SYNTHESIS AND CHARACTERIZATION OF GRAPHENE OXIDE-MOLECULARLY IMPRINTED POLYMER AND ITS ADSORPTION PROPERTIES FOR NEOPTERIN FROM AQUEOUS SOLUTION

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By

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Chair : Sazlinda Binti Kamaruzaman, PhD
Faculty : Science

Neopterin is a useful biomarker for detection of malignant diseases. However, there are only a few study that has been done on the adsorption of neopterin using a graphene oxide-polymer hybrid material such as grapheme oxide-molecularly imprinted polymer (GO-MIP). The aim of this research was to synthesize and characterize GO-MIP, and the details on adsorption study toward neopterin have been thoroughly discussed. GO-MIP with neopterin as the template was synthesized via free radical polymerization method, methacrylic acid (MAA) as the monomer, ethylene glycol dimethacrylate (EGDMA) as the cross-linker, ammonium persulfate (APS) as the initiator, and 8/2 v/v ratio of dimethylsulfoxide/acetonitrile (DMSO/ACN) solution as porogen solvent. The molar ratio of target template, monomer, and cross-linker used was 1:4:16 respectively. Reflux was performed at 50 °C for 24 hours under inert nitrogen atmosphere. Neopterin binding sites were obtained by removing neopterin template from the synthesized GO-MIP via acid washing. A graphene oxide-non imprinted polymer (GO-NIP) without neopterin imprints was prepared with the same methodology. Fourier-transform infrared spectroscopy (FTIR) result for GO-MIP showed a less intense hydroxyl –OH stretching peak and a more intense carboxylic CO- stretching peak compared to GO. Elemental CHNS analysis for GO-MIP showed that the carbon wt% was 4.37% lower and the hydrogen wt% was 3.702% higher compared to GO. A thermal decomposition peak for GO-MIP can be observed at 400 °C with thermogravimetric analysis (TGA) due to the thermal degradation of MAA. Field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM) images showed the presence of neopterin binding sites and the structural morphology of GO-MIP hybrid. Neopterin adsorption study was carried out with a mixture of 10 mg GO-MIP and 1 mL neopterin 10 mg/L standard solution. The mixture was stirred at 300 rpm for 60 minutes, then filtered using nylon syringe filter and the supernatant was analyzed using high performance liquid chromatography coupled with fluorescence detector (HPLC-FLD). GO-MIP adsorbed 45.13 % of

neopterin while GO-NIP adsorbed just 23.48 %, about twice the amount of neopterin compared to its non-imprinted counterpart. GO-MIP also showed good neopterin selectivity when under the effect of analog compound 6-biopterin, where GO-MIP adsorbed 34.63 % of neopterin but just 18.64 % 6-biopterin. The static adsorption mechanism was studied using Langmuir and Freundlich isotherms, while the adsorption kinetics was studied using Lagergren pseudo-first-order and pseudo-second-order kinetics models. The adsorption mechanism and kinetics were best described using Freundlich isotherm (R^2=0.9917) and Lagergren pseudo-second-order (R^2=0.9874), respectively. The adsorption capacity at equilibrium was found to be 0.4749 mg/g with the adsorption parameters as described (10 mg GO-MIP, 1 mL neopterin 10 mg/L). The neopterin adsorption method validation was performed via a non-matrix-matched calibration method. The limit of detection (LOD) and the limit of quantitation (LOQ) of the proposed adsorption method were determined to be 0.9126 mg/L and 3.042 mg/L respectively with linearity range (LR) of 1-10 mg/L. The synthesized GO-MIP showed promising adsorption performance towards neopterin and could be developed into a neopterin sensor in the future.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
Sebagai memenuhi keperluan untuk ijazah Master Sains

SINTESIS DAN PENCIRIAN GRAFIN OKSIDA-POLIMER MOLEKUL PENCETAKAN DAN PENJERAPAN TERHADAP NEOPTERIN DALAM LARUTAN AKUEUS

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Neopterin adalah sejenis biopenanda yang berguna untuk pengesanan penyakit malignan. Sungguhpun begitu, hanya sedikit kajian telah dijalankan ke atas penjerapan neopterin menggunakan bahan hibrid grafin oksida-polimer seperti grafin oksida-polimer molekul pencetakan (GO-MIP). Tujuan kajian ini adalah untuk mensintesis dan mencirikan GO-MIP dan butiran lengkap ke atas kajian penjerapan terhadap neopterin telah dibincangkan dengan teliti. GO-MIP dengan neopterin sebagai templat telah disintesis melalui kaedah radikal pempolimeran bebas, asid metakrilik (MAA) sebagai monomer, etelina glikol dimetakrilik (EGDMA) sebagai pemaut silang, ammonium persulfat (APS) sebagai pemula, dan nisbah 8/2 v/v larutan dimetilsulfoksida/acetonitril (DMSO/ACN) sebagai pelarut porogen. Nisbah molar sasaran templat, monomer, dan pemaut silang yang telah digunakan adalah 1:4:16 masing-masing. Reflux telah dijalankan pada 50 °C untuk 24 jam di bawah atmosfera nitrogen lengai. Tapak pengikat neopterin telah diperolehi dengan merobohkan templat neopterin daripada GO-MIP yang telah disintesis melalui pembasuhan asid. Grafin oksida tiada peneraan polimer (GO-NIP) tanpa peneraan neopterin telah disediakan dengan kaedah yang sama. Keputusan spektroskopi transformasi Fourier inframerah (FTIR) untuk GO-MIP telah menunjukkan puncak regangan hidroksida –OH yang kurang amat dan puncak regangan yang lebih amat untuk karboksilik CO- berbanding dengan GO. Analisis unsur CHNS untuk GO-MIP telah menunjukkan bahawa peratusan karbon wt% adalah 4.37 % lebih rendah dan peratusan hidrogen wt% adalah 3.702 % lebih tinggi berbanding dengan GO. Puncak penguraian terma untuk GO-MIP boleh dicerap pada 400 °C dengan analisis gravimetrik termo (TGA) disebabkan oleh penguraian terma MAA. Imej-imej mikroscopi imbasan elektron pancaran medan (FESEM) dan mikroskopi penghantaran elektron (TEM) telah menunjukkan kehadiran tapak pengikatan neopterin dan morfologi berstruktur hibrid GO-MIP. Kajian penjerapan neopterin telah dijalankan dengan campuran 10 mg GO-MIP dan 1 mL 10 mg/L larutan standard neopterin. Campuran telah dikacau pada

300 rpm selama 60 minit, kemudian telah dituras menggunakan turasan picagari nilon dan supernatant telah dianalisa menggunakan kromatografi cecair prestasi tinggi dengan pengesan pendafluor (HPLC-FLD). GO-MIP telah menjerap 45.13 % neopterin sementara GO-NIP telah menjerap hanya 23.48 %, kira-kira dua kali jumlah neopterin berbanding dengan kaunterpart tiada peneraan. GO-MIP juga telah menunjukkan kepilihan neopterin yang bagus di bawah kesan sebatian analog 6-biopterin, di mana GO-MIP telah menjerap 34.63 % neopterin dan 18.64 % 6-biopterin. Mekanisma penjerapan statik telah dikaji menggunakan Isoterm Langmuir dan Freundlich, dan penjerapan kinetik telah dikaji menggunakan model kinetik Lagergren pseudo-tertib pertama dan pseudo-tertib kedua. Mekanisma penjerapan dan kinetik telah diperihalkan menggunakan Isoterm Freundlich (R^2=0.9917) dan Lagergren pseudo-tertib kedua (R^2=0.9874) masing-masing. Keseimbangan muatan penjerap telah ditemui pada 0.4749 mg/g dengan parameter penjerap seperti yang diperihalkan (10 mg GO-MIP, 1 mL neopterin 10 mg/L). Kaedah pengesahan penjerapan neopterin telah dilakukan melalui kaedah kalibrasi bukan matrik yang sama. Had pengesanan (LOD) dan had quantitatif (LOQ) kaedah penjerap yang dibangunkan adalah 0.9126 mg/L dan 3.042 mg/L masing-masing dengan julat kelinearan (LR) 1-10 mg/L. GO-MIP yang telah disintesis telah menunjukkan prestasi penjerapan yang berprestasi terhadap neopterin dan boleh dibangunkan sebagai sensor neopterin pada masa akan datang.

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LIST OF ABBREVIATIONS

MIT	Molecular Imprinting Technology
GO-MIP	Graphene Oxide-Molecularly Imprinted Polymer
MAA	Methacrylic Acid
EGDMA	Ethylene Glycol Dimethacrylate
APS	Ammonium Persulfate
GO-NIP	Graphene Oxide-Non Imprinted Polymer
FTIR	Fourier-Transform Infrared Spectroscopy
GO	Graphene Oxide
TGA	Thermogravimetric Analysis
FESEM	Field Emission Scanning Electron Spectroscopy
TEM	Transmission Electron Spectroscopy
HPLC-FLD	High Performance Liquid Chromatography-Fluorescent Detector
LOD	Limit of Detection
LOQ	Limit of Quantitation
LR	Linearity Range
GTP	Guanosine Triphosphate
RIA	Radioimmunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
HPLC	High Performance Liquid Chromatography
MIP	Molecularly Imprinted Polymer
SPE	Solid Phase Extraction
RAFT	Reversible Addition and Fragmentation Chain Transfer
UV-Vis	Ultraviolet-Visible Spectrometer
ATR	Attenuated Total Reflectance
q_e	Adsorption Capacity at Equilibrium
C_o	Initial Concentration
C_e	Equilibrium Concentration
q_m	Maximum Adsorption Capacity

K_L	Langmuir Constant
K_F	Freundlich Constant
n	Freundlich Adsorption Favorablility
q_t	Adsorption Capacity at Time t
k_1	Pseudo-First-Order Rate Constant
k_2	Pseudo-Second-Order Rate Constant
FLD	Fluorescent Detector
σ	Standard Error of Estimate
s	Slope of Calibration
DTA	Differential Thermal Analysis
R^2	Correlation of Coefficient

CHAPTER 1

INTRODUCTION

1.1 Research background

Molecular imprinting technology (MIT) involves the development of a polymer with molecularly imprinted binding sites of specific functional group, shape and size distribution which are highly selective toward the target template molecule, namely a MIP (Chen, Wang, Lu, Wu, & Li, 2016). The fundamental working principle of MIP is very akin to the lock-and-key mechanism famously found in enzymatic reactions. By introducing the desired template molecule into the polymer during polymerization process, the template molecule would leave behind empty cavities upon template removal which serves as highly selective molecular binding sites for the template (Haupt, Linares, Bompart, & Tse, 2013). Due to the ease of preparation, flexibility, and effectiveness of MIP, they are widely researched and utilized in various molecular imprinting studies, adsorption studies of specific compounds, molecular detection studies, and more (Altintas et al., 2015; Boulanouar, Mezzache, Combès, & Pichon, 2018; Daoud Attieh, Zhao, Elkak, Falcimaigne-Cordin, & Haupt, 2017; Erdőssy, Horváth, Yarman, Scheller, & Gyurcsányi, 2016; Zamora-Gálvez et al., 2016).

One of the most common MIP synthesis method is the free radical polymerization method. The method is based on Mosbach's idea of a "biochemist's" approach where most biological molecular recognition are non-covalent in nature (Arshady & Mosbach, 1981; Wackerlig & Lieberzeit, 2015). Thus, a self-assembled MIP synthesis method which promotes non-covalent interaction between functional monomer and template molecule is desired. Due to its simplicity and flexibility, MIP synthesized using free radical polymerization method allows for the use of various functional monomers as well as template molecules.

In order to further improve and enhance the performance of MIP, modification or functionalization of MIP has been heavily explored in recent years. One of the most common modification of MIP is the addition of GO into the polymer, forming a GO-MIP hybrid material as a result. Consisting of sp^2 hybridized carbon atoms arranged in a honeycomb lattice structure, GO is a monolayer graphitic nanomaterial which possesses a range of highly labile oxygen functional groups with high specific surface area and high Young's modulus measurement (Tan et al., 2017). Due to its high specific surface area, the introduction of monolayer GO allows the fabrication of MIP across the surface of the nanomaterials, bypassing the general limitations of bulk polymerization (Luo et al., 2017). This results in the optimization of the MIP structure, where the template molecules reside at the surface or near the surface of the material instead of being trapped inside a typical bulky MIP (Anirudhan, Deepa, & Stanly, 2019).

In this study, a GO-MIP would be synthesized with neopterin selected as the desired template molecule. Neopterin is a chemical compound which belongs to the chemical class known as pteridines. It is a product from the catabolic reaction of guanosine triphosphate (GTP), a type of purine nucleotide, occurring in human macrophages. The production of neopterin is stimulated by cytokine interferon-gamma, a type of cytokine which are produced by T helper cells. Since the primary function of T helper cells revolve around cellular or adaptive immune response activation, elevated concentrations of neopterin are usually detected in patients suffering from but not limited to diseases such as HIV-1, coronary atherosclerotic heart disease, chronic obstructive pulmonary disease, renal allograft rejections, and tuberculosis (Abdel, Adawy, & Sayed, 2016; Carey et al., 2013; Cesur et al., 2014; Lyu, Jiang, & Dai, 2015). Owing to its unique biosynthesis mechanism involving cytokine interferon-gamma stimulation, neopterin has become a vital biomarker for laboratory detection and diagnosis of various malignant diseases (Sucher et al., 2010).

1.2 Problem statement

Although free radical polymerization is easy to execute and the synthesized MIP is suitable to a wide range of functional monomers and template molecules, MIP synthesized with free radical polymerization method suffers from a few major drawbacks. Due to the uncontrollable nature of free radical polymerization, the binding site distribution of the synthesized MIP is heterogeneous, with varying affinities towards the target template. This results in nonhomogeneous molecular cavities, thus leading to slower response time due to the difference in affinities of binding sites, and low reproducibility of binding performance (Zeng, Wang, Liu, Kong, & Nie, 2012). Besides, MIP synthesized with conventional free radical polymerization suffers from incomplete template removal and poor binding site accessibility due to the imprinted sites located deep within the bulky polymer structure (Luo, Gao, Tan, Wei, & Liu, 2016).

Another major problem regarding this research is the lack of neopterin adsorption study with the use of GO-MIP or any other functionalized variants of MIP in general. One of the related research is the work published by Del Sole and co. in 2013, in which it detailed the determination of neopterin with the use of MIPs synthesized using various monomers (Del Sole et al., 2013). Another research of interest is done by Sharma and co. in 2016, where they developed a potentiometric chemosensor for neopterin detection using electrochemically synthesized MIP as the sensing unit (Sharma et al., 2016). However, no report regarding GO-MIP synthesis and adsorption study with neopterin as the template molecule is published at the time of concluding the laboratory work and writing this thesis.

1.3 Research objectives

The overall aim of this research is to synthesize and characterize a GO-MIP using neopterin as the template molecule for neopterin adsorption study. The research objectives are as follows:

a) To synthesize GO-MIP using neopterin as the template molecule and characterize GO-MIP with various characterization instruments.
b) To study the neopterin adsorption behavior on GO-MIP using Langmuir and Freundlich adsorption isotherms with HPLC-FLD as the method of detection.
c) To determine the neopterin adsorption kinetics on GO-MIP using Lagergren pseudo-first-order and pseudo-second-order kinetic models with HPLC-FLD as the method of detection.

1.4 Scope of research

Synthesis and preparation of a GO-MIP using selected materials was studied. The synthesized GO-MIP was characterized using FT-IR, CHNS elemental analysis, TGA, FESEM, and TEM. A neopterin adsorption method was developed using the synthesized GO-MIP as the adsorbent. A simple HPLC-FLD method for the detection of neopterin was developed. The adsorption performance of GO-MIP towards neopterin was studied via comparison with an analogue compound, 6-biopterin. The adsorption mechanism and kinetics of neopterin onto GO-MIP was studied with the use of Langmuir isotherm, Freundlich isotherm, and Lagergren kinetic models.

1.5 Significance of research

In recent years, the use of MIP or functionalized MIPs to determine various organic compounds has been widely discussed. However, there has yet to be a publication detailing the synthesis of a GO-MIP for neopterin adsorption study. In a similar fashion, multiple assay studies of neopterin have been published utilizing various sample preparation and HPLC methods but not GO-MIP. Thus, a research about neopterin adsorption on GO-MIP seems feasible.

In this study, a GO-MIP using neopterin as the target template was synthesized with free radical polymerization method, where the disadvantages of MIP synthesized using free radical polymerization would be solved with the addition of GO, and adsorption study of neopterin onto GO-MIP were studied extensively with the use of HPLC-FLD. This research will showcase a simple free radical synthesis method for GO-MIP preparation with the use of neopterin as the template molecule. The adsorption study of neopterin onto GO-MIP will provide a significant insight into the adsorption performance, as well as the adsorption behavior and kinetics of the process. In short, this research will open the path to future adsorption studies of GO-MIP with neopterin as the template molecule.

1.6 Thesis outline

This thesis consists of 5 chapters. Chapter 1 covers research background, problem statement, research objectives, scope of research, and significant of research. Chapter 2 covers the literature review of this thesis, with detailed discussion on neopterin and its role as a biomarker in diagnosis of various diseases, detection methods of neopterin including immunoassay and liquid chromatography methods, and detailed discussion on MIP as well as GO-MIP hybrid material. Chapter 3 describes the preparation of GO powder, synthesis of GO-MIP, preparation of neopterin standard solution, adsorption method of neopterin using GO-MIP as the adsorbent, and adsorption study with Langmuir, Freundlich, and Lagergren kinetics. Chapter 4 details the experimental result and data of GO-MIP synthesis and characterizations, the adsorption study including adsorption selectivity and contact time optimization, adsorption mechanism analysis from Langmuir, Freundlich, and Lagergren kinetics model, as well as method validation data including LOD and LOQ. Chapter 5 summarizes the thesis by presenting the overall conclusions and also suggestions for future research directions.

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