THE INTERACTION OF SKIN SENSITIZERS WITH GOLD NANOPARTICLES AND CYSTEINE MODIFIED SCREEN PRINTED CARBON ELECTRODE ANALYZED USING IMPEDANCE TECHNIQUE

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DEDICATION

This thesis is especially dedicated to my beloved,

Mama, aunt Aloyah, and bestfriends

(Afzan, Siti Asmida, Noraayu, and Maisarah).

Also, to myself,

You have done a good job and really deserved this.

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ABSTRACT

Skin sensitization is defined as an allergic response to a skin sensitizer upon contact with the skin. Skin sensitization is induced through covalent binding of a skin sensitizer to skin proteins (haptenation process). Skin sensitization is usually studied using wet chemistry method. Previously, surface plasmon resonance (SPR) was used to study skin sensitization to speed up the analysis but SPR biosensor was costly and is less sensitive towards low molecular weight compounds. Due to the limitation of using SPR technology for skin sensitizer analysis, the use of disposable screen printed carbon electrode (SPCE) modified with gold nanoparticles (AuNPs) and cysteine, analyzed using electrochemical impedance spectroscopy (EIS) has been proposed. The objective of this study was to investigate the interaction of skin sensitizers with AuNPs and cysteine modified SPCE using impedance technique. EIS was carried out to measure the changes in charge transfer resistance of skin sensitizer ($\Delta R_{CT}^{sensitizer}$) as a result of different binding rates of affinity skin sensitizers to cysteine. SPCE modified through electrodeposition of AuNPs/thiourea/self–assembly of AuNPs/cysteine (designated as ETSC) was selected as the best electrode for the detection of skin sensitizers. Investigation on the effect of potency of skin sensitizers on $\Delta R_{CT}^{sensitizer}$ readings suggested that $\Delta R_{CT}^{sensitizer}$ readings were directly proportional to the strength of the skin sensitizers with strong/extreme skin sensitizers displaying higher $\Delta R_{CT}^{sensitizer}$ readings compared to moderate and weak/non skin sensitizers. Fractional coverage area (θ_{IS}^{P}) of ETSC modified SPCE exposed with maleic anhydride (extreme/strong sensitizer) was estimated as 0.98 with r_a and $2r_b$ of 2.50 μm and 15.99 μm, respectively. The θ_{IS}^{P} for ETSC modified SPCE exposed with isoeugenol (moderate sensitizer) and glycerol (weak/non sensitizer) were estimated as 0.9536 and 0.8757, respectively with r_a and $2r_b$ of 4.73 μm and 21.96 μm; and 7.08 μm and 40.20 μm, respectively. Kinetic study showed that adsorption of skin sensitizers on ETSC modified SPCE followed Langmuir isotherm with a binding rate constant of $5.00\times10^{+03}\ M^{-1}$ for maleic anhydride, $2.00\times10^{+03}\ M^{-1}$ for isoeugenol and $1.67\times10^{+02}\ M^{-1}$ for glycerol. The interaction of the skin sensitizers to the ETSC modified SPCE was probed using Fourier transform infrared spectroscopy and atomic force microscopy method and the results showed discernible differences between different classes of skin sensitizers. The data obtained from human analysis, this work, human cell line activation test (h–CLAT), direct peptide reactivity assay (DPRA), KeratinoSens^{TM} and SPR biosensor matched the categorization of local lymph node assay (LLNA–a gold standard in skin analysis testing) in the following descending order: 96 %, 92 %, 82 %, 70 % (DPRA and KeratinoSens^{TM}) and 12 %. With only 8 % mismatched with the data obtained using LLNA, EIS method employing ETSC modified SPCE used in this research has the potential to be employed as a screening tool for the identification of skin sensitizers.

ABSTRAK

Pemekaan kulit didefinisikan sebagai satu tindak balas alergi apabila pemeka kulit bersentuhan dengan kulit. Pemekaan kulit terjadi melalui ikatan kovalen antara pemeka kulit dengan protein kulit (proses haptenasi). Kebiasaannya, pemekaan kulit dikaji menggunakan kaedah kimia basah. Sebelum ini, resonans plasmon permukaan (SPR) digunakan untuk mengkaji pemekaan kulit bagi mempercepatkan analisis tetapi biosensor SPR adalah mahal dan kurang peka terhadap sebatian yang mempunyai berat molekul yang rendah. Disebabkan penggunaan teknologi SPR untuk analisis pemekaan kulit terbatas, penggunaan elektrod karbon bercetak skrin (SPCE) pakai buang diubah dengan nanopartikel emas (AuNPs) dan sisteina, dan analisis menggunakan spektroskopi impedansi elektrokimia (EIS) telah dicadangkan. Objektif kajian ini adalah untuk mengkaji hubungan pemeka kulit dengan AuNPs dan sisteina SPCE terubahsuai menggunakan teknik impedans. EIS dijalankan untuk mengukur perubahan rintangan pemindahan cas pemeka kulit ($\Delta R_{CT}^{sensitizer}$) yang disebabkan oleh kadar pengikatan afiniti pemeka kulit yang berbeza terhadap sisteina. SPCE terubahsuai melalui elektropemendapan AuNPs/tiourea/swa–himpunan AuNPs/sisteina (ETSC) dipilih sebagai elektrod terbaik untuk pengesanan pemeka kulit. Penyiasatan ke atas kesan potensi pemeka kulit terhadap $\Delta R_{CT}^{sensitizer}$ mencadangkan bacaan $\Delta R_{CT}^{sensitizer}$ berkadar langsung kepada kekuatan pemeka kulit dengan pemeka kulit yang kuat dengan mempamerkan bacaan $\Delta R_{CT}^{sensitizer}$ tertinggi berbanding dengan pemeka kulit yang sederhana dan lemah/bukan pemeka. Kawasan penutupan pecahan (θ_{IS}^{P}) daripada SPCE ETSC terubahsuai yang didedahkan dengan maleik anhidrida (pemeka kulit kuat) dianggarkan 0.98 dengan r_a dan $2r_b$ ialah 2.50 μm dan 15.99 μm. θ_{IS}^{P} daripada SPCE ETSC terubahsuai terdedah dengan isoeugenol (pemeka kulit sederhana) dan gliserol (pemeka kulit lemah/bukan) yang dianggarkan menjadi 0.9536 dan 0.8757 dengan r_a dan $2r_b$ masing–masing pada 4.73 μm dan 21.96 μm; dan 7.08μm dan 40.20μm. Kajian kinetik menunjukkan penjerapan pemeka kulit mengikuti isoterma Langmuir dengan kadar pengikat malar $5.00\times10^{+03}\ M^{-1}$ untuk maleik anhidrida, $2.00\times10^{+03}\ M^{-1}$ untuk isoeugenol dan $1.67\times10^{+02}\ M^{-1}$ untuk gliserol. Hubungan antara pemeka kulit dengan SPCE ETSC terubahsuai telah disiasat menggunakan kaedah spektroskopi inframerah transformasi Fourier dan mikroskop gaya atom dan perbezaan yang ketara dapat dilihat daripada kumpulan pemeka kulit yang berbeza. Data diperoleh daripada analisis manusia, kerja ini, ujian pengaktifan titisan sel manusia (h–CLAT), biosensor SPR, asai kereaktifan peptida secara langsung (DPRA) dan KeratinoSens™ sepadan dengan kategori daripada asai limfa nodus lokal (LLNA – ialah piawai emas dalam ujian analisis kulit) dengan turutan yang menurun: 96 %, 92 %, 82 %, 70 % (DPRA dan KeratinoSens™) dan 12%. Memandangkan data hanya 8% yang tidak sepadan dengan data yang diperolehi menggunakan LLNA, kaedah EIS menggunakan SPCE ETSC terubahsuai yang digunakan dalam kerja ini mempunyai potensi untuk digunakan sebagai saringan awal bagi pengenalpastian pemeka kulit.

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LIST OF ABBREVIATIONS

Aθ	–	Inactive Area
AC	–	Alternating Current
ACD	–	Allergic Contact Dermatitis
AD	–	Applicability Domains
AFM	–	Atomic Force Microscopy
AOP	–	Adverse Outcome Pathway
ARDA	–	Amino Acid Derivative Reactivity Assay
ARE	–	Antioxidant/Electrophile Response Element
AuNPs	–	Gold Nanoparticles
ATP	–	Adenosine Triphosphate
BBC	–	British Broadcasting Corporation
BrdU	–	5–bromo–2–deoxyuridine
CAS	–	Chemical Abstracts Service
CCR	–	Correct Classification Rate
CE	–	Counter Electrode
CIR	–	Cosmetic Ingredient Review
CPE	–	Constant Phase Element
CTFRA	–	Cosmetic, Toiletries and Fragrance
CV	–	Cyclic Voltammetry
Das	–	Defined Approaches
DC	–	Dendritic Cells
DMSO	–	Dimethyl Sulfoxide
DNA	–	Deoxyribonucleic Acid

DPRA	–	Direct Peptide Reactivity Assay
EASA	–	Electrophilic Allergen Screening Assay
EC3	–	Emulsion Concentrate 3
EC	–	SPCE/Electrodeposition of AuNPs/Cysteine
ECVAM	–	European Center for Validation of Alternative Methods
EDX	–	Energy Disperse X–Ray Spectroscopy
ELISA	–	Enzyme–Linked Immunosorbent Assay
ESC	–	SPCE/Electrodeposition of AuNPs/SAM of AuNPs/Cysteine
ESSC	–	SPCE/Electrodeposition of AuNPs/ SAM of AuNPs/SAM of AuNPs/Cysteine
ETSC	–	SPCE/Electrodeposition of AuNPs/Thiourea/SAM of AuNPs/Cysteine
ETSTSC	–	SPCE/Electrodeposition of AuNPs/ Thiourea/SAM of AuNPs/Thiourea/SAM of AuNPs/Cysteine
EIS	–	Electrochemical Impedance Spectroscopy
ESAC	–	EURL ECVAM's Scientific Advisory Committee
EU	–	European union
EU–NETVAL	–	European Network of Laboratories for the Validation of Alternative Methods
EURL ECVAM	–	European Union Reference Laboratory for Alternatives to Animal Testing
FCA	–	Freund's Complete Adjuvant
FDA	–	Food and Drug Administration
FESEM	–	Field Emission Scanning Electron Microscopy
FITC	–	Fluorescein Isothiocyanate

FRA	–	Frequency Response Analysis
FTIR–ATR	–	Fourier–Transform Infrared Spectroscopy – Attenuated Total Reflectance
GARD skin	–	Genomic Allergen Rapid Detection
GHS	–	Globally Harmonized System
GLY	–	Glycerol
GPES	–	General Purpose Electrochemical System Software
GPMT	–	Guinea Pig Maximization Test
GPS	–	GARD Prediction Signature
hCLAT	–	Human Cell Line Activation Test
HPLC	–	High–Performance Liquid Chromatography
ICATM	–	International Cooperation on Alternative Test Methods
ICCR	–	International Cooperation on Cosmetics Regulation
ICCVAM	–	Interagency Coordinating Committee on the Validation of Alternative Methods
IgE	–	Immunoglobulin E
IHCP	–	International Health And Consumer Protection
IL–8 Luc assay	–	Interleukin–8 Reporter Gene Assay
InChI™	–	IUPAC International Chemical Identifier
INRA	–	Institute National de la Recherche Agronomique
ISO	–	Isoeugenol
IUPAC	–	International Union of Pure and Applied Chemistry
JaCVAM	–	Japanese Center for the Validation of Alternative Methods

JSAAE	–	Japanese Society for Alternatives to Animal Experiments
KeratinoSens™	–	ARE–Nrf2 Luciferase Test Method
KoCVAM	–	Korean Center for the Validation of Alternative Methods
LC	–	Langerhans Cell
LLNA	–	Local Lymph Node Assay
LuSens	–	Luciferase Gene Assay
LVMH	–	Louis Vuitton Moet Hennessy
MA	–	Maleic Anhydride
MEPs	–	Member of the European Parliament
MHC	–	Major Histocompatibility Complex
MIE	–	Molecular Initiating Event
mLLNA	–	Murine Local Lymph Node Assay
MOC	–	Memorandum of Cooperation
NICEATM	–	Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	–	National Institute of Environmental Health Sciences
NTP	–	National Toxicology Program
OECD	–	Organisation for Economic Cooperation and Development
PBTG	–	Performance–Based Test Guideline
QSAR	–	Quantitative Structure–Activity Relationship
r_a	–	Active Sites With Radius
$2r_b$	–	Distances Between Two Adjacent Active Sites
R_a	–	Surface Roughness
R_d	–	Diffusion Resistance

RE	–	Reference Electrode
REACH	–	Registration, Evaluation, Authorisation, and Restriction of Chemicals
R_{CT}^{EC}	–	Charge transfer resistance for EC
R_{CT}^{ETSC}	–	Charge Transfer Resistance for ETSC
R_{CT}^{ETSTSC}	–	Charge Transfer Resistance for ETSTSC
$R_{CT}^{EC+Maleic\ Anhydride}$	–	Charge Transfer Resistance for EC with Maleic Anhydride
$R_{CT}^{ETSC+Maleic\ Anhydride}$	–	Charge Transfer Resistance for ETSC with Maleic Anhydride
$R_{CT}^{ETSTSC+Maleic\ Anhydride}$	–	Charge Transfer Resistance for ETSTSC with Maleic Anhydride
rLLNA	–	Reduced LLNA
Rs	–	Solution Resistance
RSD	–	Relative Standard Deviation
SAM	–	Self–Assembled Monolayer
SEURAT–1	–	Safety Evaluation Ultimately Replacing Animal Testing
SDD	–	Silicone Drift Detector
SI	–	Stimulation Index
SMILES	–	Formula, Simplified Molecular–Input Line–Entry System
SPE	–	Screen–printed electrode
SPCE	–	Screen–Printed Carbon Electrode
SPM	–	Scanning Probe Microscopy
SPR	–	Surface Plasmon Resonance
SVM	–	Support Vector Machine
TCPN	–	Tetrachloroisophthalonitrile

TG	–	Test Guideline
TPSA	–	Topological Polar Surface Area
UPMU	–	University Laboratory Management Unit
U–SENS™	–	U937 Cell Line Activation Test
UTM	–	Universiti Teknologi Malaysia
WE	–	Working Electrode
Z_D	–	Diffusion Impedance

LIST OF SYMBOLS

σ	–	Warburg Coefficient
ε	–	Epsilon
C_i	–	Inhibitor Concentration
D	–	Diffusion Coefficient
i_0	–	Current Exchange Density
F	–	Faraday Constant
K_b	–	Binding Constant
K_d	–	Dissociation Constant
R	–	Gas Constant
m	–	Slope
n	–	Number of Electrons Involved
T	–	Temperature
W	–	Warburg Impedance
Z (ω)	–	Impedance
θ_{IS}^{P}	–	Fractional Surface Coverage for EIS
ΔG	–	Gibbs Free Energy Changes
ΔH	–	Binding Enthalpy Changes
ΔH_{ads}	–	Heat of Adsorption Changes
ΔR_{CT}	–	Charge Transfer Resistance Changes
$\Delta R_{CT}^{sensitizer}$	–	Charge Transfer Resistance Changes for Sensitizer

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Cosmetics and personal care products are any substances that are used for body cleaning and promoting attractiveness of oneself. Safety analysis on ingredients of cosmetics and personal care products should always be made, to ensure the safety of these products (Bil et al., 2017). Determination of toxicity potential of the ingredients is the first step in hazard assessment of cosmetics and personal care products. The European Economic Community of Cosmetic Directives has suggested the following tests are needed for the safety evaluation of the ingredients of cosmetics and personal care products. The safety evaluations are including acute toxicity, percutaneous absorption, skin irritant, eye irritant, skin sensitization, and photosensitization, subchronic toxicity, mutagenicity and genotoxicity, phototoxicity and photoirritation, photomutagenicity, photogenotoxicity, metabolism studies, and long term toxicity studies (Basketter et al., 2006).

In this study, the focus is on the investigation of the skin sensitizing potential of a cosmetic ingredient. Skin sensitization is defined as an allergic response to any substance (known as skin sensitizers) upon contact with the skin. The haptenation of skin sensitization is bound by covalent bonding of hapten (skin sensitizers) to skin proteins (as a first key event) (cysteine or lysine residues) which leads to activation of keratinocytes (as a second key event). The third key event is the activation of dendritic cells, which is caused by hapten–protein complexes as well as by signaling from activated keratinocytes. Dendritic cells subsequently migrate out of the epidermis to the local lymph node which to T–lymphocytes (T–cells) (as a fourth key event) (Wang et al., 2017).

Animal testing was completely banned in the European countries starting in March 2013. The cosmetics that were tested on animals are prohibited from entering the market. As an effort towards reducing animal testing, the scientific validity of the Local Lymph Node Assay (LLNA) had been endorsed by the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) of Scientific Advisory Committee in 2000 (Alexandre et al., 2011). Ryan et al. (2000) have reported that LLNA is a predictive test to screen the skin sensitizers that have potential in causing skin sensitization by utilizing guinea pig and human. The LLNA is an *in vivo* method and has been considered as a reduction method to reduce the number of animals required by 17 % to evaluate the positive response towards allergenic contact sensitizing activity (Basketter et al., 2018).

However, animal testing has become controversial due to ethical issues. Hence, many efforts have been spent on alternatives to animal testing. Non–animal testing methods for the identification of potential skin sensitizers test substances such as studies based on dendritic cell activation (Forreryd et al., 2018), protein binding (Wareing et al., 2017), and keratinocytes activation (Klicznik et al., 2018) have been developed. However, the validated alternative non–animal testing methods have not been validated as stand–alone replacements for the animal test.

Currently, the EURL ECVAM committee has validated alternative non–animal testing methods for replacement of the animal testing, namely, direct peptide reactivity assay (DPRA), ARE–Nrf2 Luciferase Test Method (KeratinoSens^{TM}), U937 cell line activation test (U−SENS), Interleukin–8 reporter gene assay (IL–8 Luc assay) and human cell line activation test (h–CLAT). At least 2 out of 3 negative results are needed to meet regulatory requirements as a single alternative non–animal testing method has not been able to provide enough information due to the complexity of the skin sensitization endpoint in comparison to the animal testing methods data (Alexandre et al., 2011).

In general, the validated alternative non–animal testing methods have exhibited good prediction when compared to human and LLNA data. The '2 out of 3' prediction model achieved accuracies of 90 % and 79 % when compared to the human and LLNA data, respectively (Urbisch et al., 2015). The electrophilic reactivity property of skin sensitizers can be used as a skin sensitizers screening tool (Urbisch et al. 2016). Merckel et al (2013) have reported that the strongest nucleophiles potential in an amino acid is the sulfhydryl group of cysteine, imidazole group of histidine, and ε–amino group of lysine.

Investigators have always been interested in pursuing a method in measuring a chemical's reactivity based on quantitative peptide–based reactivity assay that would have utility for screening a chemical's skin sensitization potential as defined in the Local Lymph Node Assay (LLNA) (Gerberick et al., 2009; Troutman et al., 2011; Cho et al., 2019). DPRA is an *in chemico* test method that addresses peptide reactivity that is postulated to be the molecular initiating event of skin sensitization (first key event). Reactivity is measured by analyzing the interaction between the substances or skin sensitizers to the synthetic heptapeptides such as cysteine and lysine. This test method was adopted in 2015 under the standard test method Organisation for Economic Cooperation and Development (OECD) 442C. Over the years, the modifications of DPRA have been studied by the investigators such as precipitation of the chemicals (Yamamoto et al., 2019), *in silico* methods with QSAR Toolbox and TIMES SS (Urbish et al., 2016) and using high–pressure liquid chromatography (Zhang et al., 2018).

In 2009, researchers from the Institute National de la Recherche Agronomique (INRA) has worked with Louis Vuitton Moet Hennessy (LVMH) to fabricate a skin sensitizer based on electrophilic assay (cysteine, lysine, and histidine) (Achilleos et al., 2009). Surface Plasmon Resonance (SPR) biosensor was used to calculate the interaction between a ligand and an immobilized analyte (Ahmed et al., 2010) using the direct binding of protein residues as a biosensor. The

result is observed directly through the changes in the refractive index at the surface of the biosensor. Kinetic measurements obtained using SPR can measure real–time binding value (Liu et al., 2014). However, the disadvantages of SPR include long response time requiring a high volume of the sample, high regeneration time, and costly detection technique (Nguyen et al., 2015).

Due to the limitation of SPR technology, in this research work, electrochemical impedance spectroscopy (EIS) was proposed as a potential technique to study the interaction of skin sensitizers with nucleophilic amino acids based on peptide binding reactivity. Disposable screen printed carbon electrode modified with gold and cysteine was proposed to be utilized to address the issue of cost and selectivity. Until this work was written, no study on the use of EIS to study peptide binding reactivity with skin sensitizers was reported. The benefits of EIS include high sensitivity, low cost, label–free strategy, and simplicity as compared to the SPR technique (Radhakrishnan et al., 2014). EIS measures current–voltage ratio events without disturbing the properties of the analyte (Chen et al., 2013).

The fabrication of the disposable screen printed carbon electrode (SPCE) was done using gold nanoparticles (AuNPs) and cysteine. Nanotechnology can improve the detection performance of biosensors. A great number of nanomaterials, such as nanoparticle, carbon nanotube and polymer nanotube have been applied in the development of biosensors (Lu et al., 2017, Li et al., 2017).

1.2 Problem Statements

DPRA is a skin sensitizer screening technique based on peptide–based reactivity assay. The current limitation of the DPRA technique is that it is a wet chemistry method and the analysis is time–consuming (Roberts et al., 2018). In 2009, Achilleos et al (2009) studied the interaction of nucleophilic amino acid residues with skin sensitizers using SPR technology in skin sensitization analysis. However,

the biosensor based on this technology still has its limitation such as its bulky size and high cost of production. Other than that, SPR has good mass sensitivity to high molecular weight molecules but low molecular weight compounds are more difficult to detect. Since skin sensitizers are low molecular weight compounds, the SPR based skin sensitization analysis have low detection accuracy.

Due to the limitation of using SPR technology for skin sensitizer analysis, the fabrication of disposable SPCE modified with AuNPs and cysteine and analysed using EIS was proposed in this study. The interaction of skin sensitizers with AuNPs and cysteine modified SPCE that leads to the haptenation mechanism was analysed using impedance technique. The category of skin sensitizer potency (extracted from LLNA data and provided in Appendix A) (Baketter et al., 2011) was analysed based on ΔRct value. Thus far, this type of technology has not been proposed and reported. Therefore, this study aimed to prove this concept.

SPCE addresses the issues of cost viability and portability with straightforward and inexpensive analytical methods (Hayat and Marty, 2014). The application of self–assembled monolayer (SAM) technique in the construction of SPCE has attracted considerable attention since it provides many advantages, such as ease of preparation, excellent stability, reproducibility, versatility, and the possibility of incorporating different chemical functionalities to produce a high molecular order of monolayers. Many researchers have used AuNPs in the fabrication of SPCE since they exhibit excellent selectivity and sensitivity for studies on electron transfer mechanism (Pooi See et al., 2011). This is because AuNPs can be self–assembled onto the modified electrode in the fabrication process.

Apart from the SAM technique, electrodeposition is another method that can be used to coat a thin film of the material of interest onto a conductive substrate surface (Li et al., 2018). It is a simple technique and is equivalent to the electroplating process (Somé et al., 2016). In this work, SAM and electrodeposition of AuNPs techniques were combined to produce modified SPCE with good

reproducibility. Cysteine was reported to be more reactive than histidine and lysine in detecting skin sensitizers using a direct binding assay (Wareing et al., 2017). Thus, in this study, cysteine was chosen as the main bio–recognition element for the modified SPCE for skin sensitization analysis.

Direct immobilisation of cysteine onto a working surface of modified SPCE was not possible; instead, AuNPs were immobilised on the working surface modified SPCE followed by self–assembly of cysteine on AuNPs (Ee et al., 2015). Cysteine is an amino acid that has a thiol group that can bind to AuNPs. The use of AuNPs and cysteine to modify SPCE has been reported by Teh Ubaidah (2014). Hence, this technique was utilized in this work to study the interaction of skin sensitizers with cysteine using EIS.

1.3 Objective

The main objectives of this research were :

1. To modify SPCE with AuNPs and cysteine for skin sensitization analysis.
2. To characterize the modified SPCE in regards to its capability in analyzing skin sensitizers.
3. To study the interaction of skin sensitizers with AuNPs and cysteine modified SPCE using impedance technique.

1.4 Hypothesis

The potency is directly proportional to the amount of charge transfer resistance (R_{CT}) value required to initiate the pathway leading ultimately to a skin sensitization event. That is, the higher R_{CT} value produced from the haptenation of cysteine–skin sensitizer, the more potent the chemical would be.

1.5 Scopes of the Research

The experiments were divided into four parts: preparation, characterization, study of interaction of skin sensitizer with the modified SPCEs, and comparison study of the EIS data with other methods.

AuNPs and cysteine were used in the fabrication of modified SPCE to detect the haptenation between the skin sensitizers and the cysteine. Three methods of modification of SPCEs with AuNPs–cysteine SPCEs were considered. The electrodeposition of AuNPs followed by self–assembly of cysteine (designated as EC); electrodeposition of AuNPs followed by self–assembly of AuNPs and cysteine (designated as ETSC); and electrodeposition of AuNPs followed by double self–assembly of AuNPs and cysteine (designated as ETSTSC). The EC, ETSC, and ETSTSC modified SPCEs were compared and analysed using EIS.

Repeatability study using maleic anhydride for EC, ETSC, and ETSTSC modified SPCE was done using ten different modified SPCEs. Stability upon storage condition over time for EC, ETSC, and ETSTSC modified SPCE was conducted at room temperature and refrigerator for a month. Surface characterization of the ETSC modified SPCE was conducted using energy disperse x–ray spectroscopy (EDX) and field emission scanning electron microscopy (FESEM).

Next, the effect of the potency of skin sensitizers on the readings of $\Delta R_{CT}^{\text{skin sensitizer}}$ for three categories of skin sensitizers potency, which were extreme/strong, moderate, and weak/non skin sensitizer was studied. The mechanism of haptenation process between skin sensitizers and the modified SPCEs was probed using fourier–transform infrared spectroscopy–attenuated total reflectance (FTIR–ATR) and atomic force microscopy (AFM) analysis. Adsorption kinetic studies and the study of the effect of concentrations of skin sensitizers on the readings of the ETSC modified SPCE were also conducted.

After that, the data obtained from other skin sensitization study methods such as LLNA, human, DPRA, KeratinoSens^{TM}, and h–CLAT were compared with the data obtained from this work. Lastly, ETSC modified SPCE was used to analyse commercial cosmetics.

1.6 Rationale and Significance

The original contribution to knowledge is the modified SPCE in this work is similar to a diagnostic kit with respect to qualitative measurements. Also, this modified SPCE is defined as user friendly that is easy to use and measure. Besides, disposable modified SPCE is provided the rapid detection with skin sensitizers at low cost compared to SPR technology. Other than that, the modified SPCE is used for routine screening purposes during early cosmetics and personal care product development. In this way, it may be useful to examine the data set between reactivity profiles for structural skin sensitizers to correlate the peptide reactivity data with a category of potency skin sensitizing.

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LIST OF PUBLICATIONS

Journal with Impact Factor

1. **Noh T. U**., & Aziz A. A. (2018). Estimation of the Surface Area of a Screen–Printed Carbon Electrode Modified with Gold Nanoparticles and Cysteine using Electrochemical Impedance Spectroscopy. *Chemical Engineering Transactions, 63*, 529–534. https://doi.org/10.3303/CET1863089. **(Q3, IF: 0.27).**

Non–Indexed Conference Proceedings

1. **Noh T. U**., & Aziz A. A. (2017). Characterization of screen printed carbon electrode modified with gold nanoparticles and cysteine. *Conference: International Postgraduate Symposium in Biotechnology 2017* (18–24).