Synthesis of molecularly imprinted polymers for the separation of gamma-oryzanol by using methacrylic acid as functional monomer

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A B S T R A C T

Molecularly imprinting technique can be used to create polymeric materials with recognition sites providing high selectivity and affinity towards template molecules. This technique was successfully applied for the MIP synthesis via thermal polymerization method at 60 °C by using methacrylic acid (MAA) as a functional monomer. In this study, we prepared molecularly imprinted polymers with different amounts of template, porogen and cross-linker for the purpose of optimizing the binding capacity towards γ-oryzanol. MINITAB 15 was employed for the analysis of variance (ANOVA) and the regression model. The polynomial regression model showed 95.3% accuracy for estimating the adsorption capacity of MIP. The analysis of variance with 95% confidence level suggested significant interaction between the amount of template and the amount of cross-linker on the adsorption capacity of MIPs. The maximum adsorption capacity of MIPs was 1.728 mg/g-adsorbent. The synthesized MIP was reused five times without significant losses of performance. The adsorption isotherm can be described by Freundlich isothermal model.

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1. Introduction

γ-Oryzanol, a group of ferulic acid esters of phytosterols and triterpene alcohols, is a naturally rich source of antioxidants and unsaturated fatty acids. It is a particularly interesting substance that is used in a wide range of applications in food, cosmetic, and pharmaceutical industries [1] due to its certain medicinal characteristics such as improving plasma lipid patterns [2], hypocholesterolemic activity [3] and the treatment of inflammatory processes. Japanese scientists have also suggested that γ-oryzanol may ease hot flashes and other symptoms of menopause due to its ability to reduce luteinizing hormone (LH) by the pituitary, and to increase the excretion of endorphins by the hypothalamus [4]. The molecular structure of γ-oryzanol is shown in Fig. 1.

γ-Oryzanol is a natural component of rice bran, wheat, and corn. Throughout the processing of rice bran oil, the composition of γ-oryzanol in different stages are 1.5–2.9 wt% for crude rice bran oil, and 0.19–0.22 wt% for chemically refined oil [2]. There are various extraction techniques to purify γ-oryzanol, such as liquid–liquid extraction, supercritical CO_2 extraction, direct solvent extraction, and a two-step crystallization process [5–8]. Some of these techniques pose several disadvantages; for example, a high operating cost for liquid–liquid extraction and supercritical CO_2 extraction, the toxicity generated by liquid–liquid extraction, the fact that the two-step crystallization process is a complex operation and an extremely time-consuming process. Because of the aforementioned drawbacks, a molecularly imprinted polymer is recommended for the separation of γ-oryzanol because of its low cost, simple preparation and operation, minimal toxicity, and high efficiency of extraction. The MIP can be reused without significant loss of performance and can be stored for several years [9].

Molecularly imprinted polymers have been used in many applications in the field of chemistry, biology, and engineering, such as pharmaceutical preparation [10] separation processes [11] and the detection of chemicals (biosensors) [12]. This is a technique that can assist the purification of a variety of target molecules by creating recognition sites in polymeric materials with high selectivity and affinity towards the template molecules. This technique is relatively simple and easy to adapt towards a wide variety of target molecules [13].

The synthesis procedure for MIP is not complicated or expensive. The synthesis of MIP involves the radical polymerization of a mixture of functional monomers, porogen, cross-linker and a radical initiator in the presence of a template. The pre-polymerization mixture is subjected to heat at the decomposition

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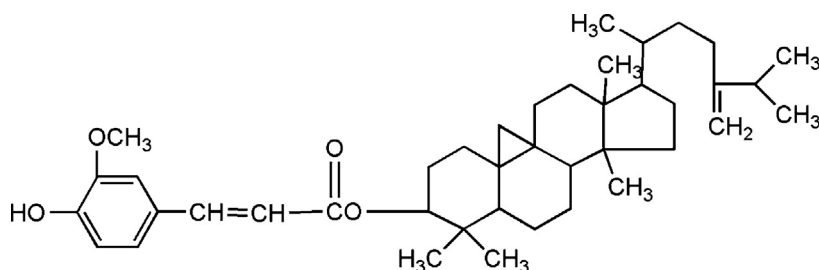E-mail address: akaewchada@yahoo.com (A. Kaewchada).

Fig. 1. Structure of γ-oryzanol.

temperature of the initiator in order to initiate polymerization. The template molecules are subsequently removed either by chemical separation or by solvent extraction, leaving binding sites with complementary size and shape exhibiting certain chemical affinity for the template molecules [14].

The goal of this work is to synthesize MIPs as adsorption materials for γ-oryzanol using methacrylic acid (MAA) as a functional monomer, toluene as a porogen, divinylbenzene (DVB) as a cross-linker and benzoyl peroxide (BPO) as an initiator. The first part of this work focuses on the effect of the amount of template, the amount of porogen and the amount of cross-linker on the adsorption capacity via statistical analysis using MINITAB 15. The reusability of MIPs was also considered.

2. Experimental

2.1. Materials

γ-Oryzanol (98% purity) was obtained from Connell Bros. Co., Ltd (Bangkok, Thailand). DVB and MAA were purchased from Sigma-Aldrich. BPO was purchased from Sandreac. Toluene was purchased from Merck. Acetonitrile was purchased from LabScan (Bangkok, Thailand). Ethanol was purchased from Merck. All chemical reagents were analytical grade or better and were used without further purification.

2.2. Instrumentation

The ultraviolet–visible (UV–VIS) measurement was performed using a UV-2100 spectrophotometer (Unic Company, USA). The variable wavelength detector was operated at 315 nm for the determination of γ-oryzanol concentration [6]. A Soxhlet apparatus was used for template removal from the synthesized MIPs. Binding experiments were performed in flasks immersed in a water bath equipped with an ultrasonic generator (28 kHz 200 W).

2.3. Sample preparation

The standard methods for the preparation of γ-oryzanol solutions were used for the study as follows: 25 mg of γ-oryzanol were dissolved in 500 ml of acetonitrile to provide a concentration of 0.05 mg/ml. Fractions of this were diluted to 0.01–0.05 mg/ml in order to calibrate the UV measurement.

2.4. Preparation of γ-oryzanol imprinted polymers

Essential components for our synthesis of γ-oryzanol imprinted polymers via thermal polymerization were a template, a functional monomer, a cross-linker, an initiator, and a porogen. The amount of each component was varied to create a full factorial experimental design of a $3 \times 2 \times 2$ structure with respect to a template, a cross-linker, and a porogen, respectively, while the amount of a functional monomer and an initiator were fixed. A full replicate of this design was carried out in order to statistically analyze the

main and interaction effects. Each polymer was assigned to a specific code such as MIP_1, MIP_{2b}, etc. The last combination was the controlled polymer synthesized without template molecules (non-imprinted polymer, NIP); see Table 1.

Following the procedure for radical bulk polymerization of MIPs and NIP, γ-oryzanol, MAA and DVB were dissolved in toluene (porogen) in a 50 ml round-bottom flask. Next, the radical initiator (BPO) was added. The solutions were vigorously stirred by means of a magnetic stirrer, providing a homogeneous solution mixture. Then, the system was purged with nitrogen gas for 3–5 min. The polymerization was carried out in a water bath for 48 h at 60 °C. The bulk rigid polymers were crushed into powder by using a mortar. These powder particles were sieved for the size range of 75–106 μm in diameter. The template (γ-oryzanol) was removed from the polymer particles by a Soxhlet apparatus (48 h, 80 ml of acetonitrile as solvent). A UV–VIS spectrophotometer was used to measure the concentration of γ-oryzanol in the washing solution to ensure complete removal of template. The removal process continued until γ-oryzanol could not be detected in extracted samples of the MIPs. NIP was prepared under the same polymerization conditions but without adding the template molecules and without the template removal step.

2.5. Equilibrium binding studies

The binding experiment was performed to evaluate the adsorption capacity of MIP and NIP particles. First, 150 mg of the dried MIP or NIP was added to 10 ml of standard solution of γ-oryzanol (0.05 mmol/l) dissolved in acetonitrile. The flask was placed in a water bath equipped with an ultrasonic generator at 25 °C for 24 h. Samples withdrawn at different incubation periods were filtered in order to analyze the concentration of γ-oryzanol in the filtrate by UV–VIS spectrophotometer. This information was used to determine the amount of γ-oryzanol bound (or unbound) to the MIP. The rebinding experiment was repeated and the average γ-oryzanol concentration was used to calculate the binding capacity.

2.6. Quantity analysis of γ-oryzanol

The amount of γ-oryzanol bound to the polymer was calculated by subtracting the unbound amount from the known initial concentration of γ-oryzanol. The straight line calibration curve passing through the origin ($R^2 = 0.9997$) for the determination of γ-oryzanol concentration via UV measurement was obtained for γ-oryzanol concentration in the range of 0.01–0.05 mg/ml [8]. This linear equation follows Beer's law (with a small offset, the absorbance is proportional to the concentration of the sample) and was used for the calculation of the γ-oryzanol concentration in the sample.

2.7. Adsorption capacity

The adsorption capacity, B, being the value of the amount of adsorbed substance obtained in a saturated solution (IUPAC