Computational Design and Synthesis of Molecular Imprinted Membrane for Selective Extraction of Quercetin

by

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LIST OF ABBREVIATIONS

4-VP	4-vinylpyridine
AA	Acrylamide
AFM	Atomic force microscopy
AIBN	2,2-azobisisobutyronitrile
APBA	3-Aminophenylboronic acid
APS	ammonium persulfate
ASA	Acetylsalicylic acid
DEG	diethylene glycol
EG	ethylene glycol
EGDMA	ethyleneglycol dimethacrylate
Fe_3O_4	Ferric oxide
$FeCl_3.6H_2O$	Ferric chloride hexahydrate
HEA	2-hydroxyethyl acrylate
HSA	Human serum albumin
L-Phe	L-Phenylalanine
MAA	Methacrylic acid
NaOAc	anhydrous sodium acetate
PAANa	sodium polyacrylate
PBI	Polybenzimidazole
PM	Primary metabolites
PVDF	Polyvinylidene fluoride
Qu-1(4VP)	Quercetin interacts with one unit 4-vinylpyridine
Qu-1AA	Quercetin interacts with one unit acrylamide

Qu-1MAA	Quercetin interacts with one unit of methacrylic acid
Qu-2(4VP)	Quercetin interacts with two units of 4-vinylpyridine
Qu-2AA	Quercetin interacts with two units of acrylamide
Qu-2MAA	Quercetin interacts with two units of methacrylic acid
Qu-3(4VP)	Quercetin interacts with three units of 4-vinylpyridine
Qu-3AA	Quercetin interacts with three units of acrylamide
Qu-3MAA	Quercetin interacts with three units of methacrylic acid
Qu-4(4VP)	Quercetin interacts with four units of 4-vinylpyridine
Qu-4AA	Quercetin interacts with four units of acrylamide
Qu-4MAA	Quercetin interacts with four units of methacrylic acid
Qu-5(4VP)	Quercetin interacts with five units of 4-vinylpyridine
Qu-5AA	Quercetin interacts with five units of acrylamide
Qu-5MAA	Quercetin interacts with five units of methacrylic acid
SA	Salicylic acid
SYN	Synephrine
THF	Tetrahydrofuran
VB-DADPM	N, N'-bis (3-vinylbenzylidene)-4,4'-diaminophenylmethane

LIST OF SYMBOLS

$\overline{Q_E}$	The average of Q_E (mg/g)
Å	Angstrom
C	Concentration of template or competitive components in a solution (mg/L)
C_E	Final concentration of solution after adsorption (mg/L)
C_I	Constant associated with the thickness of boundary layer
C_o	Initial concentration of solution before adsorption (mg/L)
k_2	Rate constant for pseudo second order kinetic model (g mg^{-1} min^{-1})
K_D	distribution coefficient (mL/g)
$K_{D(Quercetin)}$	distribution coefficient of quercetin (mL/g)
K_{Dj}	distribution coefficient of competitive component (mL/g)
K_F	Freundlich relative adsorption capacity
k_{IP}	Coefficient of intraparticle diffusion model (mg g-1 min-1/2)
K_L	Langmuir equilibrium constant
k_l	Rate constant for pseudo first order kinetic model (min^{-1})
K_S	Sips model isotherm constant (L/g)
m	Mass of the membrane (g)
n	number of monomer units involved
n	number of data points
n_F	Characteristic constant of the Freundlich model
n_S	Heterogeneity index
p	number of model's adjustable parameters
Q	Binding capacity (mg/g)
Q_E	Experimental value of binding capacity (mg/g)

$\mathbf{Q_{max}}$	monolayer adsorption capacity (mg/g)
$\mathbf{Q_t}$	Binding capacity at time, t (mg/g)
$\mathbf{Q_{THEO}}$	Theoretical value of binding capacity obtained from model (mg/g)
$\mathbf{R^2}$	correlation coefficient
$\mathbf{R_L}$	Separation factor
t	Time (min)
V	Volume of the solution (L)
α	Selectivity coefficient
α	Initial adsorption rate in Elovich model (mg g^{-1} min^{-1})
β	Extent of surface coverage and activation energy of chemisorption (g mg^{-1})
ΔE	Interaction energy (kcal/mol)
$\mathbf{\Delta H_f}$	Heat of formation (kcal/mol)
$\mathbf{\Delta H_{f,complex}}$	Heat of complex formation (kcal/mol)
$\mathbf{\Delta H_{f,monomer}}$	Heat of monomer formation (kcal/mol)
$\mathbf{\Delta H_{f,template}}$	Heat of template formation (kcal/mol)
λ	Wavelength (nm)

Reka Bentuk Perkomputeran Dan Sintesis Molekul Tercetak Membran Untuk Pengestrakan Terpilih Quercetin

ABSTRAK

Tesis ini membentangkan fabrikasi membran quercetin tercetak (QIM) untuk pengekstrakan terpilih quercetin melalui kaedah permukaan pempolimeran. Dalam pembangunan QIM, pemilihan monomer berfungsi dan pengiraan nisbah molar template-monomer yang optimum ditentukan melalui pemodelan molekul dengan menggunakan perisian HyperChem. Tiga monomer berfungsi disiasat termasuk asrilamid, asid metakrilik dan 4-vinylpyridin dan pengoptimuman kompleks pra-pempolimeran telah dijalankan pada nisbah molar 1: 1 hingga 1: 5 bagi setiap monomer. Keputusan menunjukkan bahawa nisbah molar 1: 4, yang melibatkan interaksi antara quercetin dan asrilamid berpotensi dalam menyediakan kompleks pra-pempolimeran yang wajar sebelum QIM dihasilkan. Selepas langkah ini dijalankan, QIM telah dibangunkan berdasarkan keputusan pengiraan. Dalam usaha mendepositkan lapisan tercetak pada permukaan membran, polyvinylidene fluorida (PVDF) digunakan sebagai polimer sokongan dan kemudian, akan menjalani proses pempolimeran haba dan templat disingkirkan dari matriks polimer. QIM dan membran bukan tercetak (NIM) telah disintesis untuk menilai dan mencirikan perbezaan berkenaan dengan morfologi, fungsi kimia serta tingkah laku yang mengikat mereka ke arah quercetin dan komponen lain. Pengesahan mengenai kehadiran lapisan tercetak pada membran PVDF dilakukan melalui analisis FTIR, FESEM dan AFM. Daripada keputusan yang diperolehi, dapat dirumuskan bahawa lapisan tercetak terdiri daripada laman pengenalan quercetin yang telah berjaya dibentuk dan diagihkan secara sama rata pada permukaan QIM. Untuk penilaian prestasi mengikat, eksperimen kumpulan mengikat, ujian kinetik mengikat dan pemilihan spesifik telah dijalankan. Kapasiti maksimum mengikat QIM dalam analisis kumpulan mengikat adalah 25.63 mg/g, iaitu lebih tinggi daripada kapasiti mengikat NIM, iaitu sebanyak 7.47 mg/g. Selain itu, QIM juga menunjukkan kadar penjerapan yang tinggi pada awal proses dan masa tepu QIM dicapai selepas tempoh 3-4 jam berinteraksi. Permodelan isoterma dan kinetik penjerapan menunjukkan bahawa QIM masing-masing mempunyai permukaan yang homogen dan mematuhi model kinetik Elovic. Akhir sekali, ujian pemilihan spesifik QIM disiasat dengan menggunakan sinensetin dan asid rosmarinik sebagai komponen bersaing. Ia menunjukkan bahawa QIM mempunyai keupayaan pengenalan lebih tinggi ke arah quercetin berbanding sinensetin dan asid rosmarinik.

Computational Design and Synthesis of Molecular Imprinted Membrane for Selective Extraction of Quercetin

ABSTRACT

This thesis presents the fabrication of quercetin imprinted membranes (QIM) for selective extraction of quercetin through surface polymerization method. In the development of QIM, selection of functional monomer and the optimum molar ratio of template-monomer were facilitated by the application of molecular modelling through the use of HyperChem software. Three functional monomers were investigated including acrylamide, methacrylic acid and 4-vinylpyridine and the optimization of pre-polymerization complex was conducted at molar ratio of 1:1 to 1:5 for each of the monomers. The results indicated that molar ratio of 1:4, which involving the interaction between quercetin and acylamide could provide potentially favourable pre-polymerization complex prior to the synthesis of QIM. After this step had been established, QIM were developed based on the computational results. In order to deposit the imprinted layer on the surface of membrane, polyvinylidene fluoride (PVDF) membrane was used as the polymer support and then, they were subjected to thermal polymerization process and subsequent removal of template from the polymer matrix. QIM and non-imprinted membranes (NIM) were synthesized to evaluate and characterize the differences with respect to their morphology, chemical functionality as well as their binding behaviour towards quercetin and other components. The confirmation on the presence of imprinted layer on PVDF membrane was done through FTIR, FESEM and AFM analysis. From the results obtained, it can be summarized that imprinted layer composed of recognition sites were successfully formed and distributed evenly on the surface of QIM. For the binding performance evaluation, batch binding, kinetic binding and selectivity tests were conducted. The maximum binding capacity of QIM in batch binding was 25.63 mg/g, which is higher than binding capacity of NIM with the capacity value of 7.47 mg/g. Apart from that, QIM also exhibits higher adsorption rate at the initial stage of adsorption process and the saturation time was achieved after 3-4 hours contact time. Modelling of isotherm and kinetic adsorption showed that QIM has a homogenous surface and followed Elovich kinetic model, respectively. Finally, the selectivity test of QIM was investigated by using sinensetin and rosmarinic acid as competitive components. It demonstrated that QIM showed higher recognition capability towards quercetin compared to sinensetin and rosmarinic acid.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Quercetin, a widely distributed flavonoid in various plants and human diets has drawn a substantial attention recently (Lou et al., 2016; Raie et al., 2017). As reported in numerous literatures, quercetin possesses remarkable health-promoting properties due to its excellent biological and pharmacological activities (Gonzales et al., 2015; Suganthy et al., 2016). The great appreciation on the extraction, isolation, purification and characterization of this flavonoid compound has been showed through the development of extraction technologies such as accelerated solvent extraction (Kang et al., 2016), supercritical fluid extraction (Hsu et al., 2016), column chromatography (Sun et al., 2014) and macroporous resin extraction (Wan et al., 2014).

Among those techniques, the most outstanding approach known as molecular imprinting technique (MIT), which involves the introduction of artificial recognition sites with predetermined selectivity to the molecule of interest in a synthetic polymer has been introduced (He et al., 2015). This unique technique has been widely investigated by scientific community for broad range of applications, especially in drug delivery (Luliński, 2017), artificial antibodies (Tang et al., 2017) and sensor development (Iacob et al., 2016; Uzuriaga-Sánchez et al., 2017), since it offers much lower operational cost, straight-forward preparation and high stability of molecular imprinted polymer (MIP) compared to other conventional methods (Li et al., 2017; Luliński, 2017).

Therefore, selective extraction of quercetin by molecular imprinted polymer (MIP) has tremendous meaning in nutraceutical sciences. In this work, it is expected that the synthesized MIP could potentially demonstrate the success of imprinting procedure and subsequently promote higher binding capability and selectivity properties. This chapter highlights the current limitation of molecular imprinting technique and how we intend to solve the issues through several research objectives. Besides, we also discuss the scope of study, which outlines the specific data used for this research and the theories used to interpret the data.

1.2 Problem Statement

The most popular and conventional way to prepare molecular imprinting polymer (MIP) is through bulk imprinting polymerization (Ji et al., 2014; Piacham et al., 2015; Sorribes et al., 2017). It consists of three necessary steps in developing MIP which are solution polymerization step followed by mechanical grinding and sieving of resultant bulk polymer and finally, template removal step using Soxhlet extraction (Li et al., 2013; Yan & Row, 2006). Although the preparation of MIP is much relatively straightforward, such MIP lost their 'template memory' during grinding and thus, weakens the binding capabilities of MIP to the target molecules. Furthermore, binding sites in those MIP are distributed deep inside the polymer matrix and consequently, limiting the template mobility during adsorption and desorption process (Roy et al., 2017)

In order to overcome these problems, the combination of MIT and membrane technology to produce molecular imprinted membrane (MIM) could be an incredible breakthrough in separation/extraction studies. The concept involves the localization of binding sites on the polymeric membrane surface instead of having them inside of the

polymer matrix. This unique feature associated with the flat and thin surface of the membrane would improve the accessibility of template to the binding sites and enhance the binding selectivity of MIM. Besides, the process of removing the template would be much easier and faster due to lower mass transfer resistance exhibited by MIM.

The need for having an extraction method that can offer a simple and time-consuming procedure as well as MIM with high selectivity and affinity to extract quercetin are vital.

1.3 Research Objectives

This research aims to develop quercetin imprinted membrane (QIM) which have higher binding capacity and selectivity towards quercetin. This objective is accomplished through the following specific objectives:

i. To determine the most favourable functional monomer and optimum molar ratio of pre-polymerization complexes between template-monomer using computational –aided tool (HyperChem).
ii. To synthesize quercetin imprinted membrane (QIM) and non-imprinted membrane (NIM) using surface imprinting polymerization method and characterize them using FTIR, AFM and FESEM analysis.
iii. To analyse the performance of QIM and NIM for extraction of quercetin through batch and kinetic binding as well as selectivity studies.

1.4 Scope of Research

Every research study must have some limitations that make the research more specific. Below are the scopes of the research:-

i. The computational study in this research only focuses on the template and functional monomer interaction for elucidating and modelling the interaction strengths of MIP based on the hydrogen bonding. The effect of crosslinker and solvent are neglected in order to avoid too much data in the computational system. Three types of monomers were investigated, which are acrylamide, methacrylic acid and 4-vinylpyridine as they are most commonly monomers used for the preparation of MIP.

ii. Fabrication of QIM and NIM was carried out using the surface imprinting polymerization method, where the functional monomer involved as well as the molar of quercetin to monomer are determined by the computational results. The characterization of membranes was done by analysing the changes in the chemical functionality and morphology of the membrane.

iii. The batch binding/adsorption experimental data of QIM and NIM obtained were subjected to three types of isotherm modelling which are Langmuir, Freundlich and Sips in order to investigate binding sites characteristics and binding behaviour of QIM and NIM. The reaction mechanism during adsorption process of the membranes was further evaluated through kinetic modelling analysis, in which 1st order, 2nd order and Elovich kinetic models were employed to determine which one fits the best with the experimental data.

In the selectivity study, sinensetin and rosmarinic acid were used as the competitive components. Sinensetin was chosen as it comes from flavonoid group

and it possesses a molecular structure (structural analogue) closer to quercetin. On the other hand, rosmarinic acid is also one of the most popular bioactive compounds present in herbal plants and it is originated from phenolic acid group. The selection of these components is to study whether QIM or NIM able to differentiate and recognize quercetin-structural analogue rather than quercetin itself or not.

This research can be considered as a preliminary study only in order to quantify the method to synthesize QIM and its ability to selectively recognize quercetin molecule. We have not discussed on the application of QIM in extraction system for real sample analysis.

1.5 Thesis Outline

A brief overview for the rest of the chapters are described as follows:

Chapter 2: Literature reviews

In this chapter, some reviews on the previous studies have been discussed and compared. It focuses on three main issues which are the history and importances of bioactive compounds, molecular imprinting technology background and lastly, the development of new approach of combining molecular imprinting and membrane application.

Chapter 3: Research methodology

This chapter explains the chemicals and instrumentation involved during conducting necessary experiments. All techniques used to fulfil the objectives of this

research also are clearly defined. The main techniques comprised of the evaluation of stability of pre-polymerization complex using computational approach, the preparation of quercetin imprinted membrane (QIM) and the analysis on the binding performance of QIM.

Chapter 4: Results and Discussion

This chapter starts by presenting the findings obtained from the computational study to determine which functional monomer and the suitable molar ratio of template-monomer that provide highest stability to the pre-polymerization complex. Then, the explanation on the molecular imprinting process that takes place during the synthesis of QIM begins with the help of proposed schematic of creation of quercetin binding sites in QIM.

The confirmation on the presence of an imprinted layer on the QIM surface was evaluated through FTIR, FESEM and AFM analysis. Batch and kinetic adsorption data were used to model the binding behaviour of the QIM and NIM. Meanwhile, a selectivity study was performed using rosmarinic acid and sinensetin as competitive components.

Chapter 5: Conclusion

This chapter is composed of overall conclusion on the present study and the future recommendation as well. The conclusion will be based on the successful fabrication of QIM that has a capability to selectively recognize the quercetin molecules.

CHAPTER 2

LITERATURE REVIEW

2.1 Bioactive Compounds

The natural bioactive compounds, also known as phenolic compound, which are originated from dietary plants and medicinal herbs have received phenomenal interests due to health benefits resulted from their high level of antioxidant properties (Reis Giada, 2013). These plants undergo photosynthesis process, where they generate oxygen and chemicals known as secondary metabolites by using the energy of sunlight (Akram, 2011). In general, plants produce two types of metabolites, known as primary and secondary metabolites. Primary metabolites (PM) are needed for growth and development of the plant and involved in the respiration and photosynthesis process. Examples of main PM are carbohydrates, proteins, nucleic acids and lipids (Irchhaiya et al., 2015).

Unlike PM, secondary metabolites (SM) are not involved in the growth phase of plant and they are derived from primary metabolites. SM play an important role for the plant survival against herbivores and other interspecies defense (Wink, 2016). Terpenes, phenolic compounds and nitrogen containing compounds can be considered as three important groups of SM (Verma & Shukla, 2015). However, phenolic compounds are the most widely distributed SM in the plants and contribute dominantly to the variety of pharmacological activities (Reis Giada, 2013).

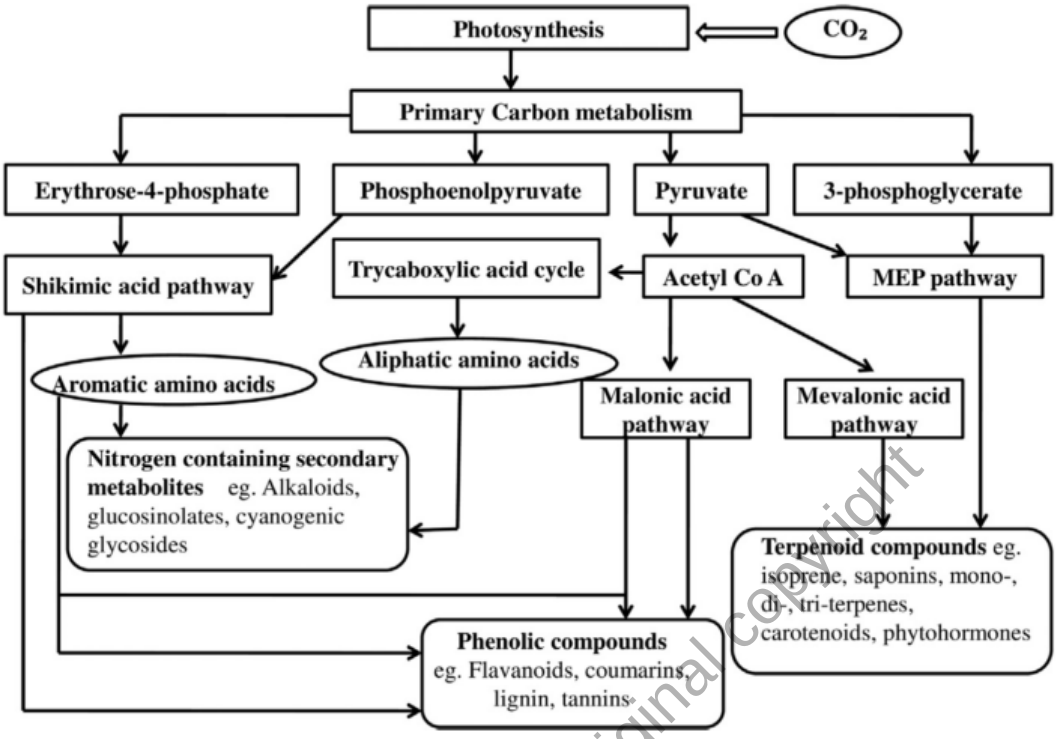

Figure 2.1: A schematic diagram of biosynthetic pathway of secondary metabolites in plants (Verma & Shukla, 2015).

Based on Figure 2.1, phenolic compounds are produced through two different metabolic pathways: the shikimic acid pathway and the malonic acid pathway. Most plants use the shikimic acid pathway where mainly, phenylpropanoids are formed, whereas the malonic acid pathway participates more significantly in fungi and bacteria compared to higher plants (Özeker, 1999; Reis Giada, 2013). Phenolic compounds may fall into two different categories which are flavonoid and non-flavonoid compounds (Działo et al., 2016). Non- flavonoid compounds consist of five subclasses: phenolic acid, lignans, stilbenes, tannins and lignins. The latter two are considered as complex organic polymers, making it hard to determine their primary carbon structures (Amoako & Awika, 2016; Le Floch et al., 2015). The distribution of phenolic compounds is presented in Figure 2.2.

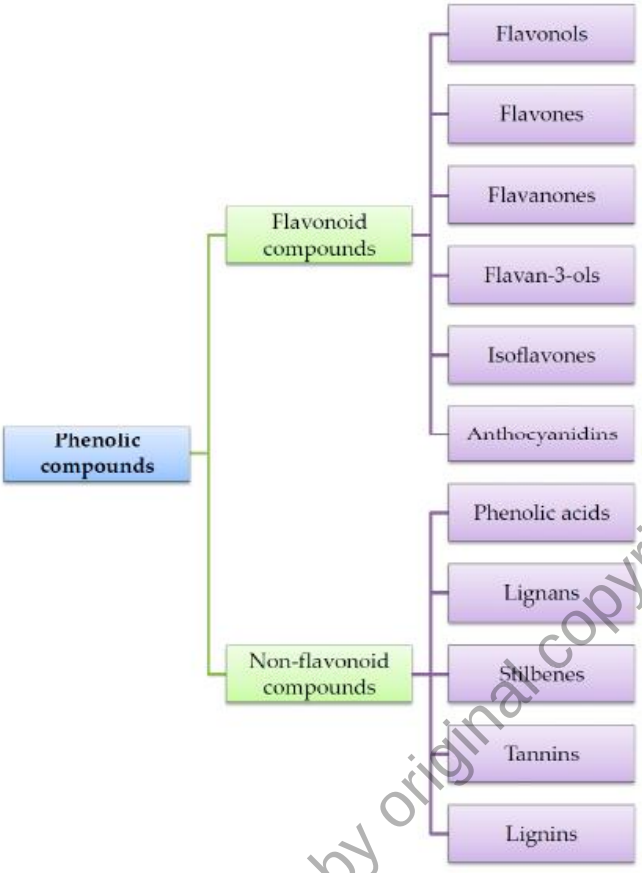

Figure 2.2: Distribution of phenolic compounds (Działo et al., 2016)

2.1.1 Quercetin as Major Component of Flavonoid Group

Flavonoid is considered to be the major representative in many plants including medicinal herbs, vegetable, fruits and human dietary foods (Sak, 2014). Flavonoids exist in various parts of plants, such as fruits, leaves, flowers, stem, root and seeds (Nabavi et al., 2012). They are distinguished mainly based on the number and pattern of hydroxyl and methyl groups in the flavonoid skeleton (Choi et al., 2002). Flavonoid structure consist of 15-carbon structure in their skeleton which contains both aromatic (A and B rings) and heterocyclic (C ring) rings as shown in Figure 2.3.