SYNTHESIS OF MOLECULARLY IMPRINTED POLYMER FOR GLUCOSE BINDING

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ABSTRACT

Molecularly imprinted polymer (MIP) is an attractive technique for the synthesis of highly selective polymeric receptors having artificial generated recognition sites. These materials were synthesized with polymerizable functional monomers and crosslinker that were surrounded around the template molecule. After polymerization, a template molecule was removed leaving in the polymer selective recognition sites with shape, size and functionalities complementary to the template. This study presents a synthesis of MIP selectively for glucose binding. Glucose phosphate salt (GPS) was used as a template molecule with poly(allylamine hydrochloride) (PAA.HCl) as a functional monomer. Three types of crosslinkers which are epichlorohydrin (EPI), ethylene glycol diglycidyl ether (EDGE) and glycerol diglycidyl ether (GDE) were studied during the MIP synthesis. MIP prepared using EPI as a crosslinking showed the highest glucose binding capacity around 0.84 mg glucose/mg dried gel. The binding capacity of MIP prepared using EGDE and GDE are 0.78 mg glucose/mg gel and 0.38 mg glucose/mg gel respectively. It is also found that the increase on GPS monomer concentration will contribute to increase in glucose binding.

ABSTRAK

Molecularly imprinted polymer (MIP) adalah salah satu teknik yang menarik dalam menghasilkan bahan polimer yang mempunyai sifat keupayaan untuk menarik sesuatu komponen secara spesifik. Bahan ini dihasilkan melalui sintesis monomer berfungsi melalui bahan pengikat di sekitar molekul template. Selepas pepmpolimeran, molekul template dikeluarkan untuk menghasilkan ruang yang selektif untuk mengikat molekul tersebut berdasarkan bentuk, saiz and kumpulan berfungsi. Dalam kajian ini, MIP disintesiskan spesifik untuk menyerap glukosa. Glucose phosphate mono-sodium (GPS) digunakan sebagai molekul template dengan poly(allylamine hydrochloride) (PAA.HCl) sebagai monomer berfungsi. Tiga jenis bahan pengikat iaitu epichlorohydrin (EPI), ethylene glycoldiglycidyl ether (EDGE) dan glycerol diglycidyl ether (GDE) telah dikaji. MIP yang terhasil daripada bahan pengikat EPI menunjukkan kadar penjerapan gula yang paling tinggi iaitu sebanyak 0.84 mg glukosa/mg MIP, diikuti oleh bahan pengikat EGDE iaitu sebanyak 0.78 mg glukosa/mg MIP dan akhir sekali bahan pengikat GDE iaitu sebanyak 0.38 mg glukosa/mg MIP. Selain itu, melalui kajian ini juga didapati bahawa semakin tinggi kepekatan molekul template iaitu GPS akan meningkatkan kadar penyerapan terhadap glukosa.

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C_i	initial sugar concentration (mg/ml)
C_f	final sugar concentration (mg/ml)
V_s	volume of test solution (ml)
m_{MIP}	mass of dried polymer (mg)
MIP	Molecularly Imprinted Polymer
GPS	Glucose Phosphate Salt
EPI	Epichlorohydrin
EGDE	Ethylene Glycol Ether
GDE	Glycerol Duglycidyl Ether
MWS	Meranti Wood Sawdust
MISPE	Molecularly Imprinted Solid-Phase Extraction
NF	Nanofiltration
RO	Reverse Osmosis
NaOH	Sodium Hydroxide
HCL	Hydrochloric Acid
(PAA.HCL)	Poly(allylaminehydrochloric)

CHAPTER 1

INTRODUCTION

1.1 Research Background

The growing interest in biotechnological production of biofuels and specialty chemicals from lignocellulosic waste or biomass is justifiable as these materials are low priced and renewable. Biomass is the term used to describe all biologically produced matter. World production of biomass is estimated at 146 billion metric tons a year, mostly wild plant growth (Ahyan Demirbas, 2001). Biomass product can be hydrolyzed into several sugar components such as xylose and glucose.

Xylose is a hemicellulosic sugar which can be economical starting raw material from biomass product for the production of a wide variety of compounds or fuel by chemical and biotechnological process. One of these compounds is xylitol that is extensively utilized in the food, pharmaceutical and odontological industries (Rafiqul & Mimi Sakinah, 2012, Nikhil et al., 2010). Xylitol is used as a sweetener and for medicinal purposes. In addition, xylose is safe for use in foods because it is contain natural healing agents that good for our health. However, in order to obtain high purity xylitol, other hemicellulosic sugars especially glucose need to be eliminates. Furthermore, the conversion of xylose into xylitol is very limited in the presence of

glucose. Glucose needs to be separated out from the xylose by any of the sugar separation method.

Molecularly imprinted polymer (MIP) is an attractive technique for the synthesis of highly selective polymeric receptors having artificial generated recognition sites. These materials were synthesized with polymerizable functional monomers and crosslinkers that are surrounded around the template molecule. After polymerization, template molecule was removed leaving in the polymer selective recognition sites with shape, size and functionalities complementary to the template (Okutucu *et al*., 2011).

Molecular imprinting technology is a rapidly developing technique for the preparation of polymers having specific molecular recognition properties for a given compound, its analogues or for a single enantiomer (Karlsson *et al*., 1999; Yin *et al*., 2005). Synthesis of MIP is a relatively straightforward and inexpensive procedure. As a technique for the creation of artificial receptor-like binding sites with a 'memory' for the shape and functional group positions of the template molecule, molecular imprinting has become increasingly attractive in many fields of chemistry and biology, particularly as an affinity material for sensors (Dickert *et al*.,; Haupt *et al*., 2000), binding assays (Chianella *et al*., 2002), artificial antibodies (eg Lavignac *et al*., 2004; Ye *et al*., 2001), adsorbents for solid phase extraction (Weiss *et al*.,; Bereczki *et al*., 2001) and chromatographic stationary phases (Hwang *et al*., 2001; Peter *et al*., 2003; Liu *et al*., 2006).

This study presents a synthesis of MIP selectively for glucose binding. Glucose phosphate salt (GPS) was used as a template molecule with poly(allylamine hydrochloride) (PAA.HCL) as a functional monomer. Three types of crosslinkers which are epichlorohydrin (EPI), ethylene glycol diglycidyl ether (EDGE) and glycerol diglycidyl ether (GDE) were studied during the MIP synthesis.

1.2 Objectives Of The Research

The objective of this research is to synthesis MIP that can specifically bind to the glucose**.**

1.3 Scope Of The Research

In order to fulfill the research objective, the following scopes of research has been outlined:

i. To synthesis MIP from poly(allylamine hydrochloride) copolymer with different cross-linker and using glucose phosphate mono-sodium (GPS) as a template molecule.
ii. To study the effect of different cross-linker such as epichlorohydrin (EPI), ethylene glycol diglycidyl ether (EGDE) and glycerol diglycidyl ether (GDE) during MIP synthesis.
iii. To study the effect of GPS concentration during MIP synthesis.

CHAPTER 2

LITERATURE REVIEW

2.1 Biomass

Biomass is a term for all organic material that stems from plants (including algae, trees and crops) (Mckendry, 2002). The biomass resource can be considered as organic matter, in which the energy of sunlight is stored in chemical bonds. When the bonds between adjacent carbon, hydrogen and oxygen molecules are broken by digestion, combustion, or decomposition, these substances release their stored, chemical energy. Biomass has always been a major source of energy for mankind and is presently estimated to contribute of the order 10–14% of the world's energy supply.

Biomass can be converted into useful forms of energy using a number of different processes. Factors that influence the choice of conversion process are the type and quantity of biomass feedstock, the desired form of the energy, i.e. end-use requirements, environmental standards, economic conditions and project specific factors (Mckendry, 2002). In many situations it is the form in which the energy is required that determines the process route, followed by the available types and quantities of biomass. Table 2.1 shows some example of biomass sources.

Table 2-1: Examples of biomass energy resources (Demirbas, 2001)

Biomass energy resources	Example
Wastes	• Agricultural production wastes • Agricultural processing wastes • Crop residues • Mill wood waste • Urban wood waste • Urban organic wastes
Forest Products	• Wood • Logging residues • Trees, shrubs and wood residues • Sawdust, bark etc. from forest clearings
Energy Crops	• Short rotation woody crops • Herbaceous woody crops • Grasses • Starch crops (corn, wheat and barley) • Sugar crops (cane and beet) • Forage crops (grasses, alfalfa and clover) • Oilseed crops (soybeen, sunflower, safflower)
Aquatic Plants	• Algae • Water weed • Water hyacinth • Reed and rushes

2.2 Ethanol Production

Ethanol can be produced from certain biomass materials which contain sugars, starch or cellulose. The best known source of ethanol is sugar cane, but other materials can be used, including wheat and other cereals, sugar beet, jerusalem artichoke and wood.

The choice of biomass is important as feedstock costs typically make up 55–80 % of the final alcohol selling price. Starch based biomass is usually cheaper than sugar based materials but requires additional processing. Similarly, cellulose materials, such as wood and straw, are readily available but require expensive preparation.

Ethanol is produced by a process known as fermentation. Typically, sugar is extracted from the biomass crop by crushing, mixed with water and yeast and kept warm in large tanks called fermenters. The yeast breaks down the sugar and converts it to methanol. A distillation process is required to remove the water and other impurities in the diluted alcohol product (10–15% ethanol). The concentrated ethanol (95% by volume with a single step distillation process) is drawn off and condensed to a liquid form.

Ethanol can be used as a supplement or substitute for petrol in cars. Brazil has a successful developed industrial scale ethanol project which produces ethanol from sugar cane for blending with petrol (Demirbas, 2001).

2.3 Xylitol Production

Xylitol is found naturally in fruits like strawberries, plums and pears, but in small quantities, which makes its extraction difficult and uneconomical. Xylitol can be produced by biological means from xylose by utilizing yeasts such as the species belonging to Candida genus, fungi such as Petromyces albertensis and also by bacteria like Enterobacter liquefaciens. According to previous study by Yuan *et al*. (2013), hemicellulose hydrolysate from corncobs without detoxification was used for xylitol production by a newly isolated and high inhibitor-tolerant yeast strain of C. tropicalis

CCTCC M2012462. Other than that, the research study by Santos *et al*. (2008) also provides a preliminary contribution to the development of a bioprocess for the continuous production of xylitol from hemicellulosic hydrolyzate utilizing Candida guilliermondii cells immobilized onto natural sugarcane bagasse fibers.

The most important method utilized in the synthesis of xylitol involves the chemical reduction of xylose, which in turn is obtained by the acid hydrolysis of xylan present in the hemicellulose of birchwood, beechwood or the structural plant tissues such as corn-stalks, wheat straw, cotton seed, peanut hulls, sugar cane bagasse, wood pulp and flax straw.

2.4 Molecularly Imprinted Polymer

Molecularly imprinting polymer (MIP) is a powerful method, which provides synthetic polymers with specific binding sites to template molecule. The synthesis of molecularly imprinted polymers involves the assembly formation of monomers around a template molecule followed by polymerization in the presence of a cross-linker. Removal of the template molecule by extraction leaves sites specific for the template molecule in both shape and chemical functionality, thus enabling subsequent recognition of the template as shown in Figure 2.1

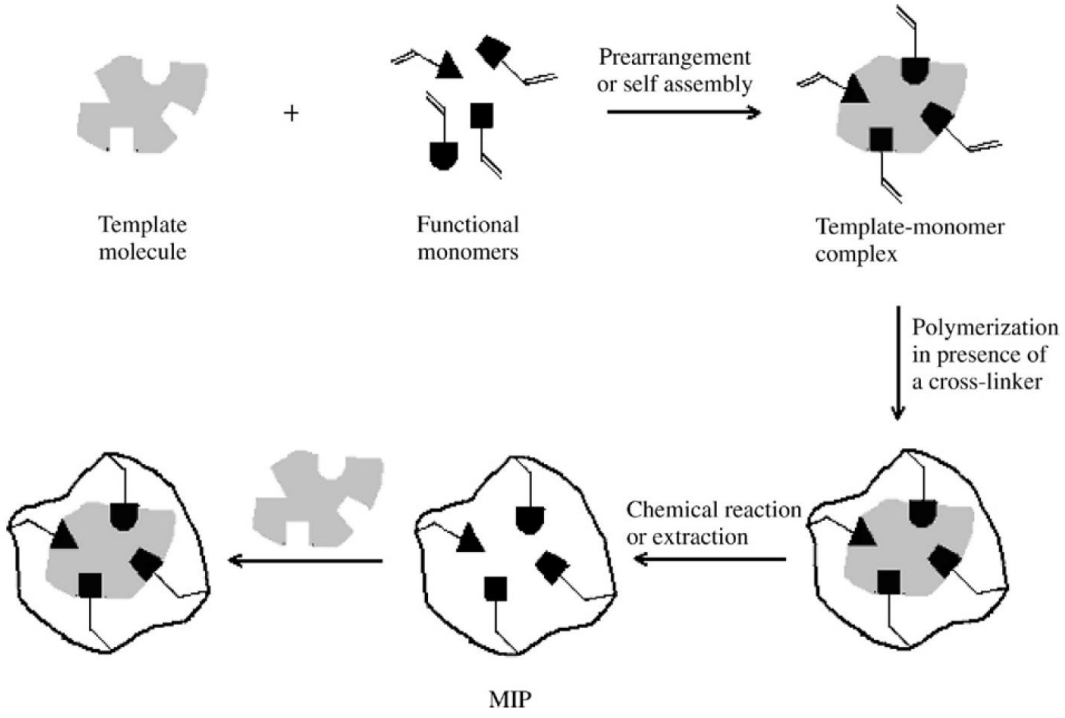

Figure 2.1: Synthesis of molecularly imprinted polymers (MIPs) and its selective recognition to target molecule (He et al. 2007).

2.5 Characteristics of MIP

2.5.1 Functional Monomers

Functional monomers are responsible for the binding interactions in the imprinted binding sites. In non-covalent molecular imprinting protocols, the monomer used in excess relative to the number of moles of template to favor the formation of template. The template to functional monomer ratios of 1:4 and upward are rather common for non-covalent imprinting.

It is clearly very important to match the functionality of the template with the functionality of the functional monomer in a complementary fashion (e.g. H-bond donor with H-bond acceptor) in order to maximize complex formation and thus the imprinting effect. When two or more functional monomers are used simultaneously in “cocktail”

polymerization, it is however also important to bear in mind the reactivity ratios of the monomers to ensure that copolymerization is feasible.

2.5.2 Template Molecules

In all imprinting process, template is one of the most important components. The template chosen must be chemically inert and stable under polymerisation conditions since all polymerisations are based on the free radical interactions. The template molecule must not participate in the radical reaction and stable upon exposure to UV or high polymerisation temperature (Cormack *et al.,* 2004). Usually, a closely structural analogue to the targeted analyte was chosen as template molecule. This is to prevent the template leaching or bleeding problem during analysis especially for quantitative analysis at trace level as not the entire template molecules are successfully extracted out from the imprinted polymer even after extensive washing (Martin *et al.*, 2004).

The following are legitimate questions to ask of a template (Cormack *et al*., 2004):

(1) Does the template bear any polymerisable groups.

(2) Does the template bear functionality that could potentially inhibit or retard a free radical polymerisation, e.g. a thiol group or a hydroquinone moiety.

(3) Will the template be stable at moderately elevated temperatures or upon exposure to UV irradiation.

2.5.3 Crosslinkers

In an imprinted polymer the cross-linker fulfills three major functions (Cormack *et al*., 2004). First of all, the cross-linker is important in controlling the morphology of the polymer matrix, whether it is gel-type, macroporous or a microgel powder. Secondly, it serves to stabilize the imprinted binding site. Finally, it imparts mechanical

stability to the polymer matrix. High cross-link ratios are generally preferred in order to access permanently porous (macroporous) materials and to be able to generate materials with adequate mechanical stability. Polymers with cross-link ratios in excess of 80% are often the norm.

2.5.4 Solvents

The solvent serves to bring all the components in the polymerization, i.e. template, functional monomer(s), cross-linker and initiator into one phase. However, it serves a second important function in that it is also responsible for creating the pores in macroporous polymers.

For this reason it is quite common to refer to the solvent as the "porogen". When macroporous polymers are being prepared, the nature and the level of the porogen can be used to control the morphology and the total pore volume. More specifically, use of a thermodynamically good solvent tends to lead to polymers with well developed pore structures and high specific surface areas, use of a thermodynamically poor solvent leads to polymers with poorly developed pore structures and low specific surface areas. Increasing the volume of porogen increases the pore volume (Cormack *et al.*, 2004).

2.5.5 Initiators

In principle, any of the methods of initiation described earlier can be used to initiate free radical polymerisations in the presence of templates. However, there may well be drivers for selecting one over another arising from the system under study.

For example, if the template were photochemically or thermally unstable then initiators that can be triggered photochemically and thermally, respectively, would not be attractive. Where complexation is driven by hydrogen bonding then lower polymerization temperatures are preferred, and under such circumstances

photochemically active initiators may well be preferred as these can operate efficiently at low temperature (Cormack *et al*., 2004).

2.5.6 Molar Ratio of Template: Monomer: Cross-linker (T: M: X)

Quality of the MIP recognition sites are highly dependable on the molar relationship between template and functional monomer. The common optimum mole ratio of template molecule, monomer and cross-linker for production of MIP is 1: 3-5: 20-30 (Komiyama *et al*., 2003). Theoretically, high molar ratio of T: M affords less than optimal complexation on account of insufficient functional monomer and too low of T: M causes non-selective binding (Andersson *et al.*, 1999). An excess of either template or functional monomer during polymerisation is unfavourable regard to selectivity (Andersson *et al.*, 1999).

MIP prepared at T: M = 1: 15 and 1: 20 exhibited poor recognition effect as it is difficult to clearly discriminate them from the corresponding blank polymers (Baggiani *et al.*, 2004). Experiments carried out by Theodoridis *et al*., 2004 showed that high molar ratio of T: M, high affinity recognition sites would be limited as the agglomeration of template in organic solvent environment could occur. Thus, polymers prepared at the ratio of 1: 2.7: 13.4 exhibited poor recognition properties compared to polymers synthesized at ratio 1: 46: 230 and 1: 4.6: 23.

2.6 Advantages and Disadvantages of MIP

Among the advantages of MIP material are (Mahony *et al.*, 2005):

i. Cost-effective alternative to biomolecule-based recognition
ii. Ease of preparation
iii. Enhanced thermal and chemical stability compare to antibodies
iv. Can be prepared in different formats (bead/block/thin film) depending on the following need of the application
v. Can be stored for years without loss of affinity for target analyte

However, MIP still has some disadvantages as follow:

i. Lower catalytic capabilities than biological counterparts
ii. Binding site heterogeneity providing a distribution of binding site affinities
iii. Template 'bleeding' requires suitable template analogue for the imprinting step and affects quantitative applications
iv. Grinding and sieving of bulk polymer for SPE/LC applications is labor-intensive and inefficient in material yield.

2.7 Applications of MIP

MIPs have been successfully applied to the pretreatment of analytes in foods, drugs, and biological and environmental samples (Chiang et al., 2007; Caro et al., 2006). MIP also has a potential application in pharmaceutical field such controlled release, drug monitoring devices and biological receptor mimetics (Allender et al., 2000).

Molecular imprinting, which allows the formation of specific recognition sites in polymer matrices also applied widely for developing robust sensors for industry, diagnostics, and environmental analysis. In these sensors, molecularly imprinted polymers are coupled with appropriate transducers for the quantitative detection. This can be shown from previous article by Isao *et al*. (1999), that describes the recent trends and some examples of sensors based on molecular imprinting.

In the last years, an area of great challenge in MIP technology is that of therapeutic agents, various MIPs have been used as unusual synthetic polymeric carriers to prepare drug delivery systems. (Sellergren *et al*., & Cunliffe *et al*., 2005; Puoci *et al*., 2008). MIPs for drug delivery applications should have specific characteristics such as the imprinted cavities should be stable to maintain the conformation in the absence of the template, but also flexible to facilitate the realization of a fast equilibrium between

the release and re-uptake of the template in the cavity (Allender *et al*., 1999, Alvarez *et al*., 2004). Furthermore, MIPs should be stable to resist enzymatic and chemical attacks and mechanical stress that can be found in biological fluids.

2.8 Sugars Separation Methods

Membrane technology has shown advantages as compared to other separation and purification techniques, including lower energy consumption, sustainable processing, simple modification of the operational variables and relatively easy scale-up. As a main section of membrane technology, pressure-driven membrane separation including microfiltration, ultrafiltration, nanofiltration and reverse osmosis had attracted great attention for their unique ability to separate and purify process streams.

Hydrolyzates separation by membrane is a promising and economic way to remove inhibitors and simultaneously concentrate sugar to a high extent. Study by Sagehashi *et al*. (2007) separated phenols and furfural from biomass-superheated steam pyrolysis-derived aqueous solution by a RO membrane NTR-759HR, and the solution was concentrated effectively during the process. Qi *et al*. (2011) investigated nanofiltration for furfural removal from monosaccharides with synthetic solution, and 66.2% of furfural removal as well as 98.5% of sugars recovery were obtained by NF90 at 3.5 MPa in diafiltration experiments.

In a study of Sjoman *et al*. (2008), nanofiltration is investigated as a possible separation method to recover d-xylose into the permeate from a hemicellulose hydrolyzate stream. In this NF process xylose is purified, i.e. the xylose content in the total dry solids of the permeate is increased as higher molar mass impurities are rejected by the NF membrane.

Since the pKa value for neutral sugars is about 12, neutral sugars can be retained in an anion exchange polymer if strongly-basic mobile phase is used. In such cases, an approximately 0.1-M sodium hydroxide solution is used as the mobile phase. In general, sugars elute in order from monosaccharides to oligosaccharides. When combined with a

gradient method that varies the concentration of sodium hydroxide, multiple components can be separated at the same time.

Size exclusion method can be used to separate sugars based on molecular weight. It provides a distribution of molecular weights ranging from several hundreds to several millions. Essentially, separation is based on molecular size, so components with the same molecular weight cannot be separated. A hydrophilic polymer is used for packing material, and only water is generally used as the mobile phase. However, a salt is sometimes added to the mobile phase for ionic or other components, which can interact with the packing material.

CHAPTER 3

METHODOLOGY

3.1 MIP Hydrogel Synthesis

The MIP was synthesized according to the method developed by Wizeman *et al*. (2001). 25% w/v of PAA.HCl solution was mixed with GPS. Under continuous stirring, NaOH was added to neutralize amines site about 2 hours. Cross-linker was then added and allows to stir for 20 minutes. The polymerization solution was poured into the petri dish to form a gel slab. After gelation, the polymer was allowed to stand overnight to ensure complete crosslinking. The polymer was then cut into 2cm x 1cm squares and washed in 1M aqueous NaOH solution for at least 48 hours to remove GPS imprint. In order to remove the remaining NaOH, the polymer was washed with deionized water in 1-2h intervals, 3-4 times per day. Finally, the completely washed gels was dried under air at 50°C in an oven. Figure 1 showed the MIP gel formed during this study.

(a) (b) (c)

Figure 3.1: MIP hydrogel synthesis for different crosslinkers, (a) EDGE, (b) EPI and (c) GDE

3.2 Verification of Imprinting Technique

The freshly synthesized hydrogel contain GPS imprint was placed in a known volume of deionized water and stirred slowly for 48 hours. An aliquot of the wash water was taken out. Then, the quantity of the phosphorus contain in the sample was determined by Hach's total phosphorus method. The pH of the diluted sample was checked to ensure it is between 6.5-7.5. The phosphorus concentration should be about 2% which is less than of what would be expected if all the GPS used in the synthesis were present in wash solution. Then, the same polymer was placed in a known volume of 1M NaOH solution and was equilibrated for 48 hours. The filtered aliquot was taken out and diluted appropriately for the Hach's total phosphorus test. The pH was adjusted by using HCL so that it is between 6.5-7.5. Again, the solution was tested for total phosphorus. According to Paraskevi *et al*., (2004), this test was useful to verified the absence of imprint removal in distilled water, indicating good template immobilization.