MULTIWALL CARBON NANOTUBE-G-(AGAROSE-G-POLYMETHYL METHACRYLATE) AND (AGAROSE-G-POLYMETHYL METHACRYLATE) SORBENTS FOR EXTRACTION OF PESTICIDES AND HEAVY METAL IONS FROM AQUEOUS MATRICES

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In the name of Allah, Most Gracious, Most Merciful

To my beloved husband Mohammad Reza Isvandzibaei, who are provided me hope with support, help and encouragement that greatly contributed to the successful completion of my studies,

To my mother, who are praying for me and who has brought, great motivation and bright inspiration into my life
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ABSTRACT

Rapid developments in agriculture and industrial sectors have increased the levels of toxic heavy metals and pesticide residues in the environment. Many sample pretreatment methods utilized in the determination of heavy metals and pesticide residues are often time consuming, labor intensive and require substantial amounts of organic solvents. Thus, new approaches in miniaturized sample preparation are imperative. In this study, new modified agarose sorbents were prepared, characterized and applied to the extraction and pre-concentration of pesticides and heavy metal ions from aqueous matrices. Poly(methyl methacrylate)-grafted-agarose (Agarose-g-PMMA) was successfully synthesized using microwave radiation and ceric ammonium nitrate (CAN) as the radical initiator. The optimum condition for graft copolymerization was found to be at 9 g monomer, 0.4 g CAN, 60 s microwave irradiation time under 700W microwave radiation for 1 g of agarose. The graft copolymer was characterized by Fourier transform infrared (FTIR) spectroscopy, CHN analysis, field emission scanning electron microscopy (FESEM), differential scanning calorimetry (DSC) analysis, thermal gravimetric analysis (TGA) and gel permeation chromatography (GPC). A micro-solid phase extraction (μ-SPE) utilizing Agarose-g-PMMA as sorbent combined with gas chromatography-microelectron capture detection was developed for the determination of selected pesticides, namely diazenon, chlorpyrifos, hexaconazole and azaconazole in water samples. Under optimized conditions, low limits of detection (LODs) (0.004 - 0.024 ng mL\(^{-1}\)) were obtained with good recoveries (82.23 - 103.58%). Agarose-g-PMMA was also employed as sorbent in dispersive micro-solid phase extraction (D-μ-SPE) combined with inductively coupled plasma-mass spectrometry for the analysis of heavy metals namely cadmium, nickel, copper and zinc in vegetables and natural water samples. Under the optimum conditions, the developed method showed excellent LODs (0.6 - 1.8 ng L\(^{-1}\)) and good relative recoveries (92.0 - 103.0%) for the analytes. The Agarose-g-PMMA was covalently-modified with multi-walled carbon nanotubes (MWCNTs) and the composite formed (MWCNTs-g-Agarose-g-PMMA) was characterized by FTIR, TGA and transmission electron microscopy (TEM). A SPE method incorporating MWCNTs-g-Agarose-g-PMMA as sorbent was developed and combined with flame atomic absorption spectrometry for the determination of lead ions in natural water samples. The method provided fast analysis and showed good sensitivity and excellent precision and suitable for extraction and pre-concentration of pesticide residues and trace metal ions in water and vegetable samples.
ABSTRAK

Pembangunan pesat dalam sektor pertanian dan perindustrian telah meningkatkan tahap logam berat toksik dan sisa racun perosak dalam persekitaran. Banyak kaedah pra-rawatan sampel yang digunakan dalam penentuan logam berat dan sisa racun perosak biasanya memakan masa yang panjang, intensif buruh dan memerlukan sejumlah pelarut organik yang banyak. Justeru, pendekatan baru dalam penyediaan sampel bersaiz kecil adalah sangat penting. Dalam kajian ini, bahan pengeras agarosa terubahsuai baru telah disediakan, dicirikan dan digunakan bagi pengekstrakan dan pra-pemekatan racun perosak dan ion logam berat di dalam matriks akueus. Poli(metil metakrilat)-cangkuk-agarosa (Agarose-g-PMMA) telah berjaya disintesis menggunakan sinaran gelombang mikro dan serik ammonium nitrat (CAN) sebagai pemula radikal. Keadaan optimum bagi ko-pempolimeran cangkuk telah didapati sebagai 9 g monomer, 0.4 g CAN, 60 s masa penyinaran gelombang mikro di bawah sinaran gelombang mikro 700 W bagi 1 g agarosa. Polimer cangkuk telah dicirikan dengan spektroskopi inframerah transformasi Fourier (FTIR), analisis CHN, mikroskopi elektron imbasan pancaran medan (FESEM), analisis kalorimetri pengimbasan pembezaan (DSC), analisis gravimetri penelapan gel (GPC). Pengekstrakan fasa pepejal mikro menggunakan Agarose-g-PMMA sebagai bahan pengeras digabungkan dengan kromatografi gas-pengesan penangkapan mikroelektron telah dibangunkan bagi penentuan racun perosak terpilih, iaitu diazenon, klorpirifos, heksakonazola dan azakonazola di dalam sampel air. Di bawah keadaan optimum, had pengesanan (LOD) yang rendah (0.004 - 0.024 ng mL^{-1}) telah diperoleh dengan perolehan balik yang baik (82.23 - 103.58%). Agarose-g-PMMA juga telah digunakan sebagai bahan pengeras dalam pengekstrakan fasa pepejal mikro (D-μ-SPE) digabungkan dengan spektrometri jisim-plasma ganding aruhan bagi analisis logam berat iaitu kadmium, nikel, kuprum dan zink di dalam sampel sayur-sayuran dan air semula jadi. Di bawah keadaan optimum, kaedah yang dibangunkan itu menunjukkan LOD yang cemerlang (0.6 - 1.8 ng L^{-1}) dan perolehan balik relatif yang baik (92.0 - 103.0%) bagi analit tersebut. Agarose-g-PMMA telah diubahsuai secara kovalen dengan nanotiub karbon dinding berbilang (MWCNT) dan komposit yang terbentuk (MWCNTs-g-Agarose-g-PMMA) telah dicirikan dengan FTIR, TGA dan mikroskopi elektron penghantaran (TEM). Kaedah SPE yang mengandungi MWCNTs-g-Agarose-g-PMMA sebagai bahan pengeras telah dibangunkan dan digabungkan dengan spektrometri penyerasan atom nyala bagi penentuan ion plumbum di dalam sampel air semula jadi. Kaedah tersebut memberikan analisis yang cepat, menunjukkan kepekaan yang baik dan kepersisian yang cemerlang, serta sesuai bagi pengekstrakan dan pra-pemekatan sisa racun perosak dan ion logam surih di dalam sampel air dan sayur-sayuran.
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6.11 Recovery percentage of lead with number of cycles using MWCNTs-g-Agarose-g-PMMA-SPE.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AA</td>
<td>Acrylic acid</td>
</tr>
<tr>
<td>APS</td>
<td>Ammonium peroxydisulfate</td>
</tr>
<tr>
<td>ATRP</td>
<td>Atom Transfer Radical Polymerization</td>
</tr>
<tr>
<td>CAN</td>
<td>Ceric ammonium nitrate</td>
</tr>
<tr>
<td>CHN</td>
<td>Carbon hydrogen and nitrogen analysis</td>
</tr>
<tr>
<td>CNTs</td>
<td>Carbon nanotubes</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethyl formamide</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
</tr>
<tr>
<td>D-μ-SPE</td>
<td>Dispersive micro-solid phase extraction</td>
</tr>
<tr>
<td>EF</td>
<td>Enrichment factor</td>
</tr>
<tr>
<td>FESEM</td>
<td>Field emission scanning electron microscope</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier Transform Infrared Spectroscopy</td>
</tr>
<tr>
<td>G (%)</td>
<td>Percentage grafting</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>GPC</td>
<td>Gel permeation chromatography</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>KPS</td>
<td>Potassium persulfate</td>
</tr>
<tr>
<td>LLE</td>
<td>Liquid-liquid extraction</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
</tr>
<tr>
<td>MMA</td>
<td>Methyl methacrylate</td>
</tr>
<tr>
<td>Mₐ</td>
<td>Number average molecular weight</td>
</tr>
<tr>
<td>Mₘw</td>
<td>Mass average molecular weight</td>
</tr>
<tr>
<td>MWCNTs</td>
<td>Multiwall carbon nanotubes</td>
</tr>
<tr>
<td>PANI</td>
<td>Polyaniline</td>
</tr>
<tr>
<td>PBMA</td>
<td>Poly(butyl methacrylate)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>PDI</td>
<td>Polydispersity index</td>
</tr>
<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
</tr>
<tr>
<td>PEMA</td>
<td>Poly(ethyl methacrylate)</td>
</tr>
<tr>
<td>PHMA</td>
<td>Poly(hexyl methacrylate)</td>
</tr>
<tr>
<td>PMMA</td>
<td>Poly (methyl methacrylate)</td>
</tr>
<tr>
<td>PNVI</td>
<td>Poly( N-vinyl imidazole)</td>
</tr>
<tr>
<td>PS</td>
<td>Polystyrene</td>
</tr>
<tr>
<td>PVP</td>
<td>Polyvinylpyrrolidone</td>
</tr>
<tr>
<td>r.p.m</td>
<td>Rotation per minute</td>
</tr>
<tr>
<td>R²</td>
<td>Coefficients of determination</td>
</tr>
<tr>
<td>RR</td>
<td>Relative recovery</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative standard deviation</td>
</tr>
<tr>
<td>SBSE</td>
<td>Stir bar sorptive extraction</td>
</tr>
<tr>
<td>SPE</td>
<td>Solid phase extraction</td>
</tr>
<tr>
<td>SPME</td>
<td>Solid phase microextraction</td>
</tr>
<tr>
<td>Tg</td>
<td>Glass transition temperature</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>μ-SPE</td>
<td>Micro solid phase extraction</td>
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LIST OF SYMBOLS

% - Percent
°C - Degree celcius
cm - Centimeter
g - Gram
g/mol - Gram per mol
kg - Kilogram
K_{o/w} - Octanol-water distribution coefficient
L - Liter
M - Molarity
mg - Milligram
min - Minutes
mL - Milliliter
mL/min - Milliliter per minute
mm - Millimeter
ng - Nanogram
nm - Nanometer
ppb - Part per billion
ppb - Part per billion
s - Second
v/v - Volume per volume
μg - Microgram
μg/L - Microgram per liter
μg/mL - Microgram per milliliter
μL - Microliter
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CHAPTER 1

INTRODUCTION

1.1 Research Background

Rapid development of industrial and agriculture are resulting in incrementing levels of heavy metals and pesticide residues in biological and environmental samples, and this pollutants have toxic effects on human health and growth of plant. These toxic heavy metals and pesticide residues can gradually pile up in the human being through the food chain. When the amounts are very high, the metals can be hazardous to human health. Biological and environmental sample matrices are complex and often contain interfering elements and organic compounds that can mask or interfere with the compounds of interest, thus, direct analysis may not be possible. The sample preparation step before chromatographic analysis is considerable intricate, and expensive.

Many routine pretreatment methods utilized in the determination of heavy metal are often time period consuming, labor intensive and substantial amount of organic solvent wasting. Sample preparations are very commonly carried out using conventional extraction methods such as liquid-liquid extraction (LLE) and solid phase extraction (SPE) to isolate, extract and concentrate target analytes. However, LLE are time consuming, labour-intensive and multi-stage operations. SPE has significant improvement over LLE by minimizing the consumption of chemicals and disposal cost of organic wastes.
Researchers have been devoted to the development of miniaturized sample preparation which resulted in significant solvent and sample savings. Apparently, the introduction and development of new materials/sorbents for microextraction have promising alternative to overcome some problems that occurred in analytical chemistry field. This microextraction is called sorbent/material-based microextraction. These relatively new techniques include such as solid phase microextraction (SPME) (Cavaliere et al., 2012), stir bar sorptive extraction (SBSE) (Maggi et al., 2008), dispersive solid phase extraction (DSPE) (Guan et al., 2013) and micro solid phase extraction (µ-SPE). Microextraction is one of the solventless microextraction techniques that dramatically simplifies the sample preparation procedures by combining the integration of sampling, isolation and enrichment in one step (Pawliszyn, 1997).

Carbon nanotubes (CNTs) have attracted special interest in multidisciplinary study and have shown potential applications in many research areas since their discovery by Iijima in 1991 (Iijima, 1991). Their nanometer size, high aspect ratios, and more importantly their excellent mechanical, electrical and conducting properties make them highly suitable in the preconcentration of metal ions and organic pollutants in the treatment of polluted biological and environmental samples.

CNTs are generally insoluble and astringently aggregate due to Van der Waals magnetization among the nanotubes, engendering a quandary when homogenous dispersion is desirable. To surmount this barrier, sundry physical and chemical approaches have been explored including direct suspension of CNTs in polymer solution via sonication, in situ polymerization in the presence of CNTs, and the chemical modification of CNTs to enhance solubilization. Overall, the grafting of CNTs to polymer through covalent functionalization appears to be one of the most attractive and efficacious ways to achieve homogenous dispersion of CNTs in polymer matrices in order to make high quality nanocomposites. This technique has been used to make nanocomposites of CNT-polymers, mostly utilizing synthetic based polymers. However, information on the utilization of natural biopolymer grafted to carbon nanotube is currently scarce and thus of interest in this study.
Generally, chemical modification is the inter-conversion or replacement of functional groups or atoms (including hydrogen) already present on the main chain (backbone) (Ebdon, 1991). This can also be described as the alteration of chemical structure of group of atoms or molecules by introducing another group of atoms or molecules via chemical means which can provide hybrid materials with improved chemical and physical properties. Chemical modification of polymer is a process which polymers/monomers are transformed into distinct copolymers via chemical means, having improved chemical and physical properties. Chemical modification of high polymers is an important aspect of polymer science which continues to receive considerable attention. One of the important aspects in the chemical modification is in the reaction of polymers leading to block and graft copolymers. An example of the formation of graft and block copolymers from synthetic polymers has been reported (Stevens, 1999). Recently, more research works have been introduced to prepare graft copolymers from natural polymers. For example, cellulose has been grafted with several monomers to improve its properties. These include homogenous graft copolymerization of styrene onto cellulose in sulphur dioxide-diethylamine-dimethyl sulphoxide solvent (Tsuzuki et al., 1980), acrylonitrile and methyl methacrylate monomers grafted onto cellulose in dimethyl sulphoxide-paraformaldehyde solvent (Nishioka & Kosai, 1981), methyl acrylate onto cellulose (Nishioka et al., 1983), hydroxyethyl methacrylate graft copolymerization onto cellulose (Nishioka et al., 1986), grafting of styrene monomer onto starch (Kaewtatip and Tanrattanakul, 2008).

Graft copolymerization is one of the interesting and promising techniques used to prepare a well-defined copolymer with novel architecture, composition and functionalities. As a result, graft copolymers have a variety of potential applications in many fields including textile industries, medical, pharmaceuticals and chromatography column (separation) technology. A graft copolymer is a macromolecular chain with one or more sort of block connected to the main chemical chain as side chain(s). Thus, grafted polymer has the general structure of polymer backbone or trunk polymer with different points along its size. An external factor is utilized to create free radical sites on this preformed polymer (polysaccharide in case of grafted polysaccharides) for starting graft copolymerization.
Graft-modification of polysaccharides is widely research in recent years to obtain macromolecular materials greater to the source polysaccharides, exhibiting more preponderant resistance to heat or abrasion, more preponderant oil/water repellant qualities, or medicament activity and higher mechanical vigor. In their native form polysaccharides are fairly resistant to degradation under shear and perform as subsidiary flocculants (Singh et al., 2000), but have poor shelf life because of their susceptibility to biodegradation. On the other hand synthetic polymers can be facilely modified, but poor shear resistant is their properties. Natural polysaccharides grafted to synthetic polymers can be changed into highly customizable matrices with hybrid properties opportune for different applications (Gref et al., 2002, Ohya, 2001).

1.2 Problem Statement

In the last two decades, efficient and sensitive analytical instrumentation have been introduced for various applications. However, sample preparation today remains considered as the most crucial step in the whole analytical process. Numerous sample preparation techniques have been developed with the following main goals: to improve the selectivity in extraction, to minimize the initial sample sizes, to facilitate the automation and to reduce or eliminate the volume of organic solvent involved in the extraction (Curylo et al., 2007; Smith, 2003).

Based on the aims of sample preparation techniques, traditional LLE method does not fulfill current requirements and it has been displaced by new extraction techniques such as SPE, D-µ-SPE, µ-SPE, magnetic solid phase extraction (MSPE) and solid phase membrane tip extraction (SPMTE). Several comprehensive reviews on the current advances in sample preparation field and emphasizing the importance of sample preparation in the analytical process have been published (Turiel and Martín-Esteban, 2010; Augusto et al., 2010).
In recent years, adsorption of toxic compounds by polysaccharides and modified polysaccharides is increasing because these sorbents are low-cost (Górecki et al., 1999). Polysaccharides have poor shelf life and bad resistant in their natural forms but they can be changed into hybrid properties under grafting for various applications. To the best of our knowledge, there has been no report on agarose (as polysaccharide) grafted with methyl methacrylate copolymer using ceric ammonium nitrate (CAN) as redox initiator with microwave assisted method were synthesized and characterized.

Raw multiwall carbon nanotube (MWCNTs) are suitable for adsorption of toxic compounds but they are generally insoluble and severely aggregated, due to Van der Waals attraction among the nanotubes, creating a problem when homogenous dispersion is desirable, thus modification of MWCNTs is important. Modifications of MWCNTs by special functional groups or polymers have been proven to be more selective than raw or oxidized MWCNTs because of their excellent properties. Thus in this study, agarose was used as new natural polymer for the modification of MWCNTs and subsequent separation of lead as heavy metal.

1.3 Objectives of the Study

This study embarks on the following objectives:

1. To prepare graft polymer based on agarose grafted with methyl methacrylate using microwave-assisted method and to characterize the properties of agarose and graft copolymer.

2. To graft the Agarose-g-PMMA with MWCNTs and to characterize the physical properties of the products obtained using FTIR, FESEM and TGA.
3. To develop Agarose-g-PMMA micro-solid phase extraction combined with gas-chromatography with micro-electron capture detector for the analysis of selected pesticides in environmental water samples.

4. To develop and apply Agarose-g-PMMA dispersive micro-solid phase extraction combined with inductively plasma-mass spectrometry for the extraction and determination of selected heavy metals in vegetables and natural waters.

5. To develop and apply MWCNTs-g-Agarose-g-PMMA-SPE combined with flame atomic absorption spectrometric detection to the determination of lead ions in vegetables and natural waters.

1.4 Scope of the Study

This study was designed to prepare agarose-g-PMMA using microwave-assisted method. Characterization of agarose-g-PMMA was by using Fourier transformed infrared (FTIR) analysis to ascertain the presence of methyl methacrylate on the agarose backbone. Glass transition temperatures were studied by means of differential scanning calorimetry (DSC), which measures endothermic and exothermic heat flow of the copolymer (in relation to their chemical structure and molecular weight) as a function of temperature/time. Thermal stability of the selected graft copolymer was studied by thermogravimetric analysis (TGA), which is based on the continuous measurement of change in weight of sample as a function of temperature. Molecular weight and molecular weight distribution of the hydrolyzed grafted poly methyl methacrylate (PMMA) were also evaluated using gel permeation chromatography (GPC). CHN analysis was also carried out to determine the percentage elemental composition of carbon, hydrogen and oxygen. Development and application of Agarose-g-PMMA sorbent based on microextraction methods were investigated. Several important extraction parameters were optimized comprehensively and analytical performances of the developed method were evaluated in the study. Agarose-g-PMMA-μ-SPE and Agarose-g-PMMA-D-μ-SPE methods were demonstrated to extract selected pesticides and heavy metals using
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