ELECTROCHEMICAL BASED SENSOR FOR DETERMINATION OF MELAMINE BY NANOPARTICLES AND IONIC LIQUID MODIFIED ELECTRODE

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BIOTECHNOLOGY RESEARCH INSTITUTE
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DECLARATION

I hereby declare that the material in this thesis is of my own effort, except for quotations, excerpts, equations, references and summaries, which have been duly acknowledged and cited clearly its sources.

29 May 2015

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CERTIFICATION

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ABSTRACT

The illegal adulteration of infant milk powder with melamine in China has resulted in chronic kidney and urinary tract failure in September 2008. To date, several analytical methods have been developed. Unfortunately, the existing methods are complicated, time consuming and costly. In this study, a novel electrochemical sensor method was developed based on the modification of gold electrode with chitosan, nanoparticles and an ionic liquid for determination in the presence of melamine. Three different nanoparticles were used, namely, zinc oxide nanoparticles (ZnONPs), calcium oxide nanoparticles (CaONPs) and gold nanoparticles (AuNPs) which nanoparticles able to detect a low concentration of melamine and cost effecftive. Combining the advantages of chitosan, metal nanoparticles and ionic liquid, the hybrid nanomaterials were effectively enhanced electron-transfer and promoted the current response of melamine ion. Cyclic voltammetry and differential pulse voltammetry were used to investigate the electrochemical behaviour with modified gold electrode using methylene blue as a redox indicator for increasing the electron transfer in electrochemical cell. The morphological characteristics of nanomaterials were observed under scanning electron microscope and transmission electron microscope. The gold electrode showed optimum response when operated at 25 ±1 ^{o}C in 50 mM Tris–HCl buffer, pH 7 and scan rate of 0.3 V/s within 30 sec. Potential peak currents were found increasing in the order of AuE> AuE/CHIT/NPs > AuE/CHIT/NPs/[EMIM][Otf]. Under optimal conditions, differential pulse voltammetry detected a wide linear range of melamine concentrations with limit of detection 4.0 x 10^{-14} M for AuE/CHIT/ZnONPs/[EMIM][Otf], 6.2 x 10^{-16} M for AuE/CHIT/CaONPs/[EMIM][Otf], and 8.0 x 10^{-18} M for AuE/CHIT/AuNPs/[EMIM][Otf], respectively. The results are suggested that the AuE/CHIT/AuNPs/[EMIM][Otf] is fast, efficient, and wider detection limit. The modified gold electrode was successfully applied for determination of melamine in pretreated milk powder product. The proposed method is fast and simple preparation procedures for analysing the melamine level. The developed method is more convenient based on practical and able to replace the traditional methods subsequently prevent any outbreak of kidney disease.

ABSTRAK

ELEKTROKIMIA BERASASKAN SENSOR UNTUK MENGESAN MELAMIN BERASASKAN NANOPARTIKEL DAN IONIC LIQUID DIUBAH SUAI ELEKTROD

Pencemaran melamin terhadap susu tepung bayi yang menyalahi undang-undang di China pada September 2008 telah menyebabkan penyakit buah pinggang yang kronik dan saluran kencing gagal berfungsi. Sehingga kini, beberapa kaedah analisis telah dijalankan. Malangnya, kaedah yang sedia ada adalah rumit, memakan masa yang lama dan melibatkan kos yang tinggi. Dalam kajian ini, satu kaedah elektrokimia telah dihasilkan berasaskan pengubahsuian elektrod emas dengan kitosan, nanopartikel logam dan cecair ionik untuk mengesan melamin. Tiga nanopartikel yang berbeza telah digunakan, iaitu nanopartikel zink oksida (ZnONPs), nanopartikel kalsium oksida (CaONPs) dan nanopartikel emas (AuNPs) yang mana dapat mengesan melamin dalam kepekatan yang rendah dan menjimatkan kos. Dengan menggabungkan kelebihan kitosan, nanopartikel logam dan cecair ionik, hibrid bahan-nano ini telah memberi kesan peningkatan dalam pemindahan-elektron dan menggalakkan tindak balas ion melamin. Kitaran voltammetri dan perbezaan pulse voltammetri telah digunakan untuk mengkaji reaksi elektrokimia dengan elektrod emas yang diubahsuai dengan menggunakan metilena biru sebagai petunjuk redoks elektrokimia bertujuan untuk meningkatkan pemindahan elektron dalam sel elektrokimia. Ciri-ciri morfologi bahan-nano diperhatikan di bawah mikroskop elektron imbasan dan mikroskop electron transmisi. Elektrod emas menunjukkan tindak balas optimum apabila beroperasi pada $25 \pm 1\ ^{o}C$ di 50 mM Tris-HCl penampan, pH 7 dan kadar imbasan 0.3 V/s dalam masa 30 saat. Kadar arus didapati meningkat daripada turutan AuE > AuE/CHIT/NPs > AuE/CHIT/NPs/[EMIM][Otf]. Dalam keadaan optimum, DPV dikesan dalam julat kepekatan melamine yang luas dengan limit pengesanan 4.0 x 10^{-14} M untuk AuE/CHIT/ZnONPs/[EMIM][Otf], 6.2 x 10^{-16} M untuk AuE/CHIT/CaONPs/[EMIM][Otf], dan 8.0 x 10^{-18} M untuk AuE/CHIT/AuNPs/[EMIM][Otf]. Keputusan di atas mencadangkan bahawa AuE/CHIT/AuNPs/[EMIM][Otf] adalah cepat, berkesan dan mempunyai julat limit pengesanan yang luas. Elektrod emas yang diubahsuai telah berjaya digunakan untuk menentukan melamin dalam pra-rawatan produk susu tepung. Kaedah yang dicadangkan adalah cepat dan prosedur penyediaan yang mudah untuk menganalisis tahap melamin dalam produk susu. Kaedah yang dihasilkan adalah lebih mudah berdasarkan praktikal dan dapat menggantikan kaedah tradisional seterusnya mengelakkan sebarang wabak penyakit buah pinggang.

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LIST OF SYMBOLS

%	Percentage
±	Plus minus
°C	Degree Celsius
μg	Microgram
μg/mL	Microgram per milliliter
μL	Microliter
μm	Micrometer
cm	Centimeter
g	Gram
h	Hour
M	Molar
mg	Milligram
mg/mL	Milligram per milliliter
min	Minute
mL	Milliliter
mM	Milimolar
mm	Millimeter
nm	Nanometer

LIST OF ABBREVATIONS

AuE	Gold electrode
AuNPs	Gold nanoparticles
CaONPs	Calcium oxide nanoparticles
CHIT	Chitosan
CV	Cyclic voltammetry
dH_20	Distilled water
DPV	Differential pulse voltammetry
[EMIM][Otf]	Ionic liquids (1-ethyl-3-methylimidazolium trifluoromethanesulfanote)
LOD	Limit of detection
MB	Methylene blue
NPs	Nanoparticles
ZnONPs	Zinc oxide nanoparticles

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Melamine (2,4,6-triamino-1,3,5-triazine) is one kind of triazine analogue together with three amino groups (Figure 1.1). Melamine will be hydrolyzed into cyanuric acid (2,4,6-trihydroxy-1,3,5-triazine), ammeline (4,6-diamino-2-hydroxy-1,3,5-triazine) and ammelide (6-amino-2,4-dihydroxy-1,3,5-triazine) under strong acid or alkaline condition (Mecker et al., 2012; Xu et al., 2009; Mauer et al., 2009). It is commonly used in the production of melamine-formaldehyde resins (MFR) to synthesis plastics, coatings, commercial filters, glue, dishware, kitchenware, adhesives, and flame retardant as well as nanomaterials since 1950s. Melamine has high-rich nitrogen content about 66 % by mass. Accordingly, it has been illegally added into dairy products by unethical company to obtain an incorrectly high readout of apparent protein content that determined by the conventional standard Kjeldahl test (Hau et al., 2009).

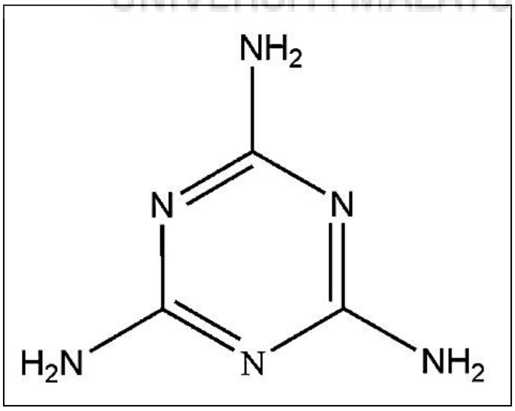

Figure 1.1: The structure of melamine.

Source: Zeng et al., 2011

In 2007, melamine became public attention in North America when there are two cases reported that involving the addition of melamine and its analogues in pet foods. Consequently, a pet food manufacturer has alerted the US Food and Drug administration to animal deaths associated with the deaths of dogs and cats in the US that is appeared the certain batches of their pet food in March 2007. In the following months, consumers and veterinarians have been reported more illnesses and deaths potentially associated with pet food (Kim et al., 2008; Sun et al., 2010; Dobson et al., 2008). In early September 2008, about 294 000 children from China are diagnosed with urinary tract stone with 50 000 infants are hospitalized and six babies death due to the melamine contamination in milk products (Lang, 2007; FDA, 2009; Gossner et al., 2009). High concentration ingestion of melamine has proven to be seriously toxic to human and can be found in the formation of insoluble melamine cyanurate crystals in kidneys caused renal failure (Kim et al., 2008; Sun et al., 2010). For this reason, there is an increasing demand to establish effective and reliable methods for the analysis of melamine in milk and other food products.

Advances in chemistry, physics, biochemistry and molecular biology have led to the development of electrochemical sensor, which can able to detect a wide range of biological elements due to their simplicity, low cost, sensitivity, selectivity, and possibility for miniaturization, portability and integration in automated devices (Farre et al., 2009). When development of electrochemical biosensor based on nanomaterials and nanocomposite membrane, a small amount of sample is possible for rapid detection of melamine.

Chitosan (CHIT) nanocomposite fibre is an interesting natural biopolymer that containing of reactive amino and hydroxyl functional groups. It has been widely used for immobilization due to high friendliness and absorption, excellent film-forming ability, high permeability, high heat-stability, mechanical strength, non-toxicity, biocompatibility, low cost and easily to get. Moreover, chemical modification of the amino groups of CHIT have provided hydrophilic environment for the biomolecules and soluble in diverse acids that able to interact with polyanions to form complexes and gels (Kim and Rajapakse, 2005; Jiang et al., 2007; Liao et al., 2005; Lin et al., 2007). However, hybrid materials based on CHIT has developed such as conducting polymers, carbon nanotubes, redox mediators, metal nanoparticles and oxide agents, due to excellent properties of individual components and outstanding synergistic effects simultaneously for electrochemical sensing platforms (Li et al., 2010a; Lin et al., 2009).

Ionic liquids (ILs) are salts with melting point below 100 ^{o}C. It's used during the past decade due to their unique properties, such as high chemical and thermal stability, relatively high ionic conductivity, low vapour pressure, wide liquid range and large electrochemical windows (Pandey, 2006; Zhang et al., 2008a). ILs usually used in homogeneous catalytic reactions because it's have high catalytic activity and good selectivity. Besides, ILs are used as supporting electrolyte and modifier in chemically modified electrode in biosensor field (Wei and Ivaska, 2008). Application of nanoparticles in micro-fabrication technology is played a vital role in the development of electrochemical biosensors due to very stable, and nanoscales molecules. Metal nanoparticles are well-known to promote faster electron transfer kinetics between electrode and compounds in the electrochemical cell (Pandey et al., 2008; Zhou et al., 2005; Kumar and Chen, 2008).

In this study, the author was used three types of nanoparticles (NPs), namely; gold nanoparticles (AuNPs), calcium oxide nanoparticles (CaONPs) and zinc oxide nanoparticles (ZnONPs). Among the metal oxide NPs, AuNPs are exploited as a potential material for sensing due to high surface area, strong adsorption ability, good biocompatibility, chemical stability, nontoxicity, and high electron communication. AuNPs are more suitable for determination of melamine *via* the development of electrochemical and colorimetric sensors (Wu et al., 2011). One of the considerable advantages of using AuNPs based assay, the molecular recognition event can be translated into visible colour changes (Li et al., 2010a). CaONPs have unique characteristics which required mild reaction conditions to produce high yields of products within short reaction times when comparing to traditional catalysts (Zabeti et al., 2009; Demirbas, 2007; Granados et al., 2007). The nanoporous ZnONPs have enhanced the surface area of electrode for strong adsorption of biomolecules (Singh et al., 2007; Wang et al., 2006).

Taking several advantages of the above characteristics, thus, the main aim of this study was to develop an electrochemical sensor based on CHIT nanocomposite film, metal nanoparticles and ionic liquid (1-ethyl-3-methylimidazolium trifluoromethanesulfonate ([EMIM][Otf]) in the presence of methylene blue as a redox indicator for determination of melamine (Figure 1.2). The experimental parameters were optimized such as pH, interaction time and scan rate, and also investigated the interaction mechanism between melamine and modified AuE. Subsequently, the proposed method was successfully applied for determination of melamine in milk products with satisfactory levels.

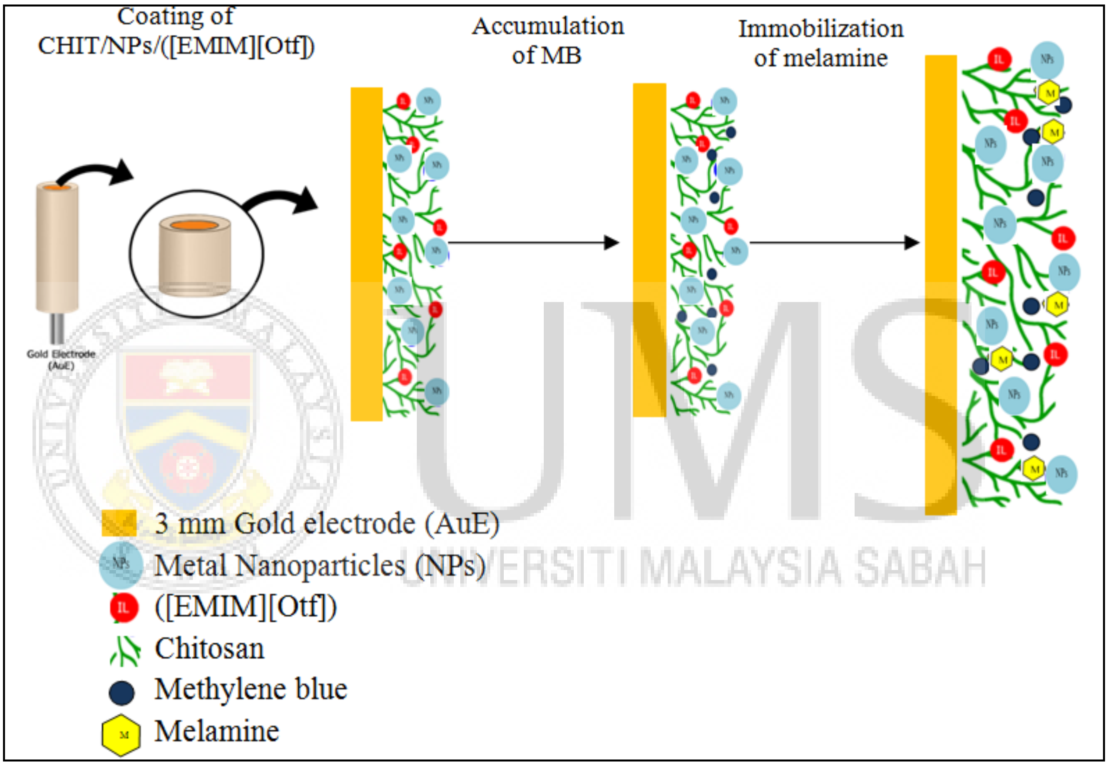

Figure 1.2: Schematic representation of the sensing processes for determination of melamine.