

EXTRACTION OF AMOXICILLIN AND ERYTHROMYCIN UTILISING  
SOPHROLIPIDS REVERSED MICELLAR SYSTEM

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EXTRACTION OF AMOXICILLIN AND ERYTHROMYCIN UTILISING  
SOPHROLIPIDS REVERSED MICELLAR SYSTEM

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A thesis submitted in fulfilment of the  
requirements for the award of the degree of  
Doctor of Philosophy (Chemical Engineering)

Faculty of Chemical and Energy Engineering  
Universiti Teknologi Malaysia

JULY 2017

To my beloved father and mother

## ACKNOWLEDGEMENTS

I would like to greatly thank my supervisor, Assoc. Prof. Dr. Siti Hamidah Mohd. Setapar for the encouragement and guidance she gave throughout the research. I also thank my co-supervisor, Assoc. Prof. Dr. Adnan bin Ripin for his insight on the study.

Special thanks to the faculty of Chemical and Energy Engineering for providing laboratories that enable the conducting of the experiments. Special thanks to Universiti Teknologi Malaysia for the scholarship provided that allowed me to continue the study.

Last but not least, this journey would not be completed without the great support and motivation from family members and friends. To them I express my gratitude.

## ABSTRACT

Downstream processing of antibiotics conventionally involves filtration, solvent extraction, and crystallization. Formation of stable emulsion during conventional solvent extraction of antibiotics causes high solvent consumption and low product yield. Recently, reverse micelle extraction has been investigated for the extraction of proteins and dyes. Reverse micelle extraction has the advantages of easy operation, high selectivity, mild operating conditions, short extraction time, preserved protein activities, reduced emulsion formation, and safer solvents, as well as having the potential for scale up, continuous operation, and solvent recycling. Most researchers use chemical surfactants for their reverse micelle extraction. In this study, sophorolipids biosurfactant was used for the first time for formation of reverse micelles to extract antibiotics. Application of biosurfactant can further improve reverse micelle extraction in terms of sustainability and environmental friendliness. Experiments were conducted to explore the reverse micelle extraction of amoxicillin and erythromycin. Solution pH was found to be the most dominant factor during both reverse micelle extraction of amoxicillin and erythromycin. Strong attractive electrostatic interactions between antibiotics and sophorolipids at solution pH below the isoelectric point of antibiotics allow more antibiotics to be extracted during forward extraction but fewer antibiotics to be released during backward extraction. Attractive electrostatic interactions which diminished at solution pH higher than the isoelectric point of antibiotics reduced forward extraction efficiency but promoted backward extraction efficiency. Both amoxicillin and erythromycin are very sensitive to surrounding pH and will degrade at solution pH outside of their stable pH ranges. Minimum amount of KCl was needed for the reverse micelle extraction of both amoxicillin and erythromycin. High KCl concentration hindered both forward and backward extraction of antibiotics. Sophorolipids is crucial in enabling the transfer of antibiotics into the isooctane organic phase. Increasing sophorolipids concentration increases the amount of antibiotics been extracted. Mass transfer studies showed that reverse micelle extraction of amoxicillin and erythromycin can be completed in very short time. Overall mass transfer coefficients of backward extraction was lower than that of forward extraction for both amoxicillin and erythromycin indicating that backward extraction is more difficult than forward extraction process. Comparisons between amoxicillin and erythromycin showed that erythromycin has better equilibrium partitioning and larger calculated overall mass transfer coefficients compared to amoxicillin. There may be some differences on the behaviours of amoxicillin and erythromycin during the reverse micelle extraction process. This reverse micelle extraction method was found to be more efficient in extracting erythromycin compared to amoxicillin. Furthermore, the study also confirmed that the quality of palm oil based sophorolipids is comparable to those of commercial sophorolipids.

## ABSTRAK

Pemrosesan hiliran antibiotik konvensional melibatkan penapisan, pengekstrakan pelarut, dan penghabluran. Pembentukan emulsi antibiotik yang stabil dalam penggunaan pengekstrakan pelarut konvensional menyebabkan penggunaan pelarut yang tinggi dan hasil produk yang rendah. Pengekstrakan misel balikan telah dikaji untuk pengekstrakan protein dan pencelup. Pengekstrakan misel balikan mempunyai kelebihan operasi yang mudah, kememilihan yang tinggi, keadaan operasi yang sederhana, masa pengekstrakan yang pendek, pemeliharaan aktiviti-aktiviti protein, pembentukan emulsi yang kurang, penggunaan pelarut yang selamat, mempunyai potensi untuk penggandaan skala, operasi secara berterusan, dan kitar semula pelarut. Kebanyakan penyelidik menggunakan surfaktan kimia untuk pengekstrakan misel balikan. Dalam kajian ini, biosurfaktan soforolipids telah digunakan untuk kali pertama untuk pembentukan misel balikan bagi mengekstrak antibiotik. Penggunaan biosurfaktan dapat meningkatkan lagi pengekstrakan misel balikan dari segi kelestarian and mesra alam. Eksperimen telah dijalankan untuk mengkaji pengekstrakan misel balikan pada amoksisilin dan eritromisin. Faktor paling dominan semasa pengekstrakan misel balikan amoksisilin dan eritromisin adalah pH larutan. Interaksi tarikan elektrostatik antara antibiotik dan soforolipids pada pH larutan yang lebih rendah daripada takat isoelektrik antibiotik membolehkan lebih banyak antibiotik diekstrak semasa pengekstrakan ke hadapan tetapi berkurangan semasa pengekstrakan ke belakang. Interaksi tarikan elektrostatik berkurangan pada pH larutan yang lebih tinggi daripada takat isoelektrik antibiotik yang mengurangkan kecekapan pengekstrakan ke hadapan tetapi meningkatkan kecekapan pengekstrakan ke belakang. Kedua-dua antibiotik adalah sangat sensitif terhadap pH persekitaran dan akan mengalami degradasi pada pH di luar julat pH stabil. Amaun minimum KCl adalah diperlukan untuk pengekstrakan misel balikan bagi kedua-dua antibiotik. Kepekatan KCl yang tinggi menghalang pengekstrakan misel balikan antibiotik. Soforolipids adalah diperlukan untuk membolehkan pemindahan antibiotik kepada fasa organik isooktana. Peningkatan kepekatan soforolipids juga akan meningkatkan jumlah antibiotik yang diekstrak. Kajian pemindahan jisim menunjukkan bahawa pengekstrakan misel balikan amoksisilin dan eritromisin boleh disiapkan dalam masa yang amat singkat. Pekali pemindahan jisim keseluruhan pengekstrakan ke belakang yang lebih rendah daripada pengekstrakan ke hadapan menunjukkan bahawa pengekstrakan ke belakang adalah lebih sukar daripada pengekstrakan ke hadapan. Kajian ini menunjukkan bahawa eritromisin mempunyai keseimbangan pembahagian dan pekali pemindahan jisim keseluruhan yang lebih baik daripada amoksisilin. Kaedah pengekstrakan misel balikan dalam kajian ini adalah lebih cekap untuk mengekstrak eritromisin berbanding dengan amoksisilin. Tambahan pula, kajian ini juga mengesahkan bahawa kualiti soforolipids berasaskan minyak kelapa sawit adalah setanding dengan soforolipids komersial.

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**LIST OF ABBREVIATIONS**

6-APA	-	6-amino penicillanic acid
Amo	-	Amoxicillin
AOT	-	Bis (2-ethylhexyl) sodium sulfosuccinate
CI	-	Counterion
CE	-	Capillary electrophoresis
CLA	-	Colloidal liquid aphrons
CMC	-	Critical micelle concentration
CTAB	-	Cetylmethylammonium bromide
DF	-	Diafiltration
DOLPA	-	Dioleylphosphoric acid
DTAB	-	Dodecyl trimethyl ammonium bromide
ELM	-	Emulsion liquid membrane
Ery	-	Erythromycin
Ery-A	-	Erythromycin A
Ery-B	-	Erythromycin B
Ery-C	-	Erythromycin C
Ery-D	-	Erythromycin D
HPGM	-	Hydroxyl-phenylglycine methyl ester
HPLC	-	High performance liquid chromatography
HTAB	-	Hexadecyltrimethyl ammonium bromide
LPE-BE	-	Liquid phase extraction with back extraction
MF	-	Microfiltration
MPOB	-	Malaysian Palm Oil Board
MWCO	-	Molecular weight cut-off
NF	-	Nanofiltration
OTC	-	Oxytetracycline
PDSE	-	Pre-dispersed solvent extraction

PGA	- Penicillin G acylase
Phe	- Phenylalanine
RO	- Reverse osmosis
RSM	- Response surface methodology
RTIL	- Room temperature ionic liquids
SDBS	- Sodium dodecylbenzene sulfonate
SDS	- Sodium dodecyl sulfate
SIC	- Sequential injection chromatography
SL	- Sophorolipids
SPE	- Solid phase extraction
TOA	- Tri-n-octylamine
UF	- Ultrafiltration
VCF	- Volume concentration factor

## LIST OF SYMBOLS

$A$	-	Interfacial area
$a_0$	-	Mean cross-sectional area
$C$	-	Concentration
$g$	-	Packing factor
$J$	-	Flux
$K$	-	Overall mass transfer coefficient
$k$	-	Film mass transfer coefficient
$l_c$	-	Length
$m$	-	Partitioning equilibrium constant
$pI$	-	Isoelectric point
$R^2$	-	Sum of squares
Subscript <sub>aq</sub>	-	In aqueous phase
Subscript <sub>b</sub>	-	During backward extraction
Subscript <sub>f</sub>	-	During forward extraction
Subscript <sub>i</sub>	-	At initial
Subscript <sub>in</sub>	-	At interface
Subscript <sub>org</sub>	-	In organic phase
Subscript <sub>w</sub>	-	Water
$t$	-	Time
$V$	-	Volume
*	-	Equilibrium
[substance]	-	Concentration of substance

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## CHAPTER 1

### INTRODUCTION

#### 1.1 Research Background

Antibiotics started to gain popularity since the introduction of germ theory of disease in the late 1800s. Throughout the centuries, various antibiotics were produced and commercialized. Several common antibiotics nowadays are penicillin G, amoxicillin, and erythromycin. During the complex bioprocesses of antibiotics production, there are three stages that need to be considered. Firstly, the seed culture should be optimized for higher antibiotic yield and large scale production (Zou *et al.*, 2011). Secondly, the main fermentation should be optimized by adjusting appropriate factors such as raw materials' concentrations, temperature, pH of culture, and aeration to obtain higher production. Thirdly, downstream processing is required to separate the desired products from other impurities. Downstream processing of antibiotics contributes to a large portion of total production costs because the product streams from broth have large volume but low concentration of antibiotics (Li *et al.*, 2004b). Conventional strategy for downstream processing of antibiotics involves filtration of broth to remove impurities especially surface active substances, solvent extraction, and crystallization to polish the product.

Reverse micelle extraction utilizes the special characteristics of reverse micelles formed by surfactant molecules in apolar solvent as a mean to selectively extract molecules which are oppositely charged with surfactant used from aqueous solution into the solvent. Its advantages are easy to scale up, high selectivity, low energy consumption, possible continuous operation, and mild thermal operating conditions (Mohd-Setapar *et al.*, 2009). Reverse micelle extraction consists of two

steps: forward extraction and backward extraction. During forward extraction, target molecules are transferred from aqueous solution into an organic phase through reverse micelles. During backward extraction, target molecules loaded in reversed micelle phase are released into a fresh aqueous phase for recovery. In some cases, only forward extraction is carried out to remove unwanted substances from water without the need of recovery.

Reverse micellar system is widely studied for downstream processing of biotechnology products. Various bio-molecules such as penicillin G (Mohd-Setapar and Mohamad-Aziz, 2013), nattokinase (Liu *et al.*, 2004), chitosanases (Chen *et al.*, 2006), polyphenol oxidase (Imm and Kim, 2009),  $\beta$ -glucosidase (Hemavathi *et al.*, 2010), lipase (Gaikawai *et al.*, 2012a), and laccase (Peng *et al.*, 2012) were effectively recovered using reverse micelle extraction. Various modifications were also studied to improve this method in term of effectiveness and environmental friendliness. For instance, mixed reverse micellar system was studied by Norazimah Mohamad-Aziz *et al.* (2013) to extract amoxicillin. The result showed reduced surfactant consumption compared to when single surfactant system was used.

Compared to chemical surfactant such as bis (2-ethylhexyl) sodium sulfosuccinate (AOT), very few studies were reported for the reverse micelle extraction of bio-molecules using biosurfactants. Rhamnolipid, a type of biosurfactant was used by Peng *et al.* (2012) to extract laccase from *C. versicolor*. The use of biosurfactant provides a more environmental friendly operation. Their study shows that significantly lower concentration of biosurfactant was needed for the extraction compared to chemical surfactants. Examination on the effects of extraction conditions shows that the process is very similar to those using chemical surfactants. The extraction yields final activity recovery of 91.1% and purification factor of 4.31 (Peng *et al.*, 2012). The solvent can also be reused for three times with minor drop in extraction efficiency. This study shows that biosurfactants has the potential to replace the chemical surfactants in reverse micelle extraction of bio-molecules.

Sphorolipids are biosurfactants commonly produced from non-pathogenic yeast *Candida bombicola*. Different structures of sphorolipids can be obtained by



using different feed stocks and cell culture but they are basically divided into two types: lactonic form and acidic form. These two forms have their own interesting features (Shah *et al.*, 2005). They can be tailored to meet specific needs making them quite flexible in applications. Researchers also studied the use of low cost raw materials for production of sophorolipids to make them more competitive especially for cosmetics and pharmaceutical applications (Deshpande and Daniels, 1995; Felse *et al.*, 2007). Examination on sophorolipids surfactant characteristics reveals that they have better, if not similar properties compared to chemical surfactants (Van Bogaert *et al.*, 2011). Thus, they have the potential to replace chemical surfactants.

## 1.2 Problem Statement

Conventionally, antibiotics are recovered through liquid-liquid extraction. A lot of solvents were tested for the extraction but many of them are not suitable due to their high solubility in water or undesirable toxic properties (Kawasaki *et al.*, 1996). One of the most commonly used solvent for antibiotics extraction to date is butyl acetate because it is biodegradable and has relatively low toxicity. However, the high boiling point of butyl acetate is said to cause subsequent processing more costly (Manic *et al.*, 2011). Besides selecting a suitable solvent, the liquid-liquid extraction process itself also faces a major difficulty: formation of stable emulsion. The emulsion is caused by the cell and finely dispersed surface active substances, especially protein and polysaccharides (Li *et al.*, 2004b). The emulsion makes the separation process harder. It causes high solvent consumption and low product yield. In some cases, de-emulsifiers are added to prevent emulsion formation. However, this will increase the production costs and bring negative impacts to the environment. Furthermore, adding de-emulsifier sometimes cannot eliminate entirely the emulsion. Conventional liquid-liquid extraction also takes long time (Le *et al.*, 2001). This prolonged contact of antibiotic molecules with organic solvent may cause irreversible damage to the antibiotic molecules, resulting in low activity recovery.

Reverse micelle extraction has great potential for the extraction of biomolecules as reported by various researchers. High recovery, often more than 90%,

can be achieved. The solvents and surfactants can be recycled and reused. This makes reverse micelle extraction a cost saving method. Besides that, it can be optimized easily by adjusting several parameters including pH, surfactant concentration, and salt concentration. This makes it a relatively easy operation. The structure of reverse micelles can effectively protect the antibiotics capped inside from direct contact with the solvents. Therefore, less degradation occurs and activity recovery can be increased. Reverse micelle extraction also takes significantly shorter time than conventional liquid-liquid extraction. The surfactants used to form reverse micelles are mostly chemical surfactants such as AOT, sodium dodecyl sulfate (SDS), and cetylmethylammonium bromide (CTAB) due to their effectiveness.

Recently, public concern regarding preservation of the environment is increasing. Although chemical surfactants can give high extraction performance, their hard to degrade characteristic and toxicity cannot be ignored. Therefore, it is desirable to replace the chemical surfactants with biosurfactants produced from renewable resources. Sophorolipids are biosurfactants produced from yeast using renewable feed stocks. They are readily biodegradable and have lower toxicity than chemical surfactants (Ma *et al.*, 2012). Their surfactant characteristics are comparable to those of chemical surfactants or even better by having lower critical micelle concentration (CMC). Therefore in this study, sophorolipids will be used for the first time to extract antibiotics from aqueous solution. Amoxicillin and erythromycin are among the most widely prescribed antibiotics (Center for Disease Dynamics, 2015). Amoxicillin is commonly used to treat respiratory infection, ear, nose and throat infection, skin infection, and urinary tract infection caused by bacteria. Erythromycin is usually used to treat respiratory tract infection, skin infection, and gastrointestinal infection caused by bacteria. It is also often used as alternative antibiotics by people who are allergic to penicillin. Amoxicillin and erythromycin are from different classes of antibiotics with their own characteristics. The effects of these differences in characteristics on reverse micelle extraction of antibiotics need to be investigated. Thus, amoxicillin and erythromycin are chosen as target antibiotics for reverse micelle extraction in this study.

### 1.3 Objectives of the Study

The aim of this study is to investigate the potential of sophorolipids reverse micelle to extract antibiotics from aqueous solution. This was completed through following objectives:

Objective 1: To study the reverse micelle extraction of amoxicillin and erythromycin using sophorolipids biosurfactant and factors affecting the extraction.

Objective 2: To investigate the significance of main factors and optimize the reverse micelle extraction of amoxicillin and erythromycin.

Objective 3: To investigate the mass transfer behaviour of amoxicillin and erythromycin in their reverse micelle extraction using sophorolipids respectively.

### 1.4 Scopes of the Study

In order to achieve the objectives of study, the scopes of research are outlined as followed:

Scope 1: Conducting forward and backward extraction of amoxicillin and erythromycin utilizing sophorolipids reversed micelles. Detailed scopes are:

- i) Using palm oil based sophorolipids to form reversed micelles for the extraction of amoxicillin.
- ii) Using commercial sophorolipids to form reversed micelles for the extraction of amoxicillin and erythromycin.
- iii) Changing aqueous phase pH, sophorolipids concentration, and salt concentration to study their effects on the extraction.

Scope 2: Using central composite design to study the significance of the factors and trends of extraction. Detailed scopes are:

- i) Using statistical analysis to obtain appropriate empirical models for reverse micelle extraction of amoxicillin and erythromycin.
- ii) Using statistical software to obtain response surfaces for the reverse micelle extraction and study the effects of main factors.
- iii) Using response surfaces to find optimum regions for reverse micelle extraction of amoxicillin and erythromycin.

Scope 3: Developing appropriate mass transfer equations to describe the reverse micelle extraction processes of amoxicillin and erythromycin.

Scope 4: Comparing the reverse micelle extraction processes between amoxicillin and erythromycin.

## **1.5 Significance of Research**

The main contribution of this research is to show the potential of sophorolipids for liquid-liquid extraction of antibiotics. Significance of affecting factors and trends of reverse micelle extraction of antibiotics using sophorolipids are revealed through this study. This study also shed some light on the kinetic of the reverse micelle extraction, which will be useful for future design of the separation process. The impacts of different antibiotic structures and characteristics on reverse micelles extraction can be seen through this research. Sophorolipids, which are environmental friendly, will be a good replacement for chemical surfactants to extract antibiotics. This should encourage more studies regarding biosurfactants for extraction of bio-molecules, promoting the development of greener processes. At the same time, it will show the usefulness of palm oil based biosurfactants.

## **1.6 Chapter Summary**

In this chapter, a brief introduction to the study is given. The objectives, scopes, and significance of the research are also presented. Detailed discussion regarding reverse micelle extraction, sophorolipids, antibiotics and downstream processing of antibiotics will be presented in Chapter 2. The materials and equipment, as well as experimental procedures will be given in Chapter 3. Results and analysis will be discussed in Chapter 4, Chapter 5, Chapter 6, and Chapter 7. Lastly, Chapter 8 will end the discussion with conclusions and recommendations.

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