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Development of mass transfer model for chromatographic separation of free lutein and fatty acids in de-esterified marigold lutein

Weerawat Clowutimon[a], *Artiwan Shotipruk*[a], *Panatpong Boonnoun*[b], *Pimporn Ponpesh*[a,*]

[a] *Chemical Engineering Research Unit for Value Adding of Bioresources, Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University, Phayathai Road, Patumwan, Bangkok, 10330, Thailand*
[b] *Department of Industrial Engineering, Chemical Engineering Program, Faculty of Engineering, Naresuan University, Phitsanulok, 65000, Thailand*

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ABSTRACT

This article was aimed to construct a mathematical mass transfer model for chromatographic separation of free lutein and fatty acids in de-esterified marigold lutein. Required model parameters: adsorption isotherm, overall mass transfer and axial dispersion coefficients, were first determined. The adsorption isotherms of the compounds were studied using batch adsorption experiments, while the axial dispersion and overall mass transfer coefficients were determined from published empirical-correlation and correlation developed experimentally from this study, respectively. The adsorption isotherms were found to be linear within the equilibrium concentration of $\leq$100 μg/ml. The isotherm constants of fatty acids were about twice those of free lutein. The isotherm constants and the mass transport parameters were then applied to the mass transfer model. The predicted results were compared with the experimental data at the optimum mobile phase velocity (0.16 cm/s). Good agreement was found, i.e., the average absolute deviations were less than 2% and 7% for semi-preparative and preparative columns, respectively.

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1. Introduction

Marigold (*Tagetes eracta* L.) is a reputed medical plant which grows as a wild and common garden plant throughout many parts of the world, including Thailand. It is one of important sources of several bioactive compounds, particularly lutein ($C_{40}H_{56}O_2$, MW 568.87 g/mol) (Gong et al., 2012; Vasudevan et al., 1997; Verghese, 1998). Increasing numbers of recent research has shown that lutein possesses strong antioxidant and anticancer properties. In addition, lutein is also known to be beneficial in preventing several human diseases, e.g., age-related macular degeneration (AMD), and protecting human eyes by filtering out the damaging blue light (Alves-Rodrigues and Shao, 2004; Khachik, 2001; Siriamornpun et al., 2012). For these reasons, extraction of marigold lutein for uses in pharmaceutical and cosmetic industries is expected to grow to US$308 million by 2018 (Lin et al., 2015).

Lutein in marigold flowers is generally found in mono- or di-esterified forms of fatty acids of which lutein palmitate is a major component (Abdel-Aal and Rabalski, 2015; Jiang et al., 2005; Piccaglia et al., 1998). In these forms, lutein is not readily absorbed by human body and its efficacy is less than 5% (Breithaupt et al., 2002; Granado et al., 2002). After solvent extraction, the extract of lutein fatty acid esters therefore needs to be converted to free lutein by reacting with an alkali solution (e.g., KOH), typically in water or alcohols with relatively low molecular weights (e.g., ethanol). After this step, the de-esterified sample requires further purification steps to remove remaining impurities, particularly fatty acids, a by-product of de-esterification process,

* *Corresponding author*.
E-mail address: pimporn.p@chula.ac.th (P. Ponpesh).
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and achieve the recommended purity level for human consumption (>95%) (Khachik, 1995). Generally, crystallization has been used to commercially purify the de-esterified marigold lutein. However, it results in rather low purity (<70% based on chromatogram peak area) (Khachik, 2001). Although the purity could be improved, up to approximately 97%, by re-crystallization, the process requires several steps, making it complicated and lowering the overall yield (Vechpanich, 2008).

Alternatively, chromatography is extensively used in purification of fine chemicals and high value compounds due to high purity and high recovery. In addition, compared to other purification techniques, this technique is easily scalable. In general, the development of an appropriate condition for chromatographic purification of a specific compound is carried out via trial-and-error in an analytical high-performance liquid chromatography (HPLC). However, the purification of the compound is usually performed in a preparative scale. Nevertheless, existing literatures related to isolation procedure of free lutein derived from different raw materials (e.g., algae and marigold flower) has been mostly reported in analytical scales only (Boonnoun et al., 2012; Shibata et al., 2004; Tsao and Yang, 2011; Tsao et al., 2004). This can pose limitations for industrial production. Thus, a process scale-up is required.

Nowadays, mathematical modelling, based on material balance and thermodynamic equilibrium of the compound between stationary phase and mobile phase, has been applied to study phenomena in large-scale chromatographic processes. In addition, it can be used to determine the optimum operating condition of the process without extensive number of experiments, resulting in considerably less time and resources. In the previous work, Choopakdee et al. (2014) developed a mass transfer model for describing the transport behaviour of marigold free lutein in a normal-phase chromatography column. Although the model could well describe the transport behaviour of the compound, other components, particularly fatty acids, have not yet been considered. In this study, we have therefore aimed to develop a mass transfer model for describing the transport behaviour of the de-esterified marigold major components: free lutein and fatty acids, in chromatography columns. Firstly, batch adsorption studies were carried out to determine the linear adsorption isotherm constants of free lutein and fatty acids on silica gel used as a stationary phase. Then, the isotherm constants and transfer parameters, i.e., mass transfer and axial dispersion coefficients, determined from appropriate correlations were applied to the mass transfer model. In addition, the comparison between the experimental data and the model prediction in both semi-preparative and preparative columns was performed to evaluate the model abilities for predicting the experimental transport behaviour of the compounds.

2. Experiments

2.1. Materials

Pulverized marigold flowers were supplied by PPT Global Chemical Company Ltd. (Rayong, Thailand). Standard chemicals of reagent grade, i.e., free lutein and palmitic acid (standard fatty acid) were purchased from Sigma-Aldrich Pte. Ltd. (Singapore). All chemicals of analytical grade for de-esterification, i.e., potassium hydroxide and ethanol, were purchased from Merck & Co. Inc. (Wilson, NC, USA). The mobile phase for HPLC analysis, i.e., acetonitrile, methanol ethyl acetate and isopropanol, were purchased from Sigma-Aldrich, Co. LLC. (St Louis, MO, USA). All the other chemicals including hexane, diethyl ether, ethyl acetate and silica gel (particle size 25–40 μm with sphericity 0.75–0.90 (Leva, 1959)) were purchased from Merck Ltd. (Bangkok, Thailand).

2.2. Preparation of de-esterified marigold lutein and purified free lutein

De-esterified marigold lutein and purified free lutein used in this study were prepared by first extracting pulverized dried marigold powder using hexane. The extract was then reacted with alkali solution (in ethanol) to convert lutein fatty acid esters to free lutein. Finally, the de-esterified marigold lutein sample was purified in a preparative chromatography column using silica gel as the stationary phase and mixture of hexane and ethyl acetate as the mobile phase. The detailed procedures were discussed elsewhere (Boonnoun et al., 2012). The de-esterified marigold lutein and purified free lutein were analyzed by UV-HPLC and ELSD-HPLC to quantify free lutein and fatty acids, respectively.

2.3. Determination of adsorption isotherms

The single-component adsorption isotherms of free lutein and fatty acids were determined by batch adsorption studies. Stock solutions of free lutein and fatty acids were prepared by dissolving known amounts of the compounds in mixtures of hexane and ethyl acetate at ratios of 70:30 v/v and 85:15 v/v, which were the mobile phase conditions in the chromatographic study. Working solutions were then prepared by diluting different volumes of the stock solutions to achieve several solutions at the desired concentrations, i.e., 5–300 μg/ml for free lutein and 100–1000 μg/ml for fatty acids. To determine the adsorption equilibria, 0.5 g of silica gel was added into each of 125-ml Erlenmeyer flasks containing 10 ml of the working solution of different concentrations. The flasks were continuously shaken in an incubator (LSI 1005R, Labtech, India) with a constant speed of 120 rpm at 30 °C for 2 h to ensure that the adsorption equilibrium was reached in each flask. After the incubator was shut off, 1 ml of the solution from each flask was collected using a filtering syringe and was subsequently analyzed by HPLC. The specific equilibrium amounts adsorbed (q_e) of free lutein and fatty acids in the stationary phase of each experiment were calculated based on Eq. (1).

$$q_e = \frac{(c_0 - c_e)\,V_s}{W} \qquad (1)$$

where c_0 and c_e are the initial concentration and the equilibrium concentration, respectively, in the mobile phase; V_s is the solution volume; and W is the silica gel weight.

2.4. Chromatographic experiments

In this study, chromatographic separation of free lutein and fatty acids was carried out in both semi-preparative and preparative columns. For the semi-preparative column, the apparatus was set as shown in Fig. 1. 18 g of silica gel was soaked in 200 ml of 70:30 v/v hexane-ethyl acetate mixture. The slurry was degassed under sonication for 30 min and was packed into the column. A flow distributor was then packed at the top of the silica gel layer to reduce the radial dispersion. 1-ml lutein samples (purified free lutein and de-esterified lutein) were loaded into the column at the top. The mobile phase, i.e., mixture of hexane and ethyl acetate, was then pumped to the column by a peristaltic pump (Masterflex, Model No. 7532-60, Cole Parmer, Thailand) at a fixed velocity to elute the sample downflow. In the isocratic elution mode, the mobile phase was a mixture of hexane and ethyl acetate at a ratio of 70:30 v/v for all elution time. On the other hand, in the gradient elution mode, the mobile phase was a mixture of hexane and ethyl acetate at a ratio of 85:15 v/v in the first 12 min and was then suddenly changed to a ratio of 70:30 v/v. The column

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