CHARACTERIZATION OF MOLECULARLY IMPRINTED POLYMER (MIP) FOR AN EXTRACTION OF CURCUMIN FROM *Curcuma longa* (Turmeric)

by

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LIST OF ABBREVIATIONS

%	-	Percent
^0C	-	Degree celcius
µg/g	-	Microgramme per gramme
µg/ml	-	Microgramme per millilitre
µm	-	Micron, micrometre
µL	-	Microlitter
a	-	Isotherm constant
AD	-	Affinity distribution
AIBN	-	Azobisisobutyronitrile
AM	-	Acrylamide
AM1	-	Semi empirical - quantum mechanics
B	-	Bound template concentration
BET	-	Brunauer, Emmett and Teller
BJH	-	Barrett–Joyner–Halenda
C_f	-	Free template concentration
C_i	-	Initial concentration
$CHCl_3$	-	Chloroform
cm^3/g	-	Cubic centimeter per gramme
d_m	-	Molecule diameter
D_p	-	Pore diameter
$E_{complex}$	-	Total energy of curcumin with functional monomer
$E_{curcumin}$	-	Total energy of curcumin
$E_{monomer}$	-	Total energy of functional monomer
EDMA	-	Ethylene glycol dimethacrylate
F	-	Final concentration
g	-	Gramme
GC	-	Gas chromatography
HOAc	-	Acetid acid
H_2O	-	Water
HPLC	-	High performance liquid chromatography
IF	-	Imprinting factor
K_0	-	Median association constant
L	-	Litre
MeCN	-	Acetonitrile
MeOH	-	Methanol
FI	-	Freundlich isotherm
LI	-	Langmuir isotherm
L-FI	-	Langmuir-Freundlich isotherm
m	-	Heterogeneity index
mg	-	Milligramme

m^2/g	-	Square meter per gramme
MIP	-	Molecularly imprinted polymer
MISPE	-	Molecularly imprinted solid-phase extraction
ml	-	millilitre
mmol	-	Millimole
mm Hg	-	Millimeters of mercury
NIP	-	Non-imprinted polymer
nm	-	Nanometer
N_t	-	Total number of binding sites
p_0	-	Saturation pressure of the gas
p	-	preassure
Q	-	Total binding amount
Q_{MIP}	-	Total binding amount of curcumin towards MIP
Q_{NIP}	-	Total binding amount of curcumin towards NIP
SD	-	Standard deviation
SEM	-	Scanning Electron Micropgraph
SPE	-	Solid-phase extraction
SPME	-	Solid-phase microextracion
THF	-	Tetrahydrofuran
UV	-	Ultraviolet
v	-	Volume
V_m	-	Quantity of the gas adsorbed at pressure
w	-	Weight
w/v	-	Weight/volume

LIST OF SYMBOLS

ΔE	-	Binding energy
Σ	-	Summation
π^*	-	Excited state
λ	-	Wave length
%	-	Percentage

ABSTRAK

PENCIRIAN POLIMER BERCETAK MOLEKUL UNTUK PENGEKSTRAKAN KURKUMIN DARI *Curcuma longa* (Kunyit)

Polimer molekul bercetak (MIP) untuk pengekstrakan selektif kurkumin telah disentesis melalui ikatan bukan kovalen di mana kurkumin digunakan sebagai templat. Polimer dibina dengan menggunakan 2 jenis monomer berfungsi iaitu asid metakrilik (MAA) dan akrilamida (AM) dengan 3 jenis pelarut iaitu klorofom ($CHCl_3$) , tetrahidrofuran (THF) dan asitonitril (MeCN). Analisis keluasan permukaan dan keporosan menunjukkan polimer yang dibina menggunakan THF memberikan saiz dimater pori tertinggi iaitu 618.43 nm. Proses pengikatan semula menunjukkan faktor molekul bercetak tertinggi adalah pada polimer yang dibina daripada MAA dan THF. Pencirian seterusnya menggunakan 3 jenis model isoterm iaitu Langmuir (LI), Freundlich (FI) dan Langmuir-Freundlich (LF-I). Solver daripada Micrsoft Excel telah digunakan untuk mengenalpasti nilai parameter dalam setiap isoterm dengan mengoptimumkan nilai R^2. Nilai R^2 yang dihitung adalah 0.91 (LI), 0.69 (FI) dan 0.96 (LF-I). Ruang bercetak, N dan kehomogenan, m telah dihitung bagi MIP dan polimer molekul tidak bercetak (NIP) menggunakan LF-I. Didapati N bagi MIP lebih tinggi iaitu 1250.62 µg/g berbanding NIP 998.35 µg/g. Analisis ini menunjukkan bahawa MIP mempunyai lebih ruang bercetak dan selektif terhadap kurkumin. Seterusnya, pengekstrakan fasa pepejal (SPE) telah dilakukan dengan menggunakan polimer seberat 150 mg. Analisis ini bertujuan untuk mengekstrak kurkumin dan hasil menunjukkan nilai permerolehan semula bagi MIP adalah 43.10 % , lebih tinggi berbanding NIP iaitu 13.46%. Bagi tujuan pengekstrakan kurkumin daripada kunyit, analisis yang sama telah dijalankan dan pemerolehan semula bagi MIP adalah sebanyak 67.76% dan NIP adalah 39.86%. Ini menunjukkan bahawa penghasilan MIP mempunyai potensi yang tinggi bagi tujuan purifikasi kurkumin dengan mengaplikasikan SPE.

ABSTRACT

CHARACTERIZATION OF MOLECULARLY IMPRINTED POLYMER FOR AN EXTRACTION OF CURCUMIN FROM *Curcuma longa* (Turmeric)

A molecularly imprinted polymer (MIP) for selective extraction of curcumin has been synthesized via non-covalent approach by using curcumin as a template. Polymerization was prepared using 2 (two) functional monomers namely methacrylic acid (MAA) and acrylamide (AM) together with 3 (three) different porogens namely chloroform ($CHCl_3$), tetrahydrofuran (THF) and acetonitrile (MeCN). Porosity and surface area analysis revealed that the polymer prepared using THF as porogen has the highest average pore diameter size i.e. 618.43 nm. Batch binding analysis revealed that the largest imprinting factor was attained by the polymer prepared using MAA and THF as functional monomer and porogen respectively. Further characterization was carried out using 3 (three) isotherm models namely Langmuir (LI), Freundlich (FI) and Langmuir-Freundlich (LF-I) isotherm. The unknown parameters in each isotherm were calculated by using Solver function in Microsoft Excel and were optimized for R^2 value. The calculated R^2 values were found to be (0.91), (0.69) and (0.96) for LI , FI and LFI respectively.. Hence, LFI was further used to calculate the binding sites (N) and homogeneity (m) of both the MIP and NIP (non-imprinted polymer). The result showed that MIP1 has more Nt (1250.62 μg/g) as compared to NIP (998.35 πg/g) suggesting that MIP has more binding sites and selective towards curcumin. A 150 mg of polymer mass was packed into SPE (solid phase extraction) cartridge and subsequently used to extract curcumin from raw turmeric extract. The recoveries were 43.10% for MIP as compared to 13.46% for NIP. This suggested that the MIP cartridge exhibited significant selectivity toward curcumin, with recoveries 67.76% and 39.86% for NIP, indicating that the synthesized MIP has the potential for curcumin purification through SPE.

CHAPTER 1

INTRODUCTION

1.1 Overview

In general agreement, bioactive compounds are essential and non-essential compound (e.g. vitamins or polyphenol) that derived naturally, are part of food chain and could give an effect on human health (Biesalski et al., 2009). These compounds may exert their effect by acting as antioxidants, activating liver detoxification enzymes, blocking the activity of bacterial or viral toxins, inhibiting cholesterol absorption, decreasing platelet aggregation, or destroying harmful gastrointestinal bacteria (Pennington, 2002). Nowadays, consumers are much aware in nutrition value and food fortification for healthcare. The abundance of traditional medicines and supplements proved that the market has high demands on natural products.

Because of the high demand, it should have a scientific way in handling and preparing bioactive compounds before it could be used for alternative products. The process of handling natural products is normally tedious and time consuming. Currently, many researches are conducted for final separation and detection steps, while less attention are paid to the development of faster, more selective cleaned up method (Möller, 2006). Sample cleaned up is very important for samples with complex matrices, such as biological fluids, food extracts and wastewater. This is because complex matrices usually contained various compounds that might suppress the targeted analytes signal. Before injection into a liquid chromatography system or other analytical

equipments, the sample matrix must be separated from the analytes of interest. Otherwise, contaminants could disturb separation and detection or even damage the analytical column. The cleaned up procedure also could enriched the analytes concentration which will improve the sensitivity.

Cleaned up method depends on types of matrices and type of targeted compound (volatile or non-volatile). Commonly, for non-volatile compound, an extraction procedure using solid-phase extraction (SPE) could be carried out. A combination of SPE with selective sorbent is a great system which could fasten up the procedure. Selective sorbent is produced using molecularly imprinted polymer (MIP) which is build based on the targeted compound. In this study, the preparation of MIP for curcumin is developed and implemented in SPE.

1.2 Molecularly imprinted polymer (MIP) as selective sorbent

Recently MIP has been broadly used as a selective sorbent or as a stationary phase for the extraction of various drugs, natural substances or traditional medicinal compounds (Rashid, Briggs, Hay, & Stevenson, 1997; Wensheng & Gupta, 2004; Yinzhe & Kyung, 2006). This technique is an adaptation from the role of antibodies - antigents concept and was developed as synthetic receptors which are suitable for separation technique. This was based on tailored selectivities and affinities. In general, an objective substrate is employed as a template molecule that binds with functional monomer during the copolymerization of the functional monomer and the cross linking agent. Movements of molecules are frozen in polymeric structures so that they are

immobilized in a desired fashion (Komiyama, Takeuchi, Mukawa, & Asanuma, 2003b). Removal of the template during washing step will leave behind cavities on the polymer matrix with the shape of the selected template. The arrangement of functional groups will result in complementary binding sites to the original template. The MIP can be prepared using covalent and non-covalent method and however the latter approach is more widely used because of its simplicity (Wang, Hong, & Row, 2004). This technique has been introduced since 1970s and developing tremendously until present. Some of the advantages of this technique are: 1) the preparation is straightforward, 2) the MIP itself is stable in terms of mechanically and thermally and 3) the MIP is reusable.

1.3 Solid-phase extraction (SPE)

Solid-phase extraction (SPE) is one of the applications that use MIP as a selective sorbent. Typically in SPE, solutes are extracted from a liquid phase into the solid phase which are readily packed with porous particles of silica with a bonded organic phase or of an organic polymer such as cross linked polystyrene (Fritz, 1999). Conventional SPE has some limitation especially in terms of specificity. With the development of MIP, the empty SPE cartridge can be packed with optimized MIP. The selective cartridge is suitable for preconcentration and sample cleaned up before analyzing using chromatographic technique. This would be a good application for specific analysis. Once MIP is optimized, it will be packed in empty SPE cartridge, followed by optimization of SPE procedures which involves conditioning the cartridge, loading

samples, washing the interferences and finally eluting the desired template. This technique also knows as molecularly imprinted solid-phase extraction (MISPE) which was firstly done to clean up pentamidine from urine (Börje, 1994). The MISPE procedures are similar to conventional SPE procedures (Caro, Marcé, Borrull, Cormack, & Sherrington, 2006). Thus, it has to be optimized to achieve maximum template recovery. The choices of solvent during MISPE procedure is crucial and need to be done carefully so that the binding interaction occurred during sample loading will not diminished and finally affected the selectiveness.

1.4 Curcumin as phytochemicals

Curcumin derived from turmeric is categorized as low molecular weight of polyphenol which is one the phytochemicals that obtained naturally in plants. Phytochemicals derived from dietary components has gained much attention to threat human diseases especially cardiovascular diseases and cancer. Curcumin has gained vast acknowledgements as an antioxidant in health and medicinal fields apart from the main role as the food colorant and preservatives (Aggarwal, Surh, & Shishir, 2007; Basile et al., 2009; Gopinath et al., 2004; Hailong & Qingrong, 2010; Manikandan, Sumitra, Gayathri, & Lonchin, 2006; Motterlini, Foresti, Bassi, & Green, 2000).

1.5 Research approach

The main objectives of this study are divided into two sections; the development studies of MIP and its application on SPE technique. The fundamental studies of MIP are important in order to produce the most effective MIP towards curcumin. A good MIP resulted with high imprinting factor which is lead to high selectivity towards target molecule. The study begins by selecting suitable components for polymerization which are functional monomer and porogen. An effective MIP is the outcome from good combination of template - functional monomer - porogen. Once an effective MIP was selected, it was than implemented in SPE. Solid-phase extraction involves four basic procedures which are conditioning, loading, washing and eluting. To achieve maximum recovery of template during SPE, it was optimized using different conditions of loading, washing, eluting solvent and mass polymer. The molecularly imprinted solid-phase extraction (MISPE) was used to extract curcumin as the bioactive compound from turmeric. Characterizations were done using analytical equipments and supported with binding isotherms.

1.6 Specific objectives

The objectives of this study are as follows:

- To develop MIP that is selective towards curcumin
- To characterize the curcumin-imprinted polymer, particularly on functional monomer and porogen
- To apply the characterized MIP for extraction of curcumin using MISPE scheme

CHAPTER 2

LITERATURE REVIEW

2.1 History of molecular imprinting

The first work on molecular imprinting was published in 1931 (Polyakov, 1931) on silica surfaces and continuously developed mainly on organic polymers. The development of silica imprinted remained silence after steady publications for 15 years. The main limitation during this period that it must used a water soluble template which must be fairly stable in acid. The new period of intensive development of molecular imprinted polymers was started in 1972 (Klotz & Takagishi, 1972; K. G. Wulff & Sarhan, 1972). They independently reported preparation of organic polymer with predetermined ligand selectivities. Briefly, the method is known as "controlled distance method", which involved copolymerization between D-glyceric-(*p*-vinylanilide)-2,3-O-*p*-vinylphenylboronate and divinylbenzene. Research and development related to MIP are kept on growing continuously until today with more than 4000 paper published ("MIP Database," 2010). The wide interests of scientific community to MIP technology are reflected with the number of publications as shown in Figure 2.1.