SYNTHESIS AND CHARACTERISATION OF SILICA AND MAGNETIC OXIDE NANOCOMPOSITE MODIFIED POLY (4,4'-CYCLOHEXYLIDENE BISPHENOL OXALATE) FOR DNA EXTRACTION

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By

ALBALAWI AISHAH NAWAF A

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

July 2019

DEDICATION

This work is dedicated to ALLAH tabaraka wa ta'ala,

To the spirit of my late dad, my lovely mum, to the love me husband, my kids, Jood, Wajed, Ahmed, Rital, and Mohammed, to my brothers and my sisters, all sons of my brothers and sisters and all my friends.
Alhamdullillah Rabbil alamin.

"Indeed little science distance one from Allah, deeper science takes one close to Allah"

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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July 2019

Chairman : Professor Nor Azah Binti Yusof, PhD
Faculty : Science

In the present project, a novel polymer has been synthesised as polyester- poly(4,4'-cyclohexylidene bisphenol oxalate) (PBPZO) by the condensation of oxalyl chloride with 4,4'-cyclohexylidene bisphenol, where its efficacy was tested for the solid phase extraction of DNA. Surface modification of polymer is also applied by using reinforcement (inorganic oxide) such as iron oxide nanoparticles and silica nanoparticles. Iron oxide (Fe_3O_4) with the particle size 50-100 nm, microcrystalline cellulose (MCC) with 20 µm particle size, urea, HNO_3, and NaOH have been used for the surface modification of PBPZO by physically mixing dispersed solutions. A novel PBPZO/ silica nanocomposite was prepared by two methods. The first method was by mixing PBPZO solution with fumed silica NPs that follow mixing solution method. The second method was by mixing 4, 4'-cyclohexylidenebisphenol monomer solution in the presence of three different ratios of fumed silica (11 nm) NPs: 3.7 wt.%, 7.0 wt.% and 13.0 wt.%. The product of synthesised polymer is a white powder with an average particle size of 162.45 nm, and PBPZO/ cellulose/ magnetite composite, which was black powder with magnetic properties characterised by vibrating sample magnetometer (VSM), scanning electron microscopy (SEM) and Brunauer- Emmett-Teller (BET) analysis. Furthermore, polymer nanocomposites of PBPZO/ silica were characterised by SEM analysis and BET. The average diameter of PBPZO/ silica (3.7 wt.%), PBPZO/ silica (7 wt.%), PBPZO/ silica (13 wt.%) and PBPZO/ silica solution were 67.8 nm, 61.5 nm, 60.6 nm and 55.9 nm respectively. The structure of polymer and polymer nanocomposites were investigated by Fourier-transform infrared spectroscopy (FTIR) and energy dispersive (x-ray) analysis (EDX). The thermal properties of the PBPZO, PBPZO-MCC-magnetite composite and PBPZO/ silica nanocomposites were studied by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). The solid phase application of the resulting polymer and polymer nanocomposites have been applied in DNA extraction, with the results indicating that the extraction efficiency is strongly influenced by the weight of polymer and polymer nanocomposites and binding buffer type. Among three types of buffers tested, 2 M GuHCl/ EtOH, NaCl and phosphate buffered saline (PBS) buffers, GuHCl buffer produced the most satisfactory results in terms of extraction efficiency for PBPZO and PBPZO nanocomposites that equal 2448 (ng/uL), 7237.5 (ng/uL) and 3370 (ng/uL) with percentages of DNA extraction (16.2%, 72.4%, and 24.4%) for PBPZO, PBPZO/ cellulose/ magnetite composite and PBPZO/ silica nanocomposites respectively. The results of the study indicated that the developed PBPZO, PBPZO/ cellulose/ magnetite composite, and PBPZO/ silica nanocomposites can be a potential candidate for the high efficiency extraction of DNA.

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

SINTESIS DAN PENCIRIAN SILIKA DAN MAGNETIK OKSIDA NANOKOMPOSIT TERUBAHSUAI POLI (4,4'- SIKLOHEKSILIDEN BISFENOL OKSALAT) UNTUK PENGEKSTRAKAN

Oleh

ALBALAWI AISHAH NAWAF A

Julai 2019

Pengerusi : Profesor Nor Azah Binti Yusof, PhD
Fakulti : Sains

Dalam kajian ini, suatu polimer yang novel teleh sintesis sebagai poliester- poli (4,4'-siklohexilidina bisfenol oksalat) PBPZO melalui pengkondensasian klorida oksalil dengan 4,4'-bisfenol siklohexiliden, di mana keberkesanannya telah diuji bagi pengekstrakan fasa pepejal bagi DNA. Pengubahsuaian permukaan polimer juga diaplikasikan dengan menggunakan peneguhan (oksida bukan organik) seperti nanopartikel oksida besi (NP) dan silika (NP). Oksida besi (Fe_3O_4) dengan saiz partikel 50-100 nm, selulosa mikrokristalin (MCC) dengan saiz partikel 20 µm, urea, HNO_3, dan NaOH telah digunakan bagi pengubahsuaian permukaan PBPZO melalui percampuran secara fizikal larutan terserak. Suatu PBPZO/ nanokomposit silika telah disediakan melalui dua kaedah. Kaedah pertama adalah melalui percampuran larutan PBPZO dengan NP silika wasap yang mengikuti kaedah larutan percampuran. Kaedah kedua adalah melalui percampuran 4,4'-larutan monomer bisfenol siklohexiliden dengan kehadiran tiga nisbah silika wasap berbeza (11 nm) NP: 3.7 wt.%, 7.0 wt.% dan 13.0 wt.%. Produk polimer yang disintesis ialah serbuk putih dengan purata saiz partikel 162.45 nm, dan PBPZO/ selulosa/ komposit magnetit, serbuk hitam dengan sifat magnetik dicirikan oleh getaran sampel magnetometer (VSM), mikroskopi elektron pengimbas (SEM) dan analisis BET. Di samping itu, nanokomposit polimer PBPZO/ silika telah dicirikan oleh analisis SEM dan BET. Purata diameter polimer/silika, masing-masing ialah (3.7 wt.%), polimer/ silika (7.0 wt.%), polimer/ silika (13.0 wt.%) dan polimer/ sol silika ialah 67.8nm, 61.5 nm, 60.6 nm dan 55.9 nmmasing-masing. Struktur polimer dan nanokomposit polimer telah diselidiki melalui spektroskopi infra merah jelmaan fourier (FTIR) dan analisis tenaga daya serak (sinar-X) (EDX). Sifat termal poli(bisfenol z oksalat), poli(bisfenol z oksalat)-MCC-nanokomposit magnetik dan nanokomposit polimer/silika telah dikaji melalui DSC dan TGA. Pengaplikasian fasa pepejal akibat polimer dan nanokomposit polimer digunakan dalam pengekstrakan DNA, dengan dapatan yang menunjukkan bahawa keberkesanana pengekstrakan adalah sangat dipengaruhi oleh berat polimer dan nanokomposit polimer dan jenis penimbal pengikat. Antara ketiga jenis penimbal yang diuji, penimbal 2 M GuHCl/EtOH, NaCl dan salin tertimbal fosfat (PBS), penimbal GuHCl menghasilkan keputusan yang paling memuaskan dari segi hasil dan kecekapan pengekstrakan untuk nanokomposit polimer dan polimer yang sama dengan 2448 (ng / uL), 7237.5 (ng / uL) dan 3370 (ng / uL) dengan peratusan pengekstrakan DNA (16.2%, 72.4%, dan 24.4%) untuk nanokomposit magnetik PBPZO, PBPZO/ selulosa/ magnetite dan nanokomposit PBPZO/ silica nanocomposites, masing-masing. Dapatan kajian ini menunjukkan bahawa PBPZO, PBPZO/ selulosa / komposit magnetit, dan PBPZO/ nanokomposit silika dapat menjadi calon yang berpotensi bagi keberkesanan pengekstrakan DNA.

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I would like to begin by saying ‘Bismillahir Rahmanir Raheem’ (In the name of Allah, the most Gracious and most Merciful).

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Declaration by graduate student

I hereby confirm that:

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LIST OF ABBREVIATIONS

ABS	Acetate buffer solution
AE	10 nMTris-HCl + 0.5 nM EDTA PH= 9
ChTSN	Chitosan
CTAB	Cetyltrimethyl ammonium bromide
CNTs	Carbon nanotubes
CS	Chitosan
DNA	Deoxyribonucleic acid
DE	Diatomaceous earth
DSC	Differential scanning calorimetry
DMAP	Dimethyl amino pyridine
EDS	Energy dispersive spectroscopy
EDX	Energy Dispersive X-Ray
EtOH	Ethanol
FTIR	Fourier Transform Infrared Spectroscopy
FC	free cells
GuHCl	Guanidine hydrochloride
ssDNA	Single –stranded DNA
ISO	International Organization for Standardization
NNI	National Nanotechnology Initiative
LbL	Layer-by-layer
MBs	Magnetic beads
MCC	microcrystalline cellulose
MNPs	Magnetic Nanoparticles
mRNA	Molecular ribonucleic acid

NPs	Nanoparticles
PBPZO	Poly(bisphenol z oxalate)
BPA	Bisphenol A
BPF	Bisphenol F
BPS	Bisphenol S
BPE	Bisphenol E
BPAF	Bisphenol AF
PE	polyethylene
PBS	Phosphate buffer solution
PCR	Polymerase chain reaction
PCS	Photon correlation spectroscopy
PVC	poly(vinyl chloride)
PS	polystyrene
PP	polypropylene
PLA	poly(lactic acid)
PA	polyamide
PEO	Poly(ethylene oxide)
iPP	isotactic polypropylene
RSD	Relative standard deviation
SEM	Scanning electron microscopy
SPE	Solid-Phase Nucleic Acid Extraction
SDS	Sodium dodecyl sulphate
THF	Tetrahydrofuran
WCNTs	single wall carbon nanotubes

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Bisphenol Z is one of 16 bisphenol analogues notarised, for use in industry. For example, Bisphenol A (BPA) ($C_{15}H_{16}O_2$) is applied in the production of epoxy resins and polycarbonate plastics. However, BPA can be substituted by Bisphenol F (BPF) (4,4′-methylenediphenol) ($C_{13}H_{12}O_2$), Bisphenol S (BPS) (4-hydroxyphenyl sulfone) ($C_{12}H_{10}O_4S$), and Bisphenol AF BPAF (4,4′ hexafluoroisopropylidene) diphenol ($C_{15}H_{10}F_6O_2$) in several applications such as adhesive plastics, water pipes, tissue substitutes, and coatings for food packaging (Chen et al. 2016). Bisphenol Z, 4,4′-(cyclohexane-1,1-diyl) diphenol ($C_{18}H_{20}O_2$) synthesised from the acid-catalysed reaction of phenol with cyclohexanone has been presented (Gregor 2012). Generally, the hydroxyl group of Bisphenol Z can be esterified successfully with acid chlorides or acid anhydrides to form several polymers of polyester or polyoxalate. The polymerisation of polyfunctional monomers by various condensation reactions such as polymers that contain the oxalyl groups that general prepared by the poly-condensation of oxalyl chloride, oxalic esters with diols/ oxalic acid (Pawlow et al., 1997). The polyoxalates have two adjacent carbonyl groups in the constitutionally repeat units and the molecular weight of these polymers is averagely high. Thus, they exhibit satisfactory mechanical properties, which render them appropriate to be practically utilised as biomedical applications (Rochester and Bolden 2015).

In the past few years, polymer nanocomposites (NCs) have been much studied because of their many desirable properties (mechanical, thermal, optical, electrical, structural, and biomedical), which offer many promising uses in several different areas of industrial product development (Zheng et al., 2004a). Compared to the traditional materials, the polymer NCs frequently demonstrate superior properties such as stiffness, strength, solvent dispensability, oxidative stability, thermal resistance, electrical conductivity, and biodegradability (Schmidt et al., 2002). Physically and structurally, and in terms of surface properties, NCs are highly affected by the interfacial adhesion of the organic compound and nanoparticles (NPs) dispersing in the polymer matrix. For instance, the polymer nanocomposites contain polymeric organics with metal/ inorganic nanoparticles (NPs); while, the magnetic nanocomposites are ferromagnetic or super-paramagnetic materials such as iron, cobalt, nickel, etc. with a diameter of 50-200 nm (Tadic et al., 2014). Several types of polymer nanocomposites have been investigated in previous studies such as polyester/ nanoclay (Valapa et al., 2017), silicates (Gupta et al., 2015), grapheme (Cui et al., 2016) carbon nanotubes (CNTs) (Chen et al., 2018), and magnetic polymer beads (Ramazanov et al., 2018). Magnetite nanoparticles (MNPs) have also attracted much research interest because of their nontoxicity, high coercively, their super paramagnetic properties, and biocompatibility. There have been many studies done on the preparation of a vast range of ferromagnetic and conductive polymer composites

(Reddy et al., 2009) using different strategies such as emulsion polymerisation, chemical grafting, and physical grafting.

The layered silicate or silica (Si) foam NPs are included as some of the most significant nanoscale materials in the R & D, while polymer NCs contain nano-reinforcements like nanoclays, graphite platelets, and carbon nanotubes. Many properties are responsible for the wide popularity and extensive use of these materials: their high surface energy, mild reactivity, and easily controllable chemical properties such as flammability, enthalpy of formation, oxidation states and chemical stability. Si is found naturally as quartz or sand, but in general it is commercially produced as crystals, fused quartz, colloidal silica, fumed silica, silica gel, and aerogel. These Si nanostructures containing the rings of Si-O (two-, three-, four- and six-membered) are complemented by the presence of non-bridging oxygen atoms (Wilson et al., 2013; Tosoni et al., 2010). The tubular and layered configurations have been studied theoretically and empirically through these nanostructures. The layered silicates comprise thin layers that are invariably bound together with counter-ions and the fundamental blocks are tetrahedral sheets. Their fundamental building blocks are tetrahedral sheets, with four oxygen atoms around Si and octahedral sheets with eight oxygen atoms around a metal such as aluminum (Miranda et al., 2003). The layered silica has a high aspect ratio (10-1000) and a layer thickness of 1 nm. As such, a small weight percentage of layered silica results in a significantly larger surface area when they are spread throughout the polymer matrix. On the other hand, the fumed silica aggregates have been shown with two varying fractal dimensions (Ibaseta and Biscans, 2010). The fumed Si being mesostructured (hexagonal) is a unit of matter that is soft and flexible, with high length to thickness ratio, and is chemically and thermally stable (Beech, 1988). Such properties make fumed silica especially suitable for certain applications e.g. filler material, porous coating and insulation material. Bonding these particles with polymer results in polymers with superior viscosity (Hwang and Hsu 2013a). Intercalated NCs, flocculated NCs, and exfoliated NCs are three varying kinds of interrelated polymer/ Si NCs (Ray and Okamoto, 2003).

Si NPs are associated with dispersion in many thermosetting, thermoplastic, elastomers, natural, and biodegradable polymers. This procedure is conducted based on a range of approaches such as the direct mixture of polymer and NPs (Jankong and Srikulkit 2008), intercalating polymer or prepolymer from solution (Beyer, 2002) such as melt intercalation (Solomon et al., 2001), sol-gel approach (*in situ* template synthesis or sol-gel technology) (Alexandre and Dubois, 2000), and *in situ* polymerisation method (Al-Hussaini et al., 2013). NCs properties are dependent on the properties of individual components and other factors, such as certain processes employed in NC fabrication, extent of mixing of two phases, volume fraction of NPs, kinds of filler materials including their orientations, kind of adhesion at the matrix interface, system morphology, NPs features, size and shape of NPs, and type of the interphase produced at the matrix interface materials (Jeon and Baek, 2010).

One important sector among many different applications of polymer compound and polymer nanocomposites that employ polymeric matrices includes the DNA isolation and purification process. Some of the challenges related to the utilisation of traditional approaches for DNA extraction such as phenol-chloroform extraction, alkaline extraction and etidium bromide– caesium chloride Gradient that include the high equipment costs, problems with the recovery and reproducibility of results, long and time-consuming procedures, requirement of experienced personnel, etc. (Liu et al., 2016; Tsigkou et al., 2014). To address some of these issues, the adsorption procedures that take advantage of nanotechnology and separation science principles were applied. This included the use of many different adsorbent materials solid support like glass particles (Tsigkou et al., 2014), silica-based matrices (Rulli et al., 2008), magnetic NPs (Fischer and Suttle, 2011). Therefore, where the efficiency, portability and analysis costs have to be equally balanced, the polymeric NCs are found to be potential candidates for the extraction of DNA and with many different polymers, the hybrid composites made up of PBPZO are emerging as appropriate adsorbents for the selective and high efficiency adsorption of DNA molecules of any size.

The adsorption of DNA on polymer nanocomposite (solid supported) takes place driven by the force of hydrophobic, hydrogen-bonding, and electrostatic interaction. Such bonding is associated with functional groups (–COOH, –OH, and $–NH_2$) of polymer nanocomposite that have been surface-modified including coating with inorganic materials (e.g., iron oxide, silica, and gold) for the investigation of highly efficient loading (Kang et al., 2008). Polymer nanocomposites have been distinguished from other solid support materials because of the polymers with NPs new class, which possess advanced properties e.g. a very small size and a relative larger surface area, with multiple applications. The overall characteristics and performances are determined based on the concentration and type of functional groups (e.g., carboxylic, iminodiacetate, amine, sulfonic), structure of polymer, and methods in preparing polymer nanocomposites (Akamatsu et al., 2008). Moreover, the total surface area, shape of micropores, and porosity of the polymer nanocomposites have a significant role in the mechanism of DNA isolation (Rozenberg and Tenne, 2008).

1.2 Problem Statement

Based on the background information, the following problem statement is identified as follows:

Some polymers that are slowly degrading or non-degrading, need to be prepared using novel polymers, such as polyoxalates, which possess high degradability under aqueous conditions (Garcia and Miller, 2014). Thus, is suitable for DNA applications e.g. disease therapy and drug delivery. Nearly all polyoxalates have small surface areas and microporous structures, so there is a need to modify the surface to enhance the properties of the absorption process that are filled with natural or synthetic inorganic compounds in order to improve their properties or simply to reduce cost.

The most common DNA extraction methods include liquid phase, solid support- based extraction. These methods consistently yield isolated DNA, but they differ in both quantity and quality of DNA yielded. There are multiple factors to consider when selecting DNA extraction method, including safety, time, cost, and risk of contamination. For example, liquid phase involves multiple and different chemical solvents such as phenol chloroform, which has several disadvantages. Sample contamination is also a major risk, which needs to be carefully considered, in particular, consumption of organic solvents and consumption time. On the contrary, solid phase extraction has been having the advantages such as high yield and purity of nucleic acids, sensitive, simple, equipment-free, no centrifugation, the best choice for automation, less time and steps (shorter protocol) reduced pipetting error, reusable resins and easy to use and storage reproducible.

In this current study, PBPZO polymer was subjected to synthesis with single phase organic solvent condensation polymerisation method (Sweileh and Al-Hiari, 2006).

This work has provided a process of producing polymer nanocomposites that include magnetically responsive metal oxide and microcrystalline cellulose (MCC) for the extraction of high efficiency DNA. Therefore, the study aims to analyse the structure of PBPZO that has a benzene ring and oxygen bond, which helps to form covalent bonding with the iron oxide MNPs. Moreover, subsequent adsorption of DNA by the surface modified polymer with three different buffers has also been studied.

By keeping in view the specific applications of PBPZO for extraction of DNA and solid supported properties of Si NPs, the present study is aimed at developing an ideal NC system that has a very high DNA extraction efficiency. Earlier studies dealt with the synthesis and characterisation of pure poly(4, 4'-cyclohexidene bisphenol oxalate) for the purpose of extracting DNA. Further, to enhance the DNA extraction capabilities of PBPZO by influencing its fundamental properties through the composite formation with Si, the present study was performed. In addition, this study examines the impacts of nanosilica on the polymeric matrix when added at differential ratios of fumed Si to the matrix. Thus, formed NCs were characterised thoroughly for the surface area, structure, shape, porous nature, surface morphology, etc. Finally, this was followed by the application of the NCs for the extraction of the DNA from the solution mixture, for the determination of the main factors related to the ratio of Si NCs and other processing conditions for high efficiency DNA extraction.

1.3 Objective of the Study

The focus of this study is to synthesise a new polymer material, and characterise this polymer by chemical and physical techniques and high efficiency of DNA extraction.

The specific objectives of the present study are:

i. To prepare and characterise polymer of polyester cross linked with oxalate group by condensation polymerisation and characterise PBPZO.
ii. To prepare and characterise magnetite nanoparticles (NPs) modified PBPZO with microcrystalline cellulose (MCC).
iii. To prepare and characterise silica nanocomposite modified PBPZO.
iv. To evaluate the performance of PBPZO, PBPZO-MCC-magnetite composite and PBPZO/ silica nanocomposite for DNA extraction.

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