UNIVERSITI PUTRA MALAYSIA

GREEN AND SUSTAINABLE TAPIOCA-DERIVED CARBON DOTS IMMOBILISED ON SCREEN PRINTED ELECTRODE FOR DETECTION OF HEAVY METALS USING ELECTROCHEMICAL METHOD

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By

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Chairman : Professor Zurina Zainal Abidin, PhD
Faculty : Engineering

The environment is increasingly polluted by heavy metals and myriads of harmful contaminants that cause irreversible damage to living things. Reliable measurement and detection of pollutants can minimize heavy metal pollution, this can be accomplished through the adoption of carbon dots (CDs). Biomass waste has been the popular choice of CDs source, but it can be contaminated due to its nature (i.e sourced from waste). To improve the sensitivity and accuracy of a rapid detection or measurement of heavy metals pollution, the need for CDs with greater purity and structural homogeneity from a clean source such as tapioca cannot be overemphasized. Herein, CDs were synthesized from tapioca by a hydrothermal process based on Photoluminescent quantum yield (PLQY). Variables such as temperature, dosage, time, and amount of solvent were explored. CDs synthesis further explored the application of response surface methodology (RSM) and subsequent development of artificial neural network (ANN) platform for achieving reliable and efficient CDs. Characterization of the optimized CDs was done by atomic force microscopy (AFM), high-resolution transmission microscopy (HRTEM), energy dispersive spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), and zeta potential. The sensitive and simultaneous detection of a ternary mixture of cadmium (Cd^{2+}), lead (Pb^{2+}), and copper (Cu^{2+}) in an aqueous solution was successful by utilizing modified SPCE/AuNP/CDs electrode. Modification by gold nanoparticles was undertaken via electrodeposition. Differential pulse voltammetry and cyclic voltammetry were deployed for the analysis of the analytes. A cyclic voltammetry analysis was employed using a potential range between − 0.8 to + 0.2 V at a scan rate of 100 mV/s. Differential pulse voltammetry technique was applied through the electrode for sensitive and selective determination of Cu^{2+}, Pb^{2+}, and Cd^{2+} at a concentration range of 0.01 to 0.27 ppm. Tolerance for the highest possible concentration of foreign substances such as Mg^{2+}, K^{+}, Na^{+}, NO^{3-} and $SO^{4}{}_{2}^{-}$ was observed with a relative error of less than ± 3%. The sensitivities of the modified electrode were 0.17, 0.42, and 0.18

ppm/μA for cadmium, lead, and copper, respectively. The limits of detections achieved for cadmium, lead, and copper were 0.0028, 0.0042, and 0.014 ppm respectively. In conclusion, the modified SPCE provides a cost-effective, dependable, and stable means of detecting heavy metal ions (Cu^{2+}, Pb^{2+}, and Cd^{2+}) in an aqueous environment.

Abstrak tesis yang dikemukakan kepada Senat of Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

DOTS KARBON YANG DIPERBATASAN HIJAU DAN TAPIOCA YANG DILAKSANAKAN PADA ELEKTRON DIPERLUKAN LESEN UNTUK MENGURANGKAN LAMAN HEAVY MENGGUNAKAN METODE ELECTROCHEMICAL

Oleh

MUSA YAHAYA PUDZA

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Alam sekitar semakin tercemar oleh logam berat dan pelbagai bahan cemar berbahaya yang menyebabkan kerosakan yang tidak dapat dipulihkan pada benda hidup. Pengukuran dan pengesanan bahan pencemar yang boleh dipercayai dapat meminimumkan pencemaran logam berat yang dapat dicapai melalui penggunaan Carbon Dots (CDs). Sisa biomassa telah menjadi pilihan sumber CDs yang popular tetapi boleh menjadi toksik kerana sifatnya (seperti bersumber dari sisa). Untuk meningkatkan kepekaan dan ketepatan pengesanan cepat atau pengukuran pencemaran logam berat, keperluan untuk CDs dengan kemurnian yang lebih besar dan homogenitas struktur dari sumber makanan bersih seperti ubi kayu tidak dapat terlalu ditekankan.

Di sini, CDs disintesis dari ubi kayu melalui laluan hidrotermal berdasarkan hasil kuantum Photoluminescent (PLQY), menggunakan pemboleh ubah seperti suhu, dos, masa, dan jumlah pelarut. Sintesis Carbon Dots meneroka penerapan metodologi permukaan tindak balas (RSM) dan pengembangan platform rangkaian neural tiruan (ANN) seterusnya untuk mencapai CDs yang boleh dipercayai dan cekap. Pencirian lebih lanjut CD yang dioptimumkan dengan mikroskopi kekuatan atom (AFM), mikroskopi transmisi resolusi tinggi (HRTEM), spektroskopi penyebaran tenaga (EDS), spektroskopi fotoelektron sinar-X (XPS), dan potensi zeta. Pengesanan sensitif dan serentak campuran kadmium terner (Cd^{2+}), plumbum (Pb^{2+}), dan tembaga (Cu^{2+}) dalam larutan berair berjaya menggunakan elektrod SPCE/AuNPs/CDs yang diubah suai. Pengubahsuaian oleh nanopartikel emas dilakukan melalui elektrodeposisi. Voltammetri nadi pembezaan dan voltammetri siklik digunakan untuk analisis analit. Analisis voltammetri siklik digunakan menggunakan julat potensi – 0.8 hingga +0.2 V pada kadar imbasan 100 mV/s. Teknik voltammetri nadi pembezaan diterapkan melalui elektrod untuk penentuan sensitif dan selektif Cu^{2+}, Pb^{2+}, dan Cd^{2+} pada julat kepekatan 0.01 hingga 0.27 ppm. Toleransi untuk kepekatan tertinggi bahan asing seperti Mg^{2+}, K^{+}, Na^{+}, NO_3^{-} dan SO_4^{2-} diperhatikan dengan ralat relatif kurang dari ± 3%. Sensitiviti elektrod yang diubah masing-masing adalah 0.17, 0.42 dan 0.18 ppm/μA untuk kadmium, plumbum, dan tembaga. Had pengesanan yang dicapai untuk kadmium, plumbum, dan tembaga masing-masing 0.0028, 0.0042, dan 0.014 ppm. Kesimpulannya, SPCE yang diubah suai menyediakan kaedah menjimatkan ion logam berat (Cu^{2+}, Pb^{2+} dan Cd^{2+}) yang efektif, boleh dipercayai, dan stabil dalam persekitaran berair.

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LIST OF SYMBOLS

α	Alpha
β	Beta
°C	Degree celsius
%	Percentage
µL	Microliter
µm	Micrometer
µmoles	Micromoles
APS	Ammonium persulfate
$CaCl_2$	Calcium chloride
mA	MilliAmps
µA	micro Amps

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ANN	Artificial Neural Network
CCD	Central Composite Design
CDs	Carbon Dots
EDTA	Ethylenediaminetetraacetic acid
EDTA	Ethylene-diamine-tetraacetic acid
DI	Deionized Water
DOE	Design of Experiment
EDX	Energy Dispersive X-ray Spectroscopy
FESEM	Field Emission Scanning Electron Microscope
FTIR	Fourier Transform Infrared Spectroscopy
G	Gram
HRTEM	High-Resolution Transmission Electron Microscope
PPM	Parts per million
L	Litre
M	Molar
RSM	Response Surface Methodology

CHAPTER 1

INTRODUCTION

1.1 Research Background

Carbon Dots (CDs) are dimensionless nanoparticles classified as carbon nanomaterials, less than ten nanometers (<10 nm) in size and are considered the latest class of nanoparticle (Biswajit et al., 2019; Sun, et al., 2015; Puvvada et al., 2012; David et al., 2017). CDs derived from organic substances are essential aspects of science with vital applications in computer science and electrical engineering (Mark 2007; Zhu et al., 2016; Xu et al., 2013; Shi et al., 2013). CDs have characteristics such as being environmentally friendly, easy to synthesize, non-blinking, high biocompatibility, durability, high photostability, quenchable (on/off) emission with excitation wavelength that can be functionalized based on desired applications and are easily dissolved in water (Sun et al., 2006). The attributes of CDs made it an interesting substance to be investigated (Xiauyou et al., 2004; Wang et al., 2009; Sun et al., 2006).

Conversely, semiconductor nanocrystals or quantum dots (QDs) are nanoparticles with a diameter ranging from 1-10 nm, but, unfortunately, are toxic and expensive (Titirici et al., 2015). QDs are conventional fluorescent dyes with unique optical properties, however, CDs derived from organic source with physicochemical and functional characteristics are the best option due to their high degree of biocompatibility, cost-effectiveness, non-toxic and had been successfully applied in bioimaging, biosensing (Cao et al 2012; Wang et al., 2013 & 2014; Zhou et al., 2016), agricultural diagnosis (Tothill et al., 2011), pharmaceuticals (Gaddam et al., 2014; Ali et al., 2017), solar cells (Yang et al., 2015), electrochemical functions (Lim et al., 2015; Li et al 2010; Hsu et al., 2012), wastewater treatment (Zhu et al., 2014), photocatalysis and chemical sensing (Saud et al., 2015; Etacheria et al., 2015).

There are numerous techniques for synthesizing CDs, these include; arc-discharge (Lim et al., 2015; Xu et al., 2004), laser ablation (Sun et al., 2006; Li et al., 2011; Yu et al., 2016), chemical oxidation (Fang et al., 2012; Sun et al., 2013; Zhou et al., 2016; Gaddam et al., 2017) and electrochemical synthesis (Li et al., 2010; Zhou et al., 2007; Yao et al., 2014; Zhang et al., 2014; Deng et al., 2014; Yang et al., 2015). However, several factors need to be considered when adopting a synthesis route to obtain CDs (Wang et al., 2014). During the carbonization process in producing CDs, there is a possibility of carbonaceous aggregation occurring. This challenge is remedied by synthesis techniques such as hydrothermal route (Mahardika et al., 2017; Sahu et al., 2012; Wei et al., 2017; Ali et al., 2017; Luyao et al., 2017), organic pyrolysis, and microwave-assisted process (Wang et al., 2012; Liu et al., 2014; Choi et al., 2017; Bhattacharyya et al., 2017; Ke et al., 2014). The aforementioned techniques possess the ability to control the size and uniformity of CDs in solvents.

Applications of agro-based wastes such as; cooking oil waste, pomelo peel, egg-white and egg-yolk, orange juice as well as eggshells have been used to synthesize CDs (Mahardika et al., 2017; Lu et al., 2012; Wang et al., 2012; Sahu et al., 2012; Ke et al., 2014). Contrary to solving the issues of the reuse of waste material (biomass), the process creates possible contaminants as a by-product (Józef et al., 2020). Based on ethical considerations, biomass is better applied for non-pristine applications such as fuel and other bulk applications. When biomass waste is adopted in producing CDs, the yield lacks purity and structural homogeneity (Haochi et al., 2019). The usage of clean, non-toxic materials in the synthesis of CDs generates higher purity of CDs for greater performance with high sensitivity, hence the need for clean production of CDs cannot be overemphasized (Titirici et al., 2015). Besides, in some applications such as food, medical health, biosensing, bioimaging, drug delivery where human and environmental safety and health cannot be compromised, the necessity for cleaner and high-purity CDs is paramount.

Heavy metal contamination in natural environments is increasing due to industrial activities. Generally, metal ions can be classified into essential and non-essential ions. Non-essential heavy metals, such as cadmium (Cd), mercury (Hg), arsenic (As), and lead (Pb), even at trace amount exposure, are highly toxic and carcinogenic (Aaron et al., 2018). Although essential metals like copper (Cu) and zinc (Zn) are required to support biological activities, these essential metals are toxic when in excess (Gedda, et al., 2016). Furthermore, they pose a severe threat to human health and the environment due to their non-biodegradability in nature and accumulation in the food chain (Aaron et al., 2018).

Therefore, it is essential to quantify these metals at trace levels in the environment, food, and drinking water (Environmental Protection Agency 2016). Traditional quantitative methods, such as atomic absorption/emission spectroscopy, inductively coupled plasma/atomic emission spectrometry, and cold vapor atomic fluorescence spectrometry (CVAFS) have been extensively applied to monitor metal ions (Homaei 2017; Shi et al., 2015). Although these techniques are highly selective and sensitive, they require sophisticated and expensive instrumentations, complicated chemical processes for detecting and extracting metal ions from aqueous systems (Meng et al., 2017).

In this research, fluorescent CDs are synthesized by the hydrothermal route and applied on an analytical screen-printed carbon electrode (SPCE) for highly selective and sensitive detection of heavy metal ions such as cadmium, lead, and copper.

1.2 Problem Statement

The presence of lead and copper in potable water is a result of the corrosion that takes place in lead and copper plumbing materials (EPA 2016). Other sources of human contact with lead are soil, dust, and/or air, which can result in adverse health effects, particularly for young children. Infants and children exposed to lead may experience delays in physical and mental development and may show deficits in attention span and learning disabilities (Zuzana et al., 2017). Copper exposure can cause stomach and intestinal distress, liver, and kidney damage which also leads to complications of Wilson's disease in genetically predisposed people (Ying et al., 2017).

Previously, various types of heavy metal ions have been detected in solutions through various means such as fluorescence quenching and chemiluminescence capillary electrophoresis, but these detection techniques have the disadvantages of adopting complex materials with extensive procedures that led to inaccuracies in the results besides being expensive. The electrochemical technique provides a sensitive and accurate means of sensing heavy metal ions and several other pollutants in aqueous media as validated by researchers (Syahraini et al., 2018). This is due to reliable results with minimal step requirements for sensing procedures (Akajionu et al., 2017). The technique of electrochemical sensing also provides the liberty of choosing electrode materials to be utilized by the researcher based on flexibility and availability (Rudra et al., 2017; Lei et al., 2019).

Hence, highly selective and sensitive detection of heavy metal in an aqueous system is desirable and can be made possible by adopting fluorescent CDs synthesized from a simple inexpensive method, onto an electrochemical-based sensor. Preferably, a single synthesis step is desirable to obtain homogenous water-soluble CDs with no need for further purification. This work focus on the use of a fast, simple, and cost-efficient method of hydrothermal synthesis process (Musa et al., 2019; Sun et al., 2015; Zhu et al., 2016; Xu et al., 2013). The hydrothermal route can potentially shorten the reaction time to minutes (Bao et al., 2015; Sun et al., 2015; Cao et al., 2012; Gaddam et al., 2014; Zhu et al., 2016) and eliminates steps involving toxic or dangerous materials, whilst being scalable and easy to operate at the same time (Subhash et al., 2020; Long et al., 2016).

Additionally, it is essential to adopt an eco-friendly and sustainable process to produce a high-quality clean nanoparticle (Das et al., 2018). Plants seem to be the best candidates to synthesize a clean, stable nanoparticle (CDs) at a rate faster than from microorganisms. *Lawsonia inermis* (Henna) plant has been reported as a carbon source to produce CDs for antibacterial studies (Shahshahanipour et al., 2019), while CDs from apple juice were utilized for imaging of mycobacterium and fungal cells (Mehta et al., 2015).

This research utilized tapioca as a precursor material for the synthesis of CDs. Tapioca can be obtained worldwide at low cost with well-defined properties in a wide variety of types and large quantities without being in direct competition with the food industry. Tapioca starch consumption in industrial applications has been more related to economics than to any unique functionality. The paper manufacturing industry and textile industries are significant users of starch (William et al., 2009). The functionality of food is the capacity possessed by food substances other than its nutritional attribute, which has a great impact on its application. The functional characteristic determines its utilization for scientific applications (Hasmadi et al., 2020).

The exploitation of tapioca is necessary to explore resources from fresh precursors (*i.e.* devoid of contamination) such as food materials. The use of biomass compromises the need for a clean resource in fabricating sensitive sensors especially for use in medical and food applications. Tapioca is a crop with a scientific attribute (with high carbohydrate content). CDs from tapioca avoids the need for doping by sulfur and nitrogen and thus eliminates the need to deal with a waste by-product as a result of using waste material. Unlike CDs derived from biomass that are believed to be more sustainable, they require the inclusion of doping agents (S and N) to enhance their surfaces for subsequent applications. The inclusion of S and N in enhancing the functionality of CDs contravenes the purpose of sustainable applications of biomass for the synthesis of CDs

Tapioca is presented as an excellent clean candidate for fluorescent CDs to meet the stringent requirement of various applications (Haiqin et al., 2019; Peter et al., 2019).

Notably, tapioca is being processed from Cassava which is known to have hydrogen cyanide (approximately 50 mg per kg of the fresh root of sweet Cassava) (Ratnayake, and Jackson 2003; Ijioma et al., 2016). Generally, cassava must be processed or cooked to bring the hydrogen cyanide to an acceptable low level for further consumption. Nigerian food and agriculture organization (FAO) stated that Cassava must follow appropriate processing to meet the safety requirement for food usage. Ijioma et al (2016) reported a value of 0.70 - 1.01 mg of hydrogen cyanide per kg of tapioca after processing which is well below the acceptable level of consumption of 2.0 mg/HCN/100g as recommended by Standard Organization of Nigeria. This proved that the toxicity of tapioca is very low and can be considered negligible (Ijioma et al., 2016).

Futhermore, based on proximate analysis of tapioca (Table 1.1), it contains fat, fiber, protein, ash, carbohydrate and very low moisture content when compared to other types of flour such as rice, potato, green gram, and wheat flour (Hasmadi et al., 2020). Low moisture content in tapioca also mean a decrease in the amount the hydrogen cyanide (Hasmadi et al., 2020). Besides that, low moisture suppresses microbial growth and give relatively longer shelf life. Based on these data, it can be assumed that toxicity of tapioca is very low and insignificant. Moreover, in this work, in order to produce carbon dots, the tapioca will be subjected to high temperature hydrothermal treatment conditions which means that it will be further processed and this will futher eliminate the presence of toxicity (hydrogen cyanide). More details will be provided in later section about the hydrothermal process in production of carbon dots.

Table 1.1 : Tapioca proximate analysis (Source; Hasmadi et al., 2020)

Analysis	Percentage (%)
Total dietary fiber	2.09 ± 0.01
Crude fat	0.68 ± 0.01
Crude protein	2.69 ± 0.02
Ash	2.19 ± 0.16
Carbohydrate	27.02 ± 1.14
Moisture content	5.97 ± 1.16

1.3 Significance of the Study

The environment is becoming increasingly polluted by heavy metals and myriads of harmful contaminants. Thus, measures must be taken to detect and prevent environmental pollution by removing or controlling these species in waste streams before their release into the ecosystem. To accomplish this goal, more accurate and rapid detection, measurement techniques, and devices must be provided. A sensor device of atomic and molecular recognition technologies targeted at the highly selective and sensitive detection of heavy metal ions at a relatively low price have been utilized. In this study, an enhanced Screen-Printed Cabon Electrode (SPCE) was modified for the selective and simultaneous detection of heavy metal ions (cadmium, lead, and copper) in aqueous media.

Research into the development of a water-quality measuring tool with which to quickly and accurately determine the potability of water at a low cost for the public was actively pursued. The development of a portable water-quality sensor was the target in the study.

1.4 Research Objectives

The main objective of this work is to develop a one-step hydrothermal route to prepare fluorescent Carbon Dots (CDs) from tapioca for heavy metal sensing applications. The sub-objectives are as follows:

1. Synthesis of tapioca-derived fluorescent CDs by hydrothermal route based on Photoluminescent quantum yield (PLQY), adopting variables such as temperature, dosage, time, and amount of solvent.
2. To apply response surface methodology (RSM) and subsequently develop an artificial neural network (ANN) platform for achieving reliable and efficient CDs.
3. To evaluate the properties of the optimized CDs by atomic force microscopy (AFM), high-resolution transmission microscopy (HRTEM), energy dispersive spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), and zeta potential.

4. To develop the electrochemical sensor using CDs and gold nanoparticles (AuNPs) to modify a screen-printed carbon electrode (SPCE) through the electrochemical deposition approach. Optimization of the experimental parameters of the modified sensor to apply for the detection of heavy metal ions (cadmium, lead, and copper) in aqueous media.

1.5 Scope of the Study

The study aims to synthesize CDs from tapioca by the design of experiment through response surface methodology (RSM) and to develop an artificial neural network (ANN) as a platform for predicting photoluminescent quantum yield by imputing the numerical experimental factors such as temperature (75-175^{0}C), dosage (0.1-0.5g), time (45-105min) and the solvent ratio of water/Acetone/NaOH (8-40ml). The attributes of CDs were characterized to determine CDs constituent elements (XPS and EDS), size and particle dimension (HRTEM), particle morphology and particle counts (AFM), functional group probe (FTIR), and photoluminescent quantum yield.

The synthesized fluorescent CDs were applied as a modifying substance on the screen-printed carbon electrode, in the detection of heavy metal ions (Cadmium, Lead, and Copper) in an aqueous solution. The modified electrode was explored for electrochemical performance, the effect of scan rate, pH, foreign ions, repeatability, and the effect of storage on the modified electrode.

1.6 Thesis Outline

This study is entirely dedicated to the synthesis of tapioca-derived CDs for application in the detection of environmental pollutants (heavy metals ions). Chapter one dwells upon the research background, problem statement, the significance of the study, research objectives, and scope to which the study has been limited. Chapter two delved into past and recent studies regarding the synthesis and application of CDs and their impacts on the scientific and social environment, more so the need to proceed with the current research in other to bridge the identified gaps.

Chapter three deals with the procedures and methods that have been successfully applied to accomplish the study that includes the applications of response surface methodology and artificial neural network to provide the best parameter points for CDs synthesis. The synthesized CDs were further subjected to various characterization analyses to determine their attributes for subsequent application in heavy metal detection via the electrochemical method. Chapter four is all about the findings and the discussions regarding what has been observed while conducting the study, followed by chapter five, which summarizes the study and provides future trends in the field of CDs and applications in environmental studies.

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