.- 4. The voltammogram shows that by repeating the measurement of 1 mM hydroquinone in 20 cycles, there was no significant change in both anodic (E_{pa}) and anodic (I_{pa}) peak potential. The stability of electrocatalytic activity from the electrodes modified with Ferrocene indicates that the Ferrocene contained in the working electrode is insoluble and stable.

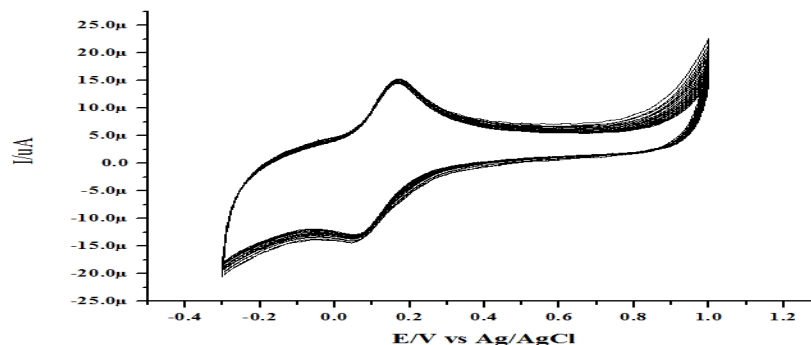


Fig.-4: Cyclic Voltammogram of 1 mM Hydroquinone Voltammogram using Fc/CPE Electrode with 20 Cycles of Repeated Measurements

Optimization of Working Electrode Composition

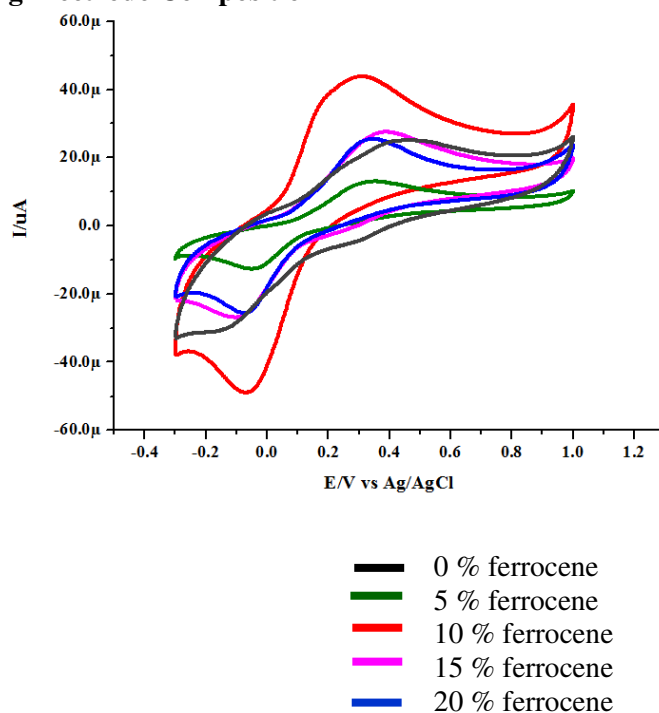


Fig.-5: Cyclic Voltammogram Influences the Composition of the Working Electrode on the Measurement of 1 mM Hydroquinone Solution in pH 7 PBS with a Scan Rate of 100 mV/s

The voltammogram in Fig.- 5 shows that the amount of Ferrocene in the working electrode composition influences the voltammetric response analysis of 1 mM hydroquinone in pH 7 of phosphate buffer. The addition of ferrocene in the working electrode increases the anodic current peak value and reaches the maximum value of 10 % ferrocene composition so that this composition was selected as the electrode in the subsequent analysis. These results indicate that a higher amount of Ferrocene in the working electrode produces higher peak currents of the anodic and cathodic since they act as electron transfer mediators. In addition to the peak current height, the reversibility of the reaction at the electrode surface must be considered. A voltammogram with a composition of 0% ferrocene gives a peak current higher than a peak current of 5% ferrocene, but has lower reversibility than an electrode with a composition of 5% ferrocene. Reversibility of the reaction can be seen from the distance between the anodic (E_{pa}) and cathodic peak potential (E_{pc}). Adjacent distances indicate increasingly close to the reversible reaction. However, it is also notable that if the composition of the working electrode is added with Ferrocene of more than 10 %, then the peak current decreases. It happened since there was an excessive amount of Ferrocene that therefore causes the carbon contained in the electrode composition to become less which leads to the decreasing electrical conductivity of the working electrode. The decreasing conductivity of the electrode affects the transfer of electrons on the surface electrode.

Effect of Scan Rate

The effects of scan rates were studied in the range of 10 to 250 mV / s. The cyclic voltammogram of the scan rate effect on 1 mM hydroquinone analysis is shown in Fig.-6.

Based on the voltammogram, a higher scan rate means higher production of I_{pa} and I_{pc} and both have a linear correlation. The linear relationship between scan rate and I_{pa} and I_{pc} is indicated by the value of the correlation coefficient (R^2) which is close to 1 (Fig.-7). This correlation is in accordance with the theory that claims a faster scanning process during analyte analysis results in faster transfer of electrons to the electrode surface. As a consequence, the resulting current is also higher. The linear curve between $\log v$ and $\log I_{pa}$ with a slope of 0.4477, this value approaches 0.5 (Fig.-8) shows the control-diffusion reaction

on the surface of the electrode. According to the theory, the slope value that is equal to 1 indicates adsorption process whereas if the slope value is between 0.5 and 1 hence it indicates the mixture of diffusion and adsorption processes.²¹

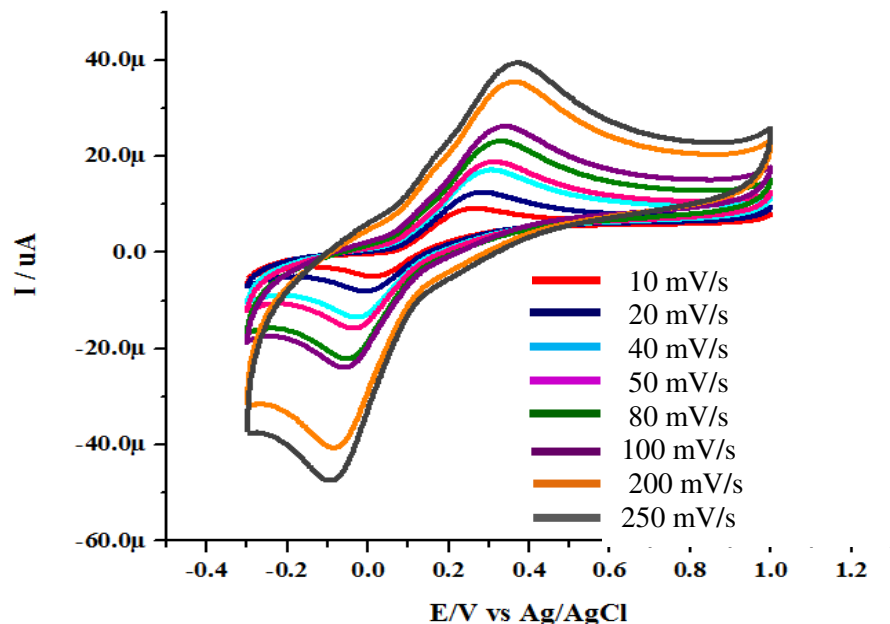


Fig.-6: Cyclic Voltammogram of the Scan Rate Effect on 1 mM Hydroquinone Measurement in pH 7 with 10 % Fc/CPE Electrode

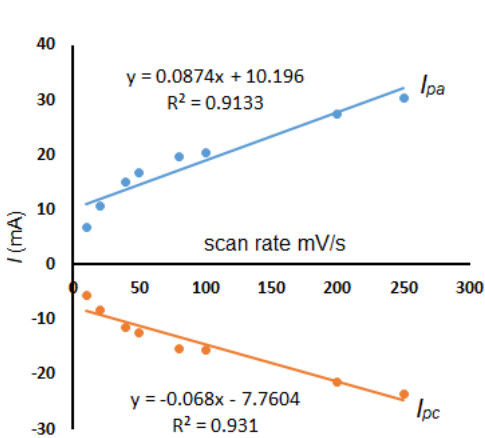


Fig.-7: The Relationship Between Curve I_{pa} and I_{pc} with the Scan Rate

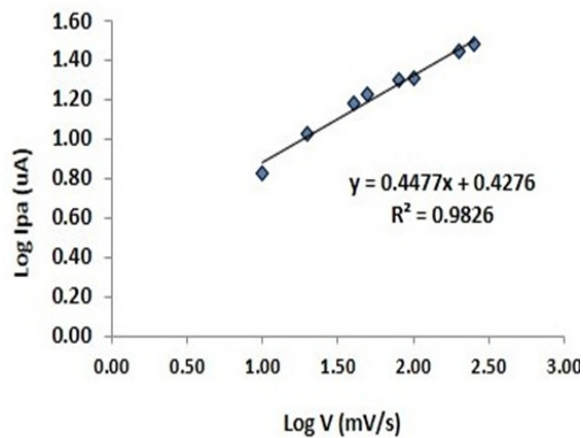


Fig.-8: The Relationship Between Curve $\log v$ with $\log I_{pa}$

The Effect of pH on the Electrochemical Properties of Hydroquinone

Figure-9 shows how the pH of the solution affects the anodic peak current (I_{pa}) and the cathodic peak current (I_{pc}) of 1mM hydroquinone. I_{pa} and I_{pc} values increase from pH 3 and reach a maximum point at pH 7. It can also be seen that at a pH more than 7, the value of I_{pa} and I_{pc} decreases. This is because the increasing pH causes the amount of hydroxide ion in the solution to also increase, thereby reducing the hydroquinone adsorption capacity on the surface electrode.²² In addition, the decrease in I_{pa} and I_{pc} is also due to the alkaline pH conditions of hydroquinone that go through an autoxidation reaction with oxygen molecules that in the end creates brownish 2-hydroxy-p-benzoquinone compounds.²³ The hydroquinone oxidation reaction can be seen in Fig.-10.

Besides being seen from the highest value of I_{pa} and I_{pc} , it is also noTable- that on the surface of the working electrode with its pH reaches 7, a reaction occurs. This reaction shows the most reversible

results. This can be seen from the value of I_{pa}/I_{pc} which is the closest to 1. However, the overall reaction on the surface of Ferrocene/CPE electrode in hydroquinone analysis with a range of pH 3 to 8 shows a quasi-reversible reaction, because the value of ΔE is in the range $61/n \text{ mV} < \Delta E_p < 212$.²⁴

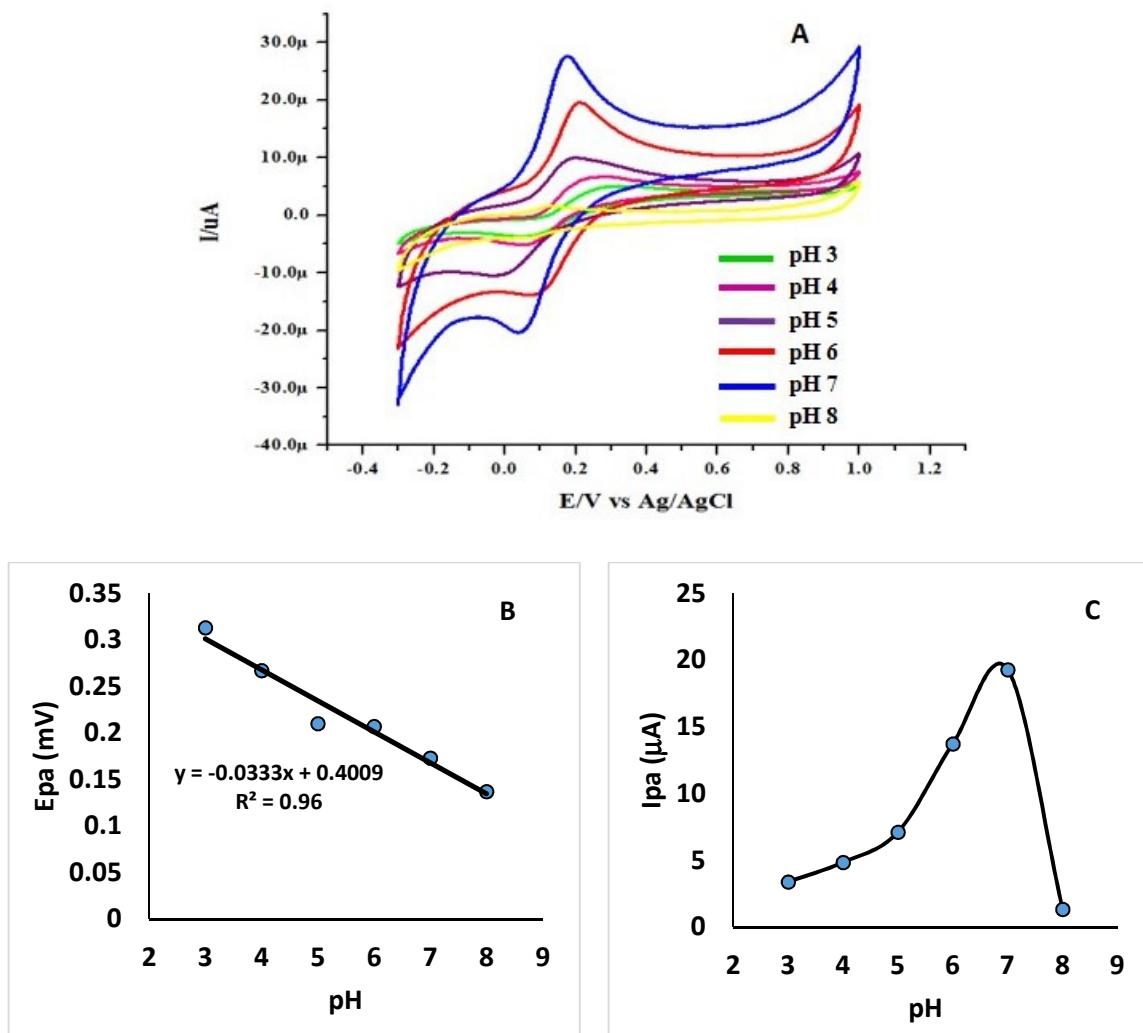


Fig.-9: (A) Cyclic Voltammogram Effect of pH Buffer on the Electrochemical Properties of 1 mM Hydroquinone at a scan rate of 10 mV / s, (B) the Effect of pH on Peak Potential Hydroquinone, and (C) the Effect of pH on I_{pa} Hydroquinone

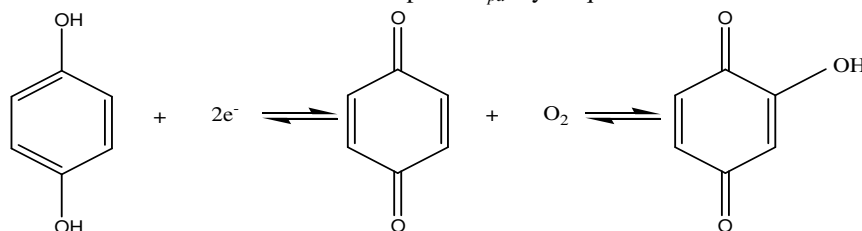


Fig.-10: Reaction of Hydroquinone Oxidation at Alkaline pH

Hydroquinone Linear Sweep Voltammetry (LSV)

Figure-11 shows the LSV HQ voltammogram at various concentrations while the calibration curves are in the concentrations of 0.20 -10.00 μM. The sensitivity value is shown from the slope of the linear

regression equation. The figure indicates that each change in the concentration of 1 μM hydroquinone results in a change of current in as much as 10.436 μA . Detection limit value in hydroquinone analysis which used Ferrosen/CPE electrode is 0.06 μM . Table-2 shows the LOD hydroquinone of this study compared to electrodes from several literature.

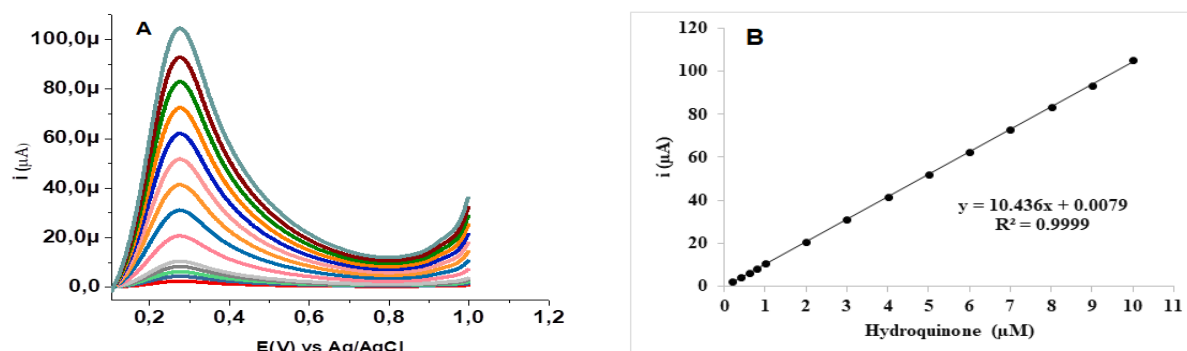


Fig.-11: (A) LSV Hydroquinone Voltammogram at a Concentration of 0.2 – 10 μM with a Scan Rate of 100 mV / s and (B) a Curve of the Relationship between Concentration and Current.

Table-2: Comparison of Hydroquinone Measurements with Various Electrodes

Electrode	Technique	Range	Sensitivity	LOD
MET/AuNPs/GCE ¹⁰	DPV	8 – 400 μM	0.47 $\mu\text{A}/\mu\text{M}$	0.12 μM
PEDOT/CNT/CPE ¹¹	DPV	1.1 – 125 μM	0.25 $\mu\text{A}/\mu\text{M}$	0.30 μM
Au-GR/CILE ¹²	DPV	0.06 – 800 μM	0.10 $\mu\text{A}/\mu\text{M}$	0.018 μM
IL/CPE ¹⁴	CV	0.01-10 mM	0.09546 mA/mM	$8.1 \times 10^{-7}\text{M}$
(ZLH-F)/ MWCNTg ¹⁵	SWV	10-1000 μM	12.787 $\mu\text{A}/\text{M}$	5.7 μM
Ferrocene/CPE (this work)	LSV	0.2-10 μM	10.436 $\mu\text{A}/\mu\text{M}$	0.06 μM

Selectivity

For arbutin analysis, the electrode selectivity test was carried out by using Fc/CPE in pH 3 solution. In this study, the concentration of arbutin varied from 0.2 to 10 μM . The resulting voltammogram is shown in Fig.-12. Based on the voltammogram, arbutin gives two peak potentials namely the potential of 0.213 V and 0.660 V. Those two are the potential peaks of hydroquinone and arbutin which are completely separated. It was possible to happen since, in the water, arbutin undergo hydrolysis reactions to hydroquinone (Fig.-13). Thus, it can be concluded that Fc/CPE electrodes have good selectivity since it is able to distinguish hydroquinone and arbutin compounds. These electrodes can be used for hydroquinone or arbutin analysis, but cannot be used for the simultaneous analysis of both in mixtures.

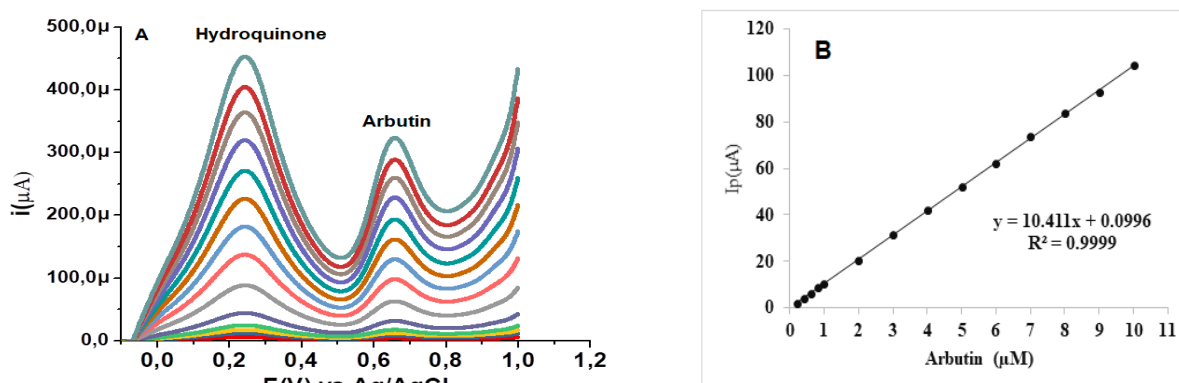


Fig.-12: LSV Voltammogram of Arbutin Variation in the Concentration that uses Fc / CPE Electrodes with a Scan Rate of 100 mV/s and Buffer Solution of pH 5. Voltammogram Gives Two Potential Peaks at 0.213 (Hydroquinone) and 0.660 (Arbutin) (A). The Curve of the Relationship between the Concentration of Arbutin and Peak Current (B)

Analysis of Hydroquinone and Arbutin that Are Added in Cosmetics

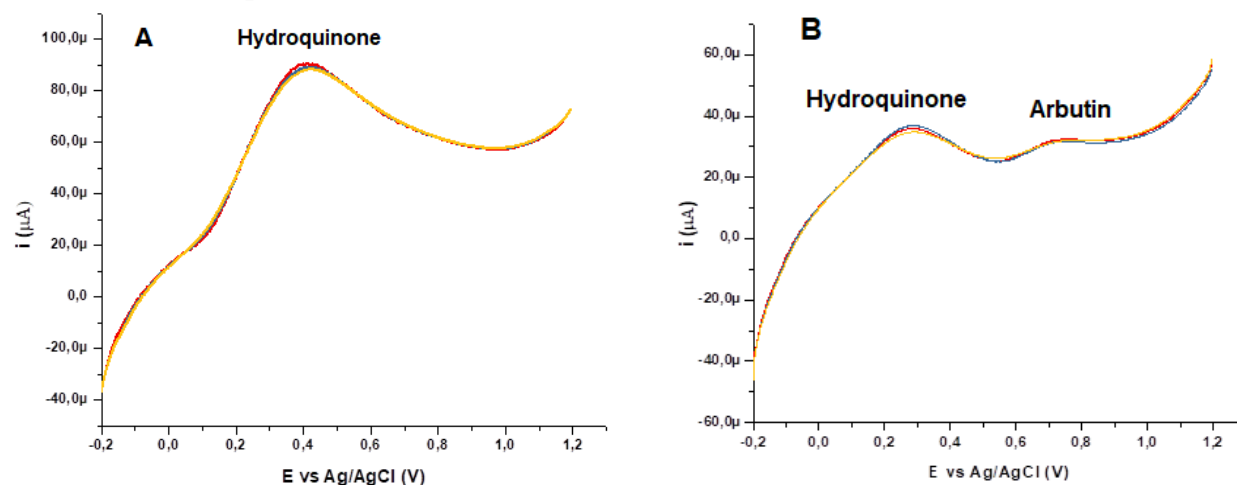


Fig.-13: LSV Voltammogram Analysis of Cosmetic Samples that are Spiked with Hydroquinone (A), and Arbutin (B) using Fc/CPE

The cosmetic samples used in the study were came from particular brand body lotion creams that did not contain skin whitening agents, both HQ and/or arbutin, and then spiked with hydroquinone so that the concentration percentage reached 2%, while the other samples were added with arbutin so that the concentration percentage reached 4%. Afterward, the samples were diluted quantitatively. The measurements were made three times by using the LSV technique with the help of Ferrocene/CPE electrodes. The resulting voltammogram is shown in Fig.-12. Based on the 12B voltammogram, it can be seen that the arbutin compound gives two oxidation peaks. The first peak is the oxidation peak of hydroquinone which reached 0.213 V, while the second peak is the oxidation peak of arbutin which reached 0.660 V. Table-3 and 4 are the results of the analysis of hydroquinone and arbutin recovery in the cosmetic cream.

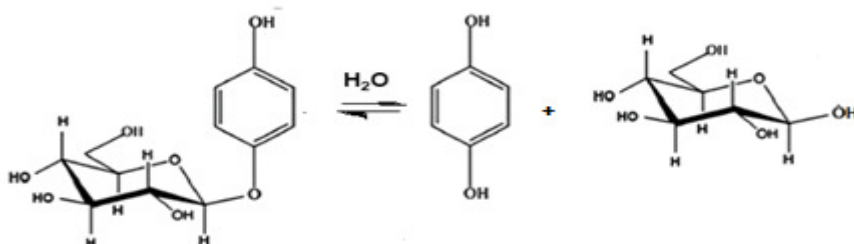


Fig.-14: Arbutin Hydrolysis to Hydroquinone Reaction

Table-3: Hydroquinone Analysis in Cosmetic Creams that were Hydroquinone-Spiked with Fc/CPE by Utilizing LSV Technique

No	Spiking HQ(g/10g)	Found (g/10g)	% Recovery
1	0.2003	0.1991	99.40
2	0.2002	0.1949	97.35
3	0.2003	0.1987	99.23

Table-4: Arbutin Analysis in Cosmetic Creams that were Arbutin-Spiked with Fc/CPE by Utilizing LSV Technique

No	Spiking Arbutin (g/10g)	Found (g/10g)	% Recovery
1	0.4001	0.3961	99.00
2	0.4002	0.3988	99.66
3	0.4001	0.3948	98.67

CONCLUSION

The results showed the composition of the electrode membrane which gave the best results was carbon, paraffin, and ferrocene each with a ratio of 6: 3: 1. Based on the voltammogram, the effect of the scan rate shows that on the electrode surface occurred an electrochemical reaction which later being followed by a chemical reaction. The pH of the solution has an effect on the cyclic hydroquinone voltammogram. pH 7 gives a voltammogram that is closer to a reversible reaction. The validity of the hydroquinone analysis method that was using carbon/ferrocene paste as the electrode shows good results. They are also proven to be selective in the analysis of a mixture of hydroquinone and arbutin. These electrodes have excellent accuracy in recovering hydroquinone and arbutin analysis in cosmetic creams. Therefore, this method is suitable to be used in skin whitening analysis, both for hydroquinone and arbutin analysis.

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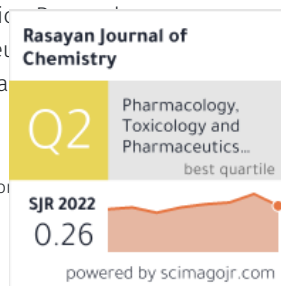
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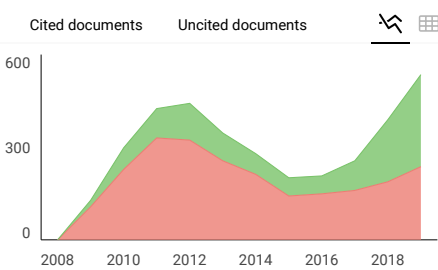
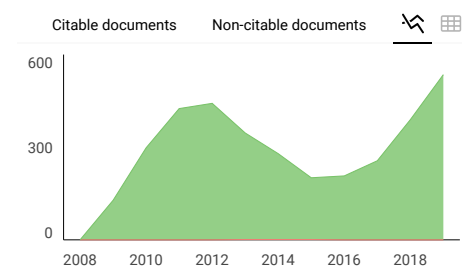
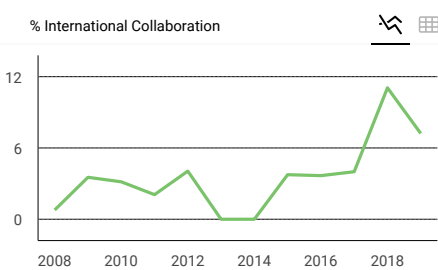
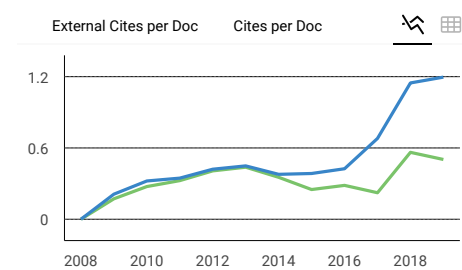
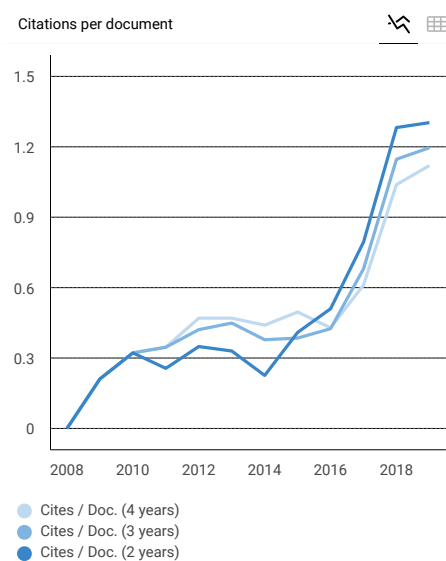
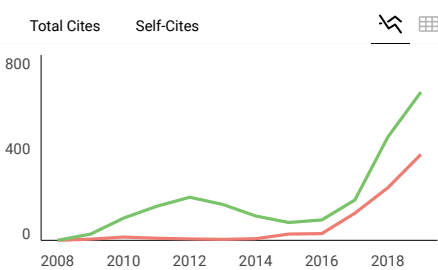
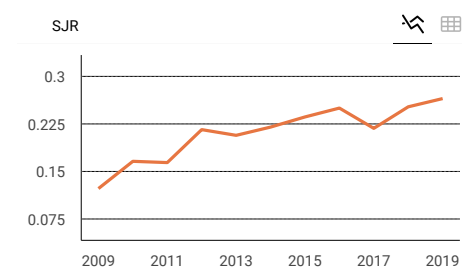
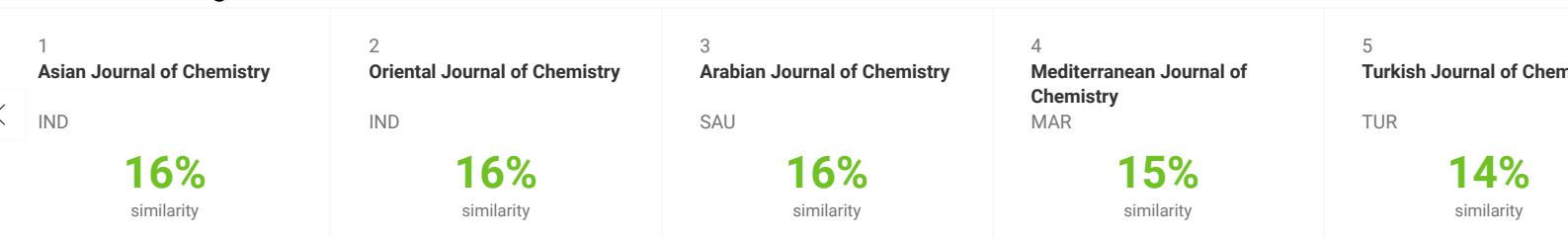
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