EFFECT OF ISOOCTANE AND TEMPERATURE ON THE SEPARATION OF LIPIDS ON PHENOGEL COLUMN

Salisa Chumsantea, Kornkanok Aryusuk, Narumon Jeyashoke and Kanit Krisnangkura
Biochemical Technology Division, School of Bioresources and Technology
King Mongkut’s University of Technology Thonburi

ABSTRACT
Phenogel column packed with styrene-divinylbenzene (ST-DVB) copolymer is designed for size exclusion chromatography (SEC). Separation of solutes on this column was controlled by the swelling of ST-DVB copolymer in which affected by the solvency of the mobile phase. In this study, effect of isooctane on the swelling of ST-DVB gel and the separation of lipids in crude rice bran wax (CRBW) were investigated. When the ST-DVB copolymer was fully swollen (in toluene), wax ester (WE), and triglyceride (TG) were co-eluted. By incorporation isooctane in the mobile phase, degree of swelling of the ST-DVB gel was decreased and the separation of WE and TG was increased. The highest resolution of WE and TG (Rs = 1.03) was observed at 70:30 (v/v) of isooctane-toluene. Furthermore, the effect of column temperature on SEC was also studied. The result showed that increasing of column temperature from 65°C to 75°C and 85°C did not affect the elution order of the analytes but analysis times were reduced.

Keyword: Crude rice bran wax, Degree swelling, High-performance size-exclusion chromatography Phenogel column

1. INTRODUCTION

The High-performance liquid chromatography (HPLC) is an analytical tool which came of age in the 1970s. HPLC was an attractive method because of its greater convenience, increased speed, ease of quantification, automation, and preparative scale-ups (William and Xianlin, 2010). Among chromatographic techniques, high-performance size-exclusion chromatography (HPSEC) is unique in that the separation of compounds is based on their molecular size, normally related to their molecular weight (MW). Phenogel column is designed for use as SEC packed with ST-DVB copolymer. Arzamendi et al. (Arzamendi, et al., 2006) monitored the transesterification products in biodiesel production (acylglycerol, fatty acid methyl ester, and methanol) via HPSEC technique. All substances were baseline separation by using two serially connected (100 and 500 Å) Phenogel columns and THF as mobile phase. Kittirattanapiboon and Krisnangkura. (Kittirattanapiboon and Krisnangkura, 2008) separated products in transesterification of biodiesel by using only one Phenogel column (100 Å). Baseline separation of acylglycerol, fatty acid methyl ester, and free fatty acid was obtained by using toluene containing 0.25% acetic acid as mobile phase.

Recently, Aryusuk, et al. (Aryusuk, et al., 2010) analyzed the composition of CRBW on a 100-Å Phenogel column by using 0.15% acetic acid in 65:35 (v/v) isooctane-toluene as mobile phase. The result showed that all substances were baseline separated. The ST-DVB with porous structures showed absorption ability of organic solvents having solubility in the range of 17.6-19.6 (MPa)1/2 (Kiatkamjornwong, et al., 1999). Whilst, the solubility parameter of ST-DVB copolymer ($\delta_2$) is 18.6 (MPa)1/2 (Kiatkamjornwong, et al., 1999). Whilst, of isooctane ($\delta_1$) and toluene are 14.3 and 18.3 (MPa)1/2, respectively. In order to study the effect of poor solvent on separation of lipids on SEC, the content of isooctane
in the mobile phase is varied. In addition, the effect of column temperature on separation was also investigated.

2. EXPERIMENT

2.1 Chemical
CRBW was obtained from Surin Bran Oil Co., Ltd. (Surin, Thailand). All standard for HPLC were from Sigma (USA). All solvents were analytical grade from Merck (Germany) and RCI-Labscan (Thailand).

2.2 Experimental

2.2.1 High performance size-exclusion chromatography (HPSEC).

The HPLC system consisted of a Waters model 510 pump (Waters Associate, Milford, MA01757, USA.), a Rheodyne model 7125 six-port injector (Cotati, CA, USA) and an evaporative light scattering detector (ELSD) model 55 from SEDEX (Sedere, Alfortville, France). Phenogel columns (300 x 7.8 mm i.d., 100Å and 50Å) were from Phenomenex (Phenomenex, Inc., Torrance, CA). The column was protected with Bondapak C18 Guard Pak (Millipore Co., Milford, MA, USA). The injector and column were set at 60°C. The ELSD drift tube was set at 30°C and the N₂ flow through the nebulizer was set at two bars. Data were collected and processed by CSW32 HPLC software (DataApex Ltd, Prague, Czech Republic).

2.3 Sample preparation

Lipids standard (1000 ppm each) and CRBW samples were dissolved in toluene. The samples were kept at 60°C before analysis.

2.4 Statistical Analysis

Repeatability was carried out by relative standard deviation (RSD) during the day from values of four replicates. Statistical analysis was performed by Microsoft Excel Version 8.0.

3. ANALYSIS

3.1 Effect of isooctane on size-exclusion chromatography

In chromatographic separation each eluent is eluted in a single peak at different retention time (t_R). But co-elution may occur if unsuitable chromatographic condition was operated. The degree to which two components are resolved is termed resolution (Rs) which calculated from the t_R and width at base (W_b) of two adjacent peaks. The acceptable separation and baseline separation require the Rs greater than 1.0 and 1.5, respectively. In this study, effect of isooctane as poor solvent on SEC was observed by varied the isooctane content. Mixture of isooctane and toluene was used as mobile phase for HPSEC system. With 100% toluene as the mobile phase, WE and TG were not separated (Fig.1). Isooctane was then added to control degree swelling of the ST-DVB gel. The ratio between isooctane content to toluene was varied from 30:70 to 70:30 (v/v).

Relationship between resolution of standard WE and TG and isooctane content in the mobile phase is showed in Fig. 2. Resolution of WE and TG was increased with isooctane content in the mobile phase greater than 30% (v/v).

![Fig. 1 Co-elution of WE and TG on a 50-Å Phenogel column eluted with 100% toluene.](image1)

![Fig. 2 Relationship between isooctane content and resolution of WE and TG.](image2)

![Fig. 3 Separation of mixed standard on a 50-Å Phenogel column eluted with isooctane-toluene (70:30 v/v).](image3)

The acceptable separation between WE and TG (Rs = 1.03) was obtained when 70:30 isooctane-toluene was used as mobile phase (Fig. 3).
3.2 Effect of column temperature on size exclusion chromatography

HPLC analysis is affected by many factors such as eluent characteristic, the flow rate, and polarity of the mobile phase, etc. Whilst, effect of temperature is not much pay attention due to room temperature is normally operated in HPLC analysis. However, solubility of wax in toluene is very low at room temperature. Determination of lipids containing WE have to operate at higher temperature. In this study, the column temperature was varied at 65°C, 75°C and 85°C. Fig. 4 showed the chromatogram of CRBW separated on 100-Å Phenogel column at various temperatures.

Fig. 4 Separation of CRBW on 100-Å Phenogel column eluted with 0.15% acetic acid in 65:35 (v/v) isooctane-toluene at different column temperature.

Although, increasing column temperature was slightly shortening the elution time. However, it was not affected the elution order of all solutes.

**CONCLUSION**

The separation of lipids in HPSEC could be improved by using poor solvent to control swelling of the gel. Separation of WE and TG was improved by the incorporation of isooctane (poor solvent) in the mobile phase. Increasing the isooctane content, the resolution of WE and TG was increased. Higher column temperature is necessary for analysis of lipid containing WE due to low solubility of wax. However, too high column temperature may affect the column quality in long term operation.

**REFERENCES**


Salisa Chumsantea received the B.Sc. (2006), M.Sc. (2008) in Biochemical Technology from KMUTT. She is a Ph.D. student, School of Bioresources and Technology, KMUTT.

Kornkanok Aryusuk received the B.Sc. (1991) in Biotechnology, M.Sc. (1999) and Ph.D. (2003) in Biochemical Technology from KMUTT. She is an Assistant Professor, School of Bioresources and Technology, KMUTT. Her current interests include fat and oil technology, GC and HPLC.

Narumon Jeyashoke received the B.Sc. (1979) in Microbiology from Kasetsart University, and M.Sc. (1983) in Industrial Microbiology from Chulalongkorn University. She is an Associate Professor and Dean of School of Bioresources and Technology, KMUTT. Her current interests include enzyme technology (lipase from rice bran) and lipid technology (analytical and extraction).

Kanit Krisnangkura received the B.Sc. (1967) in Chemistry, M.Sc. (1969) and Ph.D. (1974) in Biochemistry from Michigan State University. He is an Associate Professor, School of Bioresources and Technology, KMUTT. His current interests include lipid chemistry and lipid biotechnology, oleochemicals, GC, HPLC and mass spectrometry.

**Acknowledgments** This work was supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission.