การสกัดและการทำให้เข้มข้นขึ้นของสารแอนทราควิโนนส์จากรากของต้นยอ
โดยใช้สารละลายสารลดแรงตึงผิวชนิดไม่มีประจุ

นาย กิตติศักดิ์ เกียรติเทเวศร์

EXTRACTION AND CONCENTRATION OF ANTHRAQUINONES FROM ROOTS OF *MORINDA CITRIFOLIA* BY NON-IONIC SURFACTANT SOLUTION

Mr. Kittisak Kiathevest

A Thesis Submitted in Partial Fulfillment of the Requirements
For the Degree of Master of Engineering Program in Chemical Engineering
Department of Chemical Engineering
Faculty of Engineering
Chulalongkorn University
Academic Year 2007
Copyright of Chulalongkorn University

Thesis Title   EXTRACTION AND CONCENTRATION OF
                      ANTHRAQUINONES FROM THE ROOTS OF
                      MORINDA CITRIFOLIA BY NON-IONIC SURFACTANT
                      SOLUTION
By   Kittisak Kiathevest
Field of Study   Chemical Engineering
Thesis Advisor   Assistance Professor Artiwan Shotipruk, Ph.D.

Accepted by the Faculty of Engineering, Chulalongkorn University in Partial Fulfillment of Requirements for the Master's Degree

....Dean of the Faculty of Engineering
                (Professor Direk Lavansiri, Ph.D.)

THESIS COMMITTEE

....Chairman
        (Associate Professor Prasert Pavasant, Ph.D.)

....Thesis Advisor
        (Assistant Professor Artiwan Shotipruk, Ph.D.)

....Member
        (Associate Professor Seeroong Prichanont, Ph.D.)

....Member
        (Assistant Professor Navadol Laosiripojana, Ph.D.)

กิตติศักดิ์ เกียรติเทเวศร์ : การสกัดและการทำให้เข้มข้นขึ้นของสารแอนทราควิโนนส์จากรากของ
ต้นยอโดยใช้สารละลายสารลดแรงตึงผิวชนิดไม่มีประจุ(EXTRACTION AND CONCENTRATION
OF ANTHRAQUINONES FROM ROOTS OF *MORINDA CITRIFOLIA* BY NON-IONIC SURFACTANT
SOLUTION) อ. ที่ปรึกษา: ผศ.ดร. อาทิวรรณ โชติพฤกษ์

งานวิจัยนี้ศึกษาการสกัดและทำให้เข้มข้นขึ้นของสารแอนทราควิโนนส์จากรากของต้นยอโดยใช้
สารละลายของสารลดแรงตึงผิวชนิดไม่มีขั้ว ซึ่งในส่วนของการสกัด ใช้คุณสมบัติการรวมเป็นไมเซลล์
ของสารลดแรงตึงผิวเพื่อช่วยเพิ่มความสามารถในการละลายของสารแอนทราควิโนนส์ในตัวทำละลาย
และในการทำให้สารสกัดเข้มข้นขึ้นนั้น ทำได้โดยการให้ความร้อนเพื่อเหนี่ยวนำให้สารสกัดไมเซลล์
แยกออกเป็น 2 เฟส คือเฟสของสารลดแรงตึงผิวและเฟสน้ำ โดยสารแอนทราควิโนนส์จะละลายอยู่ใน
เฟสของสารลดแรงตึงผิว การสกัดจะทำการศึกษาที่สภาวะความดันและอุณหภูมิบรรยากาศโดยใช้สาร
ลดแรงตึงผิว 2 ชนิด คือ ไทรตัน เอ็กซ์-100 และจีนาโพล เอ็กซ์-080 เพื่อศึกษาผลของความเข้มข้นของ
สารลดแรงตึงผิวที่มีต่อผลการสกัดของสารแอนทราควิโนนส์ นอกจากนี้ยังศึกษาการสกัดโดยใช้
สารละลายสารลดแรงตึงผิวที่สภาวะกึ่งวิกฤตในระบบการไหลของตัวทำละลายอย่างต่อเนื่องที่อัตราการ
ไหลของตัวทำละลาย 4 มิลลิลิตร/นาที ในช่วงความเข้มข้นของสารลดแรงตึงผิว 1-5 % โดยปริมาตร
พบว่าสภาวะที่เหมาะสมต่อการสกัดคือ ที่สภาวะอุณหภูมิ 80 องศาเซลเซียสและใช้สารละลายสารลด
แรงตึงผิวไทรตัน เอ็กซ์-100 ความเข้มข้น 1% โดยปริมาตร จากนั้นสารสกัดที่ได้จากการสกัดที่สภาวะนี้
จะนำมาทำให้เข้มข้นขึ้น โดยศึกษาสภาวะ ความเข้มข้นของสารลดแรงตึงผิวในสารสกัด, อุณหภูมิ และ
เวลาในการทำให้เข้มข้นขึ้นที่เหมาะสม ซึ่งพบว่าคืออุณหภูมิ 75 องศาเซลเซียส, เวลา 30 นาที, และใส่
สารลดแรงตึงผิวไทรตัน เอ็กซ์-100 ลงไปเพิ่มในสารสกัดอีก 1% โดยปริมาตร ผลการสกัดที่สภาวะการ
ทำให้เข้มข้นที่เหมาะสมถูกนำไปวิเคราะห์ปริมาณสารแดมนาแคนธัลซึ่งเป็นสารต้านมะเร็งซึ่งมีมูลค่า
สูงที่สุดในกลุ่มสารแอนทราคิโนนส์ ผลการวิเคราะห์พบว่าสารแดมนาแคนธัลที่ได้จากการสกัดและการ
ทำให้เข้มข้นด้วยสารละลายลดแรงตึงผิวเกิดการสลายตัวน้อยเมื่อเปรียบเทียบกับการสกัดด้วยน้ำกึ่ง
วิกฤตที่ไม่มีสารลดแรงตึงผิว เนื่องจากสภาวะอุณหภูมิในการสกัดต่ำกว่า นอกจากนั้นในการสกัดและ
ทำให้เข้มข้นขึ้นด้วยวิธีนี้ ยังสามารถลดพลังงานที่ต้องใช้ได้อีกด้วย

ภาควิชา.......วิศวกรรมเคมี.........ลายมือชื่อนิสิต..
สาขาวิชา......วิศวกรรมเคมี.........ลายมือชื่ออาจารย์ที่ปรึกษา...............................
ปีการศึกษา........2550..............

4870223021   : MAJOR CHEMICAL ENGINEERING DEPARTMENT

KEY WORD: ANTHRAQUINONES   /   CLOUD POINT EXTRACTION /
MICELLE-MEDIATED EXTRACTION / *MORINDA CITRIFOLIA*

KITTISAK KIATHEVEST : EXTRACTION AND CONCENTRATION OF ANTHRAQUINONES FROM ROOTS OF *MORINDA CITRIFOLIA* BY NON-IONIC SURFACTANT SOLUTION. THESIS ADVISOR: Dr. ARTIWAN SHOTIPRUK.

This study investigated the use of micelle-mediated extraction (MME) of anthraquinones from the root of *Morinda citrifolia* and cloud point concentration (CPC) of the extract as effective alternatives for extraction and concentration of the product without using toxic organic solvent. Micelle-mediated extractions (MME) were carried out at ambient pressure and temperature using two types of surfactants: Triton X-100 and Genapol X-080 to determine the effect of concentrations on the percent anthraquinones extracted. In addition, micelle mediated pressurized hot water extraction (MMPHWE) was investigated in a continuous flow system at a constant flow rate of 4 ml/min surfactant solutions at the concentration between 1 and 5 %v/v. The extract from the most suitable MMPHWE, with 1% Triton X-100 solution at 80°C, was then concentrated using CPC and the effect of surfactant concentration, the incubation time and temperature was determined on the concentration efficiency. For CPC, the most suitable condition was at 75 °C and the incubation time was 30 min using Triton X-100 at 1% v/v (surfactant/the extract). The extract obtained by MMPHWE followed by CMC was analyzed with HPLC to quantify the amount of the most medicinally anti-cancer compound, damnacanthal and the result showed that of the compound degradation was reduced as the temperature required for extraction was lower compared with pressurized hot water extraction (PHWE) without use of surfactant. In addition, the amount of energy required for extraction and concentration could be greatly reduced.

Department…...Chemical Engineering……Student's signature. *kittisak Kiathevest*

Field of study…Chemical Engineering…...Advisor's signature. *Artiwan Motopas*

Academic year…….2007…………

ACKNOWLEDGMENTS

CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

CHAPTER I

INTRODUCTION

1.1 **Rationale**

Morinda citrifolia (*M. citrifolia*), a plant originated in tropical Asia or Polynesia, has been used as food and medicine for over 2000 years. All parts of this plant, which include fruits, flowers, leaves, bark, stem and roots, contain several biological activities. The main group of active compounds is anthraquinones, which have been shown to possess various therapeutic properties including anti-bacterial, anti-viral, anti-cancer activities [Chan-Blanco et al., 2006]. Among the different anthraquinones found in *M. citrifolia*, damnacanthal which is presented mainly in the root is considered the most important, due to its important activity in fighting against cancers [Hiramatsu et al., 1993].

Generally, anthraquinones can be extracted into organic solvents such as ethanol and DMSO. Pongnaravan et al. (2006) investigated a more benign alternative for extraction of anthraquinones from *M. citrifolia* roots with pressurized hot water (PHW) or subcritical water and suggested a suitable temperature for extraction of the total anthraquinones to be at 220 °C. At such temperature and adequately high pressure to maintain the water at liquid state, water behaves like organic solvent and is able to extract organic compound more readily. However, pressurized hot water extraction requires operation at high temperature, leading to high energy consumption and degradation of thermal labile organic solute. Anekpankul et al. (2007) analyzed the damnacanthal content in the pressurized hot water extracts and reported significant loss of damnacanthal when the water temperature was as high as 200 °C. Moreover, the resulted extract is generally dilute and requires concentration prior to HPLC analysis. In the previous study, concentration of the water extract was carried out by vacuum evaporation which requires large amount of energy due to high heat of vaporization of water.

An alternative extraction method involve the process called micelle mediated separation (MMS), which comprises two parts: micelle-mediated extraction (MME) and cloud point concentration (CPC). In MME, a surfactant solution whose concentration is above its critical micelle concentration (cmc) is used as an extraction solvent. At such concentration, the surfactant molecules form molecular aggregates of colloidal size,

called micelles, can extract the target solute, especially hydrophobic or non-polar solute, from plant material. After MME, the extract can be concentrated further using cloud point concentration (CPC). In CPC, either anionic, cationic, or nonionic surfactants can be employed to separate the system into two phases: the surfactant-rich phase, which contain extracted solute, and the aqueous phase, whose surfactant concentration is closed to its critical micelle concentration (cmc). The most commonly used surfactants for CPC are non-ionic surfactants, which uses heating to acquire the phase separation [Rubio et al., 2003]. The small volume of surfactant-rich phase obtained from this technique permits the extracted solution to be concentrated. The micelle-mediated separation process is simple, safe, non- toxic, and economical and has been applied for extraction and concentration of different compounds (i.e. metal ion, environmental organic compound, and biomaterial solute) from various solid matrix or water solution [Zhu et al., 2005, Shen et al., 2006, Shi et al., 2004, Abdollahi et al 2004, Fang et al., 2000].

In this study, the use of MMS was investigated to extract and concentrate the anthraquinones from the roots of *Morinda citrifolia*. Suitable conditions for micelle-mediated extraction (MME) and micelle-mediated pressurized hot water extraction (MMPHWE) that yield the maximum amount anthraquinones was firstly determined by employing two most commonly used non-ionic surfactants in separation processes: Triton X-100 and Genapol X-080. The extract obtained from the most suitable condition for MMPHWE was further concentrated using cloud point concentration (CPC). The effect of surfactant concentration, concentrate temperature, and equilibration time were determined on the efficiency of CPC. Finally, the concentrated extract by CPC was analysed with HPLC to determine the amount of damnacanthal and the results were compared with those obtained with PHWE without the use of surfactant.

1.2 Objectives

1.2.1 To investigate suitable conditions for micelle-mediated extraction (MME) of anthraquinones from *Morinda citrifolia* roots, using Triton X-100 and Genapol X-080.

1.2.2 To investigate suitable conditions for micelle-mediated pressurized hot water extraction (MMPHWE) using water and either Triton X-100 or Genapol X-080 solutions as extraction solvents.

1.2.3 To determine suitable conditions for concentration of anthraquinones obtained from MME or MMPHWE.

1.2.4 To quantify of damnacanthal in the anthraquinones extract with HPLC.

1.3 Working scopes

1.3.1 Determine the effects of concentration (0-20% v/v) of two non-ionic surfactant (Triton X-100 or Genapol X-080) and extraction on MME of anthraquinones from roots of *Morinda citrifolia* (0.5 g : 50 ml solution).

1.3.2 Determine the effects of concentration (0-5% v/v) and temperature (80, and 120 ºC) of Triton X-100 or Genapol X-080 solutions on the extraction efficiency of anthraquinones by MMPHWE.

1.3.3 Determine the effects of non-ionic surfactants concentration adds (between 0-5 % (v/v)), equilibration time (between 10-30 min), and temperature (between 70-90 °C for Triton X-100 or 50-80 °C for Genapol X-080) on CPC of the extracts obtained at the most suitable conditions observed from 1.3.1 or 1.3.2.

1.4 Expected benefits

This study provides a new and efficient alternative method for extraction and concentration of anthraquinones extracted from roots of *M. citrifolia*.

CHAPTER II

BACKGROUND AND LITERATURE REVIEWS

2.1 *Morinda citrifolia*

Morinda citrifolia is a plant originated in tropical Asia or Polynesia. The plant is locally known by various common names such as "noni" in Hawaii, "Indian mulberry" , "nuna", or "ach" on the Indian subcontinent, "mengkudu" in Malaysia, "nhau" in Southeast Asia, "painkiller bush" in the Caribbean, "cheese fruit" in Australia, or "yor" in Thailand. It is a small tree, 3-10 m tall, with wide elliptical leaves (5-17 cm length, 10-40 cm width). The noni fruit (3-10cm length, 3-6cm width) is oval and fleshy with an embossed appearance. It is slightly wrinkled and semi-translucent, and its color ranges from green to yellow, to almost white at the time of picking.

Different parts of *Morinda citrifolia*; including leaves, flowers, bark, fruit, and root, contain various phytochemicals; phenolic compound, organic acids and alkaloids. These compounds have been proven to exhibit several therapeutic effects. The fruits were used to stimulate the immune system. Juice of the fruits were claimed to relieve inflammation. The leaves were used to treat eye problems; heated leaves were used to relieve coughs, nausea, colic; and the roots were used as dye and to relieve chronic diseases such as cancer, diabetes, and cardiovascular diseases. Nowadays, there has been increased interest in extracting constituents that are responsible for the therapeutic properties of this plant. One of the most important constituents in *Morinda citrifolia* is anthraquinones, which is generally found in the leaves, barks, and mainly in the roots (Chan-Blanco et al., 2006).

Figure 2.1) *Morinda citrifolia*

2.2 Anthraquinones

Anthraquinones is a group of compounds contained in roots of *Morinda citrifolia*. Originally, it was used as yellow dye or as folkloric medicines. Subsequently, its therapeutic properties were discovered, including anti-viral, anti-bacterial, and anti-cancer activities. Basic structure of anthraquinones is shown in figure 2.2 and some important derivatives are shown in Table 2.1. Of all anthraquinones, the most important constituents in term of medicinal values is damnacanthal, which shown to be effective for fighting against cancer, preventing the growth of pre-cancerous cells, and stimulating T cell activity (Hiramatsu et al., 1993). Some physical properties of damnacanthal are demonstrated in Table 2.2.
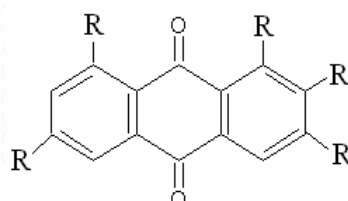
Figure 2.2) Basic structure of anthraquinones

Table 2.1: Derivatives of anthraquinones (Chan-Blanco, 2006)

No.	Structural Formula	Name
1		1,2-dihydroxyanthraquinone (alizarin)
2		3-hydroxy-2-hydroxymethylanthraquinone (lucidin)
3		morindone
4		1,3-dihydroxy-6-methyl anthraquinone
5		2-methyl-4- hydroxy-5,7-dimethoxyanthraquinone
6		3-hydroxy-1-methoxyanthraquinone-2 carboxaldehyde (damnacanthal)
7		8-hydroxy-8-methoxy-2-methyl-anthraquinone
8		2-methyl3,5,6-trihydroxyanthraquinone

Table 2.2: Properties of damnacanthal (*Biomol Research Laboratory Inc.*, 2005)

Name	3-hydroxy-1-methoxyanthraquinone-2-carboxaldehyde
Formula	$C_{16}H_{10}O_5$
Molecular weight	282.34
Solubility at 25 ºC(M)	Soluble in DMSO (25 mg/ml)

2.3 Extraction of plant materials with sub and supercritical fluid technology

Presently, the use of bioactive compound from plant materials as alternative medicines has become more popular worldwide. Conventionally, these compounds can be extracted by using organic solvents. The method is simple but the residue of toxic organic solvent may be left in the extracted product. The desire to eliminate or reduce the use of toxic organic solvent has led to increased interest in sub and supercritical fluid (SFC) technology. Supercritical fluid refers to the fluid that has pressure and temperature above its critical point, as shown in figure 2.3. Under these conditions, properties of fluid lie between those of gas and liquid. The density of SCF is close to that of liquid, the viscosity is close to that of gas, and the diffusivity lies between the two states, as shown in Table 2.3. These properties (low viscosity and relatively high diffusivity) allow SCFs to diffuse easier into plant material and give faster extraction. The most used SCF for extraction is supercritical carbon dioxide (SC-CO_2) because it has low critical temperature. Moreover, SC-CO_2 is nontoxic, environmental friendly, and is the second least expensive solvent next to water. SC-CO_2 gives usually a good extraction yield for the extraction of non-polar compounds from plant materials. However, for extraction of polar compound, the polarity of SC-CO_2 is too low to give effective extraction.
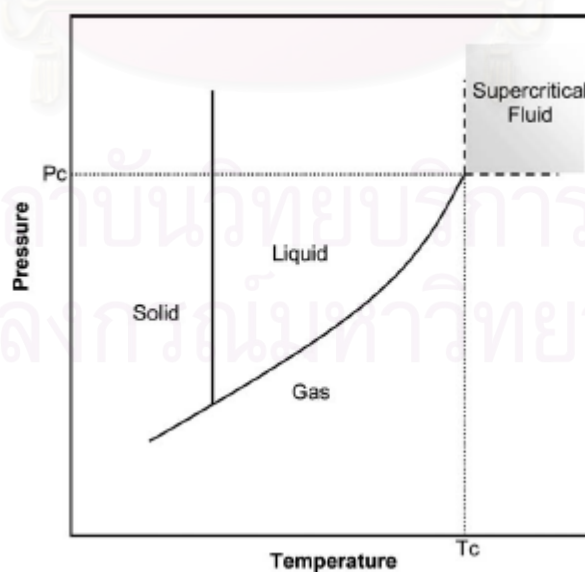
Figure 2.3) Schematic pressure-temperature diagram of critical fluid region

(Herrero, 2006)

Table 2.3: Range of values of several properties for gases, liquids and supercritical fluids

State of fluid	Density(ρ, g/cm^3)	Viscosity(μ, g s/cm)	Diffusivity(D_{AB},cm^2/s)
Liquid p = 1 atm; T = 15–30 °C	1	10^{-2}	$< 10^{-5}$
Gas p=1 atm, T=21 °C	10^{-3}	10^{-4}	10^{-1}
Supercritical p = p$_c$, T = T$_c$	0.3 - 0.8	10^{-4} -10^{-3}	10^{-4} -10^{-3}

Apart from SC-CO$_2$, an alternative for benign extraction is by using water, which is non-toxic, non-flammable, economical, and environmentally acceptable. For extraction of medicinally active compounds from natural materials, water at milder subcritical conditions rather than corrosive supercritical condition is more effective. At subcritical conditions, water has been shown to be capable of extracting a wide range of organic solutes from different matrixes.

Subcritical water (SW), sometimes called pressurized hot water (PHW), refers to liquid water at the temperatures between its boiling (100ºC) and its critical temperature (374ºC), and is the influence of under high enough pressure to maintain the liquid state (figure 2.3). At such conditions, organic solutes are much more soluble in water than at room temperature. The important factor that affects these results is the variability of the dielectric constant with temperature. Normally, water at room temperature is very polar, with a dielectric constant (ε) of approximately 80. However, the ε value is significantly decreased to less than 30 when water temperature rises to 250ºC, (see figure 2.4) which is a value similar to that of ethanol at room temperature (King et al., 2004).
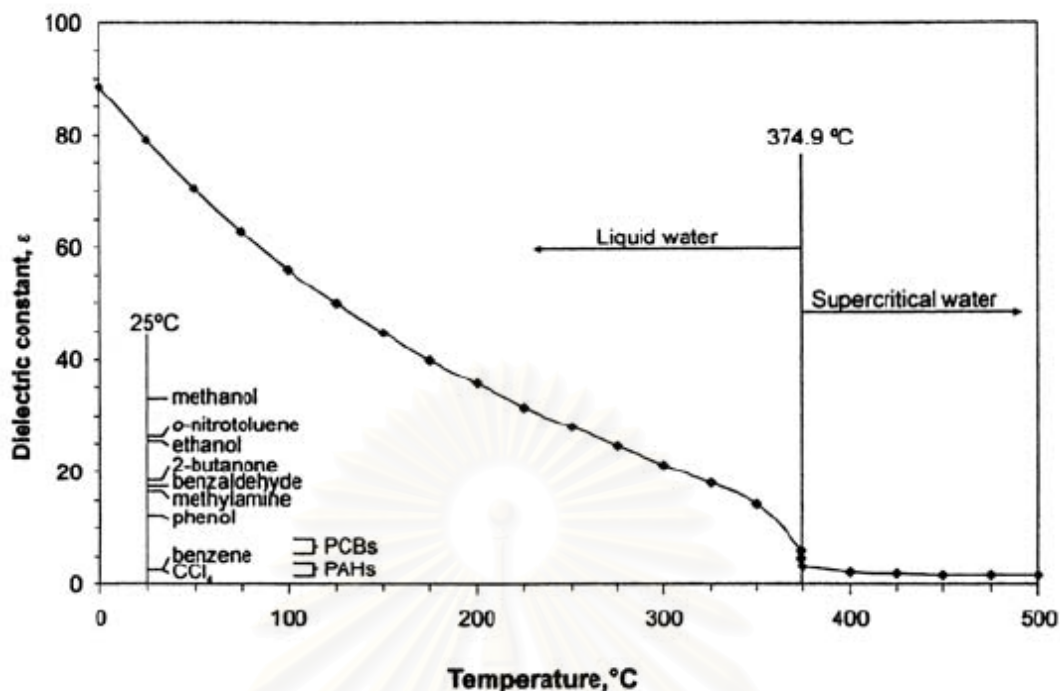
Figure 2.4) Dielectric constant of water versus temperature (King, 2004)

For extraction of anthraquinones, Pongnaravane (2006) reported the extraction of anthraquinones from the roots of *Morinda citrifolia* by using pressurized hot water. The results indicated that the amount of anthraquinones increases with increasing temperature. However, the analyte was measured as total anthraquinones by a spectrophotometer using alizarin as standard. Although the spectroscopic method is simple, it did not provide quantitative information regarding the composition and the content of different anthraquinones in the extract. When the interest is placed on a specific constituent, another analysis method should use replace. Later, Anekpankul (2006) employed HPLC for the analysis of damnacanthal, anti-cancer anthraquinones derivatives, extracted from the roots of *Morinda citrifolia* by pressurized hot water. It was found that damnacanthal degraded at temperature above 170 °C. Furthermore, for pressurized hot water extraction, the concentration of the compound in the extract is typically lower than the detection limit of HPLC. Thus, a few steps are required to concentrate the extract prior to proper HPLC analysis. The most common method to concentrate the extracted solute is evaporation. This method is acknowledged but it is time and energy consuming especially, when solute to be evaporated has high boiling point such as water. Extended process time could also lead to the loss of extracted

solute. Other concentration methods include solid-phase extraction (SPE) and solid-phase microextraction (SPME). The main advantages of solid-phase extraction are convenient with easier manipulations, fast, and sensitive, while the advantages for solid-phase microextraction are low cost, rapid concentration, and easy automation. However, their methods have some drawbacks; SPE requires eluted toxic organic solvent and SPME is difficult to operate and moderate precision extraction (Ulrich., 2000).

An interesting solution for the above mentioned problem associated with pressurized hot water extraction is to use micelle mediated separation. Enhanced extraction efficiency and solute concentration can be promoted by addition of just a small amount of surfactant polymer into the aqueous extraction system. The process is simple, requires low cost and energy, and involves non-toxic chemicals. The investigation of extraction with pressurized hot non-ionic surfactant solution followed micelle mediated concentration was carried out on ginsenosides from the roots of *American ginseng* (Choi, 2003). The author demonstrated that the addition of non-ionic surfactant in pressurized hot water as solvent to extract ginsenosides gave higher extraction yield than pressurized hot water extraction alone at the same temperature. The efficiency of the micelle mediated pressurized hot water extraction was comparable to pressurized hot methanol extraction. In addition, concentration of the extract was conducted by phase separation upon heating the extract, resulted in concentrate solute in the surfactant-rich phase. This technique was shown to increase detection sensitivity of HPLC analysis. From this study, it was shown clearly that Micelle-mediated separation (MMS) helps simplify the operating procedure by combining the extraction and concentration process of the solute into one step.

2.4 Surfactant and its role in Micelle-mediated separation.

Surfactant are amphiphilic molecules, consist of two parts; the first part is the head or hydrophilic groups, which is either strongly polar or charged, and another part is the tail, a non-polar or hydrophobic groups (see figure 2.5). Surfactants can be divided into four categories: anionic, cationic, non-ionic, and zwitterionic surfactants. Anionic surfactants have a negative charge on their hydrophilic end, including $-CO_2^-$, $-SO_3^-$, $-OSO_3^-$, and OPO_3^- and usually have the counterion as Na^+. The examples of anionic surfactant are sodium dodecyl sulfate (SDS), sodium decanesulphonate (SdeS),

and sodium dodecanesulfonic acid (SDSA). Cationic surfactant have a positive charge on their hydrophilic end, including $-N (CH_3)_3^+$, ⬡°, and usually have the counterion as Cl^- or Br^-. The examples of cationic surfactant are tricaprylmethylammonium chloride (Aliquat-336), and Cetyl trimethylammonium bromide (CTAB). Nonionic surfactants have no charge on their hydrophilic end, dominated by the ethoxylates, $-(OCH_2CH_2)_nOH)$. The examples of non-ionic surfactant are polyoxyethylene-4-lauryl ether (Brij 30), (*iso*-octyl phenoxy polyethoxy ethanol (Triton X-100), octyl phenol poly(ethylene glycol ether) (Triton X-114), and oligoethylene glycol monoalkyl ether (Genapol X-080). Zwitterionic surfactants contain two charged groups of different sign in their hydrophilic head, giving them a net charge of zero. The positive charge of Zwitterionic surfactant is almost always ammonium, while the source of the negative charge may vary (carboxylate, sulphate, sulphonate).The examples of zwitterionic surfactant are (*N*-dodecyl-*N*,*N*-dimethylammonio)undecanoate (DDMAU), and 2-decyldimethylammonioethane sulfate ($C_{10}H_{23}$ $N^+(CH_3)_2$ •CH_2•CH_2 OSO_3^- (Clint., 1992). All types of surfactants can be used in micelle-mediated separation process. However, with respect to the structure and the lower critical micellar concentration; non-ionic surfactants can bind with extracted solute better than other surfactants at the same chain length, thus they have been used frequently in the extraction/concentration process (Rubio et al., 2003). The most favorably used of non-ionic surfactants are Triton X-100, Triton X-114, and Genapol X-80 (Quina et al., 1999) whose properties are shown in Table 2.4-2.6.
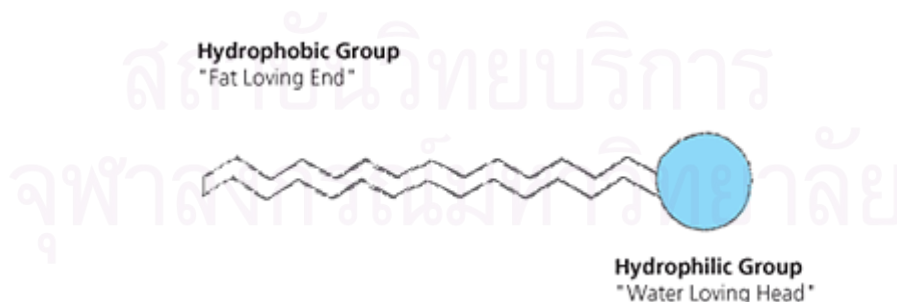
Figure 2.5) General drawing of molecular structure of surfactant

Table 2.4: Properties of Triton X-100 (*mpbio Inc.*, 2006)
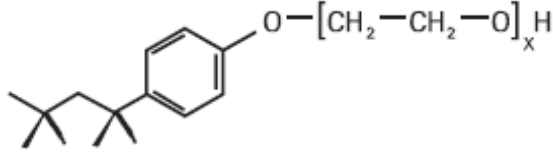
Name:	Iso-octyl phenoxy polyethoxy ethanol
Structure:	
Molecular Weight:	624-628
Density at 25°C:	8.9 lb/gal
Specific gravity:	1.07
Viscosity at 25°C:	240 cP
pH, 5% aqueous solution:	6
Cloud point temperature:	65 °C
λ_{max}:	226 nm
critical micellar concentrations:	0.25 mM

Table 2.5: Properties of Triton X-114 (*mpbio Inc.*, 2006)

Name:	Octyl phenol poly(ethylene glycol) ether
Molecular Weight	537
Density at 25°C:	8.8 lb/gal
Specific gravity:	1.058
Boiling point:	200 °C
Viscosity at 25 °C:	260 cP
Cloud point temperature:	24 °C
λ_{max}:	223 nm
critical micellar concentrations:	0.35 mM

Table 2.6: Properties of Genapol X-080 (*Sigma Aldrich Inc.*, 2006)

Name:	Isotridecyl poly(ethylene glycol) ether
Structure:	$CH_3(CH_2)_{12}\text{-}O(CH_2CH_2O)_8\text{-}H$
Molecular Weight:	553
Density at 20°C:	0.99 g/mL±0.002 g/mL
Molecular Formula:	$HO(CH_2CH_2O)_n(CH_2)_mH$
Cloud point temperature:	42 °C
λ_{max}:	< 210 nm
critical micellar concentrations:	0.06-0.15 mM

Additional of surfactant into aqueous solution is a very powerful separation technique. The effect on the separation can be viewed as consisting of two processes: micelle-mediated extraction and cloud point concentration. Both processes are described as follows.

2.4.1 Extraction organic solute by micelle-mediated extraction

Extraction by micelle-mediated extraction is carried out by means of addition of surfactant into aqueous solution. At low surfactant concentration, surfactant molecules are found as monomers. When the concentration increases above its critical micellar concentration (cmc), the surfactant molecules start to form molecular aggregates of colloidal size, called micelles. Surfactant aggregates orientate their hydrophobic tail towards the center of formation, creating a non-polar core (see figure 2.6). At this point, the solute (especially hydrophobic or non-polar solute) can be extracted from solid matrixes into aqueous micelle solution. The tendency of surfactant to form micelles depends on their cmc, and the conditions of the systems. The lower value of the cmc results in the greater tendency of surfactant to form micelles. Some of the most commonly used surfactants are shown in Table 2.7. As shown in that Table, non-ionic surfactants have low cmc values, which mean that they can bring together the solute molecules more easily than the other surfactant types (Scamehorn et al., 1989). The most favorable non-ionic surfactants used are Triton X-100, Triton X-114, PONPE 7.5, and Genapol X-80 (Quina et al., 1999). However, some non-ionic surfactants (such as

Triton X series and PONPE series) have a drawback particularly for the analysis of the interested compound is carried out with HPLC using UV detection. The peak of surfactant may overlap with the compound in UV region. To avoid this problem, another non-ionic surfactant that does not overlap with the solute might be used, in addition, the changing of UV detection to fluorescence detection or a few clean-up steps to eliminate surfactant may be considered as other alternatives.
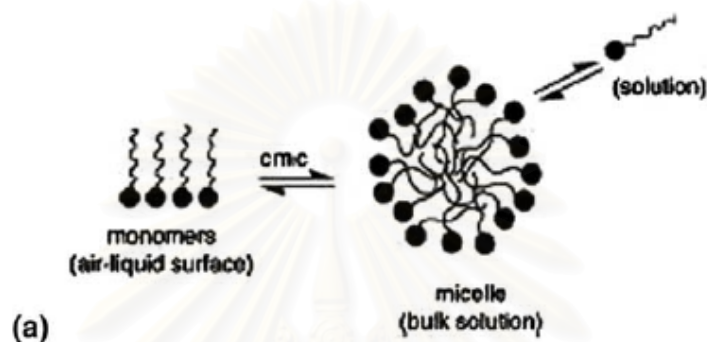
Figure 2.6) Formation of micelle above its critical micellar concentration (cmc).

Table 2.7: Commonly used of surfactants and their cmc values.

Types of surfactant	Surfactant	cmc (mM)
Anionic	SDS	3.69
	Sodium chrolate (SC)	12.6
	Sodium dodecanesulphonate (SDoS)	10
Cationic	CTAB	0.9
	TAB	3.5
Non-ionic	Triton X-100	0.25
	Triton X-114	0.35
	Genapol X-080	0.05
Zwitterionic	Pluronic P105	3
	DDMAB	4.3

2.4.2 Concentration of the extracted solution by cloud point concentration.

When the organic solute in the extracted solutions is dilute, the extracted solutions must be concentrated prior to the determination of the quantity of the solute. The common method for concentration of the extracted liquid is an evaporation. This method is well-established but its major limitation is the time and energy consuming. Moreover, the loss of extracted solute may occur. Cloud point extraction can overcome this drawback as it require shorter time and low energy and could recover the solute almost completely, particularly when the solute is non-polar (Seronero et al., 2000). In cloud point concentration phenomenon, surfactant is employed to separate the system into two phases: the organic solute is concentrated by moving from one phase to another phase. Each type of surfactant has individual systems for phase separation; non-ionic surfactant uses temperature, cationic and zwitterionic surfactant use couple with electrolytes or cosurfactant, and anionic surfactant uses acid to induce phase separation. Most commonly used surfactant for cloud point concentration is again non-ionic surfactant. The cloud point phenomenons for non-ionic surfactant occur when the temperature of aqueous micelles solution rises to the point that the solution is turbid and form two phase separation with different polarities and viscosities. The temperature that the two phase separations occur is called cloud point temperature. The two phases are surfactant-rich phase, which contain extracted solute, and aqueous phase, in which surfactant concentration is closed to its cmc. This phenomenon is reversible, thus upon cooling the solution, a single phase appears again. The schematic representation of the steps involved in the cloud-point separation is shown in Figure 2.7. The concentration efficiency of cloud point concentration depends on different parameters related to the solute, including hydrophobicity of solute, addition of salts, temperature, concentration of surfactant, centrifugation time, and equilibration time. The increasing of the hydrophobicity of the solute, temperature, and concentration of surfactant can improve the concentration efficiency (Rubio et al., 2003), while the addition of salt and centrifugation time slightly affect the concentration efficiency (Paleologos et al., 2005). However, it has been reported that the addition of salts in the process promote easy separation of the two phases. The salts, which have been used in cloud point concentration, include urea, sodium chloride, and potassium chloride. Among them,

sodium chloride is the most used due to its low cost, and environmentally friendly (Shi et al., 2004). For non-ionic surfactant, the efficiency of concentration also depends on equilibration time, the time taken for the solute to interact with micelles and to get into the surfactant-rich phase. It has been reported that the equilibration times of 20-30 min, at above its cloud point temperature, are frequently used for several cloud point processes (Rubio et al., 2003).
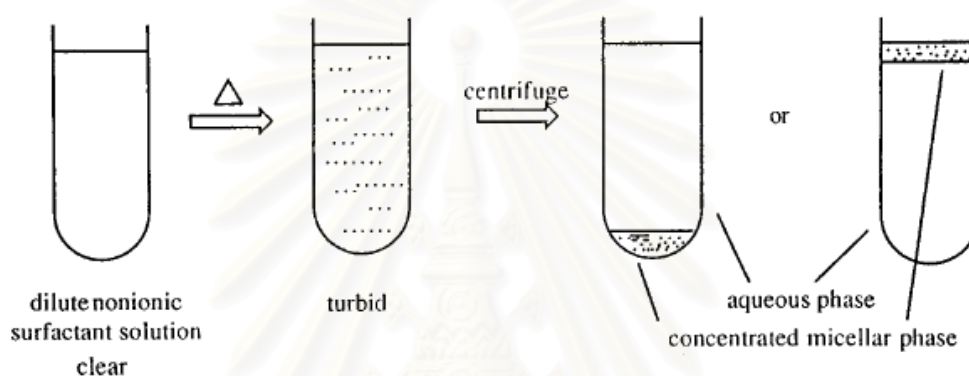
Figure 2.7) Schematic representation of steps involved in cloud-point separation

2.5 Literature reviews

Anthraquinones extraction from roots of *Morinda citrifolia* have been studied and different methods have been employed by several researchers. At the early stage, organic solvent extraction method such as maceration and soxhlet extraction with ethanol as solvent were used. However, these methods employ long extraction time to achieve high recovery. Recently, ultrasound-assisted extraction (UAE) and Microwave-assisted extraction (MAE) were introduced as alternative method to extract anthraquinones, which can reduce extraction time to about 60 min for UAE and 15 min for MAE by using the mixture of ethanol and water (50:50) at temperature of 60 °C (Hemwimol et al., 2006, 2007). Nowadays, the desire to reduce the use of the organic solvent in food and medicine processing has led to the use of water as extraction solvent. However, at room temperature water is inappropriate due to the low solubility of anthraquinones in water at this condition. To use water as a solvent for anthraquinones

extraction, pressurized hot water extraction (PHWE) was introduced and a suitable condition for extraction of the compound was suggested to be as high as 220 °C (Pongnaravane et al., 2006). However, the following study showed that the degradation of particular compound, such as damnacanthal, which is the most medicinally active anthraquinones, occurred at 170 °C (Anekpankul et al., 2007). The reviews of anthraquinones extraction are summarized in Table 2.8.

The recent published results clearly demonstrated that "micelle-mediated separation" can be used for quantitative extraction and concentration of a variety of organic compounds. Surfactant solutions have been used to extract organic solute from the solid matrixes or liquid solutions into their solutions solvent and then concentration of solute was done by using phase separation. The reviews of micelle-mediated separation of different types of surfactant are summarized in Table 2.9 to 2.12. At first, the method was applied for the removal of metal ions (i.e. nickel, zinc (II)) from liquid samples by using non-ionic surfactant (Watanabe et al., 1978). In addition, subsequent studies have applied this method for extraction or concentration various organic compounds. Some examples are the extraction of Vitamin K3 and 1,4-naphthoquinone from liquid solution by using Triton X-114, non-ionic surfactant (Abdollahi et al., 2004), the extraction of Etofenprox in environmental samples by using SDoS, anionic surfactant (Shemirani et al., 2005), the extraction of tanshinones from *Salvia miltiorrhiz bunge* by using genapol X-080, non-ionic surfactant (Shi et al., 2004). In addition, the micelle-mediated separation can be coupled with pressurized fluid extraction to combine extraction and concentration of the solute by means of cloud point concentration into one step (choi, 2003). In such study pressurized hot water with addition of non-ionic surfactant was used as solvent to extract ginsenosides from the roots of *American ginseng*. The results show that this technique gave higher extraction yield than using pressurized hot water without surfactant at the same temperature. In addition, the concentration of the extract with cloud point concentration could increase the detection sensitivity of HPLC.

In this work, we focus on the use of micelle-mediated separation to extract and concentrate the anthraquinones compound from the roots of *Morinda citrifolia*. The suitable extraction conditions that can maximize the extraction efficiency is determined. In addition, the extract would be concentrated by cloud point concentration and the

optimal concentration conditions is also examined. Furthermore, for the sample obtained at selected condition, HPLC analysis of the amount of damnacanthal anticancer anthraquinones is carried out. The materials and methods used in this study are described in chapter 3.

Table 2.8 Review on Investigation of anthraquinones extraction from the roots of *Morinda citrifolia.*

Author	Solvent	Extraction method	Range study	Optimum conditions	Analysis	Objectives
1. Hemwimol et al., 2006	acetone, acetonitrile, methanol, ethanol, ethanol: water	UAE	Extraction time (15-90 min), Temperature (25-60 °C), ultrasonic power (15.7-56.1W), ethanol: water composition (0-100 %)	Ethanol:water (50:50), T = 60 °C for 60 min.	Spectrophoto-metric at 435 nm with alizarin as standard	To determine the extraction efficiency of anthraquinones from the roots of *M. citrifolia* by means of conventional ethanol extraction per se as compared with UAE.
2. Pongnaravane et al., 2006	Water, ethanol	SWE	Temperature (120-220°C, flowrate (2-6 ml/min	T = 220 °C , flowrate = 5 ml/min	Spectrophoto-metric at 435 nm with alizarin as standard	To determine the antioxidant activity of anthraquinones extracted with PHW and compare it with that of the extracts obtained by other conventional solvent extraction methods such as extraction in ethanol with magnetic stirrer, ultrasound-assisted extraction (UAE), and Soxhlet extraction.

Author	Solvent	Extraction method	Range study	Optimum conditions	Analysis	Objectives
3. Anekpankul et al., 2007	water	SWE	Temperature (120-220°C, flowrate (2-6 ml/min	T = 170 °C , flowrate = 5 ml/min	HPLC with UV detection at 250 nm	To find the suitable conditions for subcritical water extraction of damnacathal from *Morinda critifolia* roots. To propose a mathematical model that describes the behavior of subcritical water extraction.
4. Hemwimol et al., 2007	acetone, acetonitrile, methanol, ethanol, ethanol: water	MAE	Extraction time (5-30 min), Temperature (60-120 °C), ethanol: water composition (20-80 %)	Ethanol:water (50:50), T = 60 °C for 60 min.	Spectrophoto-metric at 435 nm with alizarin as standard	To determine the extraction efficiency of anthraquinones from the roots of *M. citrifolia* by means of conventional ethanol extraction per se as compared with MAE.

Table 2.9 Review on Investigation of micelle-mediated separation using anionic surfactant

Author	Surfactant	Matrix	compounds	Range	Optimum conditions	Analysis	Objective
1. Casero et al., 1999	SDSA, SDS	water, Dried sewage sludge	pyrene, PAHS, progesterone, steroid hormones vitamin E	0-10 M HCl, T=10-90°C,0-2 M NaCl, centrifuge 500-10,000 rpm 10min-3h 0.1-9%(w/v) SDSA and SDS	4.2 M HCl, Stir 2 h at T_{ROOM} Centrifuge 1500 rpm 30min 1%w/v SDSA or SDS R_{SDS}= 80-158%, R_{SDSA}= 86-161%	HPLC with UVdetection at 220 nm	Propose the use of anionic surfactants in cloud point methodology to separate into two isotropic phase in acid medium from liquid-liquid and solid-liquid samples
2. Sicilia et al., 1999	SDSA	River water, Underground water, Network supply water	PAHs	0.2-10 M HCl, 0.2-9%(w/v) SDSA	4 M HCl stir 5 min at T_{ROOM} Centrifuge 1500 rpm 10 min 0.1%w/v SDSA %R $_{PHAs}$= 62-106%	HPLC with fluorescence detector	Use anionic surfactant determine PAHs in environmental samples
3. Sicilia et al., 2002	SDoS, SDeS, STS	water	Phenols, PAHs, Phthalic esters, Anilines, Dyes, Surfactants	0-10 M HCl, T=10-80°C,0-0.4 M NaCl, centrifuge 1500 rpm 1-60 min 0.2-10%(w/v) SDeS, SDoS, STS	4.6 M Hcl, stir 5 min,T_{ROOM}[a] Centrifuge 1500 rpm 5 min 3%w/v SDoS %R_{All} = 42.4-99.5%	Spectro-photo-metric	Study factors that affect on the anionic surfactant-mediated extraction

[a] = for SDeS, SDoS for STS use T = 50°C

Author	Surfactant	Matrix	compounds	Range	Optimum conditions	Analysis	Objective
4. Giokas et al., 2004	LA	Water	Ca^{2+}, Mg^{2+}	25-500 mg/l Ca^{2+},Zn^{2+} 12-200 mg/l Mg^{2+} 0.5-8.5 mg/l LA 0 - 2×10^{-2} M HCl, 0 – 2.5×10^{-2} M NaOH, 0.05-0.8 M $NaNO_3$ T = 5 - 80 °C	40 μL of 4 M NaOH, T=10ºC Centrifuge 4000 rpm30min 4.4 g /l LA mixed 1 min %R_{PHAs} > 95%	Spectro-photo-metric	application of liquid coacervate extraction for the preconcentration of metal ions from aqueous matrixes.
5. Mata et al., 2004	SDS	water	TBABr	0-200 mM TBABr 0-1 M SDS T = 25-85 °C	No find optimum condition	Spectro-photo-Metric, DLS	characterize the micellar properties of NaDS in presence of TBABr,
6. Jia et al., 2006	SDoS	water	Etofenprox	3-3.7 ml 12 M HCl, T=20-80°C, 0.05-0.11%(w/v) SDoS, extract time 0 - 2.5 h	3.1 ml of 12M HCl, T_{ROOM} Stir 5 min, $t_{equilibration}$ = 2 h Centrifuge 4000 rpm 5 min 0.9% w/v SDoS %R = 73.6-100.4%	HPLC with UVdetection at 230 nm	Use anionic surfactant micelle-mediated extraction to extract Etofenprox from environmental water samples

Author	Surfactant	Matrix	compounds	Range	Optimum conditions	Analysis	Objective
7. Merino et al., 2002	SDoS	Industrial solid, Dried sewage sludge, Harbour sediment	PAHS	0.2-5 M HCl, T=20-80°C, 0-2 M NaCl, 0.5-3%(w/v) SDoS, extract time 2 min-4 h	4.2 M HCl, Stir 1h,T=60°C Centrifuge 5000 rpm10min 2%w/v SDoS %R_{PHAs}= 71.8-98.4%	HPLC with fluorescence detector	Investigate factors affecting solid-liquid extraction by cloud point methodology
8. Merino et al., 2003	SDoS	Sewage sludge	Cationic surfactant	2.5-5 M HCl, T=25-80°C, 1-3%(w/v) SDoS, extract time 5-90 min	3 M Hcl, stir 1 h, T =40°C Centrifuge 420 rad/s10min 2%w/v SDoS, sample = 0.1 g %R = 91-99 %	LC-ESI-IT-MS	Develop method for determination of cationic surfactant recalcitrant to anaerobic degradation in sewage sludge
9. Ruiz et al., 2004	SDoS	Sewage sludge	Amphiphiles (LASs)	2.5-5 M HCl, T=20-70°C,, 1.5-4%(w/v) SDoS, extract time 10min-1h, sample 0.1-1 g	4 M Hcl, stir 40 min, Centrifuge 420 rad/s10min 3%w/v SDoS, T =40°C Sample =0.1 g, %R = 98 %	HPLC with fluorescence detector	Use anionic surfactant micelle-mediated extraction to extract amphiphiles from solid environmental samples and compare ability with organic-based extraction

Table 2.10 Review on Investigation of micelle-mediated separation using cationic surfactant

Author	Surfactant	Matrix	compounds	Range	Optimum conditions	Analysis	Objective
1. Man et al., 2002	Aliquat-336	water	cyanobacterial toxins, microcystins	10-75 mM Na_2SO_4, 1-15 mM Aliquat-336, 0-0.6 M NaCl	75 mM Na_2SO_4, T=25ºC 2.5 mM Aliquat-336 $\%R_{PHAs}$ = 100%	HPLC with UV detection at 238 nm	explore the feasibility of cloud-point extraction and preconcentration of a group of relatively hydrophilic polypeptide algal toxins, microcystins, from aqueous media using a cationic surfactant
2. Yu et al., 2004	Aliquat-336	water	nodularinR, cyanobacterial hepatotoxins	1-10 mM Aliquat-336 extract time 5-30 min, pH 2-12, 0-0.6 M NaCl	75 mM Na_2SO_4(pH =10), T_{ROOM}, ultrasonicate15min centrifuge 50 rpm 20 min 2.5 mM Aliquat-336 %R = 100%	HPLC with UV detection at 238 nm	reported the ability of a cationic surfactant to selectively extract anionic cyanobacterial hepatotoxins, microcystins-LR, and -YR, from natural water

Table 2.11 Review on Investigation of micelle-mediated separation using non-ionic surfactant

Author	Surfactant	Matrix	compounds	Range	Optimum conditions	Analysis	Objective
1. Ferrer et al.,1996	Triton X- 114	water	PAHS	1%w/v Triton X-114 at T=40 ºC, 10 min evaporated at 50°C + clean-up method (No Range)	1%w/v Triton X-114 at T=40 ºC, 10 min evaporated at 50°C + clean-up method R= 80-100%	HPLC with Aminco Bowman Series 2 spectrofluori meter, as detector	Propose procedure for the extraction of the sixteen PAHs classified as priority pollutants by the EPA into Triton X-l 14
2.Fernandez et al., 1998	Brij 30, Brij 97	water	PCBs	0.05-3% (w/v) Brij 30, 0.05-5% (w/v) Brij 97, T = 60-110 ºC 1-10% (w/v) KNO_3 extract time 10-25 min	2 %w/v Brij 30, Brij 97 T=100ºC for 15 min Centrifuge 1921 g 5 min %R $_{Brij\ 30}$= 82-98% , %R $_{Brij\ 97}$= 76-96%	HPLC with fluorescence detector	Report the results of a study carried out to determine the effect of these parameters on the application of cloud-point extraction for PCBs
3. Revia et al., 1999	Triton X-100	Water	fulvic acids, humic acids	pH 1-12 1-10 % wt TX-100 T = 70-90 °C Equilibration time 3-20 min Centrifuge time1-20min	pH 1 T=90ºC Centrifuge 3000 rpm10min Equilibration time 10 min 4 % wt TX-100 %R $_{fulvic\ acids}$ = 82% %R $_{humic\ acids}$ = 96%	HPLC with UV detector at 230 nm	To use the cloud point extraction technique for the preconcentration of humic substances from aqueous matrix

Author	Surfactant	Matrix	compounds	Range	Optimum conditions	Analysis	Objective
4. Purkait et al., 2006	Triton X-100, Triton X-114	water	chrysoidine dye	0.03 to 0.25 M TX-100 and TX-114 $T_{TX-100,}$=75-90°C $T_{TX-114,}$=40-55°C 0.05-0.5 M $CaCl_2$ and NaCl, pH 2-12	0.1 M Triton X-100 for T_{TX100}=75°C, pH 12 %R $_{TX100}$ = 92% 0.15 M Triton X-114 for T_{TX114}=55°C, pH 5 %R $_{TX114}$ = 99%	Spectro-metric	To use cloud point extraction to extract toxic chrysoidine dye.
5. Seronero et al., 2000	Triton X-114	water	Chlorophenols	0-1 M NaCl, T = 30-80 °C, extract time 2-45 min 0.05-1 %w/v TX-114	0.5 % w/v Triton X-114, 0.01 M H_2SO_4,0.6 M NaCl At T= 65°C for 15 min Centrifuge 1400 g 15 min %R$_{All}$ = 62-101%	HPLC with UV detector at 290 nm following with electro-chemical detector	to determine phase ratio for the surfactant Triton X-114 at different concentrations from chromatographic measurement of the analytes
6.Manzoori et al., 2002	Triton X-100	Liquid water, Serum, Human hair	Cu (II) ion	0.001-0.2 M HCl, 0.0001-0.02M DDTP 0.05-0.3%v/v TX-100, T=40-45°C	0.75M HCl, 0.5%m/v.phenol, 0.005 M DDTP, 0.1%v/v Triton X-100, T = 40°C for 20 min, Centrifuge3500rpm15min R = 97-100%	Spectro-metry	To extend the use of cloud point preconcentration technique to the determination of Cu in different samples for the first time by using DDTP as complexing agent and Triton X-100 as surfactant.

Author	Surfactant	Matrix	compounds	Range	Optimum conditions	Analysis	Objective
7. Sombra et al., 2003	PONPE 7.5	Water	Al (III) ion	0.05-0.6%w/v TX-100 pH 4.6 - 7.2 T=25-90°C, Equilibration time 2-40 min	T =90 °C, pH 6 Equilibration time 5 min Centrifuge3500rpm 5 min 4×10^{-3} M Acetate buffer 0.2%w/w TX-100 %R = 99.9%	Spectro-metry	To developed and optimized a powerful CPE-ICP-OES combined methodology for Al (III) determination
8.Nascentes et al., 2003	TX-100, SDS	Water	Co(II) ion	0-2.0% m/v SDS, 0-0.5% m/v TX-100 2- 4 M HCl, 6-10% m/v NaCl	0.2 ml 0.025%m/v 5-Br-PADAP, T_{Room} 250 ml 10% m/v TX-100, 270 ml 10% m/v SDS, 1.35 g NaCl, 4 M HCl centrifuge 1780 g 10min %R = 99-100%	Spectro-metry	To propose a cloud point extraction procedure by using both anionic (SDS) and nonionic surfactants (Triton X-100) for extraction and preconcentration of cobalt
9. Choi et al., 2003	Triton X-100	Dry roots of American ginseng	ginsenosides	0.001-10%w/vTX-100 T = 50-120 °C	Pressurized liquid extraction at 0.1 g sample, 1% TX-100+ cloud-point 4g $AlSO_4$ mixed 2 min, Centrifuge 4000 rpm10min at T=78 °C	HPLC with UV-detection at 202 nm	• compare employing an aqueous solution containing non-ionic surfactant with conventional extraction solvents (water and methanol) • compared between PLE and a conventional sample preparation method(ultrasonic-assisted extraction) for the extraction of ginsenosides

Author	Surfactant	Matrix	compounds	Range	Optimum conditions	Analysis	Objective
10.Manzoori et al., 2004	Triton X-114	Water	Cd and Ni	pH 2-9 T=40-90°C, 0-2 M NaCl, 0.025-0.2%(v/v) TX-114, 1-12 % dithizone	0.05%v/v Triton X-100, pH 7, T=40ºC, 10 min Centrifuge 3500 rpm10min 1% dithizone %R$_{Cd}$= 96.8-103.6% %R$_{Ni}$= 96.6-101%	Spectro-metric	To develop a new cloud point extraction and preconcentration method for Cd and Ni by the use of dithizone as a complexing agent prior to flame atomic absorption spectrometric determination
11. Delgado et al., 2004	Triton X-114	Sea water	PAHs	Both 0.2-2 % w/v POLE/Brij 30 and POLE/TX-114, T=71.3-92.4°C, extract time 30-90 min	1 % w/v POLE/Brij 30 or 0.8 % w/vPOLE/TX-114, T = 78°C Equilibration time 60 min %R > 72%	HPLC with fluorescence detector	To propose the use of mixtures of POLE with both Triton X-114 and Brij 30 for the improvement of the extraction and preconcentration of PAHs in seawater samples.
12. Purkait et al., 2004	Triton X-100	Wastewater	congo red	0.02-0.5 M CaCl$_2$ 0.02-0.25 M TX-100, T=70-85°C,	0.2 M TX-100, T=85ºC, Centrifuge 4000 rpm24min Equilibration time 20 min %R$_{PHAs}$ = 65-87%	Spectro-metric	To use cloud point extraction to remove cationic dye (congo red) from wastewater using TX-100 as non-ionic surfactant

Author	Surfactant	Matrix	compounds	Range	Optimum conditions	Analysis	Objective
13. Wang et al., 2006	Triton X-114	water	estrogens	0-0.7M Na$_2$SO$_4$ T = 15-50 ºC, extract time 10-120 min 0.2-2 %w/v TX-114 centrifuge time 2-20min at 3500 rpm	0.25% w/v Triton X-114, 0.4M Na$_2$SO$_4$ for 60 min T=45ºC Centrifuge 3500 rpm 5 min %R = 81.2–99.5%	HPLC with VWD detector	determination of estrogens in water by CPE using Triton X-114 as the extraction solvent
14. Zhu et al., 2005	Triton X-100	water	Cr(VI),Cr(III)	pH 2.5-9, 0-1.5 ml/10ml Br-PF T=55-100°C, 0-3.5 g-L TX-100, Equilibration time 0-30 min	0.06% Br-PF, pH 4.5, T =85ºC for 15min Centrifuge 3500rpm 5min 1.0 g/L Triton X-100 %R = 91-99 %	Spectro-metric	To proposed a new method for ETAAS speciation of chromium in environmental water samples by CPE with Br-PF as the chelating agent and Triton X-100 as the extractant.
15. Tang et al., 2005	Triton X-114	Water	As(III)	0.001 to 0.025%(w/v) APDC, pH 2.4 -11.5 T=25-80°C, 0.01-0.25%(v/v) TX-114, Equilibration time 8-10 min	0.005% (w/v) APDC, 0.125%v/v Triton X-100, pH 4.2, T=35ºC for 9 min Centrifuge 4000 rpm 5min %R> 97%	Spectro-metric	To apply CPE as a preconcentration step for ETAAS determination of As(III).

Author	Surfactant	Matrix	compounds	Range	Optimum conditions	Analysis	Objective
16. Liang et al., 2005	Triton X-100	Water	cadmium	0.1-2 mM PMBP pH 3-11, T=60-120°C, 0.1-6 g/L TX-100, Equilibration time 5-30 min	8×10^{-4} M PMBP, pH 9, T =80°C for 25min Centrifuge 3000rpm 5min 3.0 g/L Triton X-100 %R > 96 %	Spectro-metric	To optimize the use of PMBP in cloud point extraction and to assess its application to the preconcentration of cadmium prior to flame atomic absorption spectrometry using Triton X-100 as surfactant.
17. Zygoura et al., 2005	Triton X-114	water	DEHA, ATBC	pH 3-11 T = 25-50 °C, 0.5-1.5 g/L TX-114	0.8 g/L Triton X-114 for DEHA and 1.5 g/L for ATBC, pH 7, At T= 50°C for 10 min Centrifuge4000rpm 10 min %R_{All} = 62-101%	GC with FID detector	To applied cloud point extraction for the isolation of the plasticizers DEHAand ATBC from aqueous simulants and their preconcentration into the micelles of the non-ionic surfactant Triton X-114.
18.Shemirani et al., 2005	Triton X-114	water	bismuth	pH 0.5-9.5, $0.2\text{-}5 \times 10^{-6}$ M dithizone 0.02-0.25 %w/v TX-100 T=25-90°C	0.05 % w/v Triton X-100 5×10^{-6} M dithizone, sulfuric acid (pH 3.0–3.5), for T=50°C for 5 min Centrifuge 3500 rpm 10 min %R_{TX100}> 95%	ET-AAS	To introduce a reliable method for determination of bismuth in tap water and biological samples (urine and hair) by electrothermal atomic absorption spectrometry after preconcentration by the cloud point extraction technique.

Author	Surfactant	Matrix	compounds	Range	Optimum conditions	Analysis	Objective
19. Fang et al., 2000	Triton X-100, Triton X-114	Dry roots of American ginseng	ginsenosides	T = 20-65 ºC, extract time 0.5-8 h 0.5-30 %w/v TX-100 0.5-30 %w/v TX-114	10% w/v Triton X-114, Triton X-100 for T_{TX114}=20ºC, T_{TX100}=40ºC Centrifuge 3500 rpm10min extract time 3 h %R = 75-97%	HPLC with UV detector at 202 nm	employing micelle-mediated extraction as a simple and effective tool for the separation of the active ingredients from herbal products
20. Shi et al., 2004	Genapol X-80	Roots of *Salvia miltiorrhiza bunge*	tanshinones	Extraction with: 0-20% Genapol, extract time 5 - 60min	For extraction: 10% Genapol X-80, 45min liquid/solid ratio 20:1 For concentration: T =50ºC , little amount of NaCl, Centrifuge 11426 RFG(×g) for 10 min, 10% genapol X-080	HPLC with UV-detection at 254 nm	To study the feasibility of employing non-ionic surfactant solution as an alternative and effective solvent for the extraction of tanshinones from *Salvia miltiorrhiza bunge*.
21. Shen et al., 2006	Triton X-114	Tobacco samples	alkaloids	0.1-20% w/v T X-114, T=35-50ºC	5% w/v Triton X114, 100 µl of saturated NaCl, T=50ºC for 15 min Centrifuge3500rpm 10 min %R= 80.4%	GC-MS	To demonstrated employing CPE as a simple and effective alternative for recovery of alkaloids from complex solid samples followed by GC–MS analysis

Table 2.12 Review on Investigation of micelle-mediated separation using zwitterionic surfactant

Author	Surfactant	Matrix	compounds	Range	Optimum conditions	Analysis	Objective
1. Wester et al., 1998	DDMAU, DDMAB, $C_{10}E_5$ (non-ionic)	insect cells	glycoprotein D (gD-1)	2% (w/v) C E + 2% DDMAU + 2% DDMAB + 2% octyl-glucoside + 2%dodecyl-b-D-maltoside, 20 mM Tris–HCl + 2 mM PMSF + 1 mM TLCK (Do not know range))	2% (w/v) C E + 2% DDMAU + 2% DDMAB + 2% octyl-glucoside + 2%dodecyl-b-D-maltoside, 20 mM Tris–HCl + 2 mM PMSF + 1 mM TLCK, at ice bath for 1 h Centrifuge 70000 g 1h 4ºC %R $_{C10E5}$ = 72 % %R$_{DDMAU}$ = 72 %	HPIEC	• Compare effectiveness for extraction of a recombinant integral membrane protein from cells infected with recombinant baculovirus by use non-ionic and zwitterionic surfactant
2. Materna et al., 2005	OMD-9, betaine LA 47	Water	crystal violet	T=0-100 °C Extract time 20-120 min 0.005-0.01 g/l LA 47	25 g/l OMD-9 + 0.005 g/l LA 47, T=55ºC for 2 h Centrifuge 2500 rpm 10 min %R$_{spectro}$ = 92.6% %R$_{grayness}$ = 99.2%	Spectro-photo-metric, grayness method	study the dynamics of surfactant-rich phase separation from solutions containing non-ionic and zwitterionic surfactants

CHAPTER III
MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals

Triton X-100 (*iso*-octyl phenoxy polyethoxy ethanol, molecular weight: 628, λmax: 226 nm.) was purchased from Fisher Scientific, UK while Genapol X-080 (Isotridecyl poly (ethylene glycol) ether, molecular weight: 553, λmax: <210 nm.) was purchased from Fluka, USA. The critical micelle concentration of Triton X-100 and Genapol X-080 are 2.8×10^{-4} M or 0.03% water solution [Choi et al., 2003, Purkait et al., 2006] and 1.5×10^{-4} M or 0.016% water solution [www. sigma Aldrich.com]. The cloud point temperature of Triton X-100 and Genapol X-080 are 65-70 [Purkait et al., 2006] and 43 °C [www. sigma Aldrich.com]. Alizarin (1,2-Dihydroxyanthraquinone, molecular weight: 240.22, λmax: 435 nm) was purchased from Carlo Erba Reagenti Italia. Sodium chloride was purchased from Ajax Finechem, Australia and ethyl alcohol was purchased from Fisher Scientific, UK. Water used in the experiments was distilled and deionized water.

3.1.2 Plant material preparation

The roots of *Morinda citrifolia* used in this experimental were harvested, washed, and oven dried at 50 °C for 2 day. The dried sample was then ground in liquid nitrogen into small size using mortar and pestle. The ground samples were oven dried at 50 °C for 1 day, and stored in a dry place until use.

Figure 3.1 Morinda citrifolia plant (Experimental)

a)   b)
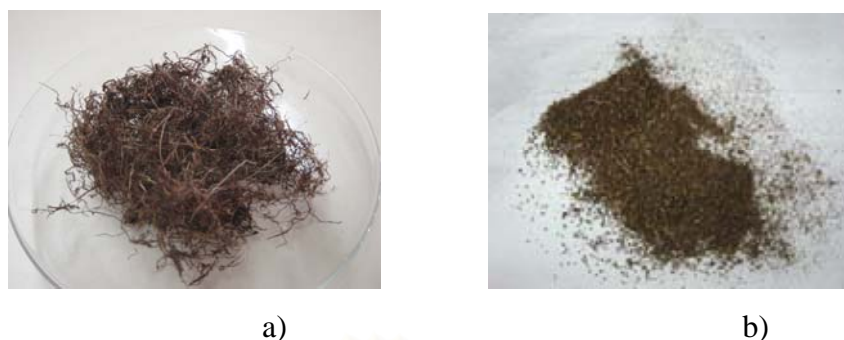
Figure 3.2 (a) Dried roots and (b) ground roots of *Morinda citrifolia*

3.2 Methods

3.2.1. Micelle-mediated extraction (MME).

For each MME experiment, 0.5 gram ground roots were placed into a 125 ml Erlenmeyer flask, containing 50 ml aqueous solutions of non-ionic surfactant (Genapol X-080 or Triton X-100). The concentrations of non-ionic surfactants in aqueous solutions were varied in the range between 0-20% (v/v). During extraction, mixing was provided by a rotary shaker at 140 rpm or an ultrasonic bath (275DAE , Crest Ultrasonics, USA, 23.5 cm × 13.3 cm × 10.2 cm with 2 38.5 kW transducers, 270 W). The extraction was carried out at room temperature (30 ˚C) for 4 h for extraction with rotary shaking. For ultrasonic extraction, the extraction time was 2 h and two sets of experiment were carried out at a controlled temperature at 30 ± 2˚C, and at the rising of temperature from 30 to 64˚C (caused by the 2 hour ultrasonic exposure.) To control the temperature of water in the ultrasonic bath, water in the ultrasonic bath was circulated and regulated at constant desired room temperature to avoid the rising of water temperature. After the extraction was completed, the root residue was separated by using a filter paper (Whatman NO.4). The extract was analyzed for the amount of total anthraquinones by using a spectrophotometer at the wavelength of 435 nm, by using Alizarin or 1, 2 dihydroxyanthraquinones as a standard.

3.2.2 Micelle-mediated pressurized hot water extraction (MMPHWE).

MMPHWE was performed using an apparatus shown in Figure 3.3. The extraction system consisted of two HPLC pumps (PU 980, JASCO, Japan) used for delivering water and solvent, a degassing instrument (ERC 3215, CE, Japan), an oven (D63450, HARAEUS, Germany), in which the extraction vessel (10 ml, Thar Design, USA) was mounted, a pressure gauge, and a back pressure regulator valve (AKICO, Japan). All connections are made with stainless steel capillaries (1/16 inch inside diameter).
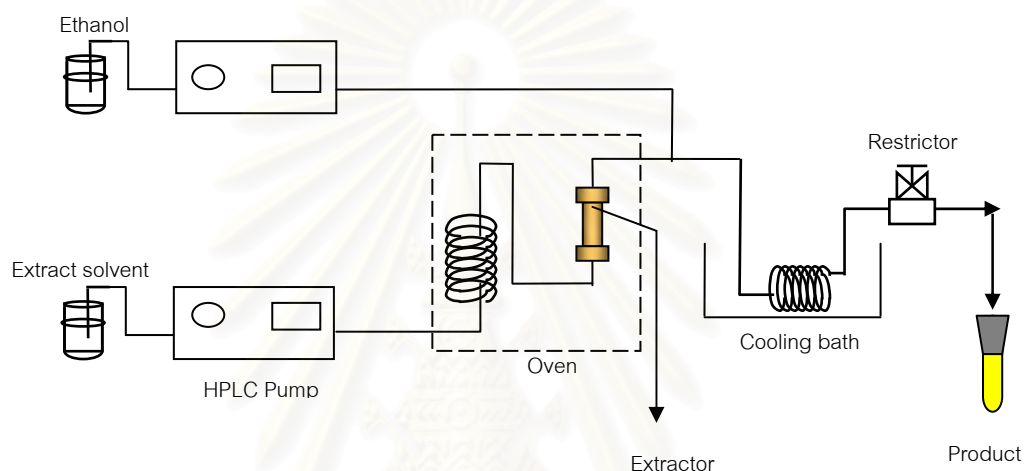
Figure 3.3 Diagram of experimental setup

Non-ionic surfactant solution was passed through a degassing equipment to remove dissolved oxygen. The solution was then delivered at a constant flow rate of 4 ml/min by the first HPLC pump to a 3-m preheating section installed in the oven to heat it to the required temperature, which then passed through the extraction vessel, preloaded with 0.5 g of ground noni roots. The pressure of the system was adjusted to the desired condition by using the back-pressure regulator valve at the outlet coil to ensure that water was in liquid state at the temperatures tested. The oven was turned on and the temperature was set to the desired operating condition. When the temperature reached the set point, the extraction started. The second pump was then turned on to deliver ethanol at constant flow rate of 1 ml/min to wash off any residual product in the outlet line behind the extractor. The extract was cooled in a coil immersed in a water bath to prevent possible product degradation, and then collected in fractions for 2 hours. The extract was analyzed spectrophotometrically at the wavelength of 435 nm, with Alizarin as a standard. The conditions tested are summarized in Table 3.1. In order to determine the extraction efficiency, the amount

of anthraquinones remained in the sample residue was determined by extracting the residue repeatedly in three 10 ml volumes of ethyl alcohol until the extract was clear.

Table 3.1 Parameter condition for MMSWE in experiment.

Solvent	0-5% (v/v) Triton X-100 or Genapol X-080 solution
Temperature	80, 120 °C
Pressure	4 MPa
Sample roots	0.5 g
Flow rate of solvent	4 ml/min
Flow rate of ethanol	1 ml/min

3.2.3 Cloud point concentration of extracted solutions.

After determining the suitable conditions for extraction, the extract obtained at these conditions were concentrated by CPC. The parameters shown in Table 3.2 were studied to determine their effects on the efficiency CPC, which is defined as the ratio of the amount of solute extract into the surfactant rich phase to the amount of solute initially contained in the extract. For non-ionic surfactant, phase separation was induced by increasing the temperature of the aqueous extract solution. CPC was performed by first adding non-ionic surfactant into 30 ml extract obtained from MMPHWE, and into this solution 0.1 g of NaCl was dissolved. The solution was then heated in water bath at constant temperature until turbidity and the consequent phase separation was observed. The complete phase separation was achieved by centrifuging at 4000 rpm for 10 min. The solution with the two phases was cooled for 5 min in ice bath or until the surfactant-rich phase become viscous. The aqueous phase was then carefully removed using a dropper with a long needle. The surfactant-rich phase was diluted with 4 ml of ethyl alcohol to reduce its viscosity and the anthraquinones concentrations in the surfactant-rich phase and aqueous phase were determined by using a spectrophotometer (Revia et al., 1998).

Table 3.2 Effect parameter for cloud point concentration in experiment.

% Triton X-100 concentration that adds to solution	0-5% (v/v)
Temperature on concentration	70, 75, 80, 85, 90 °C
Equilibration time	10, 15, 20, 25, 30 min

3.3.4 Analysis of damnacanthal by HPLC

The analysis of damnacanthal was carried out with HPLC (Prostar 240, Varian, USA), equipped with photodiode array detector (Prostar 335, Varian, USA) at room temperature using a phenomenex Luna C18, 100 A pore size, 5 μm particle size, 250mm × 4.60 mm I.D. column. The mobile phase consisted of a mixture of acetonitrile (A) and 0.5 aqueous acetic acid (B). The gradient elutions employed are shown in Table 3.3. The flow rate of the mobile phase was 1.3 ml/min, an injection volume was 50 μL, and the UV detection wavelength was 250 nm.

Table 3.3 Gradient elution for HPLC analysis of damnacanthal.

Time (min)	Acetonitrile (A)	0.5% aqueous acetic acid (B)
0-11	60-65 %	40-35 %
11-19	65-70 %	35-30 %
19-40	70-80 %	30-20%

CHAPTER IV

RESULTS AND DISCUSSIONS

This chapter presents the experimental results dealing with anthraquinones extraction and concentration with micelle-mediated separation system. Firstly, the experimental results of optimum extraction of anthraquinones with MME were presented and discussed. Secondly, the investigation of suitable extraction conditions for MMPHWE was determined. Thirdly, the suitable condition for concentration of anthraquinones by CPC was investigated. The effects on the efficiency of concentration were determined; i.e. surfactant concentration, temperature, and incubation time. And finally, the amounts of damnacanthal in anthraquinones extract after concentration by the suitable condition of CPC found from last parts, was determined and compared to previous work.

4.1 Micelle-mediated Extraction (MME).

The effect of concentrations of Triton X-100 and Genapol X-080 solutions on the percent anthraquinones recovery is shown in Figure 4.1. The results shown in Figure 4.1 are those of 4-h MME at ambient temperature provided with rotary shaking. Without the addition of non-ionic surfactant, the efficiency of anthraquinones recovery was only about 20%. The low extraction efficiency observed was due to the difference in polarity of anthraquinones and water. However, the addition of a small amount of non-ionic surfactant into water (1% both Triton X-100 and Genapol X-080) could improve the anthraquinones recovery of water significantly to about 45-50 %. This result is the effect of micelle formation of surfactant in water when the surfactant concentration in water is above its surfactant cmc (0.016% Genapol X-080 solution and 0.03% Triton X-100 solution). Above these concentrations, the surfactant molecules form molecular aggregates of colloidal size with a non-polar core, called micelles, which can extract organic solute from solid matrixes and improve the extraction efficiency. When the concentration of Triton X-100 and Genapol X-080 in water solution increased, the recovery of anthraquinones also increased as a result of the increase in the micelle formation.
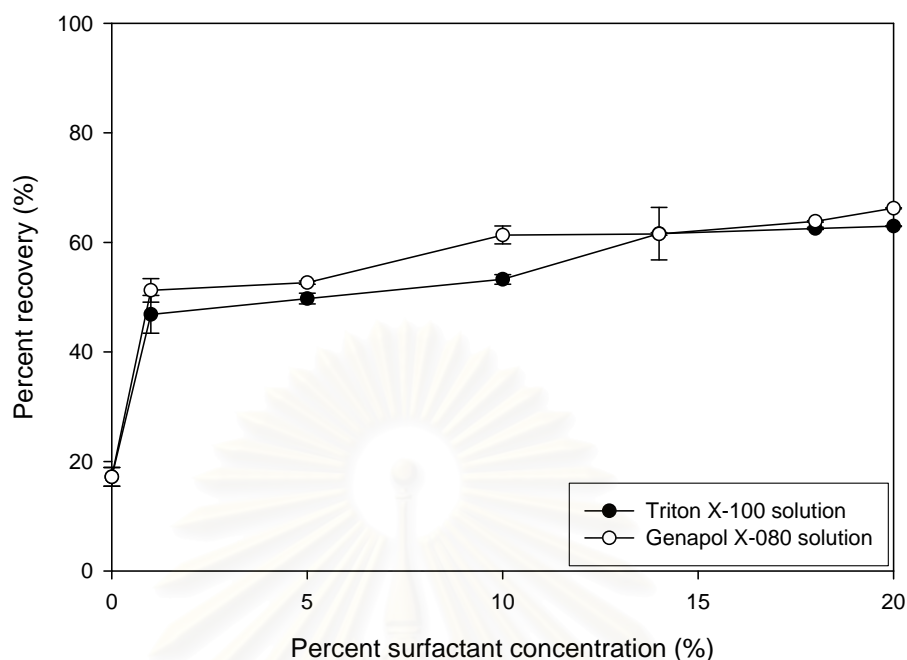
Figure 4.1 MME for 4 hrs.

Although Genapol X-080 has a lower cmc than Triton X-100, and its solution is expected to extract anthraquinones more effectively (Paleologos et al., 2005), the extraction performance of Genapol X-080 solution were found to be only slightly higher than Triton X-100 for all concentrations employed in this study. This may be related to the compatibility between the structures of the surfactant and solute, that is, Triton X-100 has an aromatic ring similar with those in anthraquinones, while Genapol X-080 does not contain such structure. The performance of extraction could also be a result of the difference in viscosities of the two surfactants, which affect the mass transfer behavior of the solute in the solution during MME. The viscosities of both surfactants in solution are presented in Table 4.1, which indicate that the viscosity of Triton X-100 is smaller than Genapol X-080 for all the concentration of surfactant solutions. This suggests that mass transfer in Triton X-100 solutions is higher than that in Genapol X-080 solutions. For MME with rotary shaking, the highest recovery was obtained with the surfactant concentration of 20% v/v in which the percent recovery after 4 h extraction was about 65%.

Table 4.1: The viscosities of surfactant solutions at various concentrations.

Surfactant concentration (%)	Viscosity of Triton X-100 (cP)	Viscosity of Genapol X-080 (cP)
0	1	1
1	1	5.22
10	7.95	20.4
20	25.8	37.8

To further improve the anthraquinones recovery, ultrasound-assisted micelle mediated extraction (UAMME) was used. Figure 4.2 shows the results of anthraquinones recovery by UAMME with Triton X-100 and Genapol X-080 solutions at various concentrations at controlled temperature of 30 ± 2ºC. The results showed that the effect of ultrasound could improve anthraquinones recovery, by lowering the extraction time when compared to shaking extraction. Without the addition of surfactant (at 0% v/v), ultrasound showed an improvement in the percent recovery of anthraquinones by at least 2 folds (from 20% for shaking for 4 h (Figure 4.1) to about 45% recovery for 2 h). This result is generally due to the cavitation effect of ultrasonic wave which facilitates the interaction between solvent and solid samples. The collapse of cavitation bubbles near tissue surfaces produces micro jet, causing tissue disruption and a good penetration of the solvent into the tissue matrix (Mason, 1996).
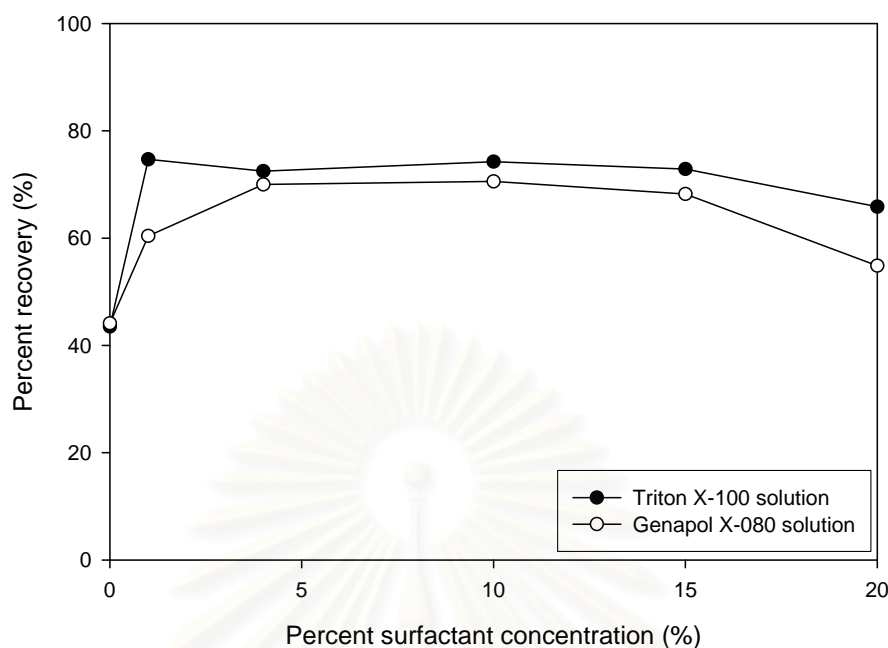
Figure 4.2 UAMME at $30 \pm 2°C$ for 2 hrs.

As seen in Figure 4.2, the addition of 1% of surfactant into the solution could improve the anthraquinones extraction from 45 to 60% recovery for Genapol X-080 and to 75% recovery for Triton X-100. A similar explanation as previously mentioned for the system with rotary shaking could be given here, that is the increase in recoveries was a result of the micelle formation of surfactant. Unlike MME with rotary shaking, UAMME with Triton X-100 solution was found to give slightly higher anthraquinones recovery than Genapol X-080, which is possibly due to the higher compatibility of surfactant micelles of Triton X as mentioned previously. In addition, the effect of ultrasound might make performance of Triton X-100 greater than Genapol X-080, due to the effect of the different viscosities of the two surfactant solutions, through which the sound travels. The effect on the extraction behavior with respect to the viscosity when ultrasound was applied perhaps is different from that without ultrasound as ultrasonic cavitation could largely depend on the properties of the media through which the sound wave travels. In this case, Triton X-100 has lower viscosity, thus acoustic cavitations occur more easily because the ultrasonic intensity applied could more easily exceed the molecular forces of the liquid. Furthermore, the lower viscosity of Triton X-100 resulted in higher diffusivity, causing the solution to easily diffuse into the pores of the plant materials (Mason, 1996). In addition, the

results in Figure 4.2 show that MME extraction with Triton X-100 and Genapol X-080 shows a similar increase in anthraquinones recovery as the surfactant concentration increased initially from 0 to 1% for triton X and from 0 to 4% for Genapol X-080. Then the recovery remained fairly constant until the concentration reached 16%, and after which the anthraquinones recovery declined. The initial increase in anthraquinones recoveries with the increase in surfactant concentrations were the result of increased micelles formation. Although the viscosity of the solution also increased, the results of enhanced extraction by micelle formation overruled the decrease in extraction efficiency as a result of increased viscosity. The two effects were counterbalanced in the concentration between 1 to 16 % and beyond which, the increase in the surfactant concentration caused the decline in anthraquinones recovery. At the concentration of 20% surfactant solutions, the extraction efficiencies of UAMME was comparable to those with shaking extraction. At this point, the viscosities of the solutions were so high that ultrasound did not have any influence on the extraction performance. From these results, the UAMME at a controlled temperature at $30 \pm 2°C$ resulted in the highest anthraquinones recovery of 75% when 1% of Triton X-100 solution was used as an extractant. However, when the temperature of extraction by ultrasound was not controlled, the higher recovery of anthraquinones was resulted as shown in Figure 4.3. The rising temperature caused the increased the anthraquinones solubility in the aqueous surfactant solution and the reduced viscosity of solution, thus enhanced the mass transfer of solute between solvent and plant materials. In addition, at elevated temperature, the decline in the anthraquinones recovery at high surfactant concentration (greater than 16%) was not observed. From the MME results previously obtained, it could be concluded that the reasonably high recovery of anthraquinones could be achieved with 1% Triton X-100 solution and that temperature was an important factor impacting the extraction efficiency of anthraquinones.
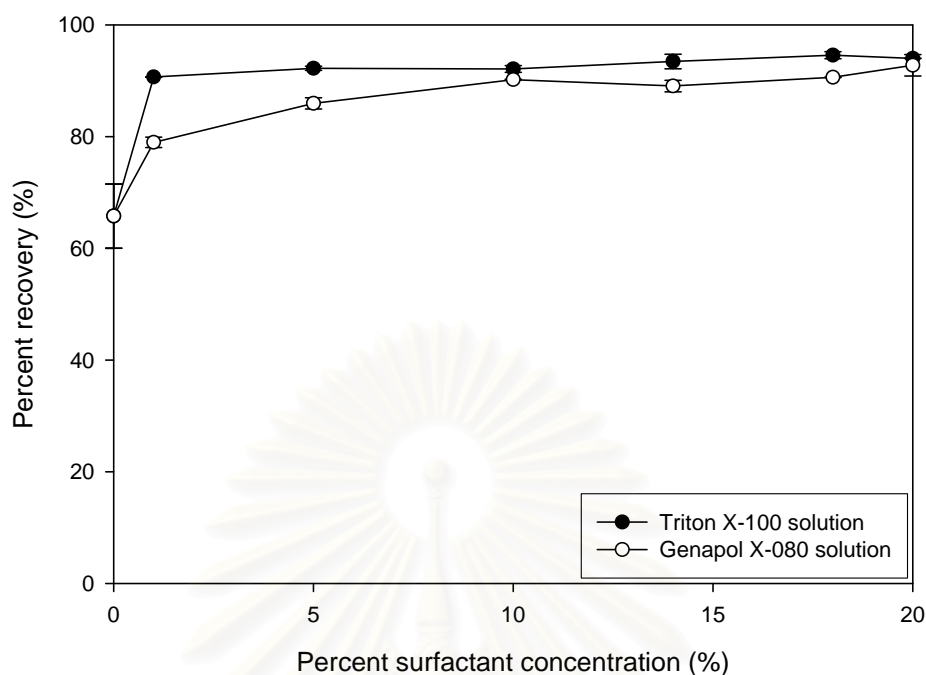
Figure 4.3 UAMME without temperature control for 2 hr.

4.2. Micelle-mediated pressurized hot water extraction (MMPHWE)

Micelle-mediated pressurized hot water extraction (MMPHWE) was carried out to investigate the effect of temperature on the efficiency of anthraquinones extraction. The extraction performance of MMPHWE with both two non-ionic surfactants: Genapol X-080 and Triton X-100, at 80 and 120 ºC, are shown in Figure 4.4 and 4.5, respectively. Figure 4.4a and 4.4b show the time course extraction curves for MMPHWE at 80 ºC with Triton X-100 and Genapol X-080, respectively which demonstrated that the extraction rates of anthraquinones increased with increasing surfactant concentration. The gradual improvement in anthraquinones recovery was observed as the concentration of Genapol X-080 increased. For MMPHWE with Triton X-100 however, the sudden increase in anthraquinones recovery was resulted when Triton X-100 concentration increased from 0 to 0.5%v/v. However, further increase in Triton X-100 concentration onwards only minimally increased the percent recovery.

The results on anthraquinones recovery of MMPHWE with Genapol X-080 and Triton X-100 after 2 h at 80 ºC are summarized in Figure 4.4c. The addition Genapol X-080 to give 0.5% aqueous solution improved anthraquinones recovery from 63.4 % to about 75% and when the concentration of increased further from 0.5

to 5%, the recovery of anthraquinones increased to about 88 %. These are a result of the effect of the micelles formation. Similarly, the addition of Triton X-100 at 0.5% v/v was shown to raise the anthraquinones recovery to 93% due to increased micelles formation, however further increase in the Triton X-100 concentration from 0.5% to 5% did not result in increased efficiency as the recovery is already close to the maximum recovery. When compare to the use of the two surfactants, it was found that the performance of Triton X-100 was greater than Genapol X-080 in every concentration even though the Genapol X-080 cmc is lower than that of Triton X-100 (Paleologos et al., 2005). Note that the extraction temperature of 80 ºC employed here was well above the cloud point temperature of Genapol X-080 which is 43ºC, which caused the cloud point phenomenon to take place in the Genapol X-080 system which separates the micelles of the surfactant during extraction, thus reduced the extraction efficiency of Genapol X-080 solution. On the other hand, the cloud point temperature for Triton X-100 is 70ºC, and therefore the extraction temperature of 80ºC was not too far from this value and the continuous flow system would disturb the equilibrium thus, the cloud point concentration did not occur to a great extent. As a result, high extraction efficiency could be achieved. At the temperature of 80ºC, the suitable condition for extraction of anthraquinones was extraction with 1% Triton X-100 solution, and at this condition, the maximum recovery of about 95% was obtained.
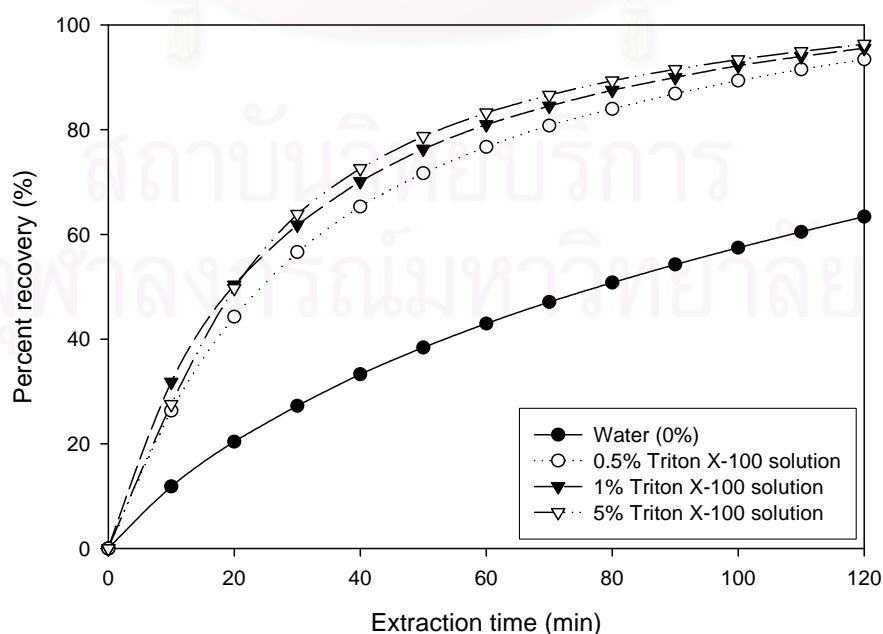
Figure 4.4a MMPHWE with Triton X-100 solutions at 80 °C.
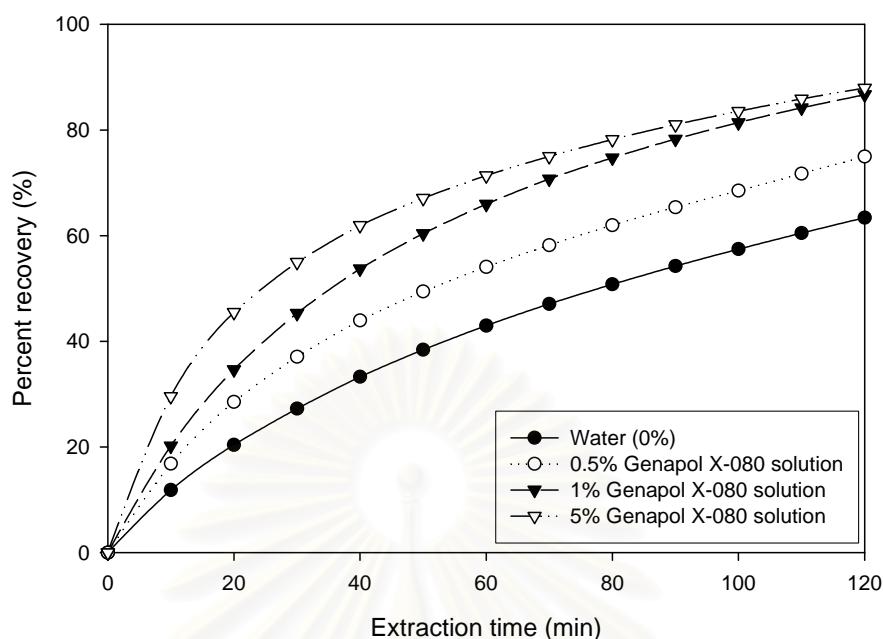
Figure 4.4b MMPHWE with Genapol X-080 solution at 80 °C.
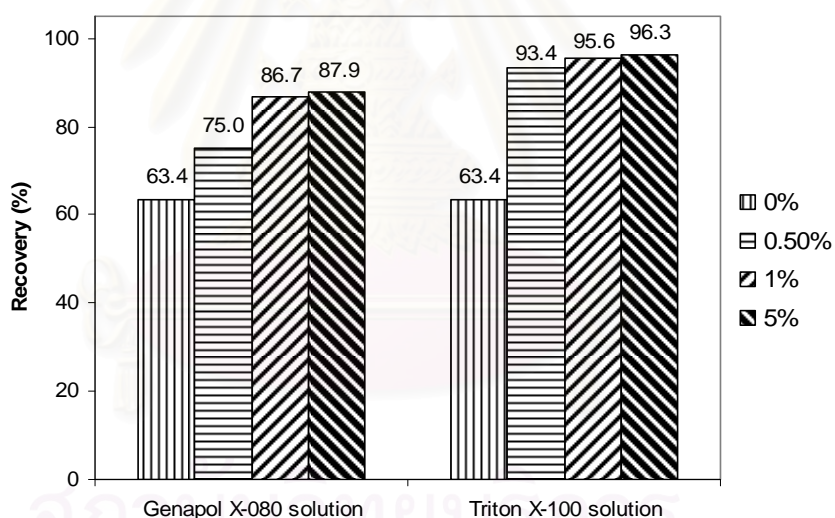
Figure 4.4c Extraction efficiencies of MMPHWE of anthraquinones at 80 °C for 2 hrs.

Figure 4.5 a and b show the extraction profile of MMPHWE with Genapol X-080 and Triton X-100 at 120°C. Without the surfactant, increasing the extraction temperature from 80 °C to 120 °C increased the anthraquinones recovery to about 90% which is a result of the decrease in water polarity. Increasing the concentration of the surfactant solution to 0.5% for Genapol X-080 and 1% for Triton X-100 increased the anthraquinones recovery, however when the concentrations increased further to 5%, the anthraquinones extracted decreased. This was due to the occurrence

of cloud point phenomena as the extraction temperature of 120 ℃ was significantly higher than the cloud point concentration temperatures for both Genapol X-080 and Triton X-100. The recovery of anthraquinones after 2 h of MMPHWE are summarized in Figure 4.5c which showed that the final anthraquinones recovery by Triton-X 100 solution was slightly higher than that by Genapol X-080 (95% vs. 90%). Nevertheless, at 120 ℃, MMPHWE with both surfactants resulted in only a slight increase in anthraquinones recovery.
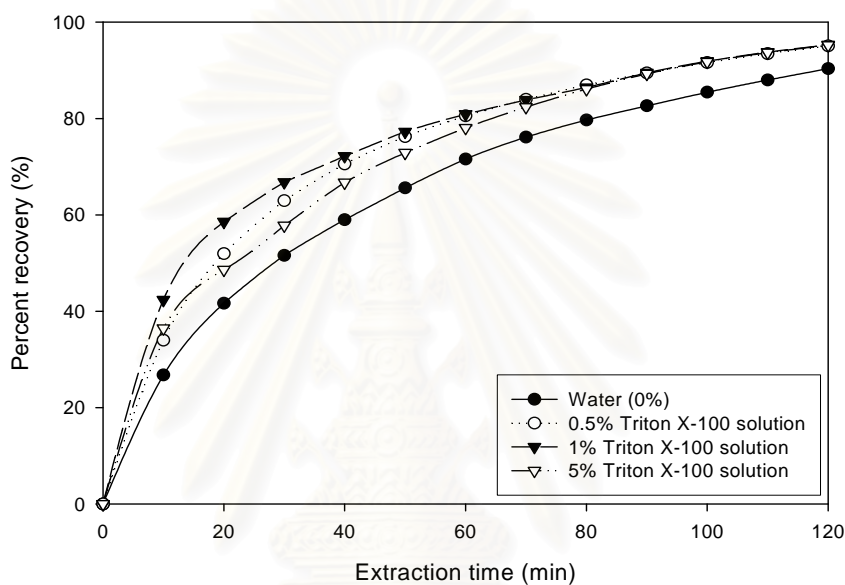
Figure 4.5a MMPHWE with Triton X-100 solutions at 120 °C.
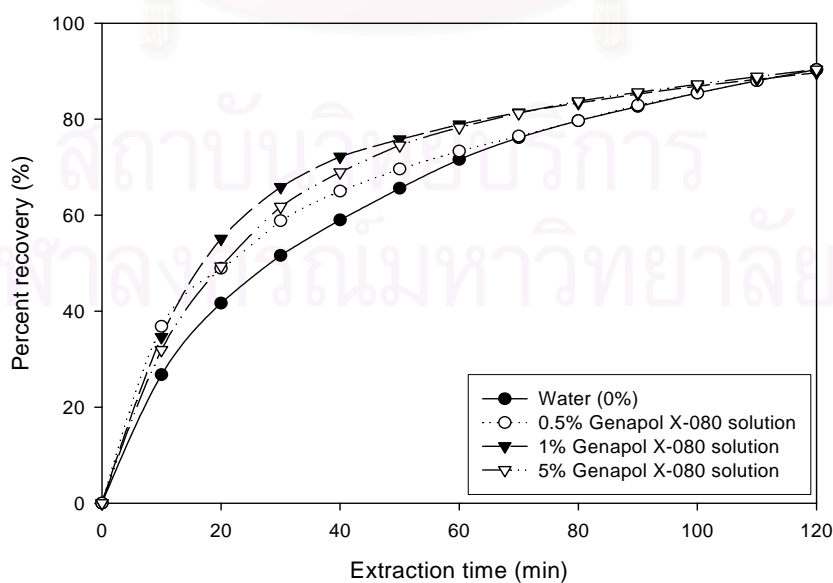
Figure 4.5b MMPHWE with Genapol X-080 solution at 120 °C.
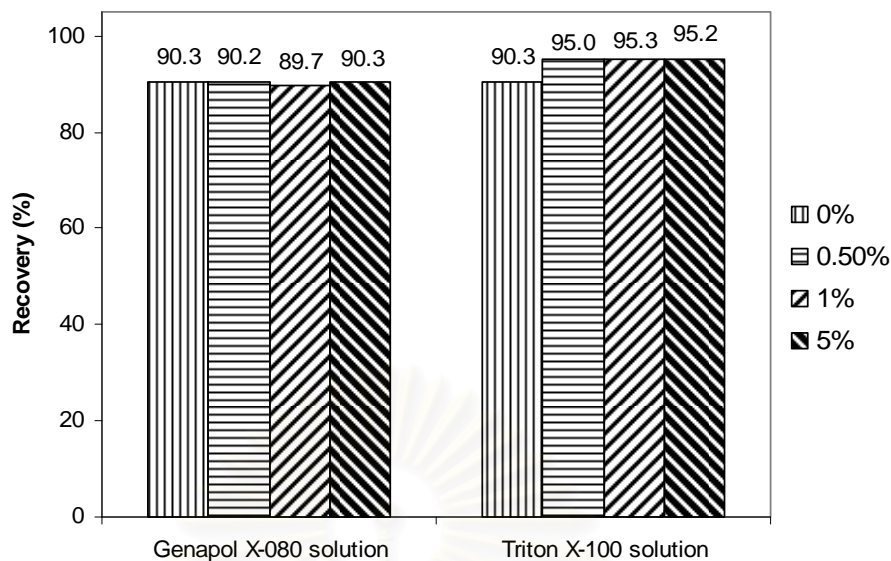
Figure 4.5c Extraction efficiencies of MMPHWE of anthraquinones at 120 °C for 2 hrs.

Based on the MMPHWE results, it may be concluded that the most suitable condition was extraction with 1% Triton X-100 solution at 80 °C which gives the maximum anthraquinones recovery of approximately 95%.

4.3. Cloud point concentration (CPC)

In this study, the effects of various parameters were determined on the efficiency of cloud point concentration, such as surfactant concentration, temperature, and incubation time. The extracts obtained after 30 min of MMPHWE at the flow rate of 4 ml/min, with 1% Triton X-100 at 80°C was used for CPC experiment.

4.3.1. Effect of surfactant concentration

First the effect of Triton X-100 concentration of extract solution was investigated and the results are shown in Figure 4.6. The incubation temperature was maintained at 90°C and the incubation time was 30 min. The x-axis represents the volume percent of the surfactant by the aqueous MMPHW extract. As shown in Figure 4.6, since the MMPHW aqueous extract already contained 1% v/v of Triton-X100, CPC could rather readily take place. Then, the recovery of anthraquinones was found to increase with the additional Triton X-100 of 1% v/v Triton X-100 to the extract solution, after which the recovery remained constant. With an increase in

surfactant concentration, the volume of surfactant-rich phase increased to maintain the material balance, as the concentration of surfactant in aqueous phase remains almost constant (Kinchuwanit et al., 2000). When the volume of surfactant-rich phase increased, higher amount of anthraquinones could be transferred to the surfactant-rich phase until the solute were exhausted. Based on this result, the effect of temperature and equilibrium time would be determined using the extract with additional Triton X-100 of 1% v/v.
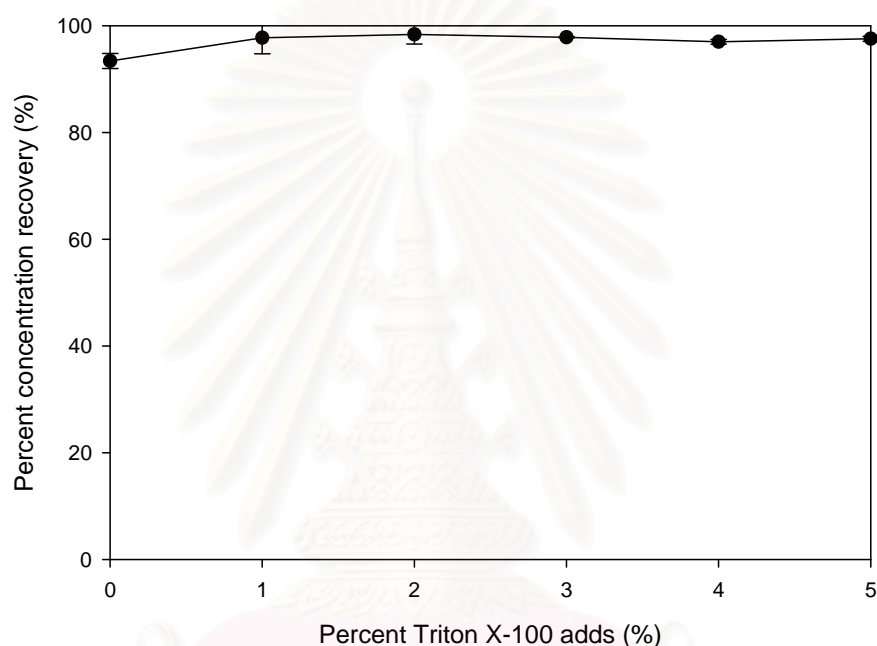
Figure 4.6 Effect of concentrations of Triton X-100 added to MMPHW extract on CPC efficiency (T=90 °C, incubation=30 min).

4.3.2. Effect of temperature

The effect of temperature between 70 to 90°C was investigated for the CPC of anthraquinones from the aqueous MMPHW extract with Triton X-100. The results are shown in Figure 4.7. In this experiment, the concentration of Triton X-100 added was 1% v/v and the equilibrium time was 30 min. The result in Figure 4.7 shows that the percent recovery of anthraquinones increased when the temperature increased from 70 to 75 °C, after which the recovery remained fairly constant. When temperature increased, the cmc also decreased, and the non-ionic surfactant solution appeared relatively more hydrophobic due to an equilibrium shift that favors dehydration of the ether oxygens [Purkait et al., 2006]. Higher temperature leads to an increase in the

number of micelles concentration. Thus, the solubilization capability of the micellar solution increased leading to an increase in the solute concentration until the solute in solution was exhausted. From this result, the temperature of 75°C was therefore chosen as an optimum temperature for anthraquinones concentration.
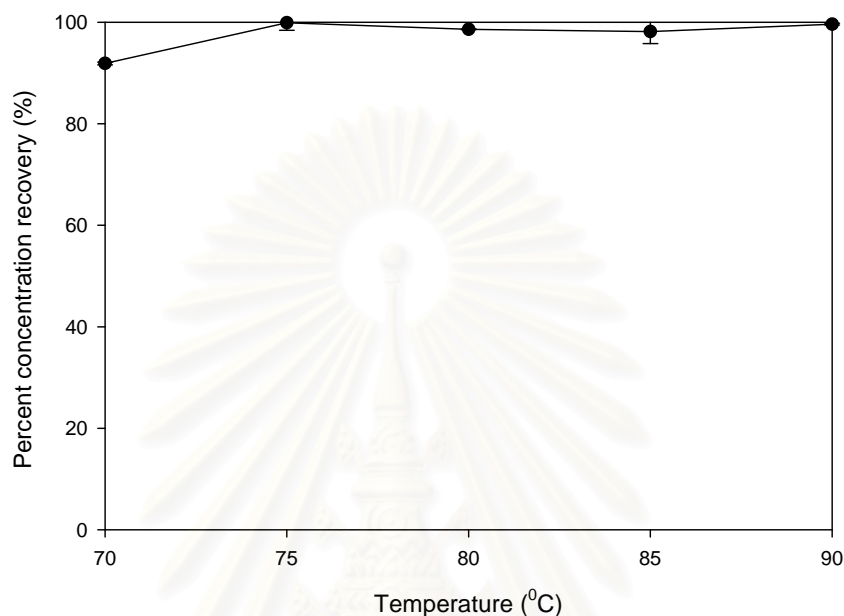
Figure 4.7 Effect of temperature on CPC efficiency (Triton X-100 added to MMPHW extract = 1%, incubation time=30 min).

4.3.3. Effect of incubation time

The optimum incubation time was determined between 10-30 min for CPC with 1 % v/v additional Triton X-100 at 75°C. The results shown in Figure 4.8 indicated that the recovery of anthraquinones increase with increasing the incubation time as the solute was allow more time to transfer into the surfactant-rich phase. The highest recovery of anthraquinones was obtained between 25 and 30 min, and almost complete CPC could be achieved after 30 min. From the CPC experiments, it could be concluded that the most suitable CPC condition for anthraquinones was concentration with 1% v/v additional Triton X-10 at 75°C for 30 min.
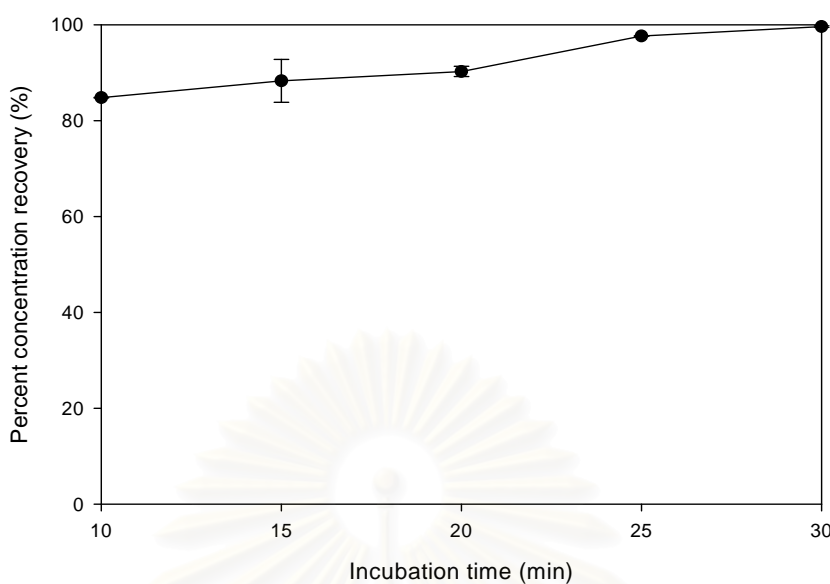
Figure 4.8 Effect of incubation time on CPC efficiency of anthraquinones (Triton X-100 added to MMPHW extract = 1%, T=75 °C.

4.4 HPLC analysis of damnacanthal.

.

The HPLC analysis was carried out to determined the amount of the anticancer compound, damnacanthal in the extract obtained with MMS at the most suitable conditions (MMPHWE with 1% Triton X-100 at 80 ℃, followed by CPC at 75 ℃, with additional 1%v/v of Triton X-100 for 30 min). The results are compared with that obtained with PHWE at 170 ℃ followed by vacuum evaporation which was reported in the previous work to give the highest recover (Anekpankul et al., 2007). In previous work, it was necessary to concentrate the water extract from PHWE and this fraction could be achieved by first evaporating off water under vacuum to dryness. The dried extract was than redissolved in dimethyl sulfoxide (DMSO) and analyzed, and the amounts of damnacanthal extract are the sum of two result fractions. The HPLC analysis of the extracted showed that retention time of the target compound damnacanthal was approximately 10.5 min. as shown in the chromatograms of the extract obtained with MMS in Figure 4.9. The quantitative analysis suggested that the amount of damnacanthal obtained with MMS was higher than that obtained with PHWE followed by vacuum evaporation. The amount of damnacanthal extract was found to be 1.422 mg/g for MMS and 0.967 mg/g for PHWE. With the MMS system,

the degradation of damnacanthal which would normally occur at high temperature with PHWE could be avoided. With the low temperature operation in MMS system, the energy consumption could be reduced and product degradation could be avoided while the process involves non-toxic solvent.
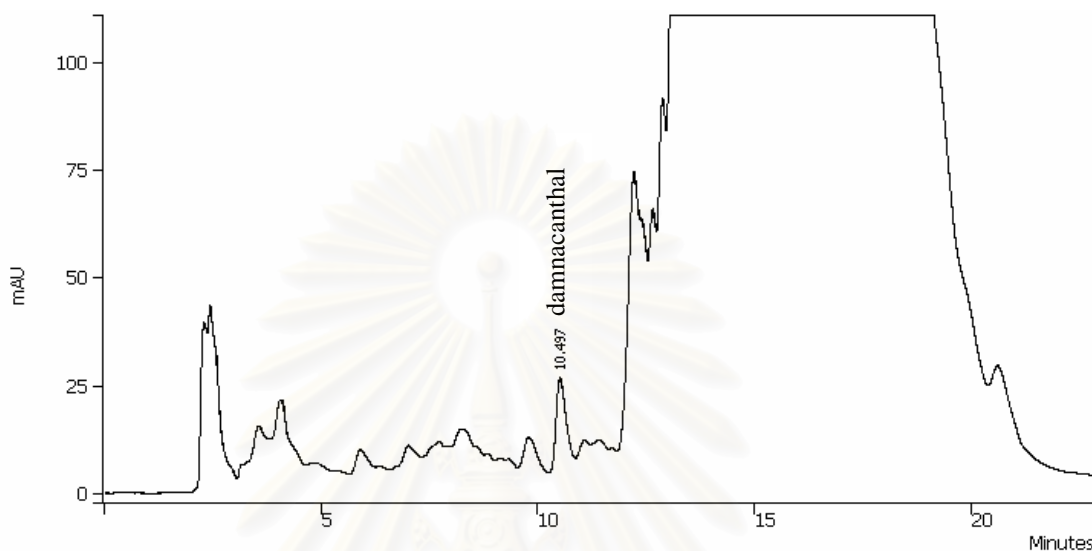
Figure 4.9 Chromatogram of *M. citrifolia* extract obtained by MMS (MMPHWE with 1% Triton X-100 solution at 80 °C, followed by CPC with additional 1% v/v of Triton X-100, T= 75 °C, and incubation time=30 min).

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

1. Micelle-mediated separation (MMS) is a promising alternative method for extraction and concentration of anthraquinones from the roots of *Morinda citrifolia*.

2. Triton X-100 is more suitable surfactant than Genapol X-080 for extraction and concentration of anthraquinones.

3. For MME under rotary shaking, the addition of surfactants into water could improve the extraction efficiency, as a result of the micelle formation of surfactant in water, and increasing in surfactant concentrations increased the recovery of anthraquinones.

4. Ultrasonic-assisted extraction (UAE) can improve the recovery of anthraquinones compare with MME with rotary shaking. In addition, UAMME at a controlled temperature of 30 °C was also found to enhance the extraction efficiency of anthraquinones. Furthermore, when the UAMME temperature increased, the recovery increased.

5. Anthraquinones recovery by UAMME at controlled room temperature did not significantly increase with increasing surfactant concentrations, as the viscosity of the solutions increase and reduced the solute-solution mass transfer.

6. The suitable UAMME condition was extraction with 1% Triton X-100 solution.

7. The most suitable condition for MMPHWE of anthraquinones was at the temperature of 80°C and using 1% Triton X-100 solution with continuous flow. The anthraquinones extraction efficiency was improved as it allowed the use of low extraction temperature and system energy.

8. MME and MMPHWE are environmental friendly methods and involve use of non-toxic solvent, and after extraction, the extract from MMPHWE

could further be concentrated by CPC, which was a simple and effective method that requires low energy.

9. For concentration of anthraquinones, the suitable condition for CPC was concentration by using 1% v/v Triton X-100/extract and at the temperature of 75 ˚C for 30 min.

10. The HPLC results show that MMS is an appropriate means for extraction and concentration of as the most active compound, damnacanthal was obtained in a high quality and quantity.

5.2 Recommendations

1. Other parameters that affect the efficiency of extraction; i.e. particle size and sample moisture content should be considered in the future work.

2. In order for the MMS techniques are highly valuable in the large-scale extraction and purification of active ingredients from herbal materials, it should be noted that a key step in the purification process would likely to be surfactant removal, which can be carried out by various methods based on exploiting the differences in size, charge, and hydrophobicity between the surfactant and extracted compounds [Furth et al., 1980]. A popular method of removing non-ionic surfactants is via hydrophobic adsorption of the surfactants with polystyrene resins [Holloway et al., 1973, Cheetam et al., 1979]. The resins are usually added batch-wise to the preparation and removed, together with the bound surfactants, simply by centrifugation or filtration. Development of the separation and purification process to remove surfactant from the extract solution should be investigated.

3. Alternative to separation of surfactant, use of natural surfactant or agent that is safe for human consumption should be investigated for MME and MMPHWE, and CPC process so that subsequent removal is unnecessary.

REFERENCES

Abdollahi, H.; and L. Bagheri. Simultaneous spectrophotometric determination of Vitamin K3 and 1,4-naphthoquinone after cloud point extraction by using genetic algorithm based wavelength selection-partial least squares regression. Analytica Chimica Acta. 514 (2004): 211–218.

Ahad Bavili Tabrizi. Cloud point extraction and spectrofluorimetric determination of aluminium and zinc in foodstuffs and water samples. Food Chemistry. 100 (2007): 1698-1703.

Anekpankul, T.; Goto, M.; Sasaki, M.; Pavasant, P.; and A. Shotipruk. Extraction of anti-cancer damnacanthal from roots of *Morinda citrifolia* by subcritical water. Separation and Purification Technology. 55 (2007): 343–349.

Biomol Research Laboratory Inc.: [online] Available from: www.biomol.com.[2005, August 30].

Carabias-Martınez, R.; Rodrıguez-Gonzalo, E.; Moreno-Cordero, B.; Perez-Pavon, J.L.; Garcıa-Pinto, C.; and E.F. Laespada. Surfactant cloud point extraction and preconcentration of organic compounds prior to chromatography and capillary electrophoresis. Journal of Chromatography A. 902 (2000): 251–265.

Carmen W. Huie. A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants. Anal Bioanal Chem. 373 (2002): 23–30.

Chan-Blanco, Y.; Vaillant, F.; Perez, A.M.; Reynes, M.; Brillouet, J.M.; and P. Brat. The noni fruit (Morinda citrifolia L.): A review of agricultural research,nutritional and therapeutic properties. Journal of Food Composition and Analysis. 19 (2006): 645-654.

P.S.J. Cheetam.; Removal of Triton X-100 from aqueous solution using amberlite XAD-2. Analytical Biochemistry. 92 (1979): 447-452.

Choi, M.P.K.; Chan, K.K.C.; Leung, H.W.; and C.W. Huie. Pressurized liquid extraction of active ingredients (ginsenosides) from medicinal plants using non-ionic surfactant solutions. Journal of Chromatography A. 983 (2003): 153–162.

Clint, J.H. Surfactant Aggregation. Chapman and Hill Inc., New York, 1992: pp 4-6.

Delgado, B.; Pino, V.; Ayala, J.H.; González, V.; and A.M. Afonso. Nonionic surfactant mixtures: a new cloud-point extraction approach for the determination of PAHs in seawater using HPLC with fluorimetric detection. Analytica Chimica Acta. 518 (2004): 165–172.

Fang, Q.; Yeung, H.W.; Leung, H.W.; and C.W. Huie. Micelle-mediated extraction and preconcentration of ginsenosides from Chinese herbal medicine. Journal of Chromatography A. 904 (2000): 47–55.

Fernandez, A.E.; Ferrera, Z.S.; and J.J.S. Rodriguez. Determination of polychlorinated biphenyls by liquid chromatography following cloud-point extracton. Analytical Chimaca Acta. 358 (1998): 145-155.

Fernandez-perez, V.; and M.D. Luque de Castro. Micelle formation for improvement of continuous subcritical water extraction of polycyclic aromatic hydrocarbons in soil prior to high-performance liquid chromatography-fluorescence detection. Journal of Chromatography A. 902 (2000): 357-367.

Ferrera, Z.S.; Sanz, C.P.; Santana, C.M.; and J.J.S. Rodriguez. The use of micellar systems in the extraction and pre-concentration of organic pollutants in environmental samples. Trends in Analytical Chemistry. 23 (2004): 469-479.

A.J. Furth.; Removing unbound detergent from hydrophobic proteins. Analytical Biochemistry. 109 (1980): 207-215.

Hemwimol, S.; Pavasant, P.; and A. Shotipruk. Ultrasound-assisted extraction of anthraquinones from roots of *Morinda citrifolia*. Ultrasonics sonochemistry. 13 (2006): 543-548.

Hemwimol, S.; Pavasant, P.; and A. Shotipruk. Microwave-assisted extraction of antioxidative anthraquinones from roots of *Morinda citrifolia*. Separation and Purification Technology 54 (2007): 44-50.

Herrero, M.; Cifuentes, A.; and E. Ibanez. Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae A review. Food Chemistry. 98 (2006): 136–148.

Hiramatsu T.; M. Imoto.; T. Koyano.; K. Umezawa. Induction of normal phenotypes in ras-transormed cells by damnacanthal from *Morinda citrifolia*. <u>Cancer Lett</u>. 73 (1993): 161–166.

P.W. Holloway.; A simple procedure for removal of triton X-100 from protein samples. <u>Analytical Biochemistry</u>. 53 (1973): 304-308.

Jia, G.; Bi, G.; Wang, Q.; Qiu,J.; Zhou, W.; and Z. Zhou. Determination of Etofenprox in environmental samples by HPLC after anionic surfactant micelle-mediated extraction (coacervation extraction). <u>Anal Bioanal Chem</u>. 384 (2006): 1423–1427.

King J.W. Critical fluid technology for the processing of lipid-related natural products. <u>C. R. Chimie</u>. 7 (2004): 647–659.

Liang, P.; Li, J.; and X. Yang. Cloud Point Extraction Preconcentration of Trace Cadmiumas 1-Phenyl-3-methyl-4-benzoyl-5-pyrazolone Complex and Determination by Flame Atomic Absorption Spectrometry. <u>Microchim Acta</u>. 152 (2005): 47–51.

Lundstedt, S.; van Bavel, B.; Haglund, P.; Tysklind, M.; and L.O. berg. Pressurised liquid extraction of polycyclic aromatic hydrocarbons from contaminated soils. <u>Journal of Chromatography A</u> 883 (2000): 151–162.

Materna, M.; and J. Szymanowski. Separation of phenols from aqueous micellar solutions by cloud point extraction. <u>Journal of Colloid and Interface Science</u>. 255 (2002): 195-201.

Manzoori, J.L.; and A. Bavili-Tabrizi. Cloud point preconcentration and flame atomic absorption spectrometric determination of Cd and Pb in human hair. <u>Analytica Chimica Acta</u> 470 (2002): 215–221.

Manzoori, J.L.; and A. Bavili-Tabrizi. The application of cloud point preconcentration for the determination of Cu in real samples by flame atomic absorption spectrometry. <u>Microchemical Journal</u>. 72 (2002): 1-7.

Manzoori, J.L.; and G. Karim-Nezhad. Development of a cloud point extraction and preconcentration method for Cd and Ni prior to flame atomic absorption spectrometric determination. <u>Analytica Chimica Acta</u>. 521 (2004): 173–177.

Merino, F.; Rubio, S.D.; and D. Perez-Bendito. Acid-induced cloud point extraction and preconcentration of polycyclic aromatic hydrocarbons from environmental solid samples. Journal of Chromatography A. 962 (2002): 1–8.

Merino, F.; Rubio, S.D.; and D. Perez-Bendito. Mixed aggregate-based acid-induced cloud-point extraction and ion-trap liquid chromatography-mass spectrometry for the determination of cationic surfactants in sewage sludge. Journal of Chromatography A 998 (2003): 143–154.

Miura, J.; Ishil, H.; and H. Watanabe. Extraction and separation of Nickel Chelates of 1-(2-Thiazolylazo)-2-Naphthol in non-ionic surfactant solution. Bunseki Kagaku. 25 (1976): 808.

Mpbio Inc.: [online] Available from: www.mpbio.com.[2006, August 30].

Nascentes, C.C.; and M.A.Z. Arruda. Cloud point formation based on mixed micelles in the presence of electrolytes for cobalt extraction and preconcentration. Talanta. 61 (2003): 759- 768.

Ohashi, A.; Ito, H.; Kanai, C.; Imura, H.; and K.Ohashi. Cloud point extraction of iron(III) and vanadium(V) using 8-quinolinol derivatives and Triton X-100 and determination of 10^{-7} mol dm^{-3} level iron(III) in riverine water reference by a graphite furnace atomic absorption spectroscopy. Talanta. 65 (2005): 525-530.

Ong, E.S.; Cheong, J.S.H.; and D. Goh. Pressurized hot water extraction of bioactive or marker compounds in botanicals and medicinal plant materials. Journal of Chromatography A. 1112 (2006): 92–102.

Ong, E.S.; and S.M. Len. Pressurized hot water extraction of berberine, baicalein and glycyrrhizin in medicinal plants. Analytica Chimica Acta. 482 (2003): 81–89.

Paleologos, E.K.; Chytiri, S.D.; Savvaidis, I.N.; and M.G. Kontominas. Determination of biogenic amines as their benzoyl derivatives after cloud point extraction with micellar liquid chromatographic separation. Journal of Chromatography A 1010 (2003): 217–224.

Paleologos, E.K.; Giokas, D.L.; and M.I. Karayannis. Micelle-mediated separation and cloud-point extraction. Analytical Chemistry 24 (2005): 426-436.

Pongnaravane, B.; Goto, M.; Sasaki, M.; Anekpankul, T.; Pavasant P.; and A. Shotipruk. Extraction of anthraquinones from roots of *Morinda citrifolia* by pressurized hot water: Antioxidant activity of extracts. <u>Journal of Supercritical Fluids</u>. 37 (2006): 390–396.

Purkait, M.K.; Banerjee, S.; Mewara, S.; DasGupta, S.; and S. De. Cloud point extraction of toxic eosin dye using Triton X-100 as nonionic surfactant. <u>Water Research</u>. 39 (2005): 3885–3890.

Purkait, M.K.; DasGupta, S.; and S. De. Performance of TX-100 and TX-114 for the separation of chrysoidine dye using cloud point extraction. <u>Journal of Hazardous Materials</u>. 137 (2006): 827-835.

Purkait, M.K.; DasGupta, S.; and S. De. Determination of design parameters for the cloud point extraction of congo red and eosin dyes using TX-100. <u>Separation and Purification Technology</u>. 51 (2006): 137-142.

Quina, F.H.; and Willie L. Hinze. Surfactant-mediated cloud point extractions: An environmentally benign alternative separation approach. <u>Ind. Eng. Chem. Res.</u>. 38 (1999): 4150-4168.

Revia, R.L.; and G.A. Makharadze. Cloud-point preconcentration of fulvic and humic acids. <u>Talanta.</u> 48 (1999): 409–413.

Rubio, S.; and D.P. rez-Bendito. Supramolecular assemblies for extracting organic compounds. <u>Analytical Chemistry</u>. 22 (2003): 470-485.

Saitoh T.; and Willie L. Hinze. Use of surfactant-mediated phase separation (cloud point extraction) with affinity ligands for the extraction of hydrophilic proteins. <u>Talanta</u>. 42 (1995): 119-127.

Sanz, C.P.; Ferrera, Z.S.; and J.J.S. Rodrıguez. Extraction and preconcentration of polychlorinated dibenzo-*p*-dioxins using the cloud-point methodology Application to their determination in water samples by high-performance liquid chromatography. <u>Analytica Chimica Acta</u>. 470 (2002): 205–214.

Scamehorn J.F.; and Jeffrey H.Harwell. <u>Surfactant-basted separation processes</u>. Marcle Dekker Inc., New York, 1994: pp 92-151.

Seronero, L.C.; Laespada, M.E.F.; Pavon, J.L P.; and B.M. Cordero. Cloud point preconcentration of rather polar compounds: application to the high-performance liquid chromatographic determination of priority pollutant chlorophenols. <u>Journal of Chromatography A</u>. 897 (2000): 171–176.

Shen, J.; and X. Shao. Determination of tobacco alkaloids by gas chromatography–mass spectrometry using cloud point extraction as a preconcentration step. Analytica Chimica Acta. 561 (2006): 83–87.

Shemirani, F.; Baghdadi, M.; Ramezani, M.; and M.R. Jamali. Determination of ultra trace amounts of bismuth in biological and water samples by electrothermal atomic absorption spectrometry (ET-AAS) after cloud point extraction. Analytica Chimica Acta 534 (2005): 163–169.

Shemirani, F.; Baghdadi, M.; and M. Ramezani. Preconcentration and determination of ultra trace amounts of arsenic(III) and arsenic(V) in tap water and total arsenic in biological samples by cloud point extraction and electrothermal atomic absorption spectrometry. Talanta. 65 (2005): 882–887.

Shi, Z.; He, J.; and W. Chang. Micelle-mediated extraction of tanshinones from *Salvia miltiorrhiza bunge* with analysis by high-performance liquid chromatography. Talanta. 64 (2004): 401–407.

Sigma Aldrich Inc.,: [online] Available from: www. sigma Aldrich.com.[2006, August 30].

Sombra, L.L.; Luconi, M.O.; Fernandez, L.P.; Olsina, R.A.; Silva, M.F.; and L.D. Martınez. Assessment of trace aluminium content in parenteral solutions by combined cloud point preconcentration* flow injection inductively coupled plasma optical emission spectrometry. Journal of Pharmaceutical and Biomedical Analysis. 30 (2003): 1451-1458.

Tang, A.N.; Ding, J.H.; and X.P. Yan. Cloud point extraction for the determination of As(III) in water samples by electrothermal atomic absorption spectrometry. Talanta. 67 (2005): 942–946.

Ulrich. Solid-phase microextraction in biomedical analysis. J. of Chromatography A. 902 (2000): 167–194.

Wang, Z.; Zhao, F.; and D. Li. Determination of solubilization of phenol at coacervate phase of cloud point extraction. Colloids and Surfaces A: Physicochem. Eng. Aspects. 216 (2003): 207- 214.

Wang, Z.; Zhao, F.; Hao, X.; Chen, D.; and D. Li. Microbial transformation of hydrophobic compound in cloud point system. Journal of Molecular Catalysis B: Enzymatic. 27 (2004): 147–153.

Watanabe, H.; and H. Tanaka. A non-ionic surfactant as a new solvent for liquid-liquid extraction of Zinc (II) with 1-(2-Pyridylazo)-2-Naphthol. Talanta. 25 (1978): 585.

Welling-Wester, S.; Feijlbrief, M.; Koedijk, D.G.A.M.; and G.W. Welling. Detergent extraction of herpes simplex virus type 1 glycoprotein D by zwitterionic and non-ionic detergents and purification by ionexchange high-performance liquid chromatography. Journal of Chromatography A. 816 (1998): 29–37.

Yu, H.X.; Man, B.K.W.; Chan, L.L.N.; Lam, M.H.W.; Lam, P.K.S.; Wang, L.; Jin, H.; and R.S.S. Wu. Cloud-point extraction of nodularin-R from natural waters. Analytica Chimica Acta. 509 (2004): 63–70.

Zenk, M.H.; El-Shagi, H.; and U. Schulte. Anthraquinone production by cell suspension cultures of *Morinda citrifolia*. Planta Med Suppl.. (1975): 79-101.

Zhu, X.; Hu, B.; Jiang, Z.; and Mingfang Li. Cloud point extraction for speciation of chromium in water samples byelectrothermal atomic absorption spectrometry. Water Research. 39 (2005): 589–595.

Zygoura, P.D.; Paleologos, E.K.; Riganakos, K.A.; and M.G. Kontominas. Determination of diethylhexyladipate and acetyltributylcitrate in aqueous extracts after cloud point extraction coupled with microwave assisted back extraction and gas chromatographic separation. Journal of Chromatography A. 1093 (2005): 29–35.

APPENDICES

APPENDIX A

EXPERIMENTAL DATA

A-1 Standard calibration curve of alizarin

Table: A-1.1 Standard calibration curve data.

Concentration of Alizarin (M)	Absorbance at 435 nm.		
	No.1	No.2	Average
2.78×10^{-4}	1.016	1.009	1.0125
1.39×10^{-4}	0.544	0.544	0.544
1.99×10^{-4}	0.728	0.722	0.725
2.78×10^{-5}	0.126	0.126	0.126
1.39×10^{-5}	0.059	0.060	0.0595
3.97×10^{-5}	0.163	0.161	0.162
9.93×10^{-5}	0.387	0.393	0.390

$$y = 0.00027x$$
$$R^2 = 0.99630$$
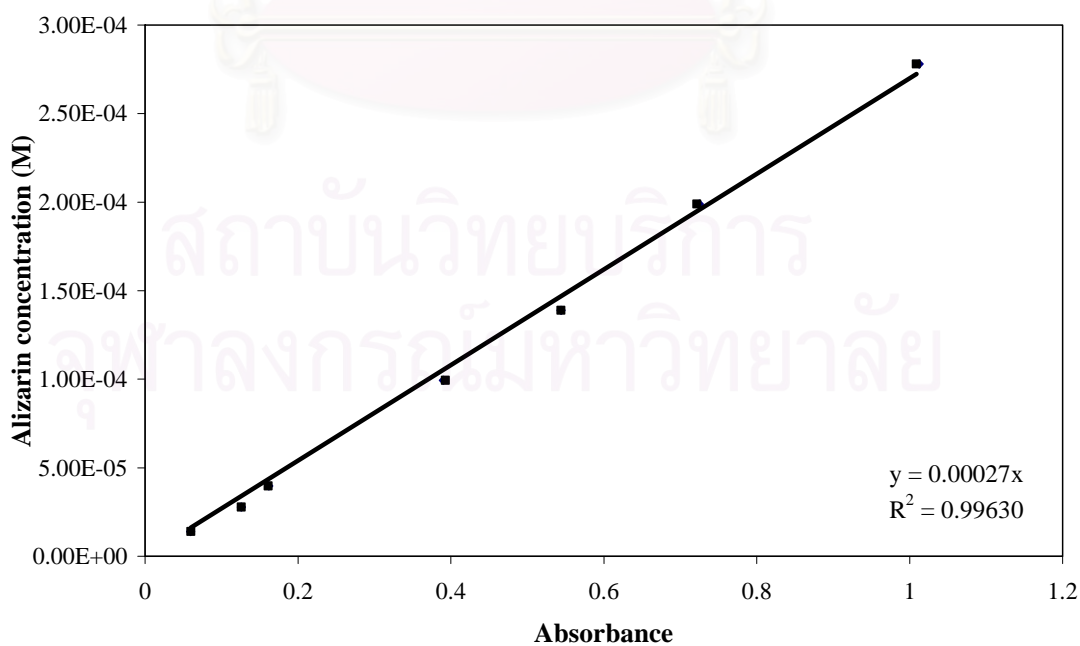
Figure A-1.1 Standard calibration curve of alizarin (average).

A-2 Standard calibration curve for HPLC analysis of damnacanthal

Table: A-1.2 Standard calibration curve data.

Concentration of damnacanthal (mg/ml)	Peak Area (UV detector at 250 nm)
0.02	7182363
0.01	3509993
0.0067	2200202
0.005	1580675
0.004	1096484

$y = 0.0000000028x$

$R^2 = 0.99162$
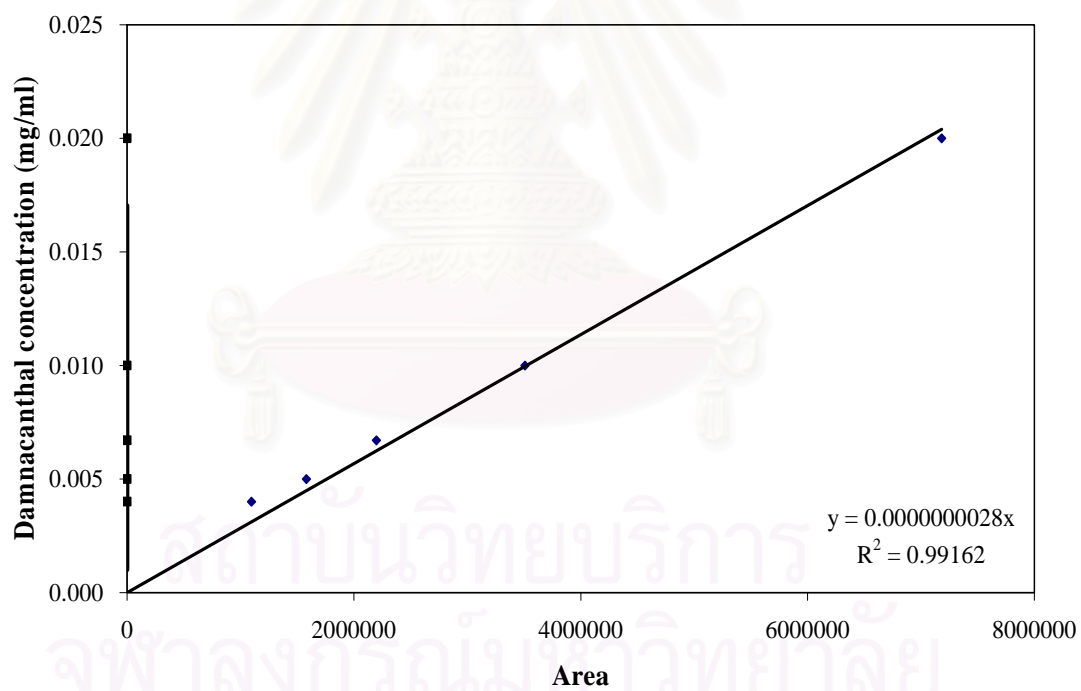
Figure A-1.2 Standard calibration curve of damnacanthal.

A-2 Experimental data of anthraquinones with micelle-mediated extraction

Table A-2.1: MME for Triton X-100 with shaking at room temperature (0.5 g roots, solvent volume 50 ml, time= 4 hrs.)

% Triton X-100 concentration in water	Recovery (%)			
	No.1	No.2	Average	Std.
0	18.40	15.98	17.19	1.710
1	44.42	49.28	51.23	3.435
5	50.44	49.04	52.64	0.995
10	52.61	53.85	61.34	0.879
14	58.18	64.97	61.54	4.803
18	62.70	62.38	63.83	0.224
20	63.00	62.90	66.22	0.072

Table A-2.2: MME for Genapol X-080 with shaking at room temperature (0.5 g roots, solvent volume 50 ml, time= 4 hrs.)

% Genapol X-080 concentration in water	Recovery (%)			
	No.1	No.2	Average	Std.
0	18.40	15.98	17.19	1.710
1	52.75	49.70	46.85	2.157
5	52.84	52.44	49.74	0.280
10	62.51	60.18	53.23	1.651
14	61.70	61.37	61.58	0.236
18	63.92	63.74	62.54	0.132
20	66.14	66.29	62.95	0.110

Table A-2.3: **UAMME for Triton X-100 at room temperature (0.5 g roots, solvent volume 50 ml, time= 2 hrs.)**

% Triton X-100 concentration in water	Recovery (%)
0	43.50
1	73.44
4	72.47
10	74.23
15	72.86
20	67.89

Table A-2.4: **UAMME for Genapol X-080 at room temperature (0.5 g roots, solvent volume 50 ml, time= 2 hrs.)**

% Genapol X-080 concentration in water	Recovery (%)
0	43.50
1	60.38
4	70.00
10	70.55
15	68.13
20	50.28

Table A-2.5: **UAMME with not control temperature for Triton X-100 (0.5 g roots, solvent volume 50 ml, time= 2 hrs.)**

% Triton X-100 concentration in water	Recovery (%)			
	No.1	No.2	Average	Std.
0	61.69	60.81	65.75	5.747
1	90.69	90.62	90.66	0.046
5	92.48	91.94	92.21	0.378
10	91.67	92.52	92.10	0.601
14	94.35	92.51	93.43	1.299
18	95.00	94.10	94.55	0.631
20	94.00	93.57	93.98	0.025

Table A-2.6 **UAMME with not control temperature for Genapol X-080 (0.5 g roots, solvent volume 50 ml, time= 2 hrs.)**

% Genapol X-080 concentration in water	Recovery (%)			
	No.1	No.2	Average	Std.
0	61.69	69.81	65.75	5.747
1	79.63	78.29	78.96	0.948
5	86.64	85.23	85.93	1.002
10	90.25	90.14	90.20	0.083
14	89.78	88.29	89.03	1.053
18	90.58	90.64	90.61	0.045
20	91.40	94.12	92.76	1.924

A-3 Experimental data of MMPHWE.

Table A-3.1: PHWE of temperature at 80°C (pressure= 4 MPa, flow rate= 5 ml/min, 0.5 g roots.)

Time (min)	Volume (ml)	Recovery (%)			Std.
		No. 1	No. 2	Average	
0	0	0	0	0	0
10	50	10.65	13.01	11.83	1.674
20	100	18.84	21.95	20.40	2.198
30	150	25.25	29.24	27.25	2.826
40	200	31.08	35.46	33.27	3.100
50	250	36.08	40.72	38.40	3.280
60	300	40.71	45.22	42.97	3.194
70	350	44.92	49.24	47.08	3.052
80	400	48.74	52.82	50.78	2.880
90	450	52.26	56.19	54.23	2.776
100	500	55.67	59.20	57.44	2.498
110	550	58.90	62.05	60.48	2.226
120	600	62.08	64.72	63.40	1.871

Table A-3.2: PHWE of temperature at 120°C (pressure= 4 MPa, flow rate= 5 ml/min, 0.5 g roots.)

Time (min)	Volume (ml)	Recovery (%)			Std.
		No. 1	No. 2	Average	
0	0	0	0	0	0
10	50	25.11	28.40	26.75	2.328
20	100	39.86	43.44	41.65	2.533
30	150	49.96	53.24	51.60	2.316
40	200	57.98	60.02	59.00	1.441
50	250	65.53	65.64	65.58	0.074
60	300	72.95	70.22	71.58	1.929
70	350	77.96	74.30	76.13	2.586
80	400	81.22	78.13	79.68	2.179
90	450	83.61	81.64	82.63	1.397
100	500	85.63	85.25	85.44	0.269
110	550	87.42	88.53	87.98	0.783
120	600	89.02	91.65	90.34	1.860

Table A-3.3: MMPHWE (surfactant: Triton X-100, concentration = 0.5%, T= 80°C, pressure= 4 MPa, flow rate= 5 ml/min, 0.5 g roots.)

Time (min)	Volume (ml)	Recovery (%)			Std.
		No. 1	No. 2	Average	
0	0	0	0	0	0
10	50	23.81	28.82	26.32	3.543
20	100	42.18	46.33	44.26	2.929
30	150	55.97	57.23	56.60	0.893
40	200	65.49	65.14	65.31	0.246
50	250	72.14	71.23	71.68	0.644
60	300	77.26	76.11	76.69	0.808
70	350	81.37	80.18	80.77	0.838
80	400	84.78	83.14	83.96	1.157
90	450	87.71	86.00	86.86	1.207
100	500	90.16	88.54	89.35	1.152
110	550	92.31	90.69	91.50	1.146
120	600	94.19	92.62	93.41	1.111

Table A-3.4: MMPHWE (surfactant: Triton X-100, concentration = 0.5%, T= 120°C, pressure= 4 MPa, flow rate= 5 ml/min, 0.5 g roots.)

Time (min)	Volume (ml)	Recovery (%)			Std.
		No. 1	No. 2	Average	
0	0	0	0	0	0
10	50	35.34	32.63	33.99	1.918
20	100	51.16	52.77	51.99	1.142
30	150	61.17	64.70	62.94	2.497
40	200	68.47	72.64	70.55	2.948
50	250	74.13	78.31	76.22	2.950
60	300	78.59	82.46	80.96	2.738
70	350	82.24	85.68	83.96	2.435
80	400	85.63	88.27	86.95	1.866
90	450	88.50	90.39	89.45	1.337
100	500	91.02	92.15	91.58	0.804
110	550	93.23	93.66	93.44	0.305
120	600	95.28	94.79	95.04	0.349

Table A-3.5: MMPHWE (surfactant: Triton X-100, concentration = 1%, T= 80°C,
pressure= 4 MPa, flow rate= 5 ml/min, 0.5 g roots.)

Time (min)	Volume (ml)	Recovery (%)			Std.
		No. 1	No. 2	Average	
0	0	0	0	0	0
10	50	33.47	30.12	31.79	2.370
20	100	50.72	49.97	50.79	0.527
30	150	61.75	61.81	61.78	0.044
40	200	69.29	70.91	70.10	1.140
50	250	74.67	77.91	76.29	2.287
60	300	79.02	82.90	80.96	2.743
70	350	82.55	86.47	84.51	2.772
80	400	85.61	89.44	87.52	2.713
90	450	88.19	91.76	89.98	2.523
100	500	90.73	93.69	92.21	2.096
110	550	92.55	95.40	93.97	2.016
120	600	94.27	96.84	95.56	1.814

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table A-3.6: MMPHWE (surfactant: Triton X-100, concentration = 1%, T= 120°C,
pressure= 4 MPa, flow rate= 5 ml/min, 0.5 g roots.)

Time (min)	Volume (ml)	Recovery (%)			Std.
		No. 1	No. 2	Average	
0	0	0	0	0	0
10	50	42.94	41.80	42.37	0.808
20	100	59.60	57.50	58.55	1.485
30	150	68.30	65.29	66.79	2.132
40	200	74.07	70.31	72.19	2.663
50	250	78.28	76.19	77.23	1.482
60	300	81.62	80.11	80.86	1.064
70	350	84.51	83.16	83.84	0.955
80	400	87.01	85.89	86.45	0.791
90	450	89.35	89.59	89.47	0.164
100	500	91.52	92.23	91.87	0.500
110	550	93.60	93.94	93.77	0.245
120	600	95.45	95.16	95.30	0.207

Table A-3.7: MMPHWE (surfactant: Triton X-100, concentration = 5%, T= 80°C,
pressure= 4 MPa, flow rate= 5 ml/min, 0.5 g roots.)

Time (min)	Volume (ml)	Recovery (%)			Std.
		No. 1	No. 2	Average	
0	0	0	0	0	0
10	50	29.78	25.29	27.54	3.175
20	100	52.30	47.09	49.69	3.681
30	150	64.91	62.77	63.84	1.516
40	200	72.97	72.18	72.58	0.560
50	250	79.04	78.43	78.73	0.430
60	300	83.58	82.86	83.22	0.505
70	350	87.00	86.20	86.60	0.569
80	400	89.72	88.95	89.34	0.550
90	450	91.88	91.14	91.51	0.526
100	500	93.81	92.95	93.38	0.613
110	550	95.38	94.48	94.93	0.636
120	600	96.85	95.82	96.33	0.724

Table A-3.8: MMPHWE (surfactant: Triton X-100, concentration = 5%, T= 120°C, pressure= 4 MPa, flow rate= 5 ml/min, 0.5 g roots.)

Time (min)	Volume (ml)	Recovery (%)			Std.
		No. 1	No. 2	Average	
0	0	0	0	0	0
10	50	37.52	35.39	36.45	1.509
20	100	49.49	47.73	48.61	1.243
30	150	58.32	57.28	57.80	0.736
40	200	66.77	66.63	66.70	0.097
50	250	72.28	73.46	72.87	0.829
60	300	76.89	79.22	78.05	1.649
70	350	81.24	83.49	82.37	1.590
80	400	85.12	87.06	86.09	1.370
90	450	88.80	89.72	89.26	0.651
100	500	91.62	91.91	91.76	0.205
110	550	93.61	93.71	93.66	0.071
120	600	95.29	95.13	95.21	0.114

Table A-3.9: MMPHWE (surfactant: Genapol X-080, concentration = 0.5%, T= 80°C, pressure= 4 MPa, flow rate= 5 ml/min, 0.5 g roots.)

Time (min)	Volume (ml)	Recovery (%)			Std.
		No. 1	No. 2	Average	
0	0	0	0	0	0
10	50	16.75	16.87	16.81	0.083
20	100	28.69	28.40	28.54	0.203
30	150	37.48	36.65	37.07	0.588
40	200	44.70	43.14	43.92	1.103
50	250	50.14	48.69	49.42	1.028
60	300	54.67	53.47	54.07	0.851
70	350	58.64	57.74	58.19	0.633
80	400	62.24	61.68	61.96	0.392
90	450	65.52	65.22	65.37	0.212
100	500	68.45	68.53	68.49	0.058
110	550	71.56	71.92	71.74	0.259
120	600	75.11	74.82	74.97	0.208

Table A-3.10: MMPHWE (surfactant: Genapol X-080, concentration = 0.5%, T= 120°C, pressure= 4 MPa, flow rate= 5 ml/min, 0.5 g roots.)

Time (min)	Volume (ml)	Recovery (%)			Std.
		No. 1	No. 2	Average	
0	0	0	0	0	0
10	50	36.50	37.13	36.82	0.441
20	100	48.60	49.23	48.92	0.444
30	150	57.94	59.69	58.82	1.238
40	200	64.54	65.35	64.99	0.506
50	250	69.54	69.67	69.60	0.092
60	300	73.28	73.35	73.32	0.056
70	350	76.30	76.62	76.46	0.225
80	400	79.54	79.80	79.82	0.188
90	450	82.76	83.01	82.89	0.178
100	500	85.19	85.68	85.43	0.344
110	550	87.62	88.42	88.02	0.568
120	600	89.76	90.81	90.29	0.741

Table A-3.11: MMPHWE (surfactant: Genapol X-080, concentration = 1%, T= 80°C,
pressure= 4 MPa, flow rate= 5 ml/min, 0.5 g roots.)

Time (min)	Volume (ml)	Recovery (%)			Std.
		No. 1	No. 2	Average	
0	0	0	0	0	0
10	50	21.00	19.39	20.19	1.141
20	100	35.18	34.09	34.64	0.775
30	150	45.76	44.86	45.31	0.640
40	200	54.32	53.15	53.73	0.829
50	250	60.95	59.84	60.39	0.782
60	300	66.47	65.45	65.96	0.726
70	350	71.29	70.17	70.73	0.794
80	400	75.18	74.29	74.73	0.630
90	450	78.64	77.91	78.28	0.511
100	500	81.74	81.05	81.40	0.494
110	550	84.48	83.91	84.67	0.405
120	600	86.97	86.37	86.67	0.423

Table A-3.12: MMPHWE (surfactant: Genapol X-080, concentration = 1%, T= 120°C, pressure= 4 MPa, flow rate= 5 ml/min, 0.5 g roots.)

Time (min)	Volume (ml)	Recovery (%)			Std.
		No. 1	No. 2	Average	
0	0	0	0	0	0
10	50	33.87	35.36	34.61	1.050
20	100	55.16	54.99	55.07	0.121
30	150	65.85	65.83	65.84	0.009
40	200	73.66	70.71	72.18	2.080
50	250	77.10	74.46	75.78	1.868
60	300	79.89	77.86	78.88	1.432
70	350	82.20	80.57	81.38	1.155
80	400	84.10	82.62	83.36	1.047
90	450	85.72	84.72	85.22	0.711
100	500	87.22	86.50	86.86	0.508
110	550	88.48	88.10	88.29	0.264
120	600	89.58	89.75	89.67	0.121

Table A-3.13: MMPHWE (surfactant: Genapol X-080, concentration = 5%, T= 80°C, pressure= 4 MPa, flow rate= 5 ml/min, 0.5 g roots.)

Time (min)	Volume (ml)	Recovery (%)			Std.
		No. 1	No. 2	Average	
0	0	0	0	0	0
10	50	30.01	29.08	29.55	0.655
20	100	45.31	45.62	45.47	0.224
30	150	54.41	55.53	54.97	0.792
40	200	61.11	62.73	61.92	1.146
50	250	66.18	67.98	67.08	1.271
60	300	70.40	72.35	71.37	1.380
70	350	74.19	75.80	74.99	1.140
80	400	77.41	78.98	78.19	1.112
90	450	80.35	81.70	81.02	0.960
100	500	83.06	84.02	83.54	0.682
110	550	85.59	86.14	85.87	0.388
120	600	87.70	88.16	87.93	0.322

Table A-3.14: MMPHWE (surfactant: Genapol X-080, concentration = 5%, T= 120°C, pressure= 4 MPa, flow rate= 5 ml/min, 0.5 g roots.)

Time (min)	Volume (ml)	Recovery (%)			Std.
		No. 1	No. 2	Average	
0	0	0	0	0	0
10	50	30.95	32.84	31.89	1.336
20	100	48.10	50.52	49.31	1.710
30	150	60.46	63.01	61.73	1.799
40	200	67.67	70.22	68.94	1.809
50	250	73.30	75.77	74.53	1.744
60	300	77.05	79.49	78.27	1.725
70	350	80.08	82.40	81.24	1.644
80	400	82.48	84.95	83.72	1.744
90	450	84.58	86.57	85.58	1.409
100	500	86.61	87.89	87.25	0.903
110	550	88.39	89.31	88.85	0.652
120	600	90.04	90.63	90.34	0.412

A-4 Experimental data for cloud point concentration of 30 ml extract with 1%Triton X-100 solution at 80 °C

Effect of percent Triton X-100 adds

Table A-4.1: CPC with effect of concentrations of Triton X-100 added to MMPHW extract (T=90 °C, incubation=30 min).

Percent Triton X-100 adds (%)	Recovery (%)				
	No.1	No.2	No.3	Average	Std.
0	93.62	91.87	94.67	93.39	1.414
1	97.85	97.80	97.65	97.76	2.990
2	98.49	98.39	98.29	98.39	1.818
3	97.85	97.86	97.85	97.85	0.103
4	96.95	97.20	96.86	97.00	0.451
5	97.57	97.41	97.66	97.55	0.460

Effect of concentration temperature

Table A-4.2: CPC with effect of temperature (Triton X-100 added to MMPHW extract = 1%, incubation time=30 min).

Temperature (°C)	Recovery (%)				
	No.1	No.2	No.3	Average	Std.
70	92.05	92.00	91.57	91.87	0.055
75	100.33	101.14	98.24	99.90	4.484
80	98.52	98.67	98.67	98.62	1.070
85	98.67	95.57	100.29	98.17	0.055
90	99.81	99.52	99.52	99.62	0.165

Effect of incubation time

Table A-4.3: CPC with effect of incubation time (Triton X-100 added to MMPHW extract = 1%, T=75 °C.)

Incubation time (min)	Recovery (%)				
	No.1	No.2	No.3	Average	Std.
10	84.76	84.86	84.76	84.79	0.055
15	93.33	86.76	84.76	88.29	4.484
20	90.00	89.33	91.43	90.25	1.070
25	97.62	97.62	97.71	97.65	0.055
30	99.81	99.52	99.52	99.62	0.165

APPENDIX B

The 16 th Thai Chemical Engineering and Applied Chemistry Conference

(TiCHE 16 th)

26-27 October 2006, Rama Gardens Hotel, Bangkok, Thailand

Extraction of Damnacanthal from Roots
of *Morinda citrifolia L.* by Subcritical Water

Kittisak Kiathewet[1], Thitiporn Anekpankul[1], Waraporn Witayasinthana[1]
Motonobu Goto[2], Mitsuru Sasaki[2], Artiwan Shotipruk[1]

1) Department of Chemical Engineering, Faculty of Engineering

Chulalongkorn University, Patumwan, Payathai, Bangkok, 10330, Thailand

Email: golfy_77@hotmail.com, neloony@hotmail.com, tutik_099@hotmail.com,

artiwan.sh@chula.ac.th

2) Department of Applied Chemistry and Biochemistry, Kumamoto University,

Kumamoto 850-8555, Japan

Email: mgoto@kumamoto-u.ac.jp, msasaki@kumamoto-u.ac.jp

1. Abstract

Roots of *Morinda citrifolia* (Noni or Yor in Thai) are the source of important compounds, anthraquinones, which have been proven to have anti-viral, anti-bacterial, anti-cancer activities. The most medicinally valuable anthraquinones in the roots of this plant is damnacanthal, which has been used for treatment of chronic diseases such as cancer and heart disease. In this study, subcritical water extraction was investigated as a benign alternative for solvent extraction of damnacanthal from the dried root of *Morinda citrifolia*. The experiments were conducted in a continuous flow system at a pressure of 4 MPa at different temperature between 150 and 220°C and water flow rates of 1.6, 2.4, 3.2 and 4 ml/min. The quantitative analysis of damnacanthal was performed with RP-HPLC with UV detection at 250 nm. The results of the study revealed that the highest amount of damnacanthal extracted with subcritical water was obtained at 170°C.

Keywords: Subcritical water; extraction;
 Morinda citrifolia;
 anthraquinones; damnacanthal

2. Introduction

For centuries, scientists and medical professionals have been investigating chemical constituents in *Morinda critifolia* (Noni or Yor in Thailand). Whole parts of this plant, which include fruits, flowers, leaves, bark, stem, and roots have been shown to contain various biological activities [1]. The roots of noni plants contain medicinally active components, namely anthraquinones, which show several therapeutic effects. These include anti-bacterial, anti-viral, and anti-cancer activities as well as analgesic effects, which make the roots potentially useful in several medical applications [2, 3]. Of the anthraquinones present in the plant, damnacanthal is the most important and has been reported to be one of the most effective anti-cancer agents.

Conventionally, anthraquinones can be extracted with ethanol. This method is simple but it requires long extraction time and solvent residue may be left in the extract. Nowadays, the desire to reduce the use of the organic solvent in food and medicine processing has led to new extraction methods; subcritical water extraction (SWE), which refers to liquid water whose temperature, lies between boiling

$(100^{o}C)$ and critical temperature $(374^{o}C)$. Under these conditions, its structure breaks up and dielectric constant of water decrease, make it less polar. This phenomenon makes it an effective solvent for several medicinal compounds.

The present study follows the previous report [4], in which show the feasibility of extracting anthraquinones from noni roots with subcritical water. The solubility of anthraquionoes in subcritical water was determined at various temperatures. The method of quantitative analysis employed in these studies was spectrophotometry, with alizarin as a reference compound, which determine the amount of total anthraquinones (included all other anthraquinones beside damnacanthal). In this work we propose to more accurately measure the amount of this target anti-cancer compound, damnacanthal, using reversed–phase high performance liquid chromatography (RP-HPLC) and determine the effects of various factors such as temperature and flow rate on extraction efficiency. In addition, the data for extraction efficiency at various flow rates were fitted with simple thermodynamic partition, equilibrium with external mass transfer resistant and

desorption models to describe the behavior of subcritical water extraction of this compound. This will provide useful information for the initial sizing and the economic evaluation of the system in a commercial scale.

3. Experimental

3.1 Plant material and chemicals

The roots of *Morinda citrifolia L* were grown locally in Thailand, The plant roots were harvested, washed, and then oven dried at $50^{o}C$ for 2 days to almost complete dryness. The dried sample was then ground to small size using mortar and pestle with liquid nitrogen. The ground samples were oven dried in at 50ºC for 1 day, and then stored in a dry place until use.

3.2 Subcritical water extraction

Subcritical water extraction was performed using an apparatus shown in Figure 3.3. The extraction system consisted of two HPLC pumps (PU 980, JASCO, Japan) used for delivering water and solvent, a degassing instrument (ERC 3215, CE, Japan), an oven (D63450, HARAEUS, Germany), in which the extraction vessel (10 ml, Thar Design, USA) was mounted, a

pressure gauge, and a back pressure regulator valve (AKICO, Japan). All connections are made with stainless steel capillaries (1/16 inch inside diameter).

Distilled water was passed through a degassing equipment to remove dissolved oxygen. The degassed water was then delivered, at a constant flow rate with the first HPLC pump, to a 3-m preheating section installed in the oven to heat it to the required temperature, which then passed through the extraction vessel, preloaded with 1 g of ground noni roots. The pressure of the system was adjusted to the desired condition (4 MPa) by using the back-pressure regulator valve at the outlet coil to ensure that water was in liquid state at the temperatures tested. The oven was turned on and the temperature was set to the desired operating condition. When the temperature reached the set point, the extraction started. The second pump was then turned on to deliver ethanol at constant flow rate to wash off any residual product in the outlet line behind the extractor. The extract was cooled in a coil immersed in a water bath to prevent possible product degradation, and was then collected in fractions. The conditions tested are summarized in Table 1.
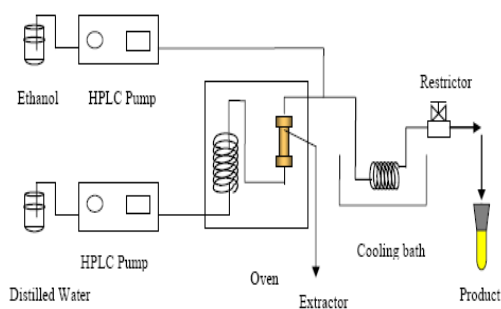

Figure1. Diagram of experimental setup.

Parameter	Condition
Temperature	150, 170, 200 and 220°C
Flow rate	2, 3, 4, and 5 ml/min
Pressure	4 MPa
Approximate roots size	0.37 mm

Table 1: Parameter condition in experiment.

3.3 Sample preparation for RPHPLC

Subcritical water extracts were evaporated under vacuum to dryness, and re-dissolved in distilled water. This resulted in two parts: solid precipitate and water soluble part which were centrifuged at 3000 rpm for 15 minutes to separate the supernatant from the precipitate. DMSO was added to the precipitate from the previous step, and the mixture was then sonicated for 15 minutes. Both supernatant and precipitate-DMSO fractions contain the active compound of interest. The supernatant and the precipitate-DMSO fraction were filtered through a membrane filter (0.45μm, Millipore,

USA) before being subjected to HPLC analysis.

3.4 Analysis RP-HPLC procedure

The HPLC apparatus consisted of pump (Prostar 240, Varian, USA), equipped with photodiode array detector (Prostar 335, Varian, USA). The analysis was carried out at room temperature on a phenomenex Luna C18, 100 A pore size, 5 μm particle size, 250mm × 4.60 mm I.D. column. The mobile phase used was modified from that described by Dabiri et al., 2004, which consisted of a mixture of (70:30) acidic methanol (50 mM TFA)-buffer (50 mM KH_2PO_4, pH = 3). The flow rate of the mobile phase was 1 ml/min and an injection volume of 50 μL was used. The UV detection wavelength was 250 nm.

3.5 Extraction models

When consider extraction in continuous flow passing short extraction vessels, it is not necessary to consider the concentration profile within the bed. The extraction models become simpler. In this case, there are simple models have been used to describe the extraction curves depending on whether the extraction is controlled by diffusion out of the solid matrix or by partitioning of solute between matrix and solvent

3.5.1. Extraction controlled by partitioning of solute between matrix and solvent

This model is based on a single thermodynamics partitioning coefficient (K_D) defined as

$$K_D = \frac{Concentration\ of\ analyte\ in\ the\ matrix}{Concentration\ of\ analyte\ in\ the\ extraction\ fluid} : at\ equilibrium$$

For this model, it is assumed that the initial desorption step does not significantly affect the extraction rate. In addition the subsequent fluid-matrix partitioning is assumed to be rapid. Essentially, the mass of analyte in each unit mass of extraction fluid and the mass of analyte remaining in the matrix at that period in the extraction time is calculated for the entire extraction time based on the K_D value determined for each compound. Therefore, if the K_D model applies to a certain extraction, the shape of an extraction curve would be defined by:

$$\frac{S_b}{S_0} = \frac{\left(1 - \frac{S_a}{S_0}\right)}{\left(\frac{K_D m}{(V_b - V_a)\rho} + 1\right)} + \frac{S_a}{S_0}$$ …….. (1)

Note that K_D model does not include extraction time, but only relies on the volume of extractant fluid used (assuming a constant sample size). Therefore, doubling the extraction fluid flow rate should double the extraction rate versus time. if the extraction is

strictly controlled by partitioning equilibrium, K_D values for all flow rates must be equal.

3.5.2 Extraction controlled by diffusion out of the matrix

When the flow of fluid is fast enough for the concentration of a particular solute to be well below its solubility limit, the rate-determining process is the rate of diffusion out of the matrix. Kinetic models such as one-site and two-site models were considered

3.5.2.1 One-site kinetic desorption model

the solution is described as the hot-ball model because of the analogy of the mathematical solutions with those for a hot spherical object being dropped into cold water, given by Carlslaw and Jaeger's equation [5]. The ratio of the mass of diffusing substance leaving the sample to the initial mass of solute in the sample, S_t/S_0 is given by

$$\frac{S_t}{S_0} = 1 - \frac{S_r}{S_0} = 1 - e^{-kt} \qquad \text{……. (2)}$$

3.5.2.2. Two-site kinetic desorption model

This model is a modification of the kinetic desorption model described in the previous section. The two-site kinetic desorption model requires two steps to define an extraction curve, i.e. a certain fraction (*F*) of the analyte

desorbs t a fast rate defined by k_1, and the remaining fraction (*1-F*) desorbs by a slower rate defined by k_2. The simple two-site kinetic model consists of two first order extractions:

$$\frac{S_t}{S_0} = 1 - \left[Fe^{-k_1 t}\right] - \left[(1-F)e^{-k_2 t}\right] \qquad \text{……. (3)}$$

Note that the kinetic desorption model includes no factor describing extraction flow rate, but relies solely on time. Therefore, doubling the extractant flow rate should have little effect on the extraction efficiency per unit time. We see that the kinetic desorption model does not include a factor describing extraction flow rate so value k should be the same value for all flow rate if the model is said to fit the experimental data.

3.5.3 External mass transfer model

This model describes extraction which is controlled by external mass transfer whose rate is described by resistance type model, the amount of solute extracted can be written as:

$$S_t = 1 - S_0 \exp\left(-k_e a_p t / K_D\right) \qquad \text{……. (4)}$$

Because a_p is difficult to be measured accurately, a_p and k_e are usually determined together as $k_e a_p$, which is called overall volumetric mass transfer coefficient. The factors that influence

the value of $k_e a_p$ include the velocity, u, of solvent through the extractor and the size and shape of plant sample.

4. Results and discussion

4.1 Effect of subcritical water temperature

The effect of the temperature of subcritical water on the extraction yield was determined. The product yield was found to be the highest at 170 ℃ as can be seen from Figure 2. As the temperature increased, the product solubility increased due to the decreasing dielectric constant. However, at 200 and 220 ℃, the yield was low due to the degradation of the product at high temperatures. These results indicated that when the specific compound, damnacanthal, was considered with HPLC analysis, the results differed from those suggested by previous study in which total anthraquinones was found to be the highest at the temperature of 220℃ [4]
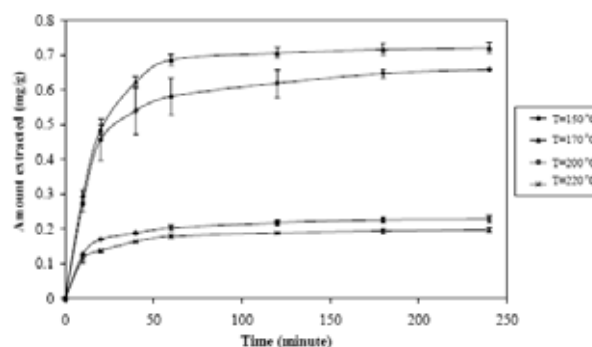

Figure 2 Effect of water temperature (Flow rate 5 ml/min at pressure of 4 MPa).

4.2 Effect of flow rate

In this study, the experimental extraction curves were obtained for the flow rate of 2, 3, 4, and 5 ml/min and for the temperatures of 170°C at the fixed pressure of 4 MPa. The results were plotted in Figure 3.3a) and 3.3b), which shows that the rate of damnacanthal extracted increases when the volumetric flow rate increased. The dependence of extraction efficiency on volumetric flow rate indicated that extraction could be controlled either by equilibrium partitioning or by external mass transfer, or the combination of the two. When the extraction yield was plotted against volume of water however, the data for all flow rates lied almost on the same curve. This result demonstrates that extraction could be controlled by a combination of different processes, and that the mechanism controlling extraction behavior may change
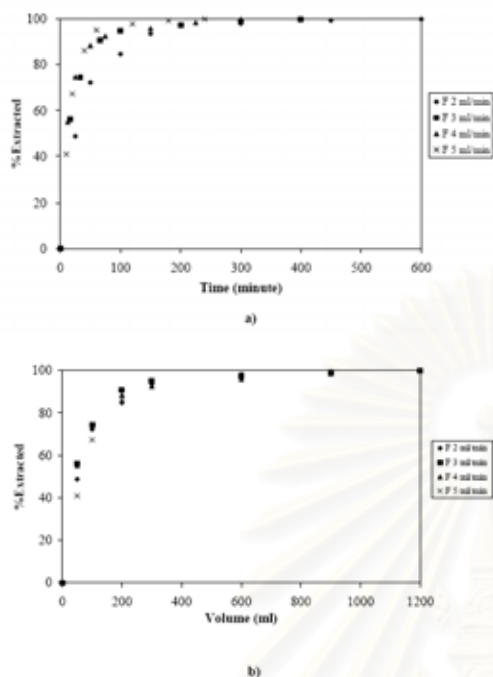
depending on the extraction flow rate conditions.

Figure 3 a) Percent extracted versus extraction time, b) Percent extracted versus volume of water (at temperature 170°C and pressure 4 MPa).

4.4. Comparison of extraction models

To compare the extraction models, the values for the model parameters such as K_D, $k_e a_p$, k_1 and k_2 are determined by Microsoft EXCEL solver. The mean percentage errors between the experimental data and the models were considered. The results are showed in Table 2, which support that K_D model was quite suitable overall for the description of extraction at different flow rates tested. On the other hand, one-site and two-site kinetic desorption

models describe the extraction data reasonably well at higher flow rates.

Table 2: Mean percent absolute errors between experimental data and extraction model results.

Model	Parameter model	%Mean absolute errors at difference flow rate (ml/min)			
		2	3	4	5
Partitioning coefficient model	K_D	2.27	2.69	3.44	2.26
One site kinetic desorption model	k	8.66	3.11	3.07	0.95
Two site kinetic desorption model	k_1, k_2	8.57	3.12	2.49	0.83
External mass transfer model	$K_D, k_e a_p$	2.27	2.35	2.97	0.95

5. Conclusions

In summary, subcritical water provides a promising alternative for extraction of the anti-cancer damnacanthal from roots of *Morinda citrifolia L.* At the temperature of 170 ºC the yield was the highest. At temperature of 200 and 220 ºC, the decomposition of damnacanthal occurred. Overall, a mathematical model base on the combination of partition coefficient (K_D) and external mass transfer gave a good description of damnacanthal extraction by subcritical water, while the kinetic model described the extraction reasonably at higher flow rates.

.

Acknowledgements

Nomenclature

$1-F$ = fraction of the analyte released slowly

F = fraction of the analyte released quickly

k = a first order rate constant describing the extraction (min^{-1})

k_1 = first-order rate constant describing the quickly released fraction (min^{-1})

k_2 = first-order rate constant describing the slowly released fraction (min^{-1})

$k_e a_p$ = overall volumetric mass transfer coefficient.

m = mass of the extracted sample (mg).

S_a = cumulate mass of the analyte extracted after volume V_a (mg/g)

S_b = cumulate mass of the analyte extracted after volume V_b (mg/g)

S_0 = initial total mass of analyte in the matrix (mg/g)

S_t = mass of the analyte removed by the extraction fluid after time t (mg/g)

t = time (min)

ρ = density of extraction fluid at given conditions (mg/ml)

References

[1] Yang, M.Y., West, B.J., Jensen, C.J., Nowicki, D., SU, C., Palu, A.K., and Anderson, G. *Morinda citrifolia* (Noni): A literature review and recent advances in Noni research. Acta Pharmacologica Sinica 12 (2002):1127 -1141.

[2] Hiramatsu, T., Imoto, M., Koyano, T., and Umezawa, K. Induction of normal phenotypes in ras-transormed cells by damnacanthal from *Morinda citrifolia*. Cancer Letters 73 (1993): 161-166.

[3] Asahina, A.Y., Ebesu, J.S., Ichinotsubo, D., Tongson, J., and Hokoma, Y. Effect of okadaic acid (OA) and noni fruit extraction in the synthesis of tumor necrosis factor-α (TNF-α) by peripheral blood mononuclear (PBN) cells *in vitro*. In Proceedings International Symposium of Ciguatera and Marine Natural Products (1994): 197-205.

[4] Shotipruk, A., Kiatsongserm, J., Pavasant, P., Goto, M., and Sasaki, M., Subcritical weater extraction of anthraquinones from the roots of *Morinda citrifolia*. Biotechnology Progress 20 (2004): 1872-1876.

[5] Westwood, S.A. <u>Supercritical fluid extraction and its use in chromatographic sample preparation</u>. 1st ed. London: Blackie Academic & Professional, 1993.

VITA

Mr. Kittisak Kiathevest was born on September 14, 1983 in Bangkok, Thailand. He received a Bachelor's Degree of Chemical Engineering from the Faculty of Engineering, King Mongkut's Institute of Technology North Bangkok in 2005. He subsequently completed the requirements for a Master's Degree in Chemical Engineering at the Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University in 2007.