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SEPARATION OF AMOXICILLIN FROM PHARMACEUTICAL WASTEWATER BY SYNERGISTIC EXTRACTION

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A Thesis Submitted in Partial Fulfillment of the Requirements
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ชัยฤกษ์ หอมศิริกมล : การแยกอะม็อกซีซิลลินออกจากน้ำทิ้งทางเภสัชกรรมโดยการสกัดแบบเสริมฤทธิ์ (SEPARATION OF AMOXICILLIN FROM PHARMACEUTICAL WASTEWATER BY SYNERGISTIC EXTRACTION) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.กษิดิศ หนูทอง, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร.นิติ สันแสนดี, 141 หน้า.

การปนเปื้อนของอะม็อกซีซิลลินจากน้ำทิ้งทางเภสัชกรรมลงในแหล่งน้ำก่อให้เกิดผลเสียต่อระบบนิเวศน์และอาจเพิ่มความลำบากในการรักษาการติดเชื้อจากแบคทีเรียในมนุษย์ เยื่อแผ่นเหลวที่พยุงด้วยเส้นใยกลวงเป็นวิธีการที่มีศักยภาพในการใช้งานเพื่อแยกและนำกลับอะม็อกซีซิลลินได้ภายในหน่วยปฏิบัติการเดียว จากสมบัติการแตกตัวของกรดของอะม็อกซีซิลลิน จึงทำการคัดเลือกสารสกัดเพื่อทดลองการสกัดอะม็อกซีซิลลินซึ่งประกอบด้วยไตรออกทิลเมทิลแอมโมเนียมคลอไรด์ (Aliquat 336) กรดไดเ-(2-เอทิลเฮกซิล)-ฟอสฟอริก (D2EHPA) ไตรบิวทิลฟอสเฟต (TBP) และไตรออกทิลเอมีน (Alamine 336) และยังใช้งานสารสกัดผสมสองชนิดระหว่างสารสกัดในข้างต้นเพื่อทดสอบความเป็นไปได้ของการสกัดอะม็อกซีซิลลินแบบเสริมฤทธิ์ ผลการทดลองพบว่าค่าพีเอชเริ่มต้นของสารละลายอะม็อกซีซิลลินเท่ากับ 10 และ การผสมระหว่างสารสกัด Aliquat 336 และ TBP (AqT) ด้วยอัตราส่วนโดยโมลเท่ากับ 10 มิลลิโมลาร์ ต่อ 2 มิลลิโมลาร์ ทำให้ได้การสกัดอะม็อกซีซิลลินแบบเสริมฤทธิ์ที่มีร้อยละการสกัดสูงสุดที่ 90.4 ± 0.39 เปอร์เซ็นต์ ขณะที่สภาวะเหมาะสมของค่าพีเอชและความเข้มข้นของสารละลายโพแทสเซียมคลอไรด์ระหว่างการนำกลับอะม็อกซีซิลลินพบว่ามีค่าเท่ากับ 5 และ 6 มิลลิโมลาร์ การทดลองต่อมาในระบบเยื่อแผ่นเหลวที่พยุงด้วยเส้นใยกลวงโดยใช้สภาวะเหมาะสมที่ได้รับในข้างต้นพบว่าได้รับร้อยละการสกัดและการนำกลับที่สภาวะคงตัวค่าเท่ากับ 31.8 เปอร์เซ็นต์ และ 9.7 เปอร์เซ็นต์ ตามลำดับเมื่อควบคุมอัตราไหลของสารละลายอะม็อกซีซิลลินและโพแทสเซียมคลอไรด์เท่ากับ 62.5 มิลลิลิตรต่อนาที โดยค่าของร้อยละการสกัดและร้อยละการนำกลับที่ค่อนข้างต่ำคาดว่าเป็นผลจากระยะเวลาการสัมผัสเพื่อทำปฏิกิริยาสั้น

ภาควิชา	วิศวกรรมเคมี	ลายมือชื่อนิสิต	.....
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CHAIYARERK HOMSIRIKAMOL: SEPARATION OF AMOXICILLIN FROM PHARMACEUTICAL WASTEWATER BY SYNERGISTIC EXTRACTION. ADVISOR: ASST. PROF. KASIDIT NOOTONG, Ph.D., CO-ADVISOR: NITI SUNSANDEE, D.Eng., 141 pp.

The contamination of amoxicillin from pharmaceutical wastewater into aquatic environment adversely affects ecosystem as well as the increasing the treatment difficulty of bacterial infection in human. Hollow fiber supported liquid membrane (HFSLM) is a promising method for amoxicillin removal and recovery in a single unit. Based on the polyprotic property of amoxicillin, several extractants are selected to perform the reactive liquid-liquid extraction of amoxicillin including trioctylmethylammonium chloride (Aliquat 336), di-(2-ethylhexyl)-phosphoric acid (D2EHPA), tributyl phosphate (TBP), trioctylamine (Alamine 336) as well as the binary mixture of the mentioned extractant as means to achieve synergistic amoxicillin extraction. The results of the experiment indicated that the initial pH of amoxicillin solution should be adjusted to 10. The mixture of Aliquat 336 and TBP (AqT) prepared at the molar ratio of 10:2 provided the synergistic amoxicillin extraction with the maximum extraction percentage of 90.4 ± 0.39% while the optimal pH and concentrations of KCl stripping solution should be maintained at 5 and 6 mM, respectively. The obtained condition was subsequently applied to HFSLM, resulting in the steady state extraction and stripping percentages of 31.8% and 9.7%, respectively, when the flow rates of feed and stripping streams were 62.5 mL/min. Low magnitudes of extraction and stripping percentages were likely related to short contact time.

Department:	Chemical Engineering	Student's Signature .....
Field of Study:	Chemical Engineering	Advisor's Signature .....
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a	=	thermodynamic activity
A	=	the 1st parameter of regression functions
A_{272}	=	absorbance at 272 nm
AmD	=	the mixture of Alamine 336 and D2EHPA
$Amox^+$	=	the protonated form with a positive charge of amoxicillin
Amox	=	the zwitterionic form of amoxicillin
$Amox^-$	=	the deprotonated form with a negative charge of amoxicillin
$Amox^{2-}$	=	the deprotonated form with two negative charges of amoxicillin
[Amox]	=	the final concentration of amoxicillin in aqueous phase (mg/L)
$[Amox]_s$	=	the final concentration of amoxicillin in the stripping aqueous phase (mg/L)
$[Amox]_0$	=	the initial concentration of amoxicillin in the extracted aqueous phase (mg/L)
AmT	=	the mixture of Alamine 336 and TBP
AOT	=	sodium di-2-ethylhexylsulfosuccinate
AqD	=	the mixture of Aliquat 336 and D2EHPA
AqT	=	the mixture of Aliquat 336 and TBP
B	=	the 2nd parameter of regression functions
BLM	=	bulk liquid membrane
BOD	=	biological oxygen demand (mg/L)
C	=	the 3rd parameter of regression functions
COD	=	chemical oxygen demand (mg/L)
D	=	distribution coefficient

DT	=	the mixture of D2EHPA and TBP
D2EHPA	=	di-(2-ethylhexyl)-phosphoric acid
ELM	=	emulsion liquid membrane
Ex	=	extractant
[Ex]	=	concentration of extractant (mM)
HFSLM	=	hollow fiber supported liquid membrane
K_a, pK_a	=	the acid dissociation constant
K_E	=	the equilibrium complexation constant
n	=	the number of extractant taking part in complexation with a mole of amoxicillin
S	=	synergistic coefficient
SE	=	standard error
SLM	=	supported liquid membrane
TBP	=	tributyl phosphate
UV-vis	=	ultraviolet-visible light
X	=	predictors (i.e. point of time)
Y	=	responses (i.e. extraction and stripping percentages)

Greek letter

γ	=	activity coefficient

Superscript

ø	=	referring to the standard state of dilute solution

CHAPTER 1
INTRODUCTION

1.1 Background and rationale

The presence of antibiotics in the wastewater generated from pharmaceutical plants can negatively affect aquatic ecosystem. Prolonged exposure of bacteria to residual antibiotics in wastewater even at small levels can result in the activation of antibiotic resistant genes (Baquero et al., 2008; Wright, 2010) and consequently increases the difficulty or even failed treatment of numerous bacterial infection (Davidson et al., 2002). Some residual antibiotics were toxic towards microalgae and bacteria responsible for the natural bioremediation in aquatic environment as well as causing the loss in aquatic biodiversity (Secondes et al., 2014). Moreover, the treatment efficiency of biological wastewater treatment plant was adversely affected by the presence of antibiotics in incoming wastewater (Gartiser et al., 2007). Based on the reasons described, separation of antibiotics from disposed effluent stream should be intensively considered.

Amoxicillin, a broad-spectrum β-lactam and penicillin-type antibiotic, is widely prescribed for treating bacterial infections. Amoxicillin blocks transpeptidase and transglycosylase enzymes causing the destruction of bacterial peptidoglycan cell wall (Kaur et al., 2011). Plianbangchang et al. (2010) reported that amoxicillin was the second most prescribed medication in Thailand distinct hospitals while Issarachaikul (2013) showed that amoxicillin was the most prescribed antibiotics for upper respiratory tract infection and acute bronchitis in King Chulalongkorn Memorial Hospital, Thailand. The Government Pharmaceutical Organization (GPO) of Thailand supplies amoxicillin in the form of 500 mg capsule with the average production rate of 100,000 capsules per day. After the production process, approximately 60 L of water is used daily for flushing and cleaning of containers and machines, thereby generating wastewater containing amoxicillin, which can trigger

negative impacts on the environment. Therefore, the treatment of pharmaceutical wastewater containing amoxicillin was warrant before discharging the effluent into natural aquatic environment.

Many methods are available for the degradation and separation of amoxicillin from wastewater that include ozonation (Andreozzi et al., 2005), chlorination (Navalon et al., 2008), Fenton and photo-Fenton (Trovo et al., 2008; Elmolla and Chaudhuri, 2009), adsorption (Homem et al., 2010; Moussavi et al., 2013), semiconductor photocatalysis (Klauson et al., 2010) and hollow fiber supported liquid membrane (HFSLM) (Pirom et al., 2014). Ozonation, chlorination, Fenton/photo-Fenton and semiconductor photocatalysis utilize the oxidizing properties of ozone (O_3), chlorinated species, hydrogen peroxide (H_2O_2) and water splitting-generated hydroxyl radical ($OH^{\cdot}$) to decompose amoxicillin molecules. However, these methods suffer from high toxicity of generated byproducts as well as inefficient performance when applying to wastewater containing high organic contents (Homem and Santos, 2011). Adsorption can overcome the limitation of ozonation and chlorination but involves the regeneration of solid adsorbents as well as proper disposal of solid residues that incurred addition expense (Homem and Santos, 2011).

Hollow fiber supported liquid membrane (HFSLM) has emerged as a promising method for amoxicillin removal from wastewater as well as the recovery of dilute compounds from upstream process. The advantages associated with HFSLM include low energy consumption, less organic solvent used as compared to the conventional liquid-liquid extraction and the complete operation in single-stage configuration (Kislik et al., 2010). HFSLM is a separation system containing three phases: feed, stripping and membrane. Feed and stripping phases are usually aqueous solutions while membrane phase is composed of an organic solution embedded in the pores of hydrophobic hollow fiber polymeric tubes (Schulz, 1988). The organic solution is a mixture of one or more extractants and diluent. Extractants are the carriers responsible for the transport of specific solutes (i.e., amoxicillin) from the feed phase to the stripping phase via chemical complexing reaction at the interfacial surface

(Kocherginsky et al., 2007). Fig. 1.1 displays the schematic diagram of the principle of separation in HFSLM.

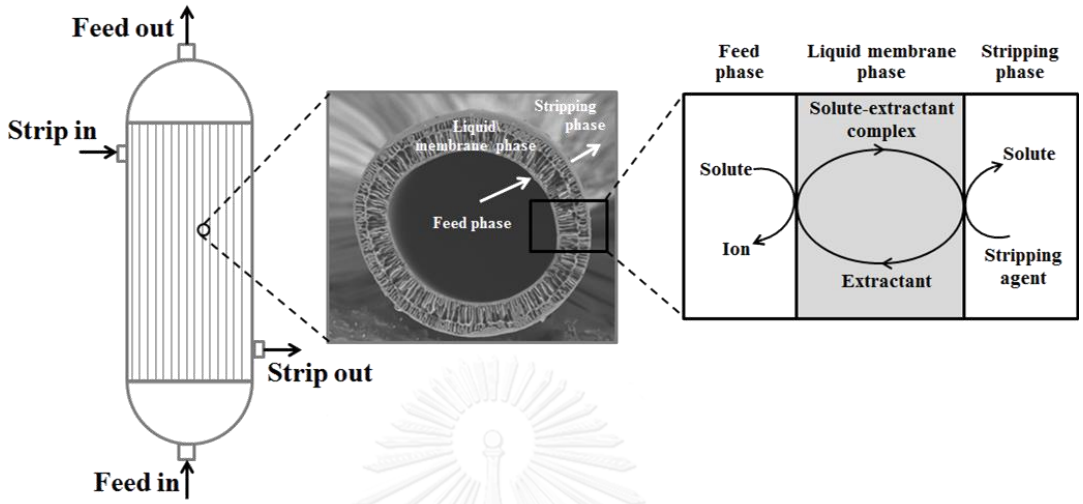

Figure 1.1 The principle of separation in HFSLM (Wannachod et al., 2014)

In the case of amoxicillin separation, the extraction and stripping efficiency of HFSLM are related to several factors such as types of extractant and stripping solution, initial pH of amoxicillin aqueous phase as well as flow rates of feed and stripping solution. Pirom et al. (2014) reported that trialkylmethylammonium chloride (Aliquat 336) at 6 mmol/L was able to dissolve in 1-decanol and be used during the extraction of amoxicillin in HFSLM, resulting in the maximum extraction efficiency of 85.21% when the initial pH of amoxicillin feed and flow rate were maintained at 8.0 and 100 mL/min, respectively. Strong electrostatic interaction between negative charge of amoxicillin and positive charge of Aliquat 336 promoted the performance of extraction.

It should also be pointed out that amoxicillin is a polyprotic compound possessing three acid dissociation constants, namely pKa_1 = 2.68 at the carboxyl group, pKa_2 = 7.49 at the amine group and pKa_3 = 9.63 at the phenol group (Andreozzi et al., 2005). Due to different charged properties of amoxicillin at different pH values, the idea of

applying other types of extractant, such as those acidic and neutral extractants, in addition to the conventional Aliquat 336 which is a basic-typed extractant should be explored. Based on our literature review, the use of other extractants containing negative or neutral charges as well as the influence of operating conditions of HFSLM on the ability to separate amoxicillin from pharmaceutical wastewater remained limited. Among commercial extractants besides Aliquat 336, di-(2-ethylhexyl)-phosphoric acid (D2EHPA), tributyl phosphate (TBP) and Alamine 336 are of our interest. These commercial extractants are typically employed in hydrometallurgy, biotechnology and wastewater treatment (Wasewar et al., 2002; Suren et al., 2012). D2EHPA, an organophosphoric acid, is an acidic carrier capable of extracting organic compounds containing amine group (Galaction et al., 2008). The amine group of amoxicillin molecule can be protonated into ammonium group ($-NH_3^+$) at specific pH range in order to attract with protonated D2EHPA. TBP is a neutral phosphorous-bonded oxygen-composing extractant, which was reported to be efficient for carboxylic acid extraction (Mei et al., 2002). Alamine 336, a mixture of tertiary amines, can also extract organic acids via acid-amine ion pair formation and acid-acid complexing reaction (Hong et al., 2001). Based on their properties, TBP and Alamine 336 are conceivable to separate amoxicillin, which consists of a carboxyl group. Apart from the single extractant systems, the use of binary extractant mixture was shown to improve the extraction efficiency relative to using the individual extractant, the effect known as synergistic extraction (Kislik, 2012). There also appears to be a lack of studies on the synergistic amoxicillin extraction, which may provide new type of extractants to improve amoxicillin removal efficiency from aqueous solution.

Based on the reasons mentioned, this research intends to study the separation of amoxicillin from pharmaceutical wastewater by using different extractants including Aliquat 336, D2EHPA, TBP, Alamine 336 and the binary combination of those mentioned extractants. Additional information to be used during the HFSLM operation including amoxicillin feed pH and flow rates will be presented.

1.2 Objectives

1.2.1 To determine the feasibility of Aliquat 336, D2EPHA, TBP and Alamine 336 as well as the binary system of the mentioned extractants in separating amoxicillin from pharmaceutical wastewater.

1.2.2 To demonstrate the separation of amoxicillin from pharmaceutical wastewater by employing HFSLM

1.3 Scopes of study

This study can be broadly classified into three main sections. The first section involves the reactive liquid-liquid extraction of amoxicillin with single extractant systems. The second section focuses on synergistic extraction of amoxicillin by using the binary extractant mixture considered in the first section. The results obtained from the previous sections are used in the final section, which involves operation of HFSLM. For all sections, synthetic pharmaceutical wastewater containing 1.37 mM (500 mg/L) of amoxicillin is used. Independent variables of each section are specified as follows (Fig. 1.2):

1.3.1 Reactive liquid-liquid extraction of amoxicillin with single extractant systems

- Four extractants are used including D2EHPA, TBP, Aliquat 336 and Alamine 336.
- Initial pHs of amoxicillin solution are varied from 2 to 12 while the concentrations of extractants are varied from 2 to 15 mM.
- KCl was selected as stripping solution
- The pH of stripping solution varied from 4 to 7 while the concentrations ranged from 2 to 10 mM.

1.3.2 Synergistic extraction of amoxicillin

- The following combinations of extractants are used: Aliquat 336 and D2EHPA, Aliquat 336 and TBP, D2EHPA and TBP, Alamine 336 and TBP, and Alamine 336 and D2EHPA
- The molar following molar ratios are used to test for synergistic effect for the mentioned binary system: 1:3, 1:1, 3:1 and 5:1.
- The pH and concentrations of KCl stripping solution ranged from 5 to 7 and from 2 to 10 mM, respectively.

1.3.3 Operation of HFSLM

- Volumetric flow rates of feed and stripping solution were identical varying from 62.5 to 200 mL/min.
- HFSLM was operated in the countercurrent once-through mode.

1.4 Expected benefits

1.4.1 Optimal conditions for extraction and recovery of amoxicillin from pharmaceutical wastewater will be obtained.

1.4.2 Use of HFSLM complies with an increasing awareness of sustainable and environmental friendly production. In this case, the application of HFSLM reduces the amount of amoxicillin discharge into aquatic environment and consequently lowers the risk of promoting amoxicillin resistant genes in bacteria.

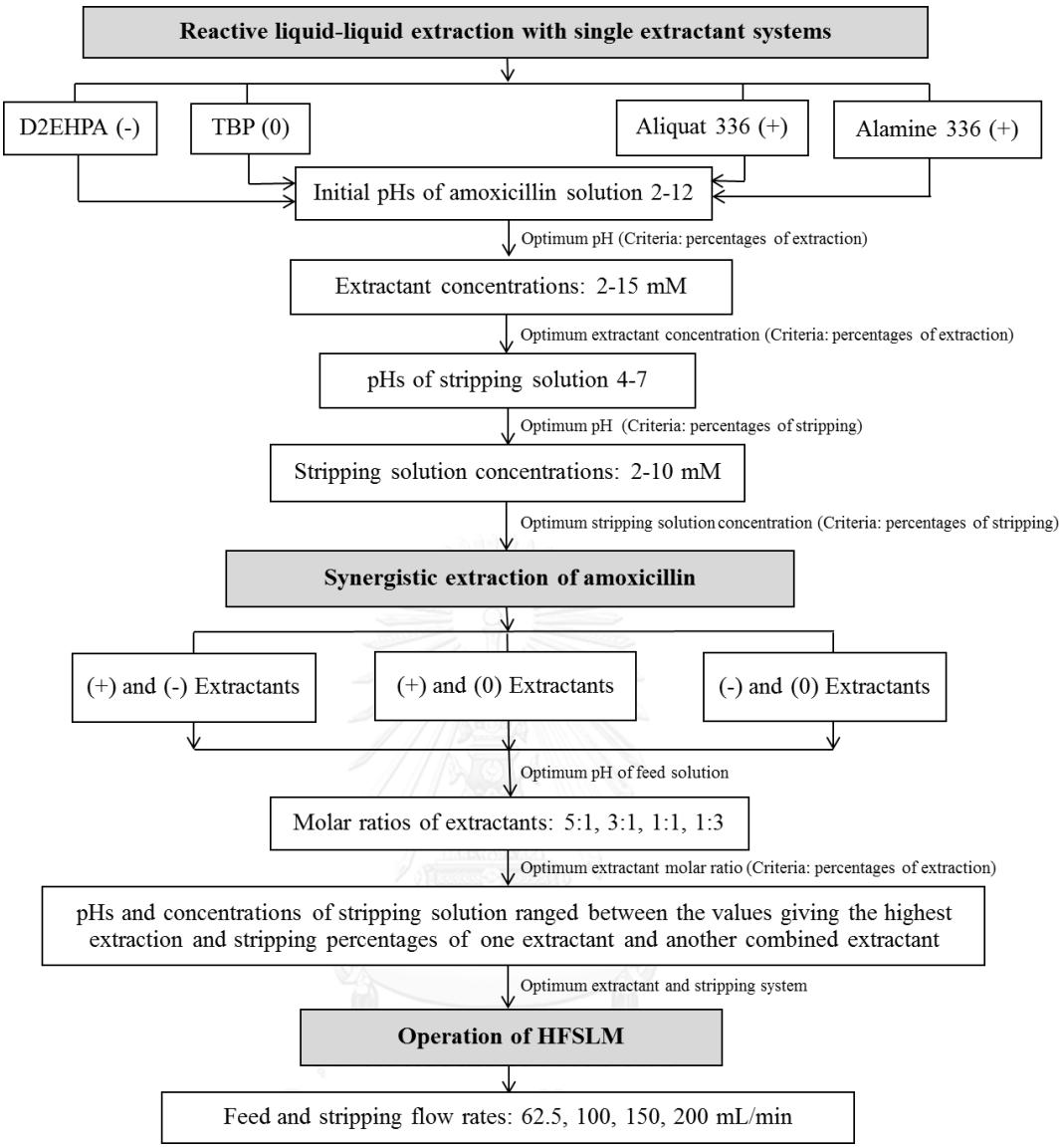

Figure 1.2 Conceptual framework of this research

CHAPTER 2
LITERATURE REVIEW

This chapter reviews the literatures and theories relating the extraction of amoxicillin and the hollow fiber supported liquid membrane (HFSLM) that may be useful in understanding this thesis. Specifically, this chapter covers the following topics: (1) general information of amoxicillin (2) removal methods of antibiotics and amoxicillin from the aquatic environment (3) principle of hollow fiber supported liquid membrane (4) properties and types of potential extractants to be employed in this research and (5) reviews of the separation of amoxicillin by HFSLM.

2.1 Amoxicillin

Amoxicillin ($C_{16}H_{19}N_3O_5S$) is classified as an antibiotic in the family of β-lactam, which includes penicillin derivatives, monobactams, carbapenems and cephalosporins. The general structures of β-lactam and amoxicillin are depicted in Fig. 2.1. Amoxicillin and other β-lactam antibiotics prevent the synthesis of peptidoglycan layer of bacterial cell wall by triggering the autolytic hydrolase to digest peptidoglycan (Kaur et al., 2011). Amoxicillin is a white or almost white powder with a smell of sulfur and is generally prescribed to treat the infections of ear, nose, throat, genitourinary tract, skin and lower respiratory tract. The amoxicillin possesses three acid dissociation constants namely pKa_1 = 2.68 at carboxyl group, pKa_2 = 7.49 at amine group and pKa_3 = 9.63 at phenol group (Andreozzi et al., 2005). The total charge of amoxicillin varies along the pH range as shown in Fig. 2.2

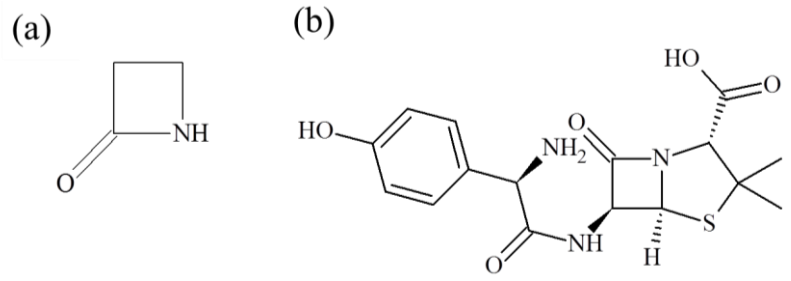

Figure 2.1 The chemical structure of (a) β-lactam ring and (b) an amoxicillin

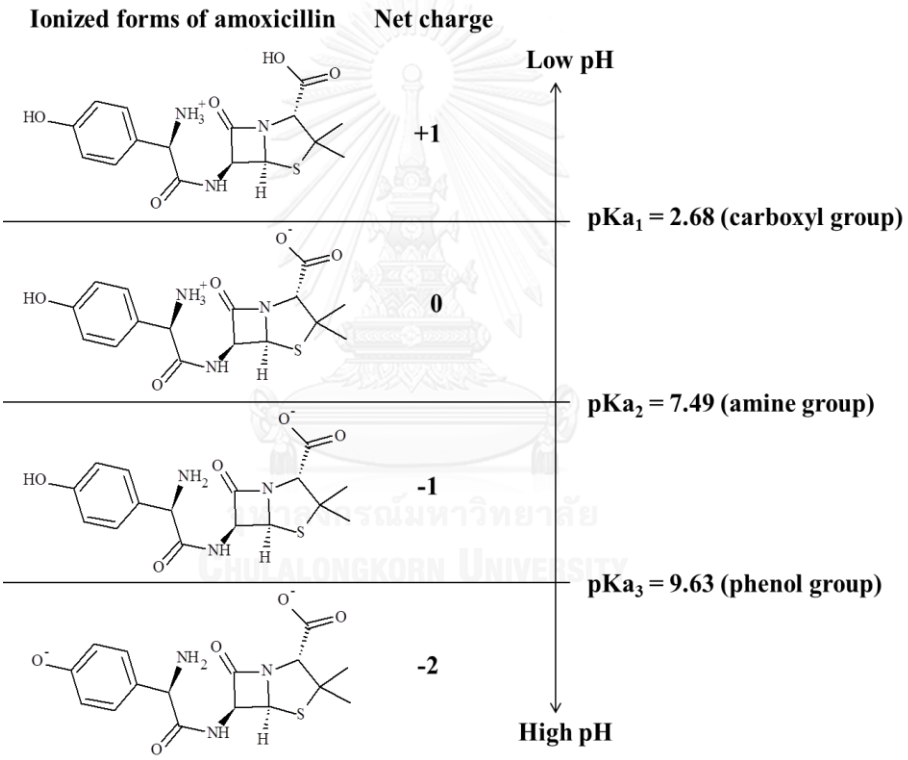

Figure 2.2 The total charge of the ionized amoxicillin along the pH scale

The presence of amoxicillin into aquatic environment was able to induce antibiotic resistant genes that consequently results in more treatment difficulty (Baquero et al., 2008). Contamination of amoxicillin can cause biodiversity loss and adversely affected the performance of biological wastewater treatment plants (Secondes et al., 2014). Pan et al. (2008) also reported that contaminated amoxicillin suppressed the

photosynthetic pathways of microalgal *Synechocystis sp*. by delaying electron transport on donor side and acceptor side. Similar observation was noted for other microalgal species (Park and Choi, 2008).

2.2 Removal methods of antibiotics from aquatic environment

There are several methods to degrade or remove antibiotics from aqueous solution. Conventional wastewater treatments such as biological processes, filtration, coagulation, flocculation and sedimentation have critical limitations to treat antibiotic contaminated wastewater. Antibiotics are toxic to microorganisms, thus resulting in low removal efficiency of activated sludge technology or biological systems (Secondes et al., 2014). Past researches reported that filtration, coagulation, flocculation and sedimentation gave the maximum removal efficiency around 30% (Adams et al., 2002; Stackelberg et al., 2007; Vieno et al., 2007). Thus, alternative processes have been developed as summarized in Table 2.1.

Table 2.1 Methods for antibiotic removal and degradation from aquatic environment

Method	Principle	Pros-Cons	References
Chlorination	Antibiotics are oxidized by chlorinated species such as hypochlorite, chlorine gas and chlorine dioxide. Oxidized antibiotics are biodegradable and ready for further biological treatments. Chlorine dioxide is most preferred due to its selectivity with pollutants via one-electron exchange reaction	*Pros*: High removal efficiency; Low cost of reagents; Suppress the presence to trihalomethanes and holoacetic acids, which are carcinogens. *Cons*: Low efficiency for the systems with high loads of organic substances; formation of carcinogen	Navalon et al., 2008; Sharma, 2008

Table 2.1 Methods for antibiotic removal and degradation from aquatic environment (continued)

Method	Principle	Pros-Cons	References
Reverse osmosis, nano and ultrafiltration	Selective semipermeable membrane retains antibiotics in the retentate. Pressurized in the membrane module, components in wastewater selectively pass solid membrane by diffusion transport. Performance greatly depends on the properties of membrane.	*Pros*: High energy efficiency; Ability to recover high value antibiotics *Cons*: Fouling and degradation of membranes; New contaminated solid residue	Benitez et al., 2011; Shahtalebi and Sarrafzadeh, 2011
Ozonation	Ozone can directly react with nucleophilic molecules or indirectly form hydroxyl radicals by degradation in water $O_3+OH^- \rightarrow O_2+HO_2^-$ $O_3+HO_2^- \rightarrow HO_2+O_3$ $HO_2 \rightarrow H^+ + O_2^-$ $O_2^- + O_3 \rightarrow O_2 + O_3^-$ $O_3^- + H^+ \rightarrow HO_3^-$ $HO_3^- \rightarrow OH^- + O_2$ Combination of ozone with UV irradiation, hydrogen peroxide or catalysts could promote the degradation efficiency and allow this process to be applied to cloudy discharge	*Pros*: Suitable for the systems with fluctuating composition and/or flow rates; High degradation efficiency *Cons*: High cost of maintenance; High energy consumption; Mass transfer limitation	Stockinger et al., 1995; Andreozzi et al., 2005

Table 2.1 Methods for antibiotic removal and degradation from aquatic environment (continued)

Method	Principle	Pros-Cons	Referenc-es
Adsorption	Antibiotics in fluid phase adhere to a solid adsorbent. Physical adsorption involves van der Waals force while chemical adsorption relates to covalent bonding. Efficiency depends properties of adsorbent and antibiotics of interest.	*Pros*: No generation of harmful metabolites; Suitable for the systems with high loads of antibiotics *Cons*: Generate new solid residue; Lack of study of continuous systems	Putra et al., 2009; Homem and Santos, 2011; Moussav-i et al., 2013
Fenton and photo-Fenton	A mixture of hydrogen peroxide and ferrous ion is utilized as a strong oxidizing reagent. Reaction mechanisms are: $Fe^{2+}+H_2O_2 \rightarrow Fe^{3+}+OH^-+OH^{\cdot}$ $Fe^{3+}+H_2O_2 \leftrightarrow H^+ + Fe(HO_2)^{2+}$ $Fe(HO_2)^{2+} \rightarrow Fe^{2+}+HO_2^{\cdot}$ $Fe(HO_2)^{2+}+H_2O_2 \rightarrow Fe(OH)(HO_2)^{+}+H^+$ $Fe(OH)(HO_2)^{+} \rightarrow Fe^{2+}+HO_2^{\cdot}+OH^-$ $OH^{\cdot}$+organic substance→ H_2O +degradation products → CO_2+H_2O Similar to ozonation, UV radiation-photo-Fenton has higher degradation efficiency owing to the extra production of hydroxyl radicals.	*Pros*: Low cost of reagents; Environmental-ly safe. *Cons*: Low efficiency for systems with high loads of organic substances and ions (Cl^-, NO_3^-, CO_3^{2-} and HCO_3^-); Strong dependence on pH.	Arslan-Alaton and Gurses, 2004; Britto et al., 2008

Table 2.1 Methods for antibiotic removal and degradation from aquatic environment (continued)

Method	Principle	Pros-Cons	References
Semiconductor photocatalysis	A semiconductor is activated by natural or artificial light to transfer electrons from valence to conduction band and permit holes to occur. The holes possess high oxidation potential leading to generation of hydroxyl radicals from water molecules. These radicals subsequently oxidize organic compounds. The organic compounds, adsorbed on the semiconductor surface, may be directly oxidized by electron transfer.	*Pros*: Ambient conditions; High removal efficiency and mineralization *Cons*: Low efficiency for the systems with high loads of organic substances: Difficulty to separate the catalyst	Elmolla and Chaudhuri, 2010a, 2010b
Photolysis	Organic compounds are forced to dissociate by using natural or artificial light. Light can directly attack aqueous organic compounds or indirectly induce the generation of radicals to oxidize substances. There are many factors influencing the performance of photolysis, for example the absorption spectrum of the target, radiation intensity, target medium and concentration of radical origins.	*Pros*: Suitable for the systems composing of photo-sensitive compounds *Cons*: Low efficiency for the systems with high loads of organic substances; Strong dependence on chemical structures of target compounds; and formation of toxic intermediates	Arslan-Alaton and Dogruel, 2004

Table 2.1 Methods for antibiotic removal and degradation from aquatic environment (continued)

Method	Principle	Pros-Cons	References
Electrochemic-al	Organic compounds undergo a direct anodic oxidation when they are adsorbed on the anode surface with the existence of an electrolyte. The occurring electron transfer between antibiotics and the electrode causes the presence of electroactive species, which indirectly oxidize pollutants in the bulk liquid.	*Pros*: Clean technology: Suitable for the systems with high loads of antibiotics *Cons*: High operating cost; Limited knowledge of the process	Hirose et al., 2005
Liquid Membrane	Antibiotics are transferred from wastewater to stripping solution by chemical reaction with an extractant dissolved in a diluent. The extractant dissolved in diluent acts as a liquid membrane which selectively allows extractant-reacting species to pass through and isolates two aqueous phases.	*Pros*: Ability to recover high value antibiotics; Energy efficiency; Able to separate antibiotics at dilute levels. *Cons*: Fouling and instability of liquid membrane	Ghosh et al., 1995; Sahoo et al., 1999; Vilt and Ho, 2010; Pirom et al., 2014

2.3 Removal methods of amoxicillin from aquatic environment

Removal of amoxicillin can be accomplished by using methods listed in the previous section. Table 2.2 displays the literature review of amoxicillin removal from aquatic environment.

Table 2.2 Literature review on methods for amoxicillin removal from aquatic environment

Author	Method	Findings
Elmolla and Chaudhuri, 2009	Fenton	• Complete degradation of amoxicillin was achieved under optimal conditions, molar ration of COD/H_2O_2/Fe^{2+} = 1:3:0.30 and pH 3, in 2 minutes when initial concentration of amoxicillin was maintained at 104 mg/L • Biodegradability (BOD_5/COD ratio) increased from about 0 to 0.37 after 60 minutes of the treatment. Moreover, COD and DOC degradation of 81.4% and 54.3% were obtained respectively. • Fenton treatment caused mineralization indicated by increasing ammonia (from 8 to 13 mg/L) and nitrate (from 0.3 to 10 mg/L) within 60 minutes.
Trovó et al., 2008	Photo-Fenton	• The amoxicillin degradation was not strongly influenced by the type of irradiation and sources, i.e. UV light and solar irradiation. • Ferrioxalate or $Fe(NO)_3$ significantly enhanced removal efficiency. • Under the optimal conditions including pH 2.5, initial concentrations of amoxicillin, ferrioxalate and hydrogenperoxide at 42 mg/L, 0.20 mM and 2.0 mM, respectively, about 90% of amoxicillin oxidation was accomplished after 1-minute irradiation. • After 10 minutes of irradiation, antibiotics were completely degraded.
Klauson et al., 2010	Semiconductor Photocatalysis	• Natural solar radiation accelerated amoxicillin degradation about three times compared to artificial UV light. • The maximum removal percentage of 85% was accomplished using pH 6, TiO_2 doped with the C atomic percentage at 37% under solar light for 2 hours. • COD removal and the formation of NO_3^-, NH_3 and SO_4^{2-} during the degradation indicated mineralization.

Table 2.2 Literature review on methods for amoxicillin removal from aquatic environment (continued)

Author	Method	Findings
Su et al., 2012	Advanced oxidation process	• The system of oxone/Co^{2+}/Ultrasound gave the best removal efficiency of amoxicillin and the lowest activated energy where the oxone is $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$. • 98.7% removal of COD was reached after 60 minutes of reaction time at 24°C using 0.095 mM of amoxicillin, 5 mM of oxone, 0.025 mM of Co^{2+} and 200 W of 20 Hz ultrasound as the optimum conditions. • The degradation data of amoxicillin using oxone/Co^{2+}/Ultrasound conformed to the first-order kinetic model.
Moussavi et al., 2013	Adsorption	• At equilibrium (after mixing at 25°C for 6 hours), adsorption efficiency of > 99% was obtained using 0.4 g /L of NH_4Cl-induced activated carbon and maintaining pH at 6. • Pseudo-second-order model properly correlated to the kinetic analysis of amoxicillin adsorption onto NH_4Cl-induced activated carbon. • Under the equilibrium condition, standard and NH_4Cl-induced activated carbon can adsorb amoxicillin at the maximum capacity of 262 and 437 mg/g, respectively.
Derakhsheshpor et al., 2013	Nanofiltration	• Longer UV irradiation time during the synthesis of polysulfone membrane led to the smaller surface pore size. • The decrease of coagulation bath temperature and the addition of higher molecular weight of poly(ethylene glycol) increased flux and amoxicillin separation through the membrane. • Membrane synthesis under strong basic condition caused electrostatic repulsion between solute and membrane and corresponding increased amoxicillin permeability.

Table 2.2 Literature review on methods for amoxicillin removal from aquatic environment (continued)

Author	Method	Findings
Chuo et al., 2014	Extraction using mixed reverse micelles	• During forward extraction, the optimum conditions were 5.5:1 molar ratio of AOT/Tween 85, 102.57 g/L of total surfactant concentration, pH 1.90 and 8.54 g/L of KCl which resulted in 95.54% of extraction efficiency. • During backward extraction, the optimum conditions were stripping pH 6.58, 15 minutes of extraction time and 11.02 g/L of KCl providing 90.79% recovery of amoxicillin. • Forward extraction required less amount of surfactant when mixed reverse micelles were used and Tween 85 conserved the antibiotic activity of amoxicillin.
Pirom et al., 2014	Hollow fiber supported liquid membrane	• Under the optimum conditions (pH 8, 6 mM of amoxicillin, 6 mM of NaCl, 6 mM of Aliquat 336 and 100 mL/min of flow rate), extraction and recovery percentages of amoxicillin reached 85.21% and 80.34%, respectively. • The aqueous-phase mass transfer coefficient and organic-phase mass transfer coefficient were found 3.57×10^2 and 0.70×10^2 cm/s, respectively. • The correlation between the developed diffusion flux model and experimental data was satisfactory.

Note: COD is Chemical Oxygen Demand (mg/L), BOD is Biological Oxygen Demand (mg/L), AOT = sodium di-2-ethylhexylsulfosuccinate, and UV = Ultraviolet

2.4 Liquid membrane

Liquid membrane system composes of three phases: (1) feed phase which is a solution of specific solute to be separated; (2) liquid membrane phase which is the solution of extractant; and (3) stripping phase which is the solution of stripping agent used for

recovery of solutes from the liquid membrane phase. The liquid membrane phase is almost immiscible with feed and stripping phases, thus the feed and stripping phases are separated with the liquid membrane phase located between them. At the interfacial surface of feed and liquid membrane phases, a specific solute reacts with an extractant and solute-extractant complexes form inside the edge of the liquid membrane phase. Formed solute-extractant complexes diffuse through the liquid membrane phase to the interfacial surface near the stripping phase based on concentration gradients. A stripping agent reacts with the complexes and transfers the solute to the stripping phase. This basic principle is identical to the reactive extraction but liquid membrane process is non-equilibrium and provides two reactive extractions in a single unit of operation. Liquid membrane system can be divided into three types as shown in Fig. 2.3.

2.4.1 Supported liquid membrane (SLM)

SLM employs the polymeric porous supports for imbedding the liquid membrane phase inside pores by capillary force (Schulz, 1988). The interaction between the liquid membrane phase and porous supports makes liquid membrane stable and fixed. There are two types of supports classified based on water affinity: hydrophilic and hydrophobic. Hydrophilic support is able to load the aqueous liquid membrane phase inside while hydrophobic support, which is most used, sinks the organic liquid membrane phase and hardly bind any aqueous solutions. Polymeric porous supports can be fabricated into various shapes, for example, flat sheet, spiral-wound and hollow fiber. Each shape provides different properties and applications represented in Table 2.3.

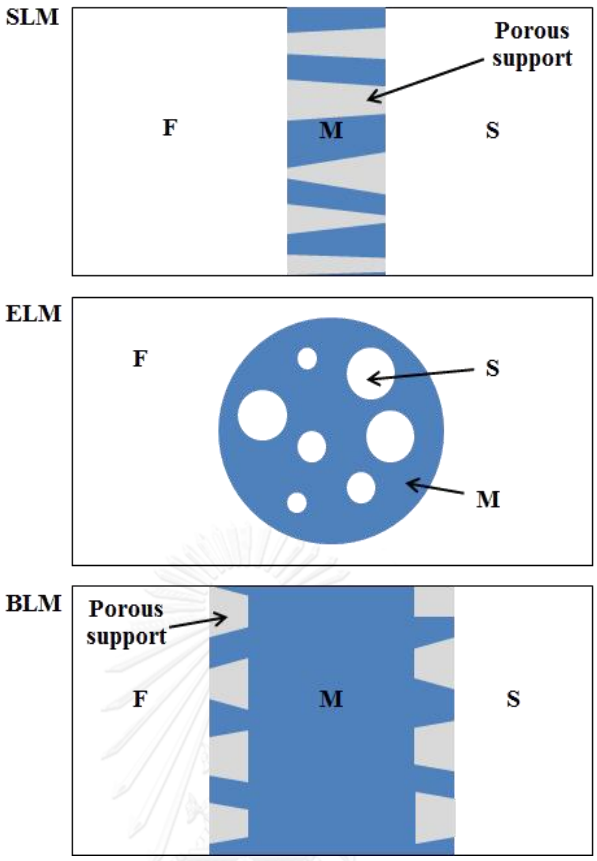

Figure 2.3 Liquid membrane systems: supported liquid membrane (SLM), emulsion liquid membrane (ELM) and bulk liquid membrane (BLM). F is the feed phase, M is the liquid membrane phase and S is the stripping phase
(Kislik, 2010)

Table 2.3 Comparison between different polymeric porous supports (Lothongkum et al., 2011)

Properties	Flat sheet	Spiral wound	Hollow fibers
Manufacturing cost	High	High	Moderate
Resistance to fouling	Good	Moderate	Poor
Parasitic pressure drop	Low	Moderate	High

Table 2.3 Comparison between different polymeric porous supports (continued)

Properties	Flat sheet	Spiral wound	Hollow fibers
High pressure operation	Difficult	Yes	Yes
Limit to specific membranes	No	No	Yes

Hollow-fiber supported liquid membrane (HFSLM) module as shown in Fig. 2.4 was applied in this work. HFSLM consists of paralleled hollow fibers loaded in a cylinder module. Inside and outside of hollow fibers are referred to as the tube and shell sides, respectively. In this research, the feed phase was fed into tube side and the stripping phase was fed into shell side under countercurrent flow condition. The HFSLM has many advantages including high rate of mass transfer per unit volume, high selectivity, ability to separate solute from very dilute solution, high volume ratio of feed solution to stripping solution, ability to apply with suspension, low fixed and maintenance cost, ease of scaling up and adaptability to different solutes, extractants and stripping agents (Parhi, 2002; Prakorn and Ura, 2003).

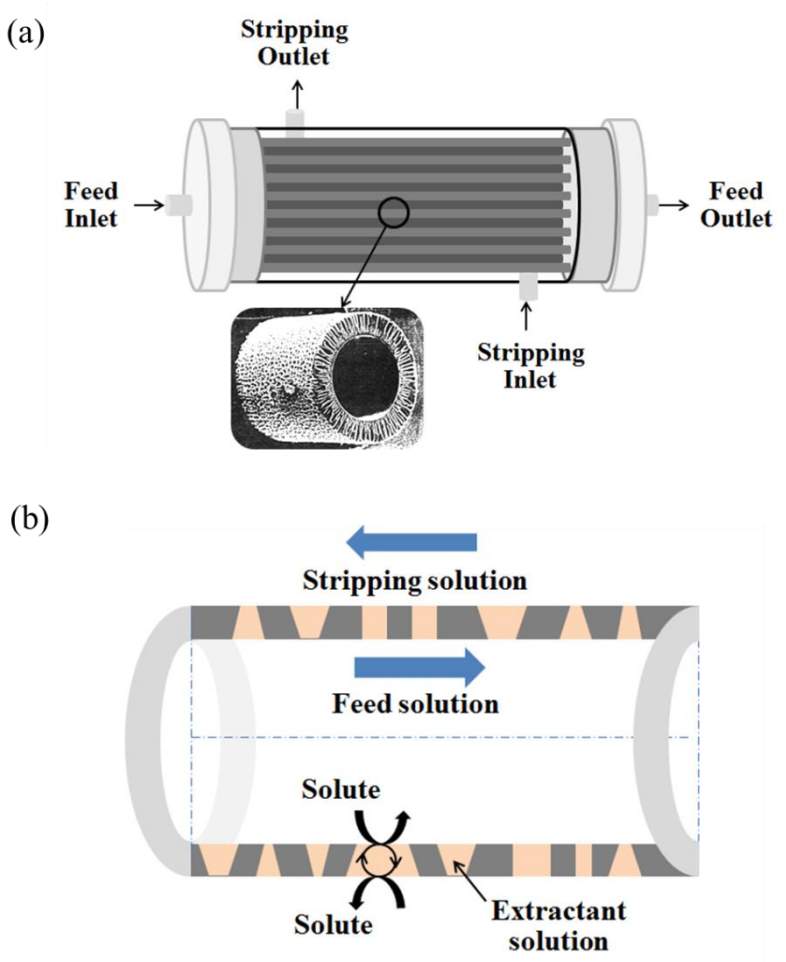

Figure 2.4 (a) HFSLM module (b) countercurrent flow configuration of HFSLM (Prakorn and Ura, 2003)

2.4.2 Emulsion liquid membrane (ELM)

The liquid membrane system becomes emulsion in ELM. Feed solution is a continuous phase and extractant solution or liquid membrane phase is a dispersion phase, which encapsulates stripping solution (Fig. 2.3). To prepare ELM, stripping solution is added to a mixture of an extractant and a surfactant. After homogenizing, the first prepared emulsion is then poured into feed solution causing the occurrence of double emulsion. There are two types of double emulsion applied to ELM: water in oil in water (w/o/w) and oil in water in oil (o/w/o). Both types of double emulsion

needs two kinds of surfactants, hydrophilic and hydrophobic surfactants, in order to stabilize a drop of aqueous and organic solutions.

2.4.3 Bulk liquid membrane (BLM)

BLM contains three phases of bulk solution (Fig. 2.3). Feed phase and stripping phases are isolated by bulk liquid membrane phase or may be additionally separated by liquid membrane-embedding porous supports. Because of the inefficiency of the conventional BLM, hybrid liquid membrane (HLM), flowing liquid membrane (FLM), membrane contactor systems and multimembrane hybrid system (MHS) are being developed to overcome drawbacks of the conventional BLM technology (Kislik et al., 2010).

2.5 Extractant types

Extractants or carriers can be divided into three types based on their functional groups: (1) acidic extractant (-COOH, =POOH,-SO_3H and chelating groups) (2) basic extractant (-NH_2, -NRH, -NR_2 and R_4N^+) and (3) Neutral or solvating extractant ($PO(OR)_3$ and R_4PO).

Acidic extractants effectively form complexes with cations while basic extractants strongly bind to anions. Chelating groups including β-diketones, hydroxyoximes, 8-hydroxyquinolones and alkylphosphorous compounds are remarkably selective extractants able to improve the selectivity of liquid membrane systems. A problem of using basic extractants is the presence of the third phase caused by the association of amines according to Eq. (2.1). To deal with this problem, a modifier, such as long-chain aliphatic alcohol is added into the liquid membrane phase (Tavlarides et al., 1987). For the neutral or solvating extractant, negative polarity of molecules plays an important role in extracting cations although there is no negative charge.

$$R_3N^+HA^- + R_3N^+HA^- \leftrightarrow (R_3N^+HA^-)_2 + R_3N^+HA^- \leftrightarrow (R_3N^+HA^-)_n \qquad (2.1)$$

Some commercial extractants are listed in Table 2.4 and chemical structures of four extractants (i.e. Aliquat 336, Alamine 336, D2EHPA and TBP) used in this work are illustrated in Fig. 2.5.

Table 2.4 Examples of commercial extractants

Extractant type	Example	Functional group
Acidic	D2EHPA	-POOH
	Crown ethers	-O-
	Neodecanoic acid	-COOH
	Cyanex 272	-POOH
	LIX 63	Hydroxyoxime
	Kelex-100	Hydroxyquinolone
Basic	Aliquat 336	R_4N^+
	Alamine 336	$-NR_2$
	Amberlite LA-2	-NRH
	Primene JMT	$-NH_2$
Neutral or solvating	Tributyl phosphate (TBP)	$PO(OR)_3$
	Tri-n-octylphosphine oxide (TOPO)	R_4PO

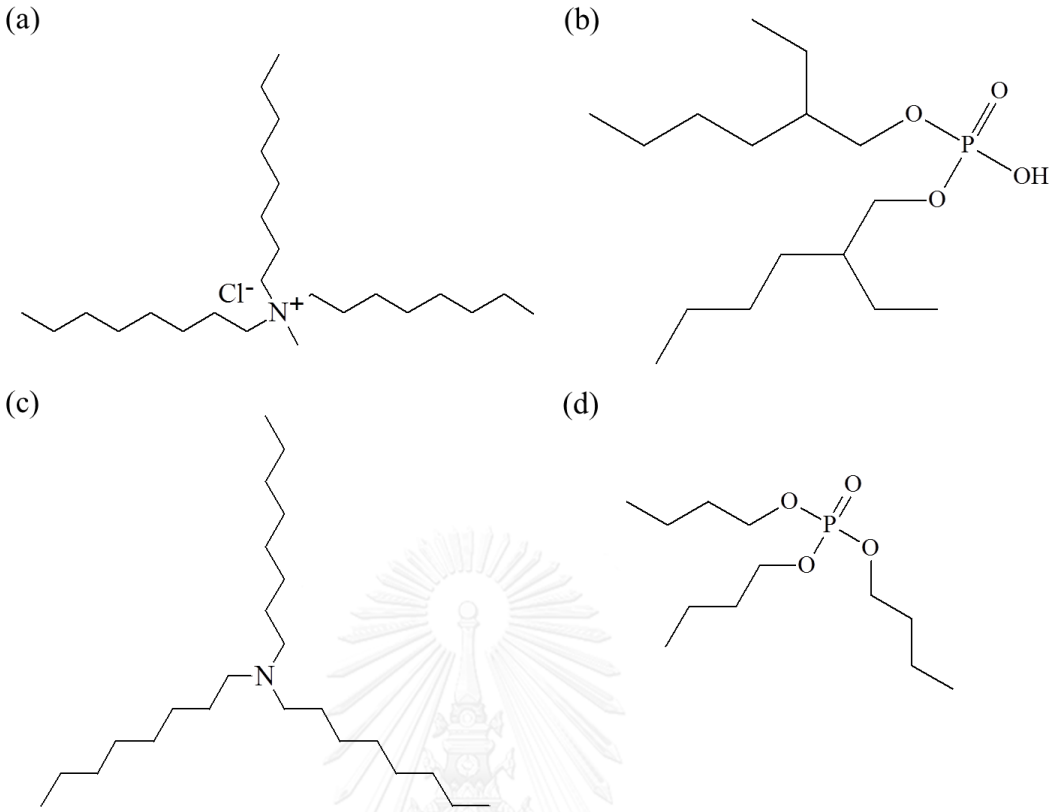

Figure 2.5 Chemical structures of extractant used in this research: (a) Aliquat 336 (b) Alamine 336, (c) D2EHPA and (d) TBP

2.5.1 Aliquat 336

Aliquat 336 or a mixture of trioctyl- and decyl-ammonium chloride is yellowish viscous liquid insoluble in water. Aliquat 336 has a permanent positive charge and able to form complexes with anions over a wider range of pH than primary, secondary and tertiary amine and yet does not deprotonate that leads to the difficulty of stripping compared to other amine reagents (Saeed et al., 2009). As a basic extractant, the extraction occurs through the ion-pair formation. The applications of aliquat 336 are listed as follows:

- Extraction of vanadium, chromium, rare earth, rhenium, arsenic, tungsten, cadmium, zinc, cobalt, gold and copper (Fontàs et al., 1999; Wassink et al., 2000; El-Nadi et al., 2009; Stojanovic et al., 2011)
- Synergistic extraction of zirconium and hafnium with TBP (Wang et al., 2014)
- Extraction of organic acids such as itaconic, propionic, acrylic, butyric, lactic and 6-aminopenicillanic acids (Yang et al., 1991; Bora et al., 1997; Keshav et al., 2008)
- Removal of phenol from waste stream (Rao et al., 2009)
- Phase transfer catalysis for etherification and esterification reactions (Yang and Lin, 2003)

2.5.2 Alamine 336

Alamine reagents compose of a basic nitrogen potentially forming amine salts with different kinds of inorganic and organic matters. Eq. (2.2) and (2.3) represent the extraction reactions of alamine series where R_3N is a tertiary amine and R is an alkyl group (Saeed et al., 2009).

$$[R_3N]_{org}+[HA]_{aq}\leftrightarrow[R_3H^+A^-]_{org} \quad (2.2)$$

$$[R_3H^+A^-]_{org}+[B^-]_{aq}\leftrightarrow[R_3H^+B^-]_{org}+[BA^-]_{aq} \quad (2.3)$$

Alamine 336, a water insoluble trioctylamine, is tertiary amine capable of being stripped by a wide variety of stripping agents such as NaCl, Na_2CO_3 and $(NH_4)_2SO_4$ or inorganic salts deprotonating the amine. Alamine 336 has been effectively applied in several fields:

- Separation of vanadium, cobalt, nickel, chromium, iron, uranium, platinum, tungsten, copper and molybdenum (Coca et al., 1990; Marchese et al., 1995; Kumar et al., 2010; Pim et al., 2014)

- Extraction of organic acids such as gluconic, citric, succinic and lactic acids (Juang and Huang, 1996; Inci, 2002; Wasewar et al., 2002)
- Recovery of mineral acids from process discharge (Eyal and Canari, 1995)

2.5.3 Di-(2-ethylhexyl)-phosphoric acid (D2EHPA)

D2EHPA is an organophosphorous compound, which can deprotonate to form anion depending on pH of the interfacial aqueous phase. D2EHPA appears as the hydrogen-bonded dimer in the organic phase. The extraction by using D2EHPA facilitates co-ordination with non-deprotonated solutes which require low-pH environment to avoid deprotonation while the presence of concentrated hydrogen ion reduces the extraction efficiency of D2EHPA. Thus, to overcome this problematic issue, the continual neutralization with bases during extraction or pretreatment of D2EHPA with NaOH or ammonia is suggested ("Chemorex D2EHPA"). The contamination of mono-(2-ethylhexyl)phosphoric acid (M2EHPA) in commercial D2EHPA at about 5%wt is considered the cause of lower degree of extraction, so other organophosphorous additives (e.g. TBP, trioctylphosphine oxide and dibutylbutyl phosphonate) are usually added to suppress that effect (Saeed et al., 2009). D2EHPA has been employed in following applications:

- Recovery of uranium, zinc, beryllium, cobalt, iron, nickel, silver and rare earth metals (Devi et al., 1998; Sakhalkar, 2002; Wongsawa et al., 2015)
- Separation of amino acids such as phenylalanine, tryptophan, aspartic and glutamic acid (Juang and Wang, 2002)
- Sorbent additive for solid-phase extraction (Kolev et al., 2009)

2.5.4 Tributyl phosphate (TBP)

TBP is one of the first phosphorous-based extractants commercially launched by Union Carbide (U.S.), Albright & Wilson (U.K.) and Daihachi Chemicals Co.

(Japan). Despite of being a neutral extractant, TBP is rather basic and forms complexes through coordinating, replacing waters and ion association. The limitations of solvating extractant are resulted by competitive extraction with anions, low solubility of organo-solute complexes in the diluent (Saeed et al., 2009). TBP has many commercial applications detailed as follows:

- Recovery of plutonium, thorium and uranium for reprocessing in nuclear plant (Pan et al., 1960; Baumgaertner and Finsterwalder, 1970)
- Recovery of molybdenum, tungsten, arsenic, platinum, iridium, chromium, titanium and gold (Wilson and Jacobs, 1961; Faye and Inman, 1963; De and Rahaman, 1964; Sato et al., 1990; Allal et al., 1997)
- Extraction of organic acids such as propionic, acetic, glycolic, lactic, pyruvic, butyric acids (Hano et al., 1990; Matsumoto et al., 2001)
- Synergist or phase modifier (Matsumoto et al., 2001)

2.6 Transport mechanisms in liquid membrane

There are six proposed transport mechanisms for describing how a solute is transferred through a liquid membrane with the graphic illustration of the mechanisms presented in Fig. 2.6 (Kislik et al., 2010). They are (1) simple transport (2) simple transport together with stripping reaction (3) facilitated transport (4) couple-counter transport (5) couple cotransport and (6) active transport

2.6.1 Simple transport

A solute molecule crosses the liquid membrane based on the different solubility associated with different phases. The solute is assumed to be in the same form in all phases due to no presence of chemical reaction. Once reaching equilibrium, concentration gradient becomes zero, resulting in the termination of transport process. To drive the remained solute in feed phase or uphill transport, stripping reaction with a stripping agent is necessary.

2.6.2 Facilitated transport

Facilitated or carrier-mediated transport involves partitioning, complexation and diffusion. Firstly, a solute from feed dissolves in liquid membrane and reacts with an extractant to from complex. The complex diffuses through the liquid membrane and reacts with stripping agent, allowing solute portioning in the stripping phase. Facilitated and simple transport can occur simultaneously with facilitated transport accelerating the over mass transfer rate.

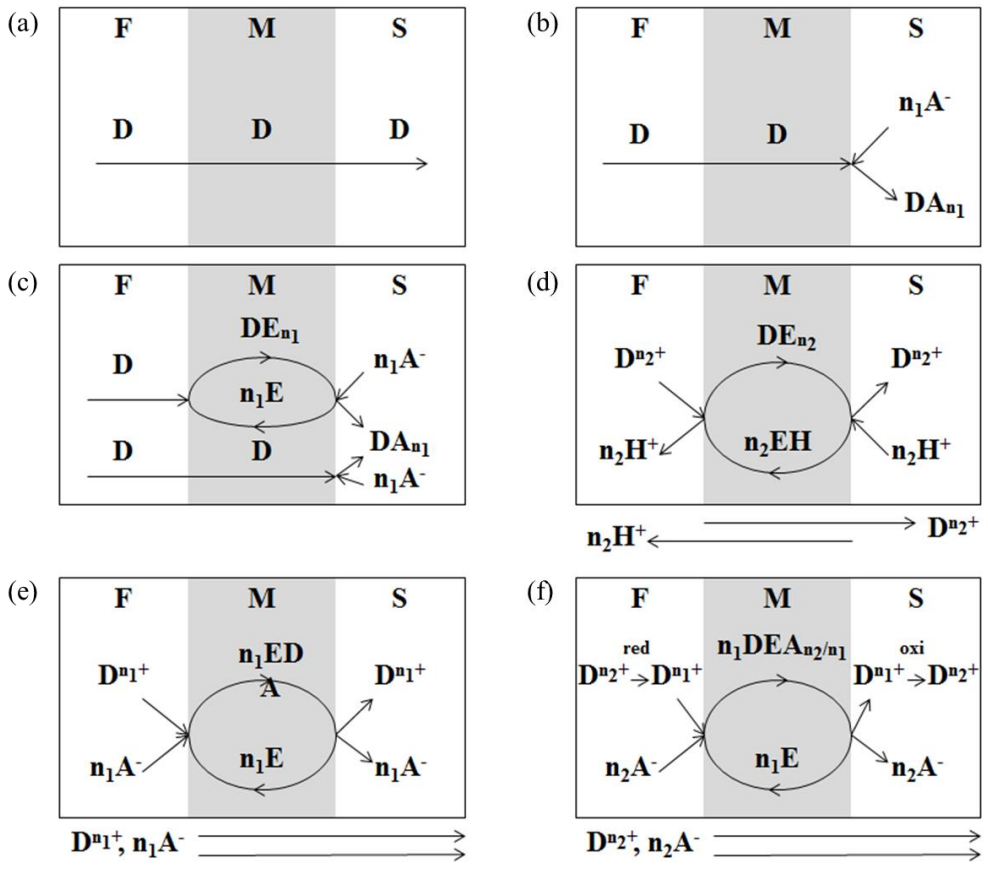

Figure 2.6 Transport mechanisms in the liquid membrane: (a) simple transport (b) simple transport together with stripping reaction (c) facilitated transport (d) coupled-counter transport (e) coupled cotransport (f) active transport. F is feed phase, M is liquid membrane phase and S is stripping phase; D is solute to be recovered; A are anions cotransported; E is extractant; red is reduction; oxi is oxidation. (Kislik et al., 2010)

2.6.3 Coupled counter- or cotransport

Depending on the acidity of liquid membrane system and the type of solute as well as extractant, coupled counter- or cotransport can be established. For couple counter-transport, ions carrying the same charge counter-currently flow through the liquid membrane system. On the contrary, the stoichiometric amount of opposite charged ions flow in the same direction to maintain the overall charge balance. Facilitated transport is always combined with coupled counter- or cotransport.

2.6.4 Active transport

Active transport refers to redox, catalytic reactions and biochemical conversions at the membrane interfacial surface resulted in higher selectivity. Almost all chemical reactions of active transport are irreversible.

2.7 Separation of β-lactam antibiotics through liquid membrane

In accordance with the reactive extraction studies of a β-lactam antibiotic published between 1980s and 1990s (Reschke and Schügerl, 1984; Harris et al., 1990; Hano et al., 1992; Bora et al., 1997), the developments in liquid membrane separation of β-lactam antibiotics emerged during late 1990s, pioneered by Regional Research Laboratory in India. At the beginning, the exploitation of liquid membrane was primarily to purify and concentrate the antibiotics produced by fermentation process without capital and energy intensive. Ghosh et al. (1996) proposed the perspective on the applicability of the liquid membrane process to separate Cephalosporin-C, the first generation of β-lactam antibiotics, from fermentation broth. The conventional methods to recover Cephalosporin-C including chromatographic and chemical processes were claimed less competitive than the operation of liquid membrane which provided low capital, operating costs, energy consumption and compact unit. The research teams of Dutta demonstrated the usage of various types of liquid membranes,

i.e. bulk liquid membrane (BLM), emulsion liquid membrane (ELM), and hollow fiber supported liquid membrane (HFSLM), for separating Cephalosporin-C from fermentation broth with and without cell of *Cephalosporium acremonium* (Ghosh et al., 1995; Sahoo et al., 1999; Sahoo et al., 2000). The investigation of several factors, including concentration of extractant, stirring speed, chloride concentration in stripping phase and solute chemical nature, was conducted (Findings summarized in Table 2.5) and the mathematical model of Cephalosporin-C concentration profile was also introduced. Acid dissociation constants of Cephalosporin-C involved in the consideration of ionic forms of drug molecules and separation efficiency in these articles. The first attempt to selectively separate Cephalosporin-C and its excess precursors was shown in Sahoo et al. (2000) where Cefalothin, Cefazolin, Cefotaxim, Cefadroxil, Cefaloridin, 7-aminocephalosporanic acid and 7-aminodesacetoxycephalosporanic acid (7-ADCA) was individually employed in BLM and their initial flux with hydrophobicity was compared. The multiple component mixture of Cephalexin, 7-ADCA, phenylglycine amide (PGA) and phenylglycine (PG) was then employed to study the selective separation by using SLM with strip dispersion (Vilt and Ho, 2010). The SLM with strip dispersion may help compensate for a major disadvantage or membrane instability of liquid membrane technology. Continuous organic phase was fed and recycled through a hollow fiber module to maintain membrane stability. Vilt and Ho (2010) investigated the effect of feed, extractant and stripping concentrations, types of buffer solution, pH of feed and strip solution as well as module specification on the separation factor.

Besides the intention to purify the β-lactam antibiotics, the integration of liquid membrane and fermentation process offered the benefit of in-situ removal of the biosynthetic products, consequently minimizing the product inhibition. Cascaval et al. (2000) established the selective extraction conditions of Penicillin V from phenoxyacetic acid using BLM and Amberlite LA-2 as a carrier due to the toxicity of Penicillin V toward strains. The conditions to be considered were pH gradient between feed and stripping phases, concentration of Amberlite LA-2 and rotational speed. Despite the increase of permeability factors across liquid membrane when the pH gradient was larger, the efficiency of selective separation was diminished. The

authors suggested using of low extractant concentration and strong mixing of the feed solution.

The application of liquid membrane to determine of four β-lactam residues, namely ampicillin, cloxacillin, penicillin V and penicillin G, in animal tissues and foodstuffs was introduced by Msagati and Nindi (2007). SLM was reported as a sample purification and enrichment method in flow systems connecting to high performance liquid chromatography coupled to a mass spectrophotometer. Donor and acceptor pH were optimized. The detection limits were revealed lower than the tolerance levels of the European Union (EU) and the American Food and Drug Administration (FDA). Msagati and Nindi (2007) indicated the necessity to monitor the degree of antibiotic contamination in food products to suppress the emergence of bacterial resistance to the antibiotics in humans. Similarly, the presence of β-lactam antibiotic from industrial wastewater in the aquatic environment induces the bacterial resistance and subsequent adverse human health effects. The potential of liquid membrane process for β-lactams removal from the discharge became promising based on satisfying results from former applications. Pirom et al. (2014) and Pirom et al. (2015) conducted the research on the separation of amoxicillin from synthetic pharmaceutical wastewater via hollow fiber supported liquid membrane (HFSLM) and studied the influence of aqueous acidity, concentrations of amoxicillin, extractant and stripping solution, flow rates and temperature on the separation performance. Nearly 90% of amoxicillin extraction percentage and 85% of stripping percentage were observed by using single extractant system of aliquat 336 and recycle mode of operation. Main conditions and conclusions of mentioned articles were reviewed and illustrated in Table 2.5.

Table 2.5 Literature review on separation of β-lactam antibiotics through liquid membrane

Author	Type of liquid membrane	Feed solution	Stripping solution	Extractant solution	Findings
Ghosh et al., 1995	SLM	10 mol/m^3 Cephalosporin-C in carbonate buffer solution (pH 10.0) with 5 - 80 mol/m^3 chloride ion	Acetate buffer solution (pH 4.0) with 5 mol/cm^3 acetate and 20 - 200 mol/cm^3 chloride ion	Aliquat 336 (From 5 to more than 800 mol/m^3) in butyl acetate	• Permeation rate of Cephalosporin-C reached a maximum at certain concentration of Aliquat 336 and decreased with increasing concentration of extractant. • The increase of chloride concentration in feed solution suppressed permeability coefficient of the drug due to lower Cl^- concentration gradient or driving force of counter transport. • Predicted permeability coefficient was always higher than the experimental value by 10-15% when concentration of Aliquat 336 was maintained below 800 mol/m^3 while the theoretical prediction was uncertain for higher

Table 2.5 Literature review on separation of β-lactam antibiotics through liquid membrane (continued)

Author	Type of liquid membrane	Feed solution	Stripping solution	Extractant solution	Findings
					concentration of Aliquat 336. • Less than unity of the surface tension of the liquid solution over the critical surface tension of the microporous support ascertained the wettability of the support by the extractant solution.
Sahoo et al., 1999	BLM and ELM (W/O emulsion)	<u>BLM</u> Fermentation broth using a strain of *C. acremonium* MTCC 886 (pH 9.0) <u>ELM</u> Filtered Fermentation broth	Citrate buffer solution (pH 5.0) and 1 M NaCl	<u>BLM</u> 5-20 mol/m^3 Aliquat 336 in butyl acetate <u>ELM</u> 10%v/v Span-80 and 1-2.5%v/v Aliquat 336 in	<u>BLM</u> • With cell mass, the transport rate and degree of extraction of Cephalosporin-C decreased compared to the synthetic buffer solution and cell-free broth. • Under liquid membrane extraction condition, cell viability was zero but β-lactam inhibitory activity was lowered

Table 2.5 Literature review on separation of β-lactam antibiotics through liquid membrane (continued)

Author	Type of liquid membrane	Feed solution	Stripping solution	Extractant solution	Findings
		in carbonate buffer (pH 9.5)		heptane : kerosene (1:1)	• The increase of stirring speed provided higher extraction and stripping rate implying that, for BLM, the interfacial ion exchange reactions were not the limiting step. <u>ELM</u> • The permeation rate increased with increasing Aliquat 336 concentration but, at high concentration of Aliquat 336 and Span-80, the mixture became gel and the separation of internal and external phases seemed difficult. • The extraction and stripping rate rose when stirring speed climbed up to 450 rpm.
Sahoo et al., 2000	BLM	Cephalosporin molecules in phosphate buffer	Citrate buffer solution (pH 5.0)	10 mM Aliquat 336 in butyl acetate	• The hydrophobicity of solutes and the initial flux across liquid membrane were strongly related in that the higher hydrophobicity the

Table 2.5 Literature review on separation of β-lactam antibiotics through liquid membrane (continued)

Author	Type of liquid membrane	Feed solution	Stripping solution	Extractant solution	Findings
		solution (pH 8.0)			cephalosporin molecule had, the faster the molecule was extracted.
Cascaval et al., 2000	BLM	0.5 g/L Penicillin V and 0.22 g/L phenoxyacetic acid (pH 2.0 – 6.0)	5% Sodium carbonate solution (pH 7.0 – 11.0)	0 – 80 g/L Amberlite LA-2 in 1,2-dichloroethane	• The larger pH gradient brought about increasing permeability of both Penicillin V and phenoxyacetic acid but lowering selectivity factors. • 10 g/L Amberlite LA-2 provided the highest selectivity factor of 6.5 when rotation speed was 500 rpm as well as pH of feed and stripping phase were maintained at pH 3 and pH 10, respectively. • Under higher rotation speed (up to 1,000 rpm), the permeability of individual solutes and separation factor increased, strong mixing was thus suggested.

Table 2.5 Literature review on separation of β-lactam antibiotics through liquid membrane (continued)

Author	Type of liquid membrane	Feed solution	Stripping solution	Extractant solution	Findings
Msagati and Nindi, 2007	SLM	β-lactam extract from liver, kidney tissue and milk sample by using acetonitrile (pH 1-8)	DI water (pH 8-12)	Undecane and di-n-hexylther (1:1)	• Extraction efficiency increased with increasing both donor and acceptor pH due to instability of ampicillin, cloxacillin, penicillin V and penicillin G in the acidic environment. • By incorporating SLM with LC-MS, the detection limit of β-lactam residues was found to be lower than the stipulated maximum residual limits imposed by EU and FDA.
Vilt and Ho, 2010	SLM with strip dispersion	A mixture of 12 mM Cephalexin, 30 mM 7-ADCA, 15 mM PG and 60 mM PGA (pH 8.0-9.5)	1 M KCl and 0.1 M various buffers (e.g. citrate, phosphate, carbonate solution, pH 6.0)	1.25-5%wt Aliquat 336 and 0.6-2.5%wt 1-decanol in Isopar L	• Although higher overall mass transfer coefficient was observed when Mini-Module was used, the degree of extraction and recovery of Cephalexin was completely comparable for Mini-Module (99.0% and 97.8%, respectively) and Extra-Flow module (98.9% and 97.6%, respectively).

Table 2.5 Literature review on separation of β-lactam antibiotics through liquid membrane (continued)

Author	Type of liquid membrane	Feed solution	Stripping solution	Extractant solution	Findings
					• Extraction and stripping percentages of >99% were obtained by using 0.05 and 0.10 M carbonate buffers while phosphate buffer and continual titration with 2 M NaOH served poor extraction and recovery. • At pH 8.0 which is the optimal pH for Cephalexin enzymatic synthesis, separation factors of 7-ADCA, PG and PDA were 6.3, 17.4 and 4.4, respectively. • At pH above 8.0, the extraction of Cephalexin decreased due to coextraction of PG while the optimal extraction of Cephalexin was observed at pH 9.5 providing that the feed solutions containing equimolar concentrations of 7-ADCA were used.

Table 2.5 Literature review on separation of β-lactam antibiotics through liquid membrane (continued)

Author	Type of liquid membrane	Feed solution	Stripping solution	Extractant solution	Findings
Pirom et al., 2015	HFSLM	6 mM Amoxicillin (pH 8.0)	6 mM NaCl (pH 6.0)	6 mM Aliquat 336 in 1-decanol	• At 318.15 K, the highest extraction percentage and stripping percentage of 89.65% and 84.70% were observed, respectively. • The amoxicillin extraction exerted the activation energy of 44.28 kJ/mol implying that the chemical reaction was the limiting step of the mass transfer of amoxicillin. • The extraction of amoxicillin was endothermic reaction revealed by the positive value of enthalpy change (24.58 kJ/mol).

Note: HFSLM is hollow fiber supported liquid membrane, SLM is supported liquid membrane, BLM is bulk liquid membrane, and ELM is emulsion liquid membrane.

CHAPTER 3
RESEARCH METHODOLOGY

This research can be classified into three main sections including the study of reactive liquid-liquid extraction of amoxicillin using single extractant, synergistic extraction of amoxicillin and operation of the HFSLM by employing the condition determined in the previous sections. Details of the experimental approach are described below.

3.1 Chemicals

Amoxicillin 500 mg capsules containing amoxicillin trihydrate were obtained from the Government Pharmaceutical Organization, Thailand. All chemicals, their purity and manufacturers are listed in Table 3.1. Chemicals were used without any pretreatment. Deionized water (DI water) was produced by filtering tab water through ThermoScientific™Barnstead™ Easypure™ II (ThermoScienticfic, USA) with the resistivity maintained at least 17.8 MΩ·cm.

Table 3.1 Lists of chemicals used in the experiment

Chemicals	Purpose	Purity (%wt)	Manufacturers
Acetic acid glacial (CH_3COOH)	pH adjustment	99.8	Qrec Chemical Co., Ltd., New Zealand
Alamine 336 ($N(C_8H_{17})_3$)	Extractant	95 - 100	Cognis Thai Co., Ltd., Thailand

Table 3.1 Lists of chemicals used in the experiment (continued)

Chemicals	Purpose	Purity (%wt)	Manufacturers
Aliquat 336 ($N^+(CH_3)(C_8H_{17})_3Cl^-$)	Extractant	88.2 - 90.6	Sigma-Aldrich Co., Ltd., USA
Amoxicillin 500 mg capsule	Wastewater preparation	n/a	Government Pharmaceutical Organization, Thailand
Bis(2-ethylhexyl)phosphate ($(C_8H_{19}O)_2PO_2H$)	Extractant	≥ 95.0	Merck Co., Ltd., Germany
1-Decanol ($C_{10}H_{22}O$)	Diluent	≥ 99.0	Merck Co., Ltd., Germany
Di-potassium hydrogen orthophosphate (K_2HPO_4)	Buffer	99.0	Ajax Finechem Pty Ltd., Australia
Nitric acid (HNO_3)	Membrane Cleaning	65	Qrec Chemical Co., Ltd., New Zealand
Orthophosphoric acid (H_3PO_4)	Buffer	85	Carlo Erba Co., Ltd., France
Potassium acetate (CH_3COOK)	Buffer	99.0	Ajax Finechem Pty Ltd., Australia

Table 3.1 Lists of chemicals used in the experiment (continued)

Chemicals	Purpose	Purity (%wt)	Manufacturers
Potassium chloride (KCl)	Stripping solution	99.8	Ajax Finechem Pty Ltd., Australia
Potassium dihydrogen orthophosphate (KH_2PO_4)	Buffer	99.0	Ajax Finechem Pty Ltd., Australia
Potassium hydroxide (KOH)	pH adjustment	85	Ajax Finechem Pty Ltd., Australia
Tributyl phosphate ($(C_4H_9O)_3PO$)	Extractant	97	Acros Organics Co., Ltd., USA

3.2 Equipments

Utilized laboratory equipments and their manufacturers are listed in Table 3.2. Liqui-Cel® liquid/liquid membrane and module (Model X50, Hoechst Celanese Corporation, NC, USA) composed of pumping system, two rotameters and four pressure gauges (Fig. 3.1). Table 3.3 displays the specification of the Celgard® hollow fibers.

Table 3.2 Lists of equipments and their manufacturers

Equipments	Manufacturers / suppliers
Multi-position magnetic stirrer (RT 10 power IKAMAG®)	IKA, Malaysia

Table 3.2 Lists of equipments and their manufacturers (continued)

Equipments	Manufacturers / suppliers
C-MAG HS 7 Hot plate magnetic stirrer	IKA, Malaysia
Thermo Scientific™ Barnstead™ Easypure™ II Ultrapure water purification systems	Fisher Scientific, USA
S20 SevenEasy™ pH meter	Mettler Toledo, USA
Cary 60 UV-vis spectrophotometer	Agilent, USA
Lab pipette and pipette tips	Thermo Scientific, USA
Liqui-Cel® liquid/liquid extraction system	Hoechst Celanese Corporation, USA
Beaker	Duran, Germany
Magnetic bar	Suksapanpanit, Thailand
Volumetric cylinder	Duran, Germany
Plastic tip	Thermo Scientific, USA
Test tube tong	Suksapanpanit, Thailand
Dropper	Suksapanpanit, Thailand
Glass bottle	Suksapanpanit, Thailand
Volumetric flask	Duran, Germany

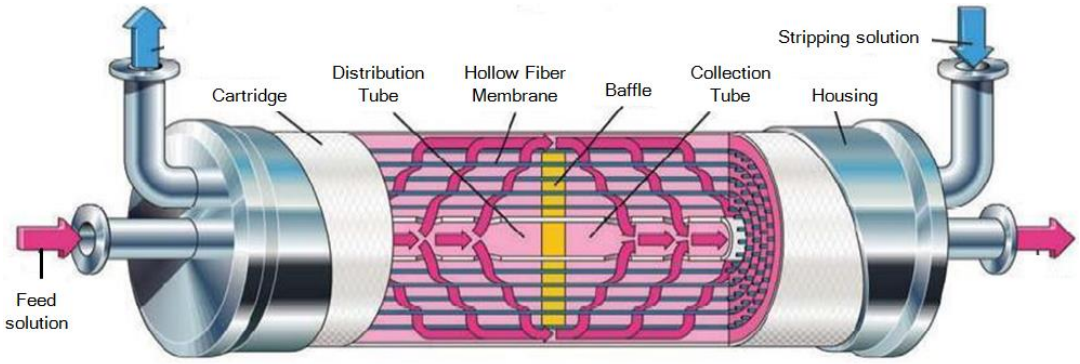

Figure 3.1 Schematic drawing of hollow-fiber supported liquid membrane module (Lothongkum et al., 2011)

Table 3.3 Specification of hollow fibers membrane (Sunsandee et al., 2014)

Properties	Specification
Hollow fiber material	Polypropylene
Number of fibers	35,000
Inner diameter of hollow fibers	2.4×10^{-4} m
Outer diameter of hollow fibers	3.0×10^{-4} m
Effective length of hollow fibers	0.15 m
Porosity	30 %
Average pore size	3.0×10^{-8} m
Effective surface area	1.4 m^2
Effective surface area per unit volume	2.93×10^{3} m^2/m^3
Tortuosity	2.6
Dimension of module (Diameter × Length)	0.0635 m × 0.2032 m
Operating temperature	1 °C to 60 °C

3.3 Reactive liquid-liquid extraction of amoxicillin by single extractant

3.3.1 Effect of initial pH of amoxicillin solution

Synthetic pharmaceutical wastewater containing amoxicillin was prepared by dissolving an amoxicillin capsule into DI water under continuous stirring at 440 rpm for 1 h by using hot plate magnetic stirrer and adjusting the total volume to attain the final concentration of 1.37 mM (500 mg/L). After filtering the prepared solution through Whatman paper (average pore size 11 μm), the pH of the solution was adjusted to the desired values ranged from 2 to 12 by adding acetic acid glacial or ammonia solution. Extracting solvents were prepared by mixing the extractant (i.e., Aliquat 336, D2EHPA, Alamine 336 or TBP) in 1-decanol to attain the extractant concentration of 6 mM. Extraction experiment was performed in triplicates by magnetic stirring the equal volumes (5 mL) of amoxicillin solution and organic extractant for 3 h on the multi-position magnetic stirrer under the room temperature (30 ± 2 °C). Extraction systems were then kept idle and avoided from light exposure for the next 19 h to allow phase separation. Carefully withdraw the liquid in lower layer approximately 4 mL and analyze photometrically by using Cary 60 UV-Vis spectrophotometer at 272 nm (USP 35, 2012). The extraction percentage of amoxicillin was calculated using Eq. (3.1) (Kislik, 2012):

$$\text{Extraction percentage (\%)} = \left(\frac{[Amox]_0 - [Amox]}{[Amox]_0} \right) \times 100 \qquad (3.1)$$

where $[Amox]_0$ is the initial amoxicillin concentration in aqueous phase (mg/L); and $[Amox]$ is the final amoxicillin concentration in aqueous phase after the completion of extraction process (mg/L).

3.3.2 Effect of extractant concentrations

The mixture containing amoxicillin and extractant (Aliquat 336, D2EHPA, Alamine 336 and TBP) was prepared according to the method presented in section 3.3.1. The pH of the mixture was maintained at the optimal value, which was determined according to section 3.3.1 while the concentrations of extractant were varied from 2 to 15 mM. Extraction experiment was conducted in triplicates for each extractant by continuous stirring at 440 rpm under room temperature (30 ± 2 °C) for 3 h and then kept idle from light exposure for the additional 19 h. Lower liquid layer approximately 4 mL was obtained and analyzed for amoxicillin concentrations using Cary 60 UV-Vis spectrophotometer at 272 nm (USP 35, 2015). The extraction percentage of amoxicillin was calculated according to Eq. (3.1) (Kislik, 2012).

3.3.3 Effect of pHs of stripping solution

Amoxicillin solution (1.37 mM) was mixed with individual extractants in 1-decanol at the optimal pH and optimal extraction concentrations, which were the results of the section 3.3.1 and 3.3.2. The mixture containing amoxicillin and extractant (3 replications) was stirred at 440 rpm under room temperature (30 ± 2 °C) for 3 h and remained idle for another 4 h to allow liquids layers to separate. Organic phase was separated from aqueous phase by using a separation funnel. Separated organic phase was stirred at 440 pm with 6 mM potassium chloride (KCl) solution whose pH varied for 3 h at the room temperature in beakers (3 replications at each pH). All experimental units remained idle for another 12 h. Liquid samples (4 mL) from the stripping phase were obtained and analyzed for amoxicillin concentrations by using Cary 60 UV-Vis spectrophotometer at 272 nm (USP 35, 2012). The percentages of stripping were calculated according to Eq. (3.2) (van der Hoogerstraete et al., 2013).

$$\text{Stripping percentage (\%)} = \left(\frac{[Amox]_s}{[Amox]_0} \right) \times 100 \qquad (3.2)$$

where $[Amox]_0$ is the initial concentration of amoxicillin in the extracted aqueous phase (mg/L); and $[Amox]_s$ is the final concentration of amoxicillin in the stripping aqueous phase (mg/L).

3.3.4 Effect of concentrations of stripping solution

Prepare amoxicillin solution and perform the reactive liquid-liquid extraction and stripping similar to the method described in the section 3.3.3 except that the concentrations of KCl stripping solution were varied from 2 to 10 mM. The pH of the stripping solution was maintained according to the result of the section 3.3.3. Amoxicillin concentrations were analyzed based on USP 35 (2012) and used to calculate the stripping percentage according to Eq. (3.2) (van der Hoogerstraete et al., 2013).

3.4 Synergistic extraction of amoxicillin

3.4.1 Synergistic extraction

An amoxicillin capsule was dissolved into DI water under continuous stirring at 440 rpm for 1 h on the hot plate magnetic stirrer and the total volume was subsequently adjusted to the final concentration of 1.37 mM. The prepared solution was filtered through Whatman paper (average pore size 11 μm) before adding ammonia solution to attain the initial pH of 10. The binary mixture extractant systems of interest were (1) Aliquat 336 and D2EHPA (AqD), (2) Aliquat 336 and TBP (AqT), (3) D2EHPA and TBP (DT), (4) Alamine 336 and D2EHPA (AmD) and (5) Alamine 336 and TBP (AmT). The binary extractant systems were prepared to achieve the following molar ratios of 0:12, 3:9, 6:6, 9:3, 10:2 and 12:0 mM in 1-decanol. The equal volumes (5

mL) of amoxicillin solution and extractant mixture (3 replicates) were mixed at 440 rpm for 3 h on multi-position magnetic stirrer under room temperature (30 ± 2 °C) before being kept idle and avoided from light exposure for further 19 h to allow phase separation. Approximately 4 mL of the top liquid layer was carefully pipetted and analyzed by using Cary 60 UV-Vis spectrophotometer at 272 nm (USP 35, 2012). Eq. (3.1) was used to determine the extraction percentages. The concentration of amoxicillin in the organic phase was determined by mass balance calculation. According to Guezzen and Didi (2012), synergistic coefficient as shown in Eq. (3.3) can be used to quantify the magnitude of synergistic extraction of the binary extractant systems:

$$S = \frac{D_{1+2}}{D_1 + D_2} \tag{3.3}$$

where S is the synergistic coefficient of the system with a mixture of extractant 1 and 2; D_1 is the distribution coefficient of the system with extractant 1; D_2 is distribution coefficient of the system with extractant 2; and D_{1+2} is distribution coefficient of the system with a mixture of extractant 1 and 2. The distribution coefficient in Eq. 3.4 was defined as (Kislik, 2012)

$$D_i = \left(\frac{[Amox]_{org}}{[Amox]} \right)_i = \left(\frac{[Amox]_0 - [Amox]}{[Amox]} \right)_i \tag{3.4}$$

where $[Amox]_{org}$ is the final amoxicillin concentration in extractant phase (mg/L); D_i is the distribution coefficient of amoxicillin extracted by using extractant i (i.e., Aliquat 336, D2EHPA, Alamine 336 and TBP). Synergism is established when $S > 1$ while $S < 1$ implies the occurrence of antagonistic effect and $S = 1$ suggests coextraction of extractants (Guezzen and Didi, 2012).

3.4.2 Effect of pH of stripping solution on the stripping percentage for synergistic extraction of amoxicillin

The amoxicillin solution was prepared and extracted using the optimal extractant mixture, which was determined in section 3.4.1. The organic phase was separated by a separating funnel and stirred with 6 mM KCl in acetate buffer whose pH was adjusted between the optimal pH, which was determined according to section 3.3.3 for the specific extractants. The mixture was then mixed in a beaker (3 replicates) at 440 rpm for 3 h at the room temperature. All experimental units remained idle for another 12 h before the liquid sample approximately 4 mL from the stripping phase was obtained and analyzed for amoxicillin concentrations by using Cary 60 UV-Vis spectrophotometer at 272 nm (USP 35, 2012). Eq. (3.2) was applied to calculate the stripping percentage.

3.4.3 Effect of concentrations of stripping solution on the stripping percentage for synergistic extraction of amoxicillin

The amoxicillin solution was prepared, extracted and stripped using the optimal conditions, which obtained based on the previous sections except that the concentrations of KCl stripping solution were varied between the optimal concentrations reported in section 3.3.4. Approximately 4 mL of liquid from the stripping phase was collected and analyzed for amoxicillin concentrations by using Cary 60 UV-Vis spectrophotometer at 272 nm (USP 35, 2012). The stripping percentage was calculated according to Equation (3.2).

3.5 Operation of HFSLM

3.5.1 Preparation of HFSLM

A mixture of AqT was dissolved in 1-decanol by maintaining molar ratio of Aliquat 335 and TBP at 10:2 mM. The binary extractant mixture was fed into the HFSLM system as illustrated in Fig. 3.2 for at least 90 minutes to ensure that the extractant solution was fully embedded into pores of the membrane due to capillary force. Excess amount of extractant solution was flushed out of the hollow membrane system by feeding deionized water into both tube and shell sides. The differences between the fresh and flushed extractant solution corresponded to the total volume of pores inside hollow fibers membrane.

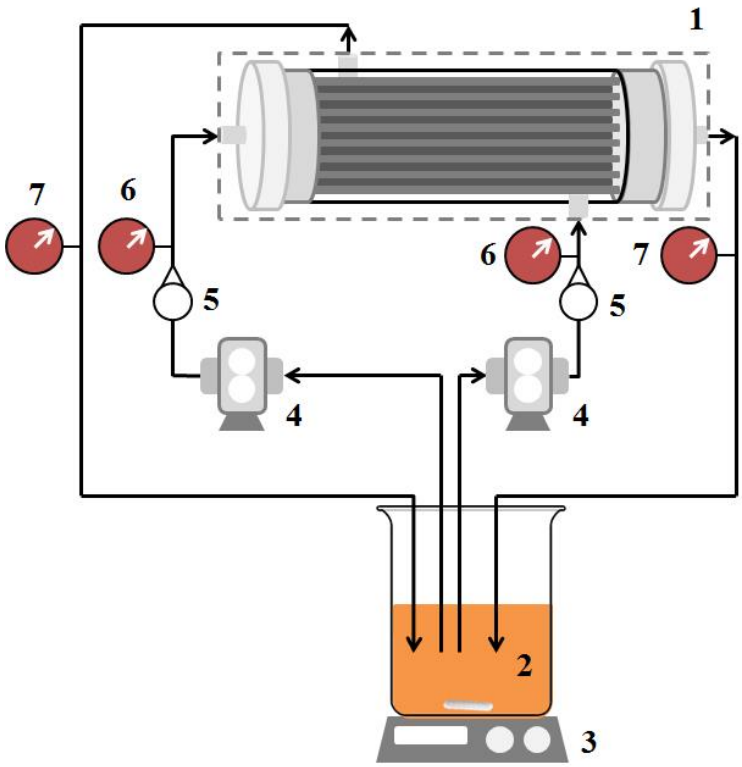

Figure 3.2 HFSLM scheme during the startup in which the extractant solution was fed into the system to form liquid membrane while the DI water was flushed to remove the excess extractant solution: (1) the HFSLM module (2) extractant solution/DI water container (3) magnetic stirrers (4) gear pumps (5) rotameters (6) inlet pressure gauges and (7) outlet pressure gauges

3.5.2 Effect of feed and stripping flow rate on the separation efficiency

Amoxicillin solution (1.37 mM) at the initial pH 10 and 6 mM KCl in acetate buffer solution at pH 5 were prepared and fed countercurrently into the hollow fiber membrane system at the tube and shell sides, respectively. Two sets of the HFSLM were used as the controls, employing 10 mM of Aliquat 336 or 2 mM of TBP as the extractants. Another set of HFSLM was assigned as the treatment, which employed the binary mixture that yield the best synergistic extraction as the extractant. The

flow rates for both feed and stripping streams were identical and varied from 62.5 to 200 mL/min. The experiment was carried out at room temperature (30 ± 2 °C) until the system reached the steady state, which was indicated by relatively constant outlet concentrations. Fig. 3.3 illustrates the diagram of the HFSLM system used in this experiment. Liquid sample (4 mL) from the outlets of amoxicillin feed and stripping stream were obtained and analyzed for amoxicillin concentrations using Cary 60 UV-Vis spectrophotometer at 272 nm (USP 35, 2012) and chemical oxygen demand (COD) based on the open reflux method 5220-B (APHA, 1998). The extraction and stripping percentages at any points of time are calculated according to Eq. (3.1) and (3.2), respectively. The steady-state extraction and stripping percentages were statistically analyzed by the nonlinear regression using Minitab 16.

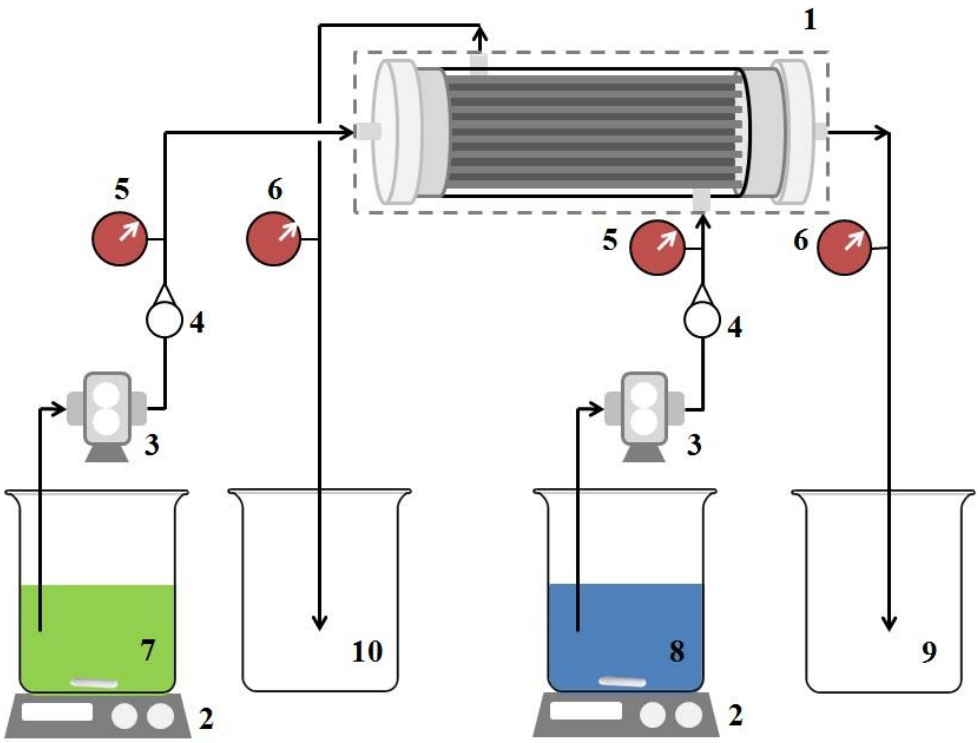

Figure 3.3 The HFSLM scheme during the steady state extraction and stripping of amoxicillin: (1) the hollow fiber supported liquid membrane module (2) magnetic stirrers (3) gear pumps (4) rotameters (5) inlet pressure gauges (6) outlet pressure gauges (7) inlet amoxicillin solution container (8) inlet stripping/potassium chloride solution container (9) outlet amoxicillin solution container and (10) outlet stripping/potassium chloride solution container

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Reactive liquid-liquid extraction of amoxicillin by single extractant

Amoxicillin can exist in positive and neutral forms according to the first and second acid dissociation constants (i.e., pKa_1 = 2.68 and pKa_2 = 7.49). However, our literature review indicated that the information on amoxicillin extraction by the positive and neutral charged extractants were not widely available. In this study, KCl was chosen as stripping solution but the data on suitable pH and concentration to be used during amoxicillin recovery remained limited.

4.1.1 Effect of initial pH of amoxicillin solution

The reactive liquid-liquid extraction of amoxicillin solution was carried out by using 6 mM of single extractant (Aliquat 336, D2EHPA, TBP and Alamine 336) dissolved in 1-decanol. The range of pH chosen, which varied from 2 to 12, covered the entire range of acid dissociation constants of amoxicillin (pKa_1 = 2.68, pKa_2 = 7.49 and pKa_3 = 9.63). 1-Decanol was chosen as the diluent in this study due to its non-toxic nature as food additive (US FDA, 2014). Fig. 4.1 illustrates the results of the reactive liquid-liquid extraction at different pH of amoxicillin solution. 1-Decanol did not interfere with amoxicillin extraction because it demonstrated insignificant extraction percentage at less than 1%. The extraction percentages increased when the pH of amoxicillin solution was raised to 10 before remained relatively constant or decreased slightly depending on the types of extractant used. The extraction percentages for Aliquat 336 were observed within the range from 1 to 5% when the pH of amoxicillin solution was between 2 and 6. The extraction percentages for Aliquat 336 showed the maximum value of 68.2 ± 1.54% when the initial pH of amoxicillin solution was maintained at 10. The significant increase of extraction percentage was related to the

presence of mainly negative-charged amoxicillin at pH greater than 7.49 that strongly attracted to the quaternary ammonium cation through ion-pair formation. Similar explanation can be applied for the amoxicillin extraction by Alamine 336 in which the highest extraction percentage (17.4 ± 2.95%) was also reported when maintaining the initial pH of amoxicillin solution at 10. Alamine 336, a tertiary amine, seemed to express weaker positive polarity than Aliquat 336, which possesses a positive charge regardless of the pH of the solution (Rao et al., 2009).

D2EHPA was present mainly in negative form when the pH of the solution was greater than 2.75 (Ulewicz and Walkowiak, 2005). Protonation of D2EHPA occurred at the interface between aqueous and organic phases after its dimer configuration was broken out (Gajda and Bogacki, 2007). The results of reactive liquid-liquid extraction of amoxicillin using D2EHPA as extractant revealed two peaks of extraction percentage at pH 3 and 10 (Fig. 4.1). These peaks corresponded to the extraction percentages of 13.2 ± 1.79% and 30.4 ± 2.54%, respectively. The extraction percentage associated with pH 3 was possibly caused by the ion-pair formation of phosphoryl anions and positive-charged amoxicillin (Pursell et al., 2003). The extraction percentages remained low at 1.6 ± 1.62% despite reducing pH of the amoxicillin solution from 3 to 2. This observation was likely explained based on the existence of deprotonated D2EHPA molecule that limited the formation of chemical complex via ion-pair route. When the pH of amoxicillin solution increased from 4 to 7, the total charge of amoxicillin gradually shifted towards neutral and then negative so that the phosphoryl anions of D2EHPA were unable to attract amoxicillin molecules effectively, hence resulting in low extraction percentages at roughly 5%. As the pH continued to increase, the existing phosphoryl anions and negative-charged amoxicillin should repel with each other but surprisingly the experimental results revealed the significant increase of extraction percentages as high as 30.4 ± 2.54% at pH 10. It was possible that the two anions formed the chemical complexes based on solvation mechanism that consequently partitioned into organic phase (Hamdo, 2011). It should be pointed out that the cloud layer, which was immiscible layer to either organic or aqueous phases, was observed for the extraction systems involving

D2EHPA at high pH (Fig. 4.2). Formation of this immiscible layer might obstruct amoxicillin molecules from entering the organic phase (Kedari et al., 2005).

Extraction percentage of amoxicillin by TBP was low and relatively constant measured at 5.6 ± 3.15% for the pH range tested (Fig. 4.1) although TBP was reported as a good extractant for organic acids (Keshav et al., 2008; Kumar and Babu, 2009; Wasewar et al., 2011). Low extraction performance by TBP relative to Aliquat 336 could be linked to the ability of TBP that suited the extraction of neutral undissociated molecules while Aliquat 336 can extract both dissociated and undissociated forms (Canari and Eyal, 2003; Wasewar et al., 2011).

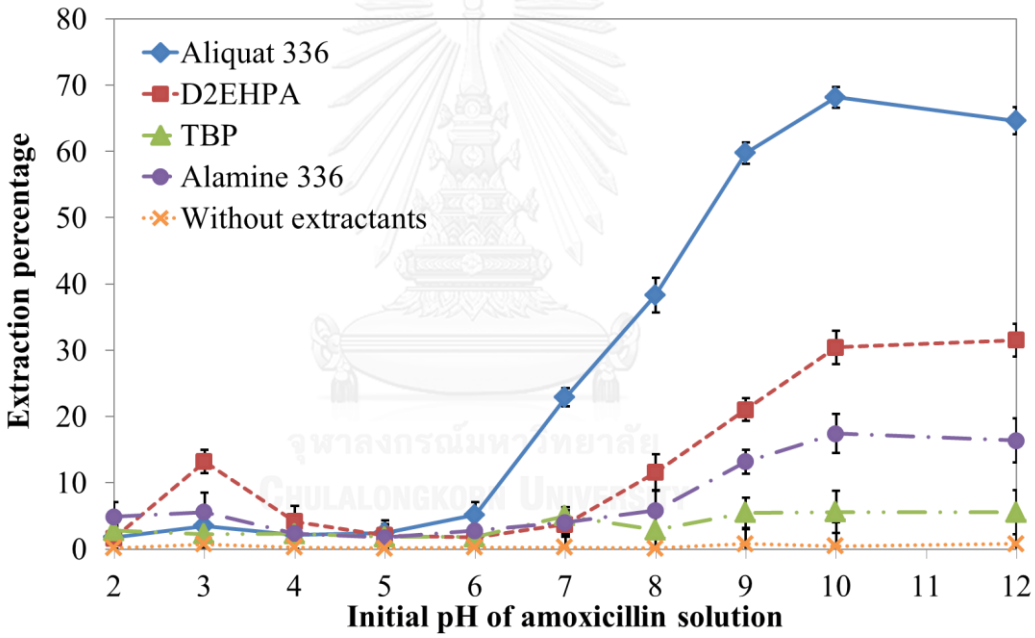

Figure 4.1 Extraction percentages of amoxicillin by using 6 mM of single extractant dissolved in 1-decanol with different initial pHs of amoxicillin solution

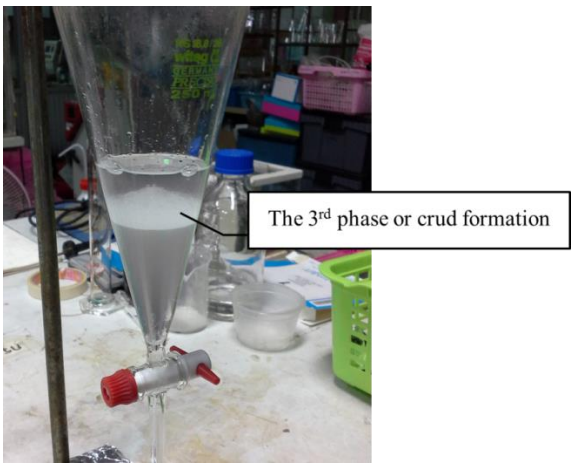

Figure 4.2 Crud formation during amoxicillin extraction by D2EHPA

4.1.2 Effect of extractant concentrations

Amoxicillin extraction was carried out by varying the concentrations of extractant from 2 to 15 mM while maintaining the initial pH of amoxicillin solution at 10. The results as shown in Fig. 4.3 indicated that Aliquat 336 was the most efficient extractant followed by D2EHPA, TBP and Alamine 336. The extraction percentage by Aliquat 336 increased rapidly with increasing extractant concentrations, reaching approximately 85% at 12 mM before remaining relatively constant. Similar observation was noted for D2EHPA, TBP and Alamine 336 except that the rate of increase was significantly slower. The extraction percentages for D2EHPA, TBP and Alamine 336 also stabilized when the extractant concentrations were greater than 12 mM. The increase of extraction percentages with respect to increasing extractant concentrations could be explained by the forward shifting of reaction according to Le Châtelier's principle when excess reactants (i.e., extractants) were added before reestablishing equilibrium. The different rising rate of extraction percentages with concentration of extractants can be explained by the mole ratio between amoxicillin and extractants participating in chemical complexes found in chemical equilibrium study detailed in Appendix D. The number of extractant taking part in complexation with one mole of amoxicillin (n) of Aliquat 336, D2EHPA and Alamine 336 were

1.40, 0.53 and 0.30, respectively. This revealed that the concentration change of Aliquat 336 had the highest impact on the value of equilibrium complexation constant (K_E) and the system opposed that change by shifting reaction forward or reverse. When concentration of Aliquat 336 which is a denominator of K_E goes up, reaction quotient falls down and the reaction would shift forward resulted in further increase of chemical complexes concentration. The higher n value is, the more concentration of chemical complexes was shifted. This is the reason why Fig. 4.3 shows the order of increasing rate of extraction percentage: Aliquat 336 > D2EHPA > Alamine 336. Equation D.19 was invalid for the complexation between TBP and amoxicillin (R^2 = 0.1481 and negative sign of K_E) implying other set of complexation reactions which required out-of-scope complicated experimental design to examine.

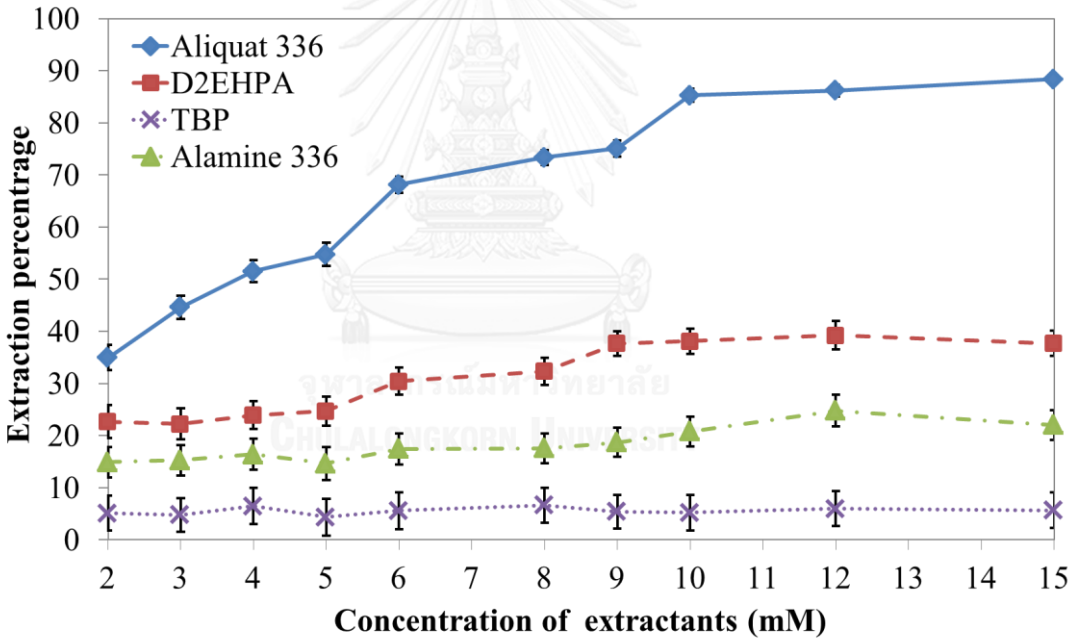

Figure 4.3 Extraction percentages of amoxicillin subjected to different extractant concentrations given that the initial pH of amoxicillin solution was maintained at 10.

4.1.3 Effect of pH of stripping solution

Amoxicillin extraction was conducted by maintaining the initial pH of amoxicillin solution (1.37 mM) at 10 and the extractant concentrations at 12 mM. Liquid from organic layer was obtained and then stripped with 6 mM of KCl solution in potassium acetate buffer maintained at the pH ranged from 4 to 7. This pH range of the buffer solution was reported to improve the stability of recovered amoxicillin (Erah et al., 1997). KCl was employed as stripping solution instead of NaCl as reported in Pirom et al. (2014). It was postulated that larger K^+ ions can enhance solubility of amoxicillin compared to smaller Na^+ ions (Feng et al., 2006) while Lenzi et al. (1975) suggested that the presence of K^+ ions was associated with lower ionic strength under the room temperature that led to higher water activity to solvate amoxicillin molecules.

The effect of pH of stripping solution on the stripping percentage for each extractant is displayed in Fig. 4.4. The decreasing trend of stripping percentages was observed with increasing pH for the system utilizing Aliquat 336, D2EHPA and TBP. Changing the charged property of amoxicillin to the opposite or neutral charges by increasing the pH of stripping solution caused the repulsion between amoxicillin and extractants and disruption of chemical complexes, and consequently pushed amoxicillin into stripping phase (Chuo et al., 2014). The highest stripping percentage were achieved by maintaining the acetate buffer at pH 5 (36.7 ± 1.0%) for Aliquat 336, at pH 4 (13.6 ± 0.65%) for D2EHPA and at pH 3 (8.4 ± 1.01%) for Alamine 336. For TBP, the highest stripping percentage (5.3 ± 1.06%) was observed at pH 7 while the lower stripping percentages were determined at approximately 2%. It should be pointed out that the stripping percentages reported were calculated according to equation 3.3, which compared the amount of amoxicillin recovered in the stripping solution with that in the feed solution. By taking the amount of amoxicillin present in the extracted solution as the basis, the efficiency of amoxicillin recovery for TBP could be as high as 90%, which were significantly higher than the remaining extractants. Effective stripping of amoxicillin when using TBP as the extractant was possible because the chemical complexation between amoxicillin and TBP involved

weaker intermolecular interactions compared to that between amoxicillin and other extractants that is usually associated with stronger electrostatic forces.

4.1.4 Effect of KCl concentration

The concentrations of KCl stripping solution were varied from 2 to 10 mM during this study. The pH of stripping solution was maintained at 5 for each concentrations tested. For Aliquat 336, the highest amoxicillin stripping percentage was determined at 39.3 ± 1.35% when KCl concentrations increased from 2 to 4 mM (Fig. 3.5). The increase in concentrations of stripping agent shifted the stripping reaction forward through an increasing thermodynamic activity of potassium chloride. The stripping percentage of amoxicillin decreased considerably after KCl concentrations increased from 4 to 10 mM. This may be linked to increasing ionic strength and salting-out effect (Kislik, 2012). Higher salt concentrations in stripping phase caused the reduction of water activity in solvating amoxicillin molecules. In contrary, the stripping percentage of amoxicillin steadily decreased in the case of D2EHPA from 31.8 ± 7.48% to 14.0 ± 3.54% when applying higher KCl concentrations. Due to the downward trend of stripping percentage, the use of KCl as stripping agent should be cautious when D2EHPA was employed as extractant. For Alamine 336, the stripping percentage of amoxicillin was relatively constant at 7.0 ± 7.48%, implying that changing KCl concentrations within the range studied had insignificant effect on stripping efficiency. For TBP, the stripping percentage was significantly lower than those of Aliquat 336 and D2EHPA with the maximum reported at 5.2 ± 1.16% when KCl concentrations was maintained at 6 mM

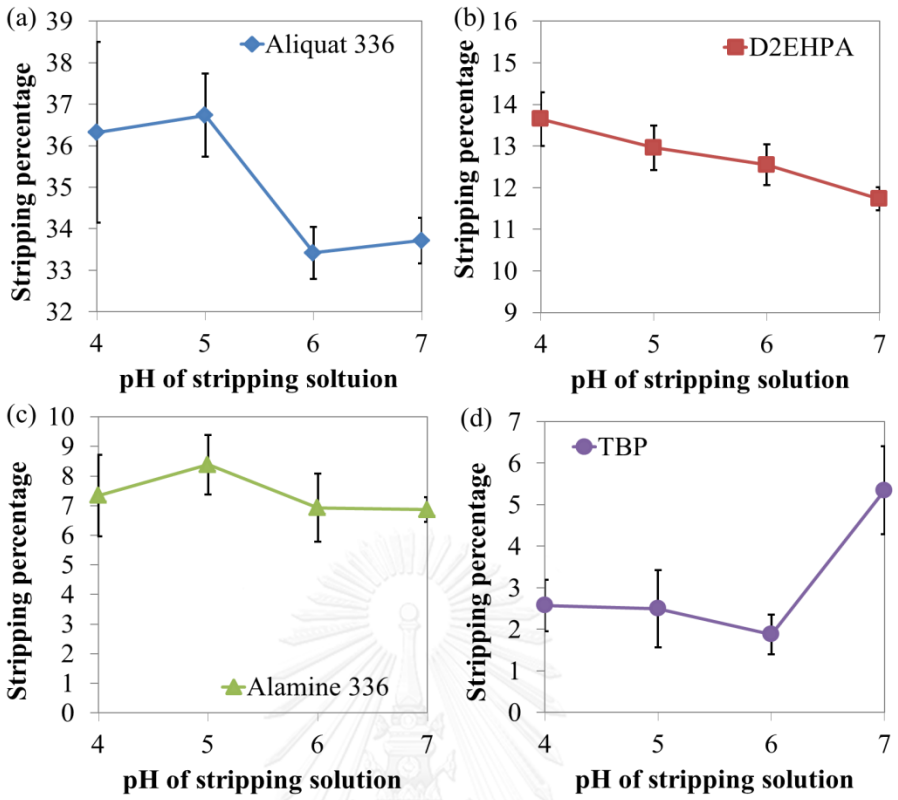

Figure 4.4 Stripping percentages of amoxicillin by using KCl as stripping solution at different pH. The stripping solution was maintained at 6 mM. Initial pH of amoxicillin solution was maintained at 10. Amoxicillin extraction was carried out by using 12 mM of Aliquat 336, D2EHPA, TBP and Alamine 336.

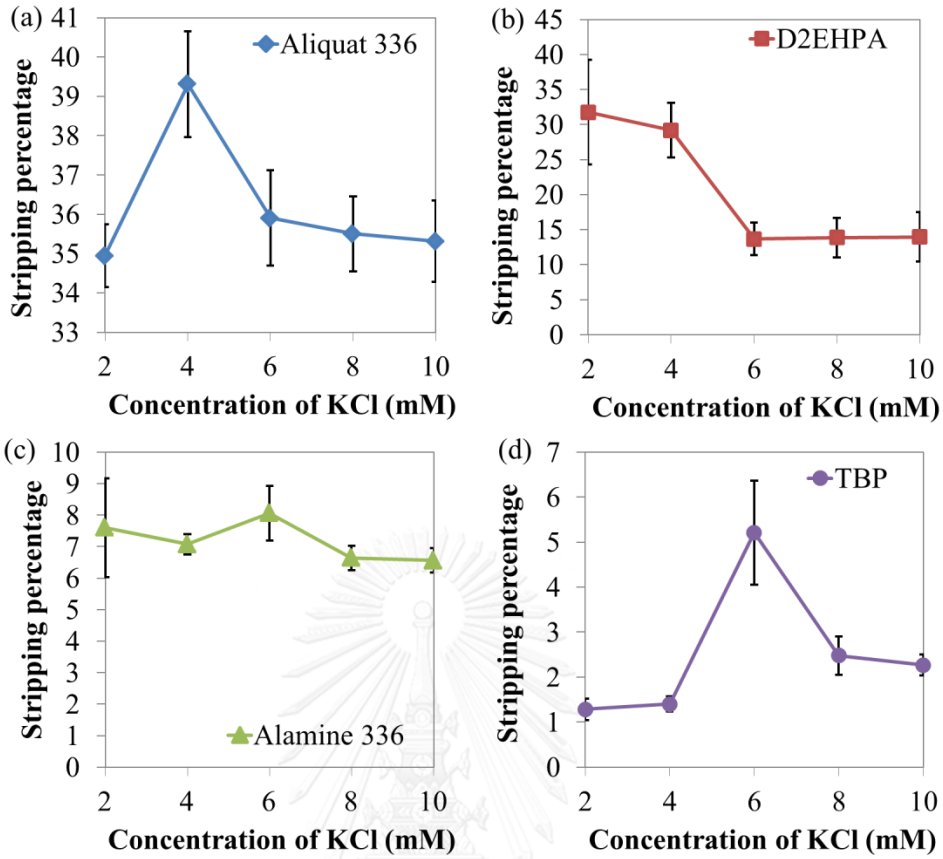

Figure 4.5 Stripping percentages of amoxicillin when varying KCl concentration. The initial pH of amoxicillin solution was set at 10 and four types of extractants at 12 mM were applied including Aliquat 336, D2EHPA, Alamine 336 and TBP

4.2 Synergistic extractant of Amoxicillin

Previous works have reported the improvement of extraction efficiency when using the mixture of extractants in comparison to that of the single extractant, the effect referred to as synergistic extraction (Singh et al., 2001; Belkhouche et al., 2005; Yunhai et al., 2011). Synergistic extraction is defined as the extraction by using the mixture of extractants that yielded the extractant efficiency exceeding that of individual extractant (Kislik, 2012). The extend of each component as indicated by the mole or mass ratio of each component in the mixture plays an important role in the effectiveness of synergistic extraction. In this section, the binary mixture made up

from extractants including Aliquat 336, D2EHPA, TBP and Alamine 336 were examined for the feasibility of synergistic extraction of amoxicillin.

4.2.1 Synergistic extraction

Synergistic extraction of amoxicillin was conducted by using the binary extractants with different molar ratios. Total concentrations of the extractants and the initial pH of amoxicillin solution were based on the results of section 4.1. That is maintaining the total concentrations of extractants and the initial pH of amoxicillin solution at 12 mM and 10, respectively. Figure 4.6 demonstrates the results of extraction percentage and synergistic coefficient during the synergistic extraction by using the binary extractant mixture. For the AqD mixture, decreasing the concentrations of D2EHPA resulted in the increasing trend of extraction percentage with the highest value determined at 70.7 ± 1.11% when the molar ratio of Aliquat 336 and D2EHPA was set at 10:2 (Fig. 4.6a). The maximum extraction percentage for AqD mixture was significantly lower than the result when Aliquat 336 was solely employed to extract amoxicillin, thereby suggesting that the synergistic extraction by means of using the AqD mixture was unlikely. This conclusion was confirmed by the magnitude of synergistic coefficient (*S*) less than 1 for all molar ratio combinations. The possible explanation for the antagonistic effect ($S < 1.0$) of amoxicillin extraction could be related to the metathesis reaction between Aliquat 336 and D2EHPA, releasing hydrochloric acid which highly disturbed the extraction of amoxicillin by blocking the active sites on the extractant (Blahusiak et al., 2011). The finding in the present study concurs with the results of Wang et al. (2014) who reported the decrease of overall extraction percentage when employing the AqD mixture as compared to the individual extractant to separate zirconium and hafnium from aqueous solution. In addition, it was reported that the separation of platinum (IV) from the diluted solution was negatively affected when the binary extractant mixture of AqD at the molar ratio of 1:5 was used (Lee et al., 2009).

DT mixture exhibited the synergistic effects on amoxicillin extraction as can be seen by the magnitudes of synergistic coefficient greater than 1.0 as well as the improved extraction percentages as compared to those from the single extracting system of D2EHPA or TBP (Fig. 4.6b). The extraction percentage increased sharply with increasing molar ratio and reached the maximum value of 52.6 ± 1.53% when maintaining the molar ratio of D2EHPA to TBP at 10:2 before starting to decrease. Guezzen and Didi (2012) speculated that the formation of DT complex containing the molecular linkage of the type RHO=P- accounted for the synergistic effect between D2EHPA and TBP. Other works also detected the formation of D2EHPA-TBP bonds by using FT-IR analysis and found the reduction of intensity in P=O vibration band, thereby suggesting the polymerization through hydrogen bonding between these two extractants, which may be the reason for synergism (Alamdari et al., 2004; Fatmehsari et al., 2009).

Synergist amoxicillin extraction was also observed for the AqT mixture as can be confirmed by the magnitude of synergistic coefficient greater than one (S = 1.45 to 1.84) for all molar ratio combinations (Fig. 4.6c). As a result, the optimal synergistic condition could be selected based on the magnitude of extraction percentage alone. The single extractant exhibited the extraction percentages of 86.2 ± 1.10% and 6.0 ± 3.36% for Aliquat 336 and TBP, respectively, whereas the use of AqT mixture resulted in the improved extraction performance with the highest extraction percentage reported at 90.4 ± 0.39% when maintaining the molar ratio at 10:2. It should also be pointed out that the minimum expense for chemicals was also associated with the optimal molar ratio as compared to the single extractant system and other molar ratio combinations. Observation of synergistic extraction can be described by the formation of bulky ion pair so-called the ion pair with solvating complexes (Gaikwad and Damodaran, 1990; Gaikwad, 2003).

Synergistic amoxicillin extraction was not observed for the cases of AmT and AmD mixtures since their synergistic coefficients were less than one (S = 0.51 to 0.71 for AmT and S = 0.62 to 0.81 for AmD). The results obtained for AmT mixture disagree with outcomes of previous researches, which reported the synergistic effect of AmT

carriers on the separation of Nb(V), zirconium(IV) and lactic acid (Mishra et al., 1989; Campderros and Marchese, 2001; Matsumoto et al., 2003). The possible reason might be related to different pH values and types of diluent used during the extraction in the current work. Synergistic extraction in the previous work mentioned seemed to require high acidity as well as excess chloride ions while the condition in our work was strongly basic. As for the antagonistic extraction of AmD mixture, similar explanation to the case of AqD could be applied. The acid-base interaction between Alamine 336 and D2EHPA was the likely cause of antagonism as suggested by Quinn et al. (2013). Another reason for low extraction percentage for the AqD mixture might be related to the impurity of commercial D2EHPA used in the experiment (Fatmehsari et al., 2009).

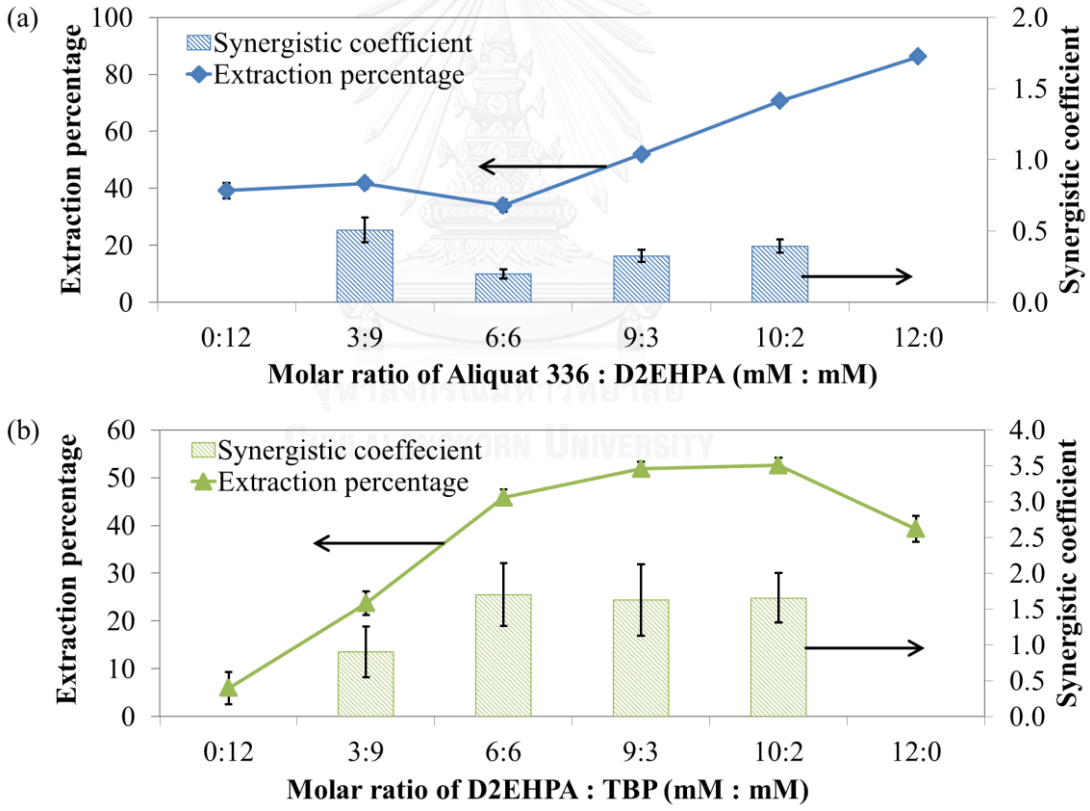

Figure 4.6 Extraction percentage and synergistic coefficient during synergistic extraction of amoxicillin by using binary mixtures: (a) AqD and (b) DT.

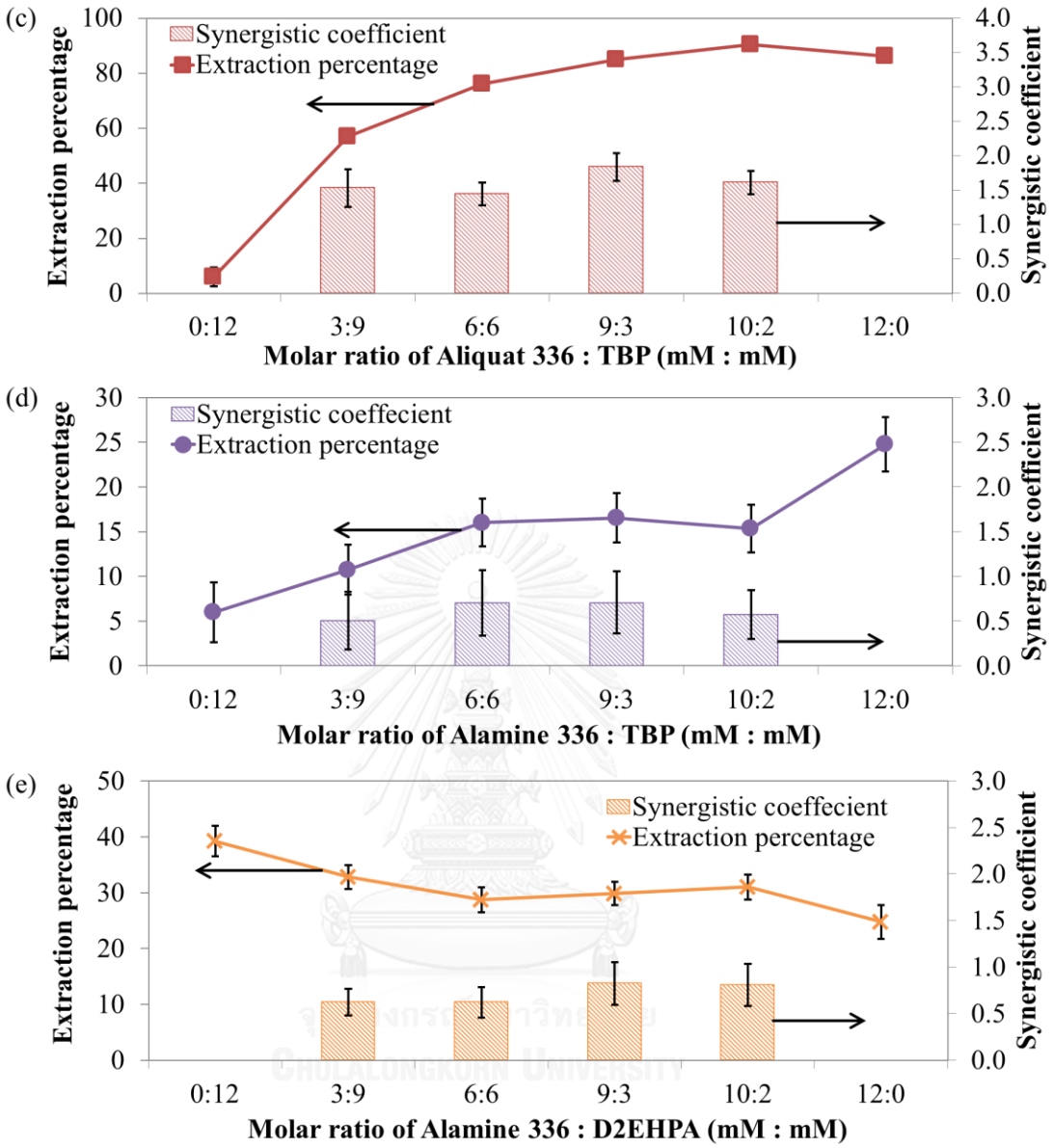

Figure 4.6 (continued) Extraction percentage and synergistic coefficient during synergistic extraction of amoxicillin by using binary mixtures:
(c) AqT (d) AmT and (e) AmD

4.2.2 Effect of pH and KCl concentration on stripping performance for synergistic extraction of amoxicillin

Amoxicillin solution was extracted by using the AqT mixture prepared at the molar ratio of 10:2. Subsequently, amoxicillin in organic phase was stripped by using 6 mM KCl solution in potassium acetate buffer with pH varied from 5 to 7. The range of the studied pH was between the optimal pH values of stripping solution for individual component in the AqT mixture. The maximum stripping percentage of 34.2 ± 0.62% was observed when the pH of stripping solution was maintained at 5 (Fig. 4.7a). The stripping percentage reached the bottom and then slightly increased as the pH of stripping solution continued to rise. It should be noted that the highest stripping percentage for the system with AqT mixture as extractant was lower than that of the system using solely Aliquat 336 (36.7 ± 1.0%). This might be attributed to stronger interaction between amoxicillin and extractant during the chemical complexation and thus making it more difficult for the stripping solution to break down the existing chemical complexes.

The effect of KCl concentration on stripping percentage of amoxicillin was also investigated in for the AqT binary mixture. It can be seen from Fig. 4.7b that varying KCl concentrations from 2 to 10 mM did not significant effect the stripping percentage. The stripping percentage remained constant at about 34% with the best stripping percentage of 34.3 ± 0.87% observed when KCl concentrations were 6 mM. The optimal stripping percentage was smaller than that from using solely Aliquat 336. Despite obtaining lower stripping percentage when applying the AqT mixture as extractant, it should be emphasized that one of the goals of this research focused on the removal of amoxicillin from aqueous solution, that is trying to obtain high value of extracting percentage.

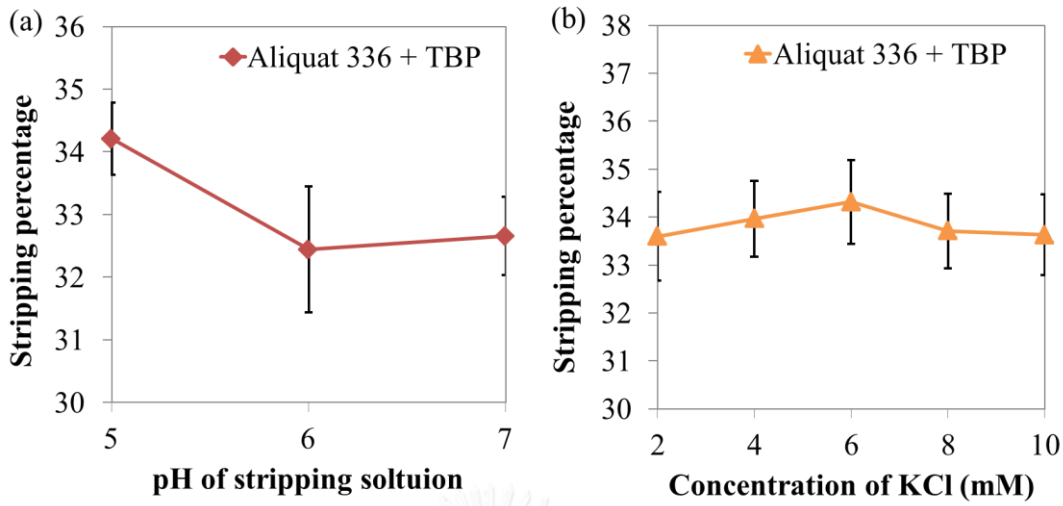

Figure 4.7 Stripping percentages of amoxicillin by using AqT mixture prepared at the molar ratio of 10:2 as the extractant: (a) varying the pH of 6 mM KCl stripping solution and (b) varying the concentrations of KCl stripping solution with the pH of KCl maintained at 5. The initial pH of amoxicillin solution was adjusted to 10.

4.3 Operation of HFSLM

In this section, the effect of flow rates of feed and stripping streams was studied. Countercurrent flow configuration of the feed and stripping streams was used. Flow rates of feed and stripping streams were identical ranging from 62.5 to 200 mL/min. It was speculated that during the operation of HFSLM, amoxicillin molecules in the feed stream diffused across the interface between feed and liquid membrane phases due to concentration gradient and subsequently reacted with the mixture of AqT to form amoxicillin-AqT complexes dissolving in the liquid membrane phase. The existing complexes then diffused across the liquid membrane-stripping interface before interrupted by the stripping reaction, resulting in the dissolution of amoxicillin into the stripping stream. Figure 4.8 and 4.9 illustrate the extraction and stripping percentage of HFSLM during the steady state operation, which was indicated by the relatively constant parameters. The steady-state values of extraction and stripping

percentages decreased from 31.8 to 20.2% and from 9.7 to 5.6%, respectively, when the flow rates were increased from 62.5 to 200 mL/min. The increase of flow rates reduced the liquid contact time for reactions although the overall film resistance to mass transfer was suppressed at higher flow rates. The estimated contact time of amoxicillin solution decreased from 6.51 to 2.04 ms when the flow rates increased from 62.5 to 200 mL/min. The time to reach steady state condition for extraction was about 25 min, which was 5 mins longer than the time required for the stripping to attain steady states. Low extraction and stripping performance observed in this work as compared to that of Pirom et al. (2014), whose work reported the extraction and stripping percentage of 85.21% and 80.34% with Aliquat 336 as the extractant, was likely linked to maintaining HFSLM in once-through mode rather than recycling. Seeing that HFSLM was operated in once-through mode. Relatively constant pH values at roughly 10 and 5 were measured at the outlets of feed and stripping streams, respectively. Constant pH profiles implied suitable condition for extraction and stripping of amoxicillin inside HFSLM.

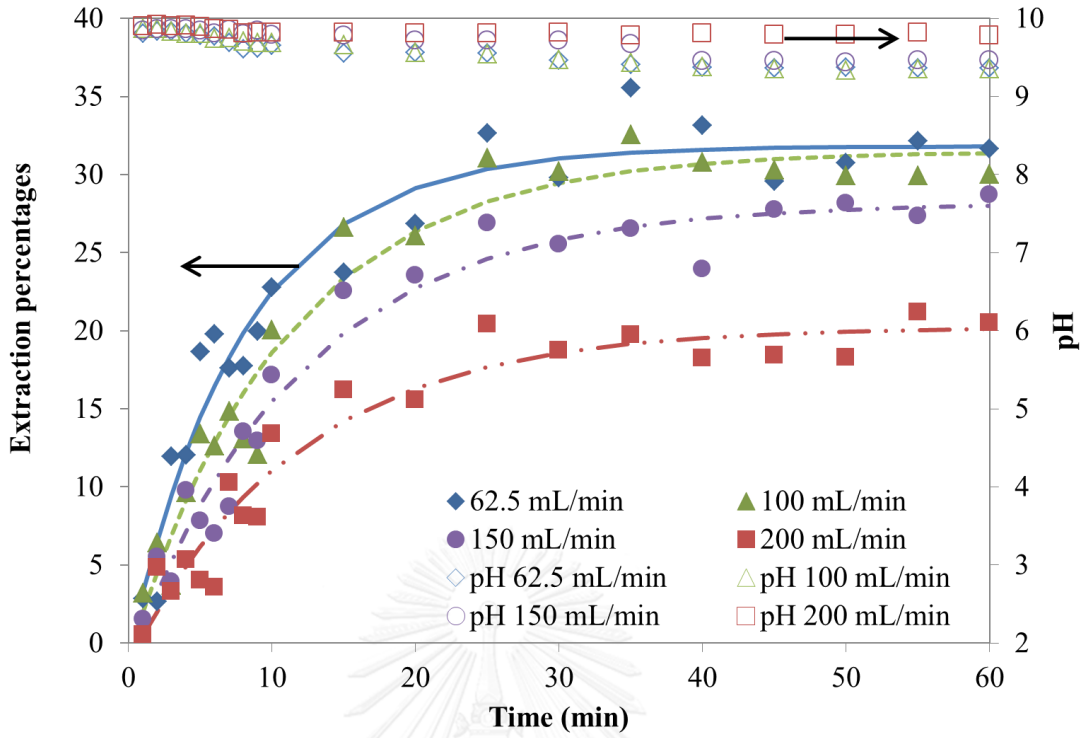

Figure 4.8 Profiles of extraction percentages and pH during the operation of HFSLM at different flow rates. Countercurrent flow and once-through mode operation of HFSLM was used during the experiment.

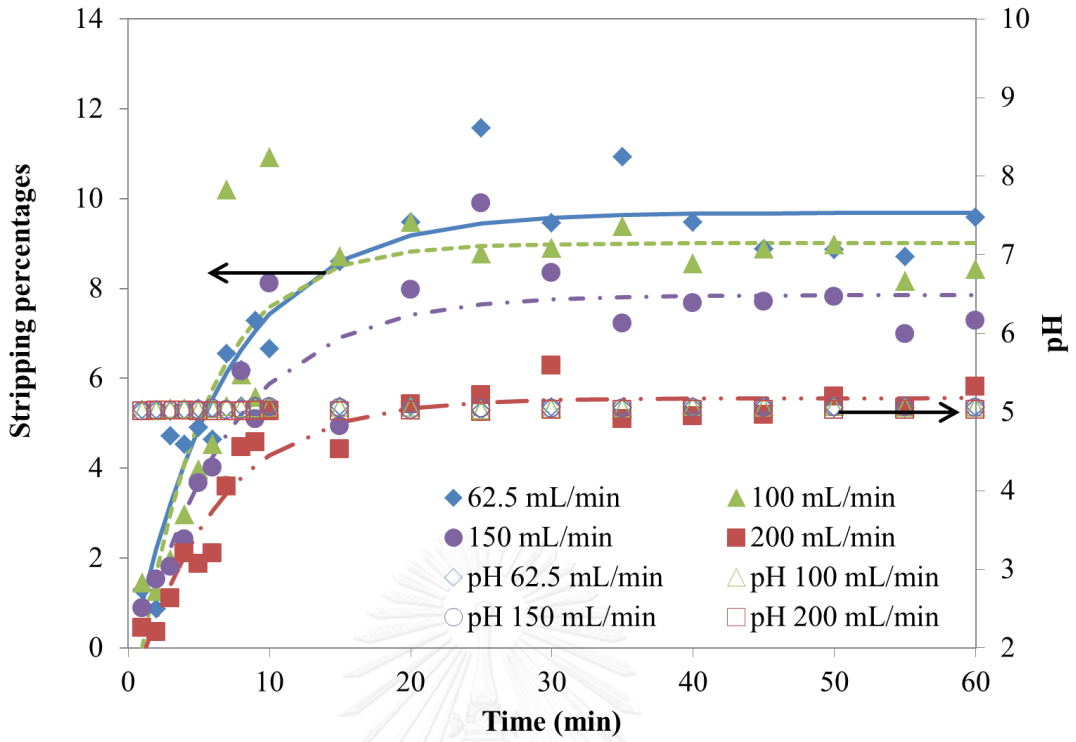

Figure 4.9 Profiles of stripping percentages and pH during the operation of HFSLM at different flow rates. Countercurrent flow and once-through mode operation of HFSLM was used during the experiment.

The COD measurement was also carried out on the prepared amoxicillin solution, liquid membrane solution, treated solution by means of reactive liquid-liquid extraction and treated solution by means of HFSLM (feed outlet). The results, as presented in Table 4.1, indicated that COD concentration of prepared amoxicillin solution was decreased by 58.6% when reactive liquid-liquid extraction was applied while surprisingly HFSLM led to an increase of COD by 93% when maintaining the volumetric flow rates at 62.5 mL/min. The reason for increasing COD in feed outlet might be linked to the contamination of liquid membrane caused by liquid shear force. It was expected that the degree of contamination was likely to increase as the flow rates of the system were increased (Kislik et al., 2010). The remaining COD at the outlet of HFSLM may be further reduced by the method of phase separation where the

settled particles are removed as sludge and floatable liquid membrane is simultaneously separated from the discharge as scum.

Table 4.1 Chemical oxygen demand (COD) for amoxicillin solution, liquid membrane and treated solution.

Sample	COD (mg/L)
Amoxicillin solution	852
Liquid membrane (AqT mixture dissolved in decanol)	117,174
Extracted amoxicillin solution by liquid-liquid extraction using AqT mixture as extractant	354
Feed outlet of HFSLM (outlet of tube side) when the system was maintained with flow rate of 62.5 mL/min	1,644

Amoxicillin extraction with Aliquat and TBP as extractants was carried out in the HFSLM when the flow rates of the amoxicillin feed and stripping streams were 62.5 mL/min. HFSLM was operated countercurrently without recycle. The steady state performance of the HFSLM was illustrated in Fig. 4.11 and 4.12. At steady state condition, 10 mM of Aliquat 336 and 2 mM of TBP yielded the extraction percentages of 23.8% and 1.6%, respectively. The synergistic coefficient of 1.42 was determined when the liquid membrane phase was AqT mixture at the molar ratio of 10:2. The magnitude of synergistic coefficient was lower than that found during the equilibrium batch extraction (S = 1.61) by 11.8% possibly due to a lack of contact time suppressing the possibility of bulky ion-pair formation between amoxicillin and AqT. According to Fig. 4.12, the steady state stripping percentages of 8.4% and 1.1% were obtained for Aliquat 336 and TBP, respectively. It was observed that the pH at inlet and outlet of both tube and shell sides were comparable and the lag time time for the system applying TBP was relatively shorter than those subjected to Aliquat 336 and AqT binary mixture. This may be attributed to the lower steric

hindrance effect or small ligand of TBP resulting in higher reaction rate of TBP-amoxicillin complexation and decomplexation (Leffler and Grunwald, 2013).

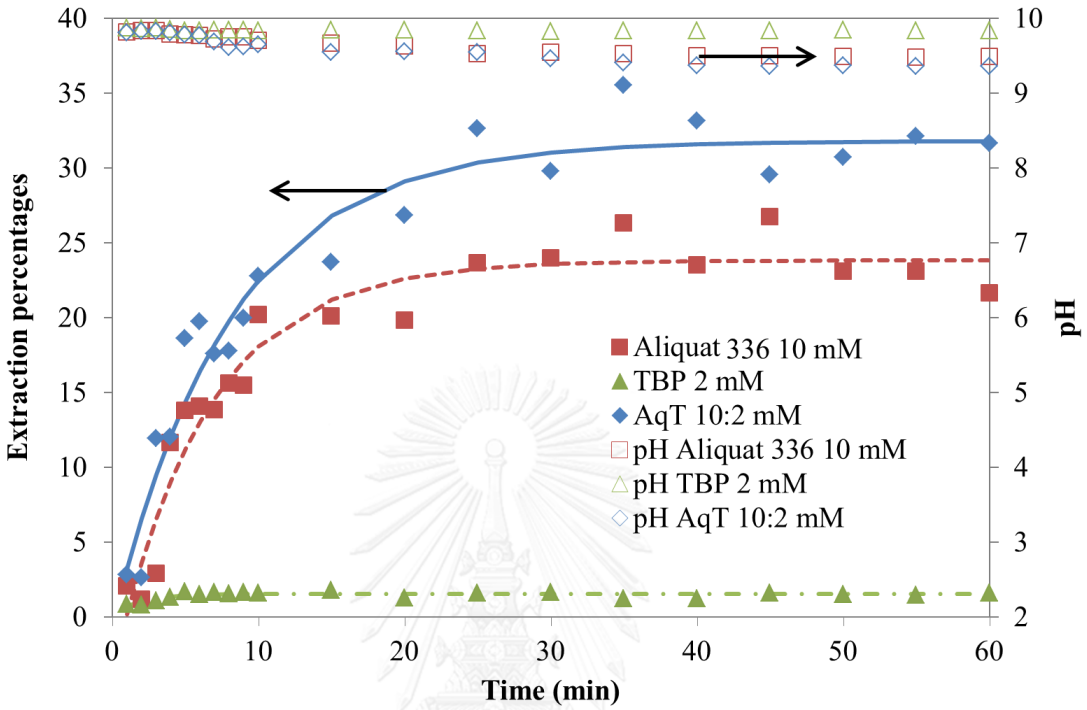

Figure 4.10 Extraction percentages and pH during the operation of HFSLM using different extractants as the liquid membrane and maintaining flow rate at 62.5 mL/min. HFSLM was operated countercurrently without recycle.

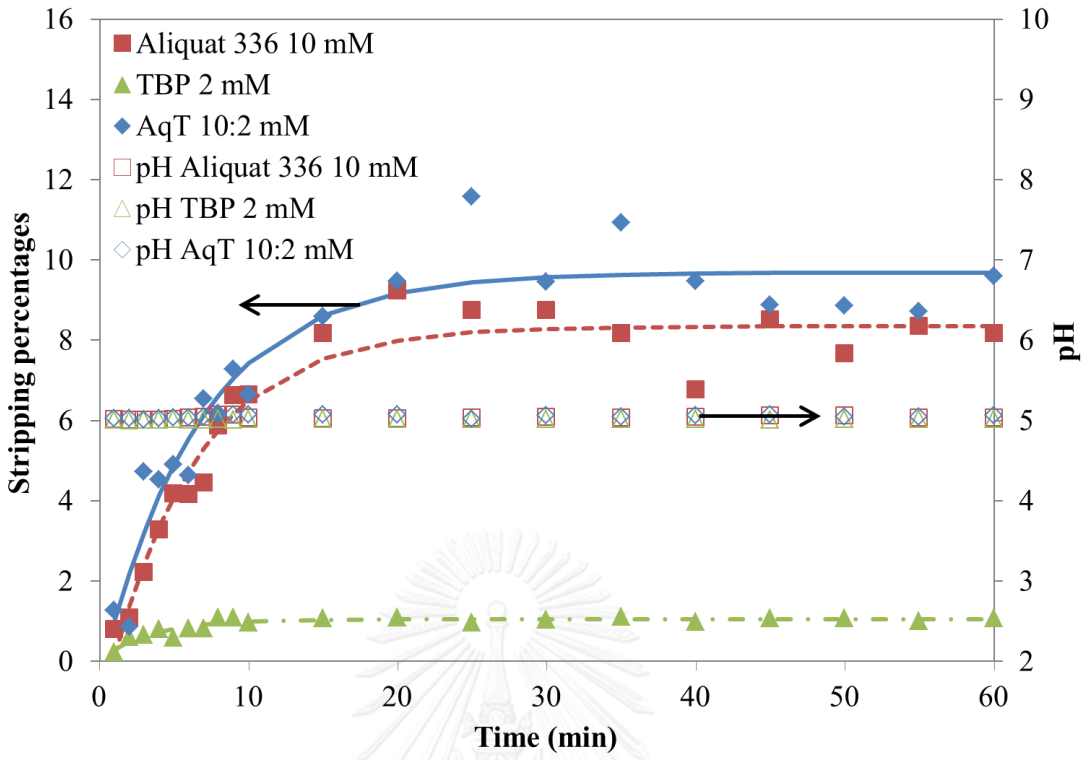

Figure 4.11 Stripping percentages and pH during the operation of HFSLM using different extractants as the liquid membrane and maintaining flow rate at 62.5 mL/min. HFSLM was operated countercurrently without recycle.

CHAPTER 5
CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The following conclusions can be drawn based on the experimental data presented in the previous chapters.

1. Commercial extractants including Aliquat 336, D2EHPA, TBP and Alamine 336 dissolved in 1-decanol were able to extract amoxicillin from aqueous solution with different degrees of success depending on the conditions maintained such as the initial pH of amoxicillin solution, concentrations of extractants, pH of stripping solution and concentrations of stripping solution.

2. The initial pH of amoxicillin solution should be maintained at 10 for all extractants employed. By maintaining the optimal pH for amoxicillin solution, it was found that the optimal extractant concentrations were 12 mM for all extractant employed with maximum extraction percentage reported at 86.2% for Aliquat 336. The reason for high extraction percentage for Aliquat 336 at high pH could be linked to the increasing number $Amox^{2-}$ that led to the formation of amoxicillin-extractant complex via ion-pair interaction. The effectiveness of amoxicillin extractant was in the following order: Aliquat 336 > D2EHPA > Alamine 336 > TBP. Stripping percentage varied with different extractants used during amoxicillin extraction. The optimal pH and concentrations of KCl stripping solution were determined at 5 and 4 mM, respectively, resulting in the maximum stripping percentage of 39.3%.

3. Synergistic extraction of amoxicillin was possible when using the binary extractant mixtures of DT and AqT. The combination of Aliquat 336 and TBP

maintaining the molar ratio of 10:2 gave the highest extraction percentage and synergistic coefficient of 90.4 ± 0.39% and 1.84 ± 0.202, respectively, due to the formation of bulky ion pair. Subsequent experiment, which employed the extracted amoxicillin solution from the optimal synergistic extraction, indicated that optimal pH and concentrations of KCl stripping should be maintained at 5 and 6 mM, respectively, leading to stripping percentage of 34.3%.

4. The optimal operating conditions obtained from the synergistic amoxicillin extraction were used to operate the HFSLM under steady state. Extractant was the AqT mixture prepared at the molar ratio of 10:2 while the stripping solution was 6 mM of KCl maintained at pH 5. Amoxicillin solution and stripping were fed countercurrently into the tube and shell sides of HFSLM, respectively at the same flow rates. The highest extraction and stripping percentage of amoxicillin were determined at 31.8% and 9.7%, respectively, when the flow rates of amoxicillin and stripping solutions were 62.5 mL/min. Lower extraction and stripping percentages compared to those from the reactive liquid-liquid extraction experiment was linked to operating the HFSLM in once-through mode that led to short liquid contact time. Moreover, it appeared that parts of the liquid membrane separated from the organic phase into the aqueous phase as suggested by the significant increase in COD in the feed outlet.

5.2 Recommendations

Based on the results of this research, the several recommendations for future work are listed.

1. *Selection of diluent*. Diluent can affect the performance of amoxicillin extraction namely chemical complexing mechanism, solubility of extractants,

stability of membrane and ease of operation in HFSLM. This work employed 1-decanol as the diluent to improve the viscosity of the extracting phase but the disadvantage was due to expensive price. Therefore, the application of other diluents and perhaps the mixtures of diluents should be considered for further study with the selection criteria include toxicity towards human and environment for both short term and long term effects, possible reactions involving amoxicillin, ease of handling and cost.

2. *Effect of temperature*. Changing temperature can affect thermodynamic and kinetic parameters of amoxicillin extraction. Moreover, the study on the effect of temperature on amoxicillin separation via HFSLM would be beneficial for formulating the mathematical model of HFSLM and advancement of HFSLM design.

3. *Mode of operation*. Other modes of operation such as semi-batch or partial recycle should be considered to increase the contact time of amoxicillin within HFSLM.

4. *Other Antibiotics and Actual Wastewater*. Separation of other beta-lactam antibiotics such as dicloxacillin and cefoxitin via HFSLM should be further investigated as well as the factors such as foreign ions and molecules, which are able to disturb the separation efficiency. In addition, the effectiveness of HFSLM operated under suitable mode should be tested with the actual wastewater containing amoxicillin. This study is necessary as other components in wastewater may adversely affect the extraction performance.

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APPENDIX

APPENDIX A
THE FORMULATIONS OF BUFFER SOLUTIONS

According to Henderson-Hasselbalch equation, 1M of phosphate and acetate buffer solutions are prepared at various pHs by dissolving the certain amount of acids and conjugate bases as shown in Table A.1-2 into DI water. To adjust the pH of phosphate buffer solution, potassium hydroxide or orthophosphoric acid is used while potassium hydroxide or acetic acid is added to acetate buffer solution for pH adjustment. The prepared phosphate and acetate buffer solutions are kept at room temperature and avoid light exposure until used.

Table A.1

Formulations of 1 liter of 1M phosphate buffer solutions at pH 2.0-10.0

Desired pH	The nearest pKa	Base (K_2HPO_4)		Acid (KH_2PO_4)	
		Moles	Grams	Moles	Grams
2.0	2.15	0.4145	72.20	0.5855	79.68
3.0	2.15	0.8762	152.62	0.1238	16.84
4.0	2.15	0.9861	171.75	0.0139	1.90
5.0	6.82	0.0149	2.60	0.9851	134.06
6.0	6.82	0.1315	22.90	0.8685	118.20
7.0	6.82	0.6022	104.88	0.3978	54.14
8.0	6.82	0.9380	163.39	0.0620	8.43
9.0	6.82	0.9934	173.04	0.0066	0.89
10.0	12.38	0.0042	0.72	0.9958	135.53

Table A.2

Formulations of 1 liter of 1M acetate buffer solutions at pH 4.0-7.0

Desired pH	The nearest pKa	Base (CH_3COOK)		Acid (CH_3COOH)	
		Moles	Grams	Moles	Grams
4.0	4.76	0.1481	14.53	0.8519	51.16
5.0	4.76	0.6348	62.30	0.3652	21.93
6.0	4.76	0.9456	92.80	0.0544	3.27
7.0	4.76	0.9943	97.58	0.0057	0.34

APPENDIX B
PREPARATION OF STANDARD CALIBRATION CURVE

Standard calibration curves of amoxicillin are prepared at particular pH in order to account for amoxicillin degradation at different aqueous acidity. After dissolving an amoxicillin capsule into DI water, adjusting the pH by adding ammonia solution or glacial acetic acid, the antibiotic solution is kept undisturbed and avoided exposure to light for 19 h before the stepwise dilution. The prepared 500 mg/L of amoxicillin solution is diluted into 375, 250, 125 and 100 mg/L. Subsequently, amoxicillin solutions at all concentrations are further diluted at dilution ratio of 4:10 to regulate the absorbance meeting the acceptable range (0.1-0.9). The concentrations of amoxicillin are calibrated by using UV-vis spectrophotometer and monitoring at 272 nm. The obtained calibration curves and equations are shown in Fig. B.1 and Table B.1, respectively.

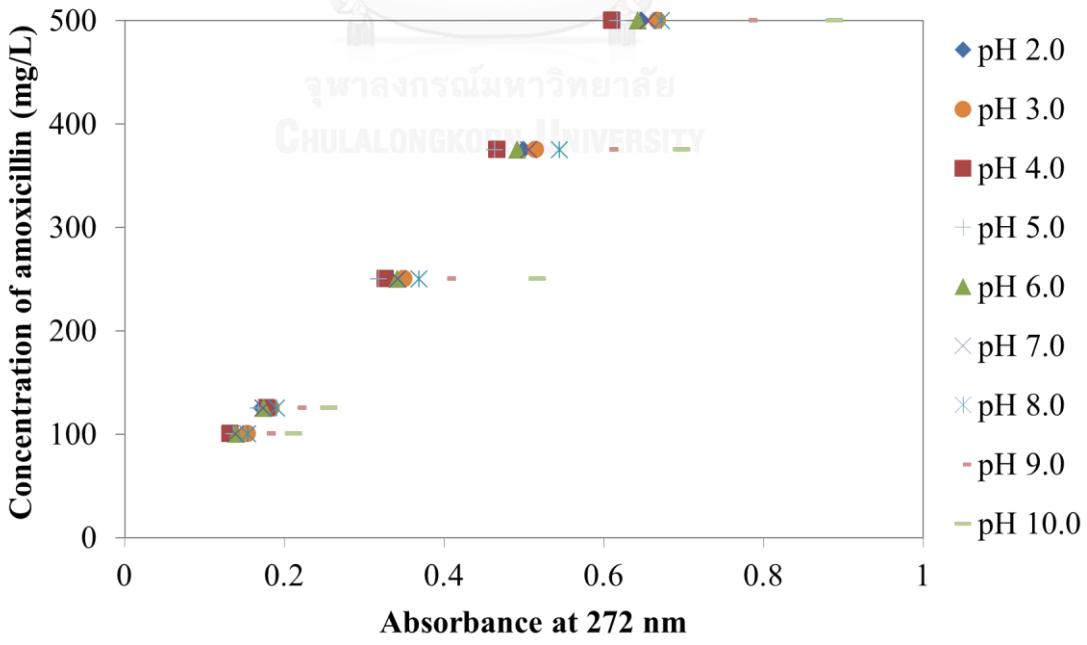

Figure B.1 Standard calibration curves of amoxicillin at pH ranged from 2.0 to 10.0

Table B.1 Calibration equations of amoxicillin at particular pH fitted to go through the origin point

pH	Calibration equation	Coefficient of determination (R^2)
2	y = 754.74x	0.9973
3	y = 731.76x	0.9960
4	y = 798.68x	0.9945
5	y = 801.06x	0.9976
6	y = 762.55x	0.9971
7	y = 747.92x	0.9983
8	y = 710.02x	0.9913
9	y = 624.06x	0.9963
10	y = 536.10x	0.9856

Fig. B.1 demonstrated the increase of absorbance with increasing initial pH of amoxicillin solution at the same concentration after the absorbance reached the bottom at pH between 4 and 5. This may be attributed to the presence of degradation products of amoxicillin whose quantity developed according to too high or too low pH and their specific wavelengths to absorb were near to 272 nm (Fig. B.2). The absorbance of amoxicillin sharply increased when initial pH increased from 8 to 10 since the basic condition catalyzed the hydrolysis reaction of amoxicillin resulted in a larger extent of degradation product formation (Connors, 1986). The least formation of degradation products at pH 5 implied that the amoxicillin molecule in aqueous solution was most stable at pH 5 conformed to the other pharmaceutical report (Kaur, et al., 2011).

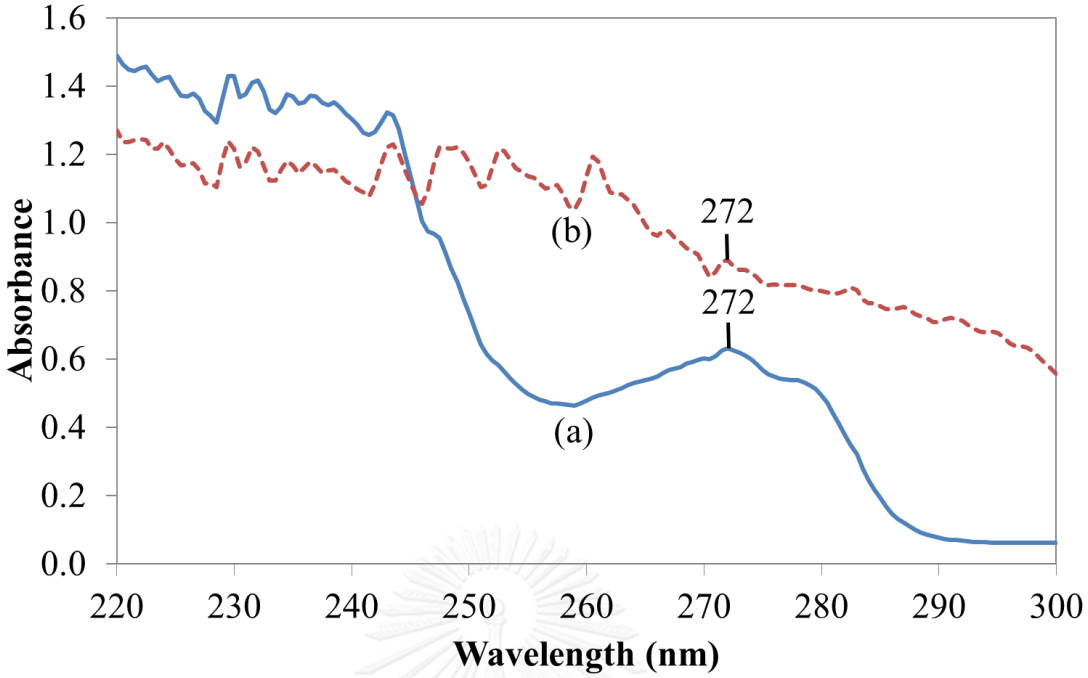

Figure B.2 UV-vis spectra of amoxicillin solutions maintained at initial pH (a) 4 and (b) 10

APPENDIX C
EXPERIMENTAL DATA

The experimental data are tabulated in Table C.1 - C.16 in chronological order according to the conceptual framework. Each data is expressed as mean ± standard deviation (n = 3). The standard deviations of calculated variables were pooled by using error propagation equations.

Table C.1 Extraction percentage of amoxicillin by using 6 mM of single extractant and varying initial pH of amoxicillin solution from 2 to 12

Initial pH	Without any extractant		
	A_{272}	*[Amox]*	Extraction percentage
2	0.6590±0.0005	499±6.7	0.2±1.69
3	0.6800±0.0016	496±8.8	0.7±2.11
4	0.6233±0.0014	499±10.2	0.3±2.38
5	0.6242±0.0008	499±6.6	0.1±1.66
6	0.6534±0.0009	499±7.2	0.2±1.79
7	0.6682±0.0016	499±6.2	0.3±1.58
8	0.7042±0.0005	499±11.7	0.1±2.69
9	0.7936±0.0030	496±9.3	0.8±2.20
10	0.9260±0.0030	498±16.2	0.4±3.59
12	0.9222±0.0012	499±15.3	0.8±3.39

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L).

Table C.1 Extraction percentage of amoxicillin by using 6 mM of single extractant and varying initial pH of amoxicillin solution from 2 to 12 (continued)

Initial pH	Aliquat 336		
	A_{272}	*[Amox]*	Extraction percentage
2	0.6481±0.0012	491±7.2	1.8±1.79
3	0.6611±0.0043	483±10.6	3.5±2.47
4	0.6116±0.0001	489±9.0	2.1±2.14
5	0.6097±0.0022	488±7.6	2.4±1.87
6	0.6212±0.0022	474±7.9	5.2±1.93
7	0.5166±0.0014	386±4.9	22.9±1.40
8	0.4347±0.0052	308±10.7	38.3±2.62
9	0.3220±0.0039	201±5.4	59.8±1.64
10	0.2962±0.0003	159±4.8	68.2±1.54
12	0.3290±0.0040	177±7.3	64.6±2.03

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L).

Table C.1 Extraction percentage of amoxicillin by using 6 mM of single extractant and varying initial pH of amoxicillin solution from 2 to 12 (continued)

Initial pH	TBP		
	A_{272}	*[Amox]*	Extraction percentage
2	0.6415±0.0036	486±8.9	2.8±2.13
3	0.6695±0.0013	489±8.5	2.3±2.05
4	0.6105±0.0007	488±9.4	2.3±2.23
5	0.6135±0.0017	491±7.2	1.8±1.79
6	0.6431±0.0011	491±7.2	1.8±1.80
7	0.6363±0.0001	475±4.8	5.0±1.33
8	0.6848±0.0020	486±12.5	2.9±2.85
9	0.7562±0.0038	473±9.4	5.5±2.25
10	0.8779±0.0002	472±13.9	5.6±3.15
12	0.8778±0.0017	472±14.8	5.5±3.32

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L).

Table C.1 Extraction percentage of amoxicillin by using 6 mM of single extractant and varying initial pH of amoxicillin solution from 2 to 12 (continued)

Initial pH	Alamine 336		
	A_{272}	*[Amox]*	Extraction percentage
2	0.6277±0.0040	476±9.1	4.9±2.18
3	0.6469±0.0074	472±12.7	5.6±2.90
4	0.6102±0.0012	488±9.8	2.4±2.31
5	0.6141±0.0009	491±6.6	1.7±1.67
6	0.6368±0.0035	486±9.0	2.8±2.16
7	0.6432±0.0032	480±7.2	4.0±1.80
8	0.6640±0.0035	471±13.2	5.8±3.01
9	0.6945±0.0012	434±7.2	13.2±1.83
10	0.7678±0.0011	413±12.7	17.4±2.95
12	0.7771±0.0041	418±14.5	16.4±3.29

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L).

Table C.1 Extraction percentage of amoxicillin by using 6 mM of single extractant and varying initial pH of amoxicillin solution from 2 to 12 (continued)

Initial pH	D2EHPA		
	A_{272}	*[Amox]*	Extraction percentage
2	0.6492±0.0004	492±6.4	1.6±1.62
3	0.5945±0.0012	434±7.0	13.2±1.79
4	0.5990±0.0048	479±10.1	4.2±2.37
5	0.6120±0.0023	490±6.5	2.1±1.65
6	0.6434±0.0001	491±6.5	1.8±1.64
7	0.6451±0.0047	481±6.3	3.7±1.62
8	0.6233±0.0061	442±11.8	11.6±2.73
9	0.6316±0.0016	395±6.3	21.1±1.67
10	0.6470±0.0012	348±10.5	30.4±2.54
12	0.6364±0.0009	342±10.2	31.5±2.50

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L).

Table C.2 Extraction percentage and distribution coefficient of amoxicillin by maintaining initial pH of amoxicillin solution at 10 and varying concentration of extractant from 2 to 15 mM

[Ex]	Aliquat 336			
	A_{272}	*[Amox]*	Extraction percentage	*D*
2	0.6047±0.0004	325±9.8	35.0±2.42	0.54±0.052
3	0.5149±0.0011	277±8.7	44.6±2.24	0.81±0.063
4	0.4507±0.0012	242±7.8	51.5±2.08	1.06±0.073
5	0.4211±0.0035	226±8.5	54.7±2.24	1.21±0.091
6	0.2962±0.0003	159±4.8	68.2±1.54	2.14±0.106
8	0.2478±0.0004	133±4.1	73.4±1.42	2.75±0.129
9	0.2317±0.0018	125±4.6	75.1±1.52	3.01±0.162
10	0.1367±0.0015	73±3.0	85.3±1.23	5.80±0.297
12	0.1283±0.0005	69±2.3	86.2±1.10	6.25±0.267
15	0.1080±0.0011	58±2.3	88.4±1.11	7.61±0.372

Note: *[Ex]* is concentration of extractant (mM), A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), *D* is distribution coefficient.

Table C.2 Extraction percentage and distribution coefficient of amoxicillin by maintaining initial pH of amoxicillin solution at 10 and varying concentration of extractant from 2 to 15 mM (continued)

[Ex]	D2EHPA			
	A_{272}	*[Amox]*	Extraction percentage	*D*
2	0.7190±0.0042	387±13.6	22.7±3.14	0.29±0.050
3	0.7230±0.0028	389±12.9	22.3±3.00	0.29±0.047
4	0.7077±0.0001	380±11.2	23.9±2.67	0.31±0.043
5	0.7002±0.0012	376±11.7	24.7±2.77	0.33±0.046
6	0.6470±0.0012	348±10.8	30.4±2.62	0.44±0.050
8	0.6294±0.0012	338±10.6	32.3±2.57	0.48±0.051
9	0.5798±0.0005	312±9.4	37.7±2.36	0.60±0.054
10	0.5756±0.0012	309±9.7	38.1±2.42	0.62±0.056
12	0.5652±0.0042	304±11.2	39.2±2.71	0.65±0.066
15	0.5795±0.0013	312±9.9	37.7±2.44	0.60±0.056

Note: *[Ex]* is concentration of extractant (mM), A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), *D* is distribution coefficient.

Table C.2 Extraction percentage and distribution coefficient of amoxicillin by maintaining initial pH of amoxicillin solution at 10 and varying concentration of extractant from 2 to 15 mM (continued)

[Ex]	TBP			
	A_{272}	*[Amox]*	Extraction percentage	*D*
2	0.8824±0.0022	474±15.1	5.1±3.38	0.05±0.037
3	0.8856±0.0007	476±14.4	4.8±3.23	0.05±0.035
4	0.8700±0.0034	468±15.6	6.5±3.48	0.07±0.039
5	0.8896±0.0033	478±15.8	4.3±3.53	0.05±0.038
6	0.8779±0.0038	472±15.9	5.6±3.54	0.06±0.039
8	0.8683±0.0021	467±14.8	6.6±3.33	0.07±0.038
9	0.8795±0.0009	473±14.4	5.4±3.24	0.06±0.036
10	0.8816±0.0022	474±15.1	5.2±3.38	0.05±0.037
12	0.8743±0.0022	470±15.0	6.0±3.36	0.06±0.038
15	0.8771±0.0023	472±15.1	5.7±3.38	0.06±0.038

Note: *[Ex]* is concentration of extractant (mM), A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), *D* is distribution coefficient.

Table C.2 Extraction percentage and distribution coefficient of amoxicillin by maintaining initial pH of amoxicillin solution at 10 and varying concentration of extractant from 2 to 15 mM (continued)

[Ex]	Alamine 336			
	A_{272}	*[Amox]*	Extraction percentage	*D*
2	0.7914±0.0005	425±12.8	14.9±2.95	0.18±0.039
3	0.7880±0.0007	424±12.8	15.3±2.96	0.18±0.040
4	0.7772±0.0007	418±12.6	16.4±2.92	0.20±0.040
5	0.7934±0.0025	427±13.9	14.7±3.17	0.17±0.042
6	0.7678±0.0011	413±12.7	17.4±2.95	0.21±0.041
8	0.7664±0.0001	412±12.2	17.6±2.84	0.21±0.040
9	0.7559±0.0002	406±12.0	18.7±2.82	0.23±0.041
10	0.7366±0.0007	396±12.0	20.8±2.82	0.26±0.043
12	0.6996±0.0038	376±13.1	24.8±3.04	0.33±0.051
15	0.7252±0.0009	390±11.9	22.0±2.80	0.28±0.044

Note: *[Ex]* is concentration of extractant (mM), A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), *D* is distribution coefficient.

Table C.3 Stripping percentage of amoxicillin by using 6 mM of KCl solution and varying pH of stripping solution from 4 to 7 after forward extraction by employing 12 mM of single extractant and maintaining initial pH of amoxicillin solution at 10

pH	Aliquat 336		
	A_{272}	*[Amox]*	Stripping percentage
4	0.2270±0.0087	182±2.3	36.3±0.58
5	0.2296±0.0027	184±4.4	36.7±1.00
6	0.2189±0.0005	167±2.5	33.4±0.62
7	0.2259±0.0006	169±2.2	33.7±0.55

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L).

Table C.3 Stripping percentage of amoxicillin by using 6 mM of KCl solution and varying pH of stripping solution from 4 to 7 after forward extraction by employing 12 mM of single extractant and maintaining initial pH of amoxicillin solution at 10 (continued)

pH	D2EHPA		
	A_{272}	*[Amox]*	Stripping percentage
4	0.0853±0.0022	68±3.0	13.6±0.65
5	0.0810±0.0021	65±2.5	13.0±0.53
6	0.0822±0.0018	63±2.2	12.5±0.49
7	0.0786±0.0008	59±1.2	11.7±0.28

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L).

Table C.3 Stripping percentage of amoxicillin by using 6 mM of KCl solution and varying pH of stripping solution from 4 to 7 after forward extraction by employing 12 mM of single extractant and maintaining initial pH of amoxicillin solution at 10 (continued)

pH	TBP		
	A_{272}	*[Amox]*	Stripping percentage
4	0.0161±0.0035	13±3.0	2.6±0.62
5	0.0156±0.0056	12±4.6	2.5±0.93
6	0.0123±0.0029	9±2.3	1.9±0.47
7	0.0358±0.0066	27±5.2	5.3±1.06

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L).

Table C.3 Stripping percentage of amoxicillin by using 6 mM of KCl solution and varying pH of stripping solution from 4 to 7 after forward extraction by employing 12 mM of single extractant and maintaining initial pH of amoxicillin solution at 10 (continued)

pH	Alamine 336		
	A_{272}	*[Amox]*	Stripping percentage
4	0.0459±0.0076	37±6.8	7.3±1.38
5	0.0524±0.0055	42±4.9	8.4±1.01
6	0.0454±0.0068	35±5.6	6.9±1.15
7	0.0460±0.0022	34±2.0	6.9±0.42

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L).

Table C.4 Stripping percentage of amoxicillin by varying concentration of KCl from 2 to 10 mM and adjusting pH of stripping solution to the optimal value after forward extraction by employing 12 mM of single extractant and maintaining initial pH of amoxicillin solution at 10

[KCl]	Aliquat 336		
	A_{272}	*[Amox]*	Stripping percentage
2	0.2184±0.0003	175±3.4	34.9±0.80
4	0.2457±0.0031	197±6.1	39.3±1.35
6	0.2244±0.0028	180±5.5	35.9±1.22
8	0.2219±0.0012	178±4.1	35.5±0.95
10	0.2207±0.0017	177±4.6	35.3±1.04

Note: *[KCl]* is concentration of potassium chloride in stripping solution (mM), A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L).

Table C.4 Stripping percentage of amoxicillin by varying concentration of KCl from 2 to 10 mM and adjusting pH of stripping solution to the optimal value after forward extraction by employing 12 mM of single extractant and maintaining initial pH of amoxicillin solution at 10 (continued)

[KCl]	D2EHPA		
	A_{272}	*[Amox]*	Stripping percentage
2	0.1986±0.0425	159±36.9	31.8±7.48
4	0.1825±0.0206	146±19.1	29.2±3.93
6	0.0854±0.0128	68±11.5	13.7±2.34
8	0.0866±0.0156	69±13.7	13.9±2.79
10	0.0873±0.0202	70±17.4	14.0±3.54

Note: *[KCl]* is concentration of potassium chloride in stripping solution (mM), A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L).

Table C.4 Stripping percentage of amoxicillin by varying concentration of KCl from 2 to 10 mM and adjusting pH of stripping solution to the optimal value after forward extraction by employing 12 mM of single extractant and maintaining initial pH of amoxicillin solution at 10 (continued)

[KCl]	TBP		
	A_{272}	*[Amox]*	Stripping percentage
2	0.0086±0.0014	6±1.2	1.3±0.24
4	0.0094±0.0009	7±0.8	1.4±0.17
6	0.0349±0.0070	26±5.7	5.2±1.16
8	0.0166±0.0025	12±2.1	2.5±0.43
10	0.0152±0.0012	11±1.1	2.3±0.23

Note: *[KCl]* is concentration of potassium chloride in stripping solution (mM), A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L).

Table C.4 Stripping percentage of amoxicillin by varying concentration of KCl from 2 to 10 mM and adjusting pH of stripping solution to the optimal value after forward extraction by employing 12 mM of single extractant and maintaining initial pH of amoxicillin solution at 10 (continued)

[KCl]	Alamine 336		
	A_{272}	*[Amox]*	Stripping percentage
2	0.0475±0.0088	38±7.7	7.6±1.57
4	0.0442±0.0011	35±1.5	7.1±0.33
6	0.0504±0.0043	40±4.2	8.1±0.86
8	0.0415±0.0016	33±1.9	6.6±0.39
10	0.0410±0.0016	33±1.8	6.6±0.39

Note: *[KCl]* is concentration of potassium chloride in stripping solution (mM), A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L).

Table C.5 Synergistic coefficient of binary extractant systems with total extractant concentration equal to 12 mM

$[Ex_1]$: $[Ex_2]$	1st Aliquat 336 + 2nd D2EHPA				
	A_{272}	*[Amox]*	Extraction percentage	*D*	*S*
3:9	0.5413±0.0025	291±9.9	41.8±1.98	0.72±0.064	0.51±0.088
6:6	0.6145±0.0012	330±10.3	33.9±2.06	0.51±0.052	0.20±0.032
9:3	0.4466±0.0007	240±7.4	52.0±1.48	1.08±0.071	0.33±0.042
10:2	0.2723±0.0023	146±5.5	70.7±1.11	2.41±0.141	0.40±0.046

Note: $[Ex_1] : [Ex_2]$ is mole ratio of 1st extractant to 2nd extractant (mM:mM), A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), *D* is distribution coefficient, *S* is synergistic coefficient.

Table C.5 Synergistic coefficient of binary extractant systems with total extractant concentration equal to 12 mM (continued)

$[Ex_1]$: $[Ex_2]$	1st D2EHPA + 2nd TBP				
	A_{272}	*[Amox]*	Extraction percentage	*D*	*S*
3:9	0.7095±0.0022	381±12.4	23.7±2.47	0.31±0.047	0.90±0.355
6:6	0.5037±0.0011	271±8.5	45.8±1.70	0.85±0.064	1.70±0.435
9:3	0.4479±0.0004	241±7.3	51.8±1.45	1.08±0.070	1.63±0.498
10:2	0.4409±0.0013	237±7.7	52.6±1.53	1.11±0.075	1.66±0.344

Note: $[Ex_1] : [Ex_2]$ is mole ratio of 1st extractant to 2nd extractant (mM:mM), A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), *D* is distribution coefficient, *S* is synergistic coefficient.

Table C.5 Synergistic coefficient of binary extractant systems with total extractant concentration equal to 12 mM (continued)

$[Ex_1]$: $[Ex_2]$	1st Aliquat 336 + 2nd TBP				
	A_{272}	*[Amox]*	Extraction percentage	*D*	*S*
3:9	0.4003±0.0018	215±7.3	57.0±1.46	1.32±0.087	1.53±0.276
6:6	0.2222±0.0010	119±4.0	76.1±0.80	3.19±0.155	1.45±0.166
9:3	0.1403±0.0009	75±2.7	84.9±0.53	5.63±0.258	1.84±0.202
10:2	0.0891±0.0010	48±1.9	90.4±0.39	9.44±0.459	1.61±0.170

Note: $[Ex_1]$: $[Ex_2]$ is mole ratio of 1st extractant to 2nd extractant (mM:mM), A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), *D* is distribution coefficient, *S* is synergistic coefficient.

Table C.5 Synergistic coefficient of binary extractant systems with total extractant concentration equal to 12 mM (continued)

$[Ex_1]$: $[Ex_2]$	1st Alamine 336 + 2nd TBP				
	A_{272}	*[Amox]*	Extraction percentage	*D*	*S*
3:9	0.8302±0.0016	446±13.9	10.7±2.79	0.12±0.039	0.51±0.324
6:6	0.7811±0.0018	420±13.3	16.0±2.66	0.19±0.042	0.70±0.365
9:3	0.7761±0.0027	417±13.7	16.5±2.74	0.20±0.044	0.71±0.347
10:2	0.7872±0.0016	423±13.3	15.4±2.66	0.18±0.041	0.57±0.275

Note: $[Ex_1]$: $[Ex_2]$ is mole ratio of 1st extractant to 2nd extractant (mM:mM), A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), *D* is distribution coefficient, *S* is synergistic coefficient.

Table C.5 Synergistic coefficient of binary extractant systems with total extractant concentration equal to 12 mM (contined)

$[Ex_1]$: $[Ex_2]$	1st Alamine 336 + 2nd D2EHPA				
	A_{272}	*[Amox]*	Extraction percentage	*D*	*S*
3:9	0.6248±0.0016	336±10.7	32.8±2.15	0.49±0.053	0.62±0.142
6:6	0.6629±0.0014	356±11.2	28.7±2.24	0.40±0.049	0.62±0.163
9:3	0.6525±0.0003	351±10.5	29.8±2.09	0.43±0.047	0.82±0.232
10:2	0.6416±0.0016	345±11.1	31.0±2.20	0.45±0.051	0.81±0.227

Note: $[Ex_1]$: $[Ex_2]$ is mole ratio of 1st extractant to 2nd extractant (mM:mM), A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), *D* is distribution coefficient, *S* is synergistic coefficient.

Table C.6 Stripping percentage of amoxicillin by using 6 mM of KCl solution and varying pH of stripping solution from 5 to 7 after forward extraction by employing 10 mM of Aliquat 336 combined with 2 mM of TBP and maintaining initial pH of amoxicillin solution at 10

pH	10 mM Aliquat 336 + 2 mM TBP		
	A_{272}	*[Amox]*	Stripping percentage
5	0.2138±0.0006	171±2.5	34.2±0.62
6	0.2125±0.0016	162±3.4	32.4±0.79
7	0.2188±0.0030	163±3.9	32.7±0.89

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L).

Table C.7 Stripping percentage of amoxicillin by varying concentration of KCl from 2 to 10 mM and adjusting pH of stripping solution to 5 after forward extraction by employing 10 mM of Aliquat 336 combined with 2 mM of TBP and maintaining initial pH of amoxicillin solution at 10

[KCl]	10 mM Aliquat 336 + 2 mM TBP		
	A_{272}	*[Amox]*	Stripping percentage
2	0.2100±0.0013	168±4.0	33.6±0.92
4	0.2123±0.0004	170±3.4	34.0±0.79
6	0.2145±0.0008	172±3.8	34.3±0.87
8	0.2107±0.0003	169±3.3	33.7±0.78
10	0.2102±0.0008	168±3.6	33.6±0.85

Note: *[KCl]* is concentration of potassium chloride in stripping solution (mM), A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L).

Table C.8 The performance of amoxicillin separation via HFSLM at volumetric flow rate ranged from 62.5 to 200 mL/min by using 10 mM Aliquat 336 mixed with 2 mM TBP as a liquid membrane

Time (min)	Flow rate = 62.5 mL/min							
	Tube side				Shell side			
	A_{272}	*[Amox]*	%Ex	pH	A_{272}	*[Amox]*	%Str	pH
1	0.8911	479	2.8	9.81	0.0079	6	1.28	5.03
2	0.8926	480	2.7	9.83	0.0053	4	0.86	5.03
3	0.8073	434	12.0	9.83	0.0291	23	4.72	5.01
4	0.8068	434	12.0	9.81	0.0279	22	4.53	5.03
5	0.7461	401	18.6	9.78	0.0302	24	4.90	5.04
6	0.7357	396	19.8	9.77	0.0286	23	4.64	5.04
7	0.7554	406	17.6	9.69	0.0403	32	6.54	5.05
8	0.7541	405	17.8	9.61	0.0381	30	6.18	5.07
9	0.7339	395	20.0	9.62	0.0449	36	7.29	5.07
10	0.7082	381	22.8	9.65	0.0410	33	6.65	5.07
15	0.6995	376	23.7	9.55	0.0530	42	8.60	5.07
20	0.6706	361	26.9	9.56	0.0584	47	9.48	5.07
25	0.6174	332	32.7	9.55	0.0713	57	11.57	5.02
30	0.6438	346	29.8	9.46	0.0583	47	9.46	5.06
35	0.5909	318	35.6	9.41	0.0673	54	10.92	5.05
40	0.6131	330	33.1	9.37	0.0584	47	9.48	5.06
45	0.6458	347	29.6	9.36	0.0547	44	8.88	5.07

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), %Ex is extraction percentage, %Str is stripping percentage.

Table C.8 The performance of amoxicillin separation via HFSLM at volumetric flow rate ranged from 62.5 to 200 mL/min by using 10 mM Aliquat 336 mixed with 2 mM TBP as a liquid membrane (continued)

Time (min)	Flow rate = 62.5 mL/min							
	Tube side				Shell side			
	A_{272}	*[Amox]*	%Ex	pH	A_{272}	*[Amox]*	%Str	pH
50	0.6350	341	30.8	9.37	0.0546	44	8.86	5.05
55	0.6223	335	32.1	9.36	0.0537	43	8.71	5.05
60	0.6266	337	31.7	9.36	0.0591	47	9.59	5.06

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), %Ex is extraction percentage, %Str is stripping percentage.

Table C.8 The performance of amoxicillin separation via HFSLM at volumetric flow rate ranged from 62.5 to 200 mL/min by using 10 mM Aliquat 336 mixed with 2 mM TBP as a liquid membrane (continued)

Time (min)	Flow rate = 100 mL/min							
	Tube side				Shell side			
	A_{272}	*[Amox]*	%Ex	pH	A_{272}	*[Amox]*	%Str	pH
1	0.9090	489	3.2	9.87	0.0091	7	1.4	5.02
2	0.8791	473	6.4	9.87	0.0079	6	1.3	5.02
3	0.9047	486	3.7	9.83	0.0124	10	2.0	5.04
4	0.8488	456	9.6	9.81	0.0187	15	3.0	5.04
5	0.8135	437	13.4	9.81	0.0250	20	4.0	5.03
6	0.8208	441	12.6	9.74	0.0286	23	4.5	5.04
7	0.8000	430	14.8	9.75	0.0643	51	10.2	5.07
8	0.8163	439	13.1	9.71	0.0384	31	6.1	5.05
9	0.8260	444	12.1	9.69	0.0352	28	5.6	5.05
10	0.7511	404	20.0	9.69	0.0689	55	10.9	5.06
15	0.6893	371	26.6	9.66	0.0550	44	8.7	5.06
20	0.6945	373	26.1	9.56	0.0598	48	9.5	5.06
25	0.6475	348	31.1	9.54	0.0554	44	8.8	5.03
30	0.6554	352	30.2	9.47	0.0561	45	8.9	5.04
35	0.6335	341	32.6	9.44	0.0592	47	9.4	5.05
40	0.6500	349	30.8	9.38	0.0540	43	8.6	5.07

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), %Ex is extraction percentage, %Str is stripping percentage.

Table C.8 The performance of amoxicillin separation via HFSLM at volumetric flow rate ranged from 62.5 to 200 mL/min by using 10 mM Aliquat 336 mixed with 2 mM TBP as a liquid membrane (continued)

Time (min)	Flow rate = 100 mL/min							
	Tube side				Shell side			
	A_{272}	*[Amox]*	%Ex	pH	A_{272}	*[Amox]*	%Str	pH
45	0.6548	352	30.3	9.35	0.0560	45	8.9	5.06
50	0.6582	354	29.9	9.34	0.0566	45	9.0	5.07
55	0.6581	354	29.9	9.35	0.0515	41	8.2	5.06
60	0.6575	353	30.0	9.35	0.0532	43	8.4	5.06

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), %Ex is extraction percentage, %Str is stripping percentage.

Table C.8 The performance of amoxicillin separation via HFSLM at volumetric flow rate ranged from 62.5 to 200 mL/min by using 10 mM Aliquat 336 mixed with 2 mM TBP as a liquid membrane (continued)

Time (min)	Flow rate = 150 mL/min							
	Tube side				Shell side			
	A_{272}	*[Amox]*	%Ex	pH	A_{272}	*[Amox]*	%Str	pH
1	0.9123	490	1.5	9.84	0.0055	4	0.9	5.01
2	0.8752	471	5.5	9.86	0.0095	8	1.5	5.02
3	0.8897	478	3.9	9.86	0.0112	9	1.8	5.02
4	0.8356	449	9.8	9.86	0.0150	12	2.4	5.02
5	0.8536	459	7.8	9.84	0.0228	18	3.7	5.03
6	0.8615	463	7.0	9.81	0.0250	20	4.0	5.04
7	0.8454	455	8.7	9.85	0.0224	18	3.6	5.03
8	0.8010	431	13.5	9.80	0.0383	31	6.2	5.03
9	0.8065	434	12.9	9.84	0.0317	25	5.1	5.06
10	0.7674	413	17.2	9.79	0.0505	40	8.1	5.06
15	0.7174	386	22.6	9.78	0.0307	25	4.9	5.05
20	0.7080	381	23.6	9.72	0.0496	40	8.0	5.03
25	0.6771	364	26.9	9.72	0.0616	49	9.9	5.04
30	0.6899	371	25.5	9.72	0.0520	42	8.4	5.04
35	0.6804	366	26.5	9.67	0.0449	36	7.2	5.04
40	0.7045	379	23.9	9.45	0.0478	38	7.7	5.05

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), %Ex is extraction percentage, %Str is stripping percentage.

Table C.8 The performance of amoxicillin separation via HFSLM at volumetric flow rate ranged from 62.5 to 200 mL/min by using 10 mM Aliquat 336 mixed with 2 mM TBP as a liquid membrane (continued)

Time (min)	Flow rate = 150 mL/min							
	Tube side				Shell side			
	A_{272}	*[Amox]*	%Ex	pH	A_{272}	*[Amox]*	%Str	pH
45	0.6692	360	27.8	9.45	0.0480	38	7.7	5.05
50	0.6652	358	28.2	9.44	0.0486	39	7.8	5.06
55	0.6731	362	27.3	9.46	0.0435	35	7.0	5.06
60	0.6604	355	28.7	9.46	0.0453	36	7.3	5.05

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), %Ex is extraction percentage, %Str is stripping percentage.

Table C.8 The performance of amoxicillin separation via HFSLM at volumetric flow rate ranged from 62.5 to 200 mL/min by using 10 mM Aliquat 336 mixed with 2 mM TBP as a liquid membrane (continued)

Time (min)	Flow rate = 200 mL/min							
	Tube side				Shell side			
	A_{272}	*[Amox]*	%Ex	pH	A_{272}	*[Amox]*	%Str	pH
1	0.9214	495	0.5	9.90	0.0028	2	0.4	5.01
2	0.8814	474	4.8	9.92	0.0022	2	0.4	5.02
3	0.8959	482	3.3	9.90	0.0069	6	1.1	5.02
4	0.8767	471	5.4	9.91	0.0131	10	2.1	5.02
5	0.8891	478	4.0	9.89	0.0116	9	1.9	5.01
6	0.8931	480	3.6	9.86	0.0131	10	2.1	5.01
7	0.8312	447	10.3	9.85	0.0223	18	3.6	5.01
8	0.8508	457	8.1	9.81	0.0278	22	4.5	5.01
9	0.8517	458	8.1	9.82	0.0285	23	4.6	5.02
10	0.8021	431	13.4	9.82	0.0329	26	5.3	5.01
15	0.7762	417	16.2	9.82	0.0275	22	4.4	5.01
20	0.7819	420	15.6	9.81	0.0337	27	5.4	5.02
25	0.7372	396	20.4	9.81	0.0350	28	5.6	5.00
30	0.7525	405	18.8	9.82	0.0391	31	6.3	5.03
35	0.7435	400	19.7	9.78	0.0317	25	5.1	5.03
40	0.7573	407	18.2	9.81	0.0321	26	5.2	5.03

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), %Ex is extraction percentage, %Str is stripping percentage.

Table C.8 The performance of amoxicillin separation via HFSLM at volumetric flow rate ranged from 62.5 to 200 mL/min by using 10 mM Aliquat 336 mixed with 2 mM TBP as a liquid membrane (continued)

Time (min)	Flow rate = 200 mL/min							
	Tube side				Shell side			
	A_{272}	*[Amox]*	%Ex	pH	A_{272}	*[Amox]*	%Str	pH
45	0.7555	406	18.4	9.79	0.0323	26	5.2	5.03
50	0.7569	407	18.3	9.79	0.0348	28	5.6	5.03
55	0.7300	392	21.2	9.82	0.0333	27	5.3	5.03
60	0.7363	396	20.5	9.78	0.0362	29	5.8	5.03

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), %Ex is extraction percentage, %Str is stripping percentage.

Table C.9 The nonlinear regression model of experimental data between extraction percentage and time

Functions	Nonlinear regression model		
	62.5 mL/min	100 mL/min	150 mL/min
Asymtotic regression $Y = A-Be^{-CX}$	$Y = 31.82 - 32.37e^{-0.12X}$	$Y = 31.51 - 32.40e^{-0.09X}$	$Y = 28.19 - 29.50e^{-0.08X}$
Michaelis-Menten $Y = AX/(B+X)$	$Y = 37.41X / (7.26 + X)$	$Y = 39.20X / (11.52 + X)$	$Y = 36.40X / (14.09 + X)$
Power $Y = AX^B$	$Y = 8.97X^{0.34}$	$Y = 6.54X^{0.41}$	$Y = 4.95X^{0.45}$

Note: Y is responses or extraction percentages (%), X is predictors or points of time (min), A is the 1st parameter, B is the 2nd parameter, C is the 3rd parameter and SE is standard error.

Table C.9 The nonlinear regression model of experimental data between extraction percentage and time (continued)

Functions	Nonlinear regression model	Total value of SE
	200 mL/min	
Asymtotic regression $Y = A\text{-}Be^{-CX}$	$Y = 20.25 - 21.55e^{-0.09X}$	8.6495
Michaelis-Menten $Y = AX/(B+X)$	$Y = 26.41X / (14.63 + X)$	9.5714
Power $Y = AX^{B}$	$Y = 3.45X^{0.46}$	13.8714

Note: Y is responses or extraction percentages (%), X is predictors or points of time (min), A is the 1st parameter, B is the 2nd parameter, C is the 3rd parameter and SE is standard error.

Table C.10 The nonlinear regression model of experimental data between stripping percentage and time

Functions	Nonlinear regression model		
	62.5 mL/min	100 mL/min	150 mL/min
Asymtotic regression $Y = A-Be^{-CX}$	$Y = 9.69 - 10.11e^{-0.15X}$	$Y = 9.01 - 11.09e^{-0.21X}$	$Y = 7.86 - 8.75e^{-0.15X}$
Michaelis-Menten $Y = AX/(B+X)$	$Y = 11.10X / (5.77 + X)$	$Y = 10.32X / (5.04 + X)$	$Y = 9.21X / (6.78 + X)$
Power $Y = AX^{B}$	$Y = 3.24X^{0.30}$	$Y = 3.29X^{0.27}$	$Y = 2.38X^{0.32}$

Note: Y is responses or stripping percentages (%), X is predictors or points of time (min), A is the 1st parameter, B is the 2nd parameter, C is the 3rd parameter and SE is standard error.

Table C.10 The nonlinear regression model of experimental data between stripping percentage and time (continued)

Functions	Nonlinear regression model	Total value of SE
	200 mL/min	
Asymtotic regression $Y = A\text{-}Be^{-CX}$	$Y = 5.56 - 6.87e^{-0.17X}$	4.0762
Michaelis-Menten $Y = AX/(B+X)$	$Y = 6.64X / (7.16 + X)$	4.7249
Power $Y = AX^{B}$	$Y = 1.60X^{0.34}$	6.2334

Note: Y is responses or stripping percentages (%), X is predictors or points of time (min), A is the 1st parameter, B is the 2nd parameter, C is the 3rd parameter and SE is standard error.

Table C.11 Steady-state extraction and stripping percentage of amoxicillin via HFSLM at different countercurrent flow rates

Flow rate (mL/min)	Steady-state extraction percentage	Steady-state stripping percentage
62.5	31.8	9.7
100	31.5	9.0
150	28.2	7.9
200	20.2	5.6

Table C.12 The performance of amoxicillin separation via HFSLM at volumetric flow rate of 62.5 mL/min by using 10 mM Aliquat 336 as a liquid membrane

Time (min)	10 mM Aliquat 336							
	Tube side				Shell side			
	A_{272}	*[Amox]*	%Ex	pH	A_{272}	*[Amox]*	%Str	pH
1	0.9105	490	2.1	9.82	0.0050	4	0.8	5.02
2	0.9187	494	1.2	9.84	0.0068	5	1.1	5.01
3	0.9029	485	2.9	9.84	0.0139	11	2.2	5.01
4	0.8215	442	11.7	9.79	0.0205	16	3.3	5.01
5	0.8014	431	13.8	9.78	0.0262	21	4.2	5.02
6	0.7988	429	14.1	9.77	0.0260	21	4.2	5.04
7	0.8011	431	13.9	9.73	0.0279	22	4.5	5.05
8	0.7846	422	15.6	9.75	0.0367	29	5.9	5.07
9	0.7860	423	15.5	9.75	0.0415	33	6.6	5.07
10	0.7418	399	20.2	9.71	0.0416	33	6.7	5.04
15	0.7429	399	20.1	9.67	0.0511	41	8.2	5.03
20	0.7453	401	19.9	9.63	0.0578	46	9.2	5.03
25	0.7099	382	23.7	9.53	0.0547	44	8.8	5.04
30	0.7068	380	24.0	9.55	0.0547	44	8.8	5.05
35	0.6851	368	26.3	9.53	0.0511	41	8.2	5.04
40	0.7113	382	23.5	9.50	0.0424	34	6.8	5.05

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), %Ex is extraction percentage, %Str is stripping percentage.

Table C.12 The performance of amoxicillin separation via HFSLM at volumetric flow rate of 62.5 mL/min by using 10 mM Aliquat 336 as a liquid membrane (continued)

Time (min)	10 mM Aliquat 336							
	Tube side				Shell side			
	A_{272}	*[Amox]*	%Ex	pH	A_{272}	*[Amox]*	%Str	pH
45	0.6810	366	26.8	9.50	0.0533	43	8.5	5.06
50	0.7152	385	23.1	9.49	0.0480	38	7.7	5.06
55	0.7149	384	23.1	9.48	0.0522	42	8.4	5.04
60	0.7286	392	21.7	9.49	0.0511	41	8.2	5.04

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), %Ex is extraction percentage, %Str is stripping percentage.

Table C.13 The performance of amoxicillin separation via HFSLM at volumetric flow rate of 62.5 mL/min by using 2 mM TBP as a liquid membrane

Time (min)	2 mM TBP							
	Tube side				Shell side			
	A_{272}	*[Amox]*	%Ex	pH	A_{272}	*[Amox]*	%Str	pH
1	0.9219	496	0.9	9.88	0.0014	1	0.2	5.01
2	0.9222	496	0.8	9.87	0.0038	3	0.6	5.00
3	0.9199	495	1.1	9.88	0.0041	3	0.7	5.01
4	0.9176	493	1.3	9.86	0.0050	4	0.8	5.01
5	0.9141	491	1.7	9.84	0.0037	3	0.6	5.02
6	0.9160	492	1.5	9.84	0.0052	4	0.8	5.01
7	0.9144	492	1.7	9.85	0.0051	4	0.8	5.01
8	0.9154	492	1.6	9.85	0.0068	5	1.1	5.02
9	0.9147	492	1.6	9.85	0.0068	5	1.1	5.01
10	0.9148	492	1.6	9.84	0.0060	5	1.0	5.02
15	0.9132	491	1.8	9.85	0.0067	5	1.1	5.02
20	0.9179	493	1.3	9.85	0.0068	5	1.1	5.02
25	0.9150	492	1.6	9.84	0.0060	5	1.0	5.02
30	0.9144	492	1.7	9.83	0.0065	5	1.0	5.02
35	0.9185	494	1.2	9.84	0.0069	6	1.1	5.02
40	0.9184	494	1.2	9.84	0.0062	5	1.0	5.02

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), %Ex is extraction percentage, %Str is stripping percentage.

Table C.13 The performance of amoxicillin separation via HFSLM at volumetric flow rate of 62.5 mL/min by using 2 mM TBP as a liquid membrane (continued)

Time (min)	2 mM TBP							
	Tube side				Shell side			
	A_{272}	*[Amox]*	%Ex	pH	A_{272}	*[Amox]*	%Str	pH
45	0.9151	492	1.6	9.84	0.0067	5	1.1	5.01
50	0.9157	492	1.5	9.85	0.0067	5	1.1	5.02
55	0.9164	493	1.5	9.84	0.0063	5	1.0	5.02
60	0.9150	492	1.6	9.84	0.0067	5	1.1	5.02

Note: A_{272} is absorbance at 272 nm, *[Amox]* is the final concentration of amoxicillin in aqueous phase (mg/L), %Ex is extraction percentage, %Str is stripping percentage.

Table C.14 The nonlinear regression model of experimental data between extraction percentage and time when flow rate of 62.5 mL/min was maintained during the operation of HFSLM

Functions	Nonlinear regression model	
	10 mM Aliquat 336	2 mM TBP
Asymtotic regression	$Y = 23.83 - 27.66e^{-0.16X}$	$Y = 1.55 - 1.33e^{-0.48X}$
$Y = A-Be^{-CX}$	(SE = 2.1146)	(SE = 0.1831)

Note: Y is responses or stripping percentages (%), X is predictors or points of time (min), A is the 1st parameter, B is the 2nd parameter, C is the 3rd parameter and SE is standard error.

Table C.15 The nonlinear regression model of experimental data between stripping percentage and time when flow rate of 62.5 mL/min was maintained during the operation of HFSLM

Functions	Nonlinear regression model	
	10 mM Aliquat 336	2 mM TBP
Asymtotic regression	$Y = 8.35 - 9.70e^{-0.17X}$	$Y = 1.05 - 1.00e^{-0.28X}$
$Y = A-Be^{-CX}$	(SE = 0.6584)	(SE = 0.0905)

Note: Y is responses or stripping percentages (%), X is predictors or points of time (min), A is the 1st parameter, B is the 2nd parameter, C is the 3rd parameter and SE is standard error.

Table C.16 Extraction synergistic coefficient of the AqT mixture as a liquid membrane during the steady-state operation of HFSLM

Liquid membrane	*D*	*S*
10 mM Aliquat 336	0.31	-
2 mM TBP	0.02	-
10:2 mM AqT	0.47	1.42

Note: D is distribution coefficient, *S* is synergistic coefficient.

APPENDIX D
EQUILIBRIUM STUDY FOR AMOXICILLIN EXTRACTION BY SINGLE EXTRACTANT

To find the number (n) of extractant (Ex) taking part in complexation with a mole of amoxicillin, the equilibrium complexation constant (K_E) represented by the complexing equation (Wasewar et al., 2002) at pH 10 is stated as follows and the nomenclature of each amoxicillin form is declared in Fig. D.1:

According to $Amox^{2-} + n\overline{Ex} \leftrightarrow \overline{Amox^{2-}Ex_n}$, (D.1)

$$K_E = \frac{a_{\overline{Amox^{2-}Ex_n}}}{a_{\overline{Ex}}^{n} a_{Amox^{2-}}} = \frac{\gamma_{\overline{Amox^{2-}Ex_n}}}{\gamma_{\overline{Ex}}^{n} \gamma_{Amox^{2-}}} \times \frac{[Amox^{2-}]^{\phi} [\overline{Ex}]^{\phi n}}{\left[\overline{Amox^{2-}Ex_n}\right]^{\phi}} \times \frac{\left[\overline{Amox^{2-}Ex_n}\right]}{[Amox^{2-}][\overline{Ex}]^{n}} \quad (D.2)$$

where a is thermodynamic activity, γ = activity coefficient, the overbar refers to the species in the organic phase, superscript ϕ refers to the standard state of dilute solution. The solution was assumed to behave as if it were ideal. In the other words, the interactions between chemical species within a same phase were negligible, so activity coefficients are unity and Equation D.2 consequently becomes:

$$K_E = \frac{\left[\overline{Amox^{2-}Ex_n}\right]}{[Amox^{2-}][\overline{Ex}]^{n}} \quad (D.3)$$

$$K_E[\overline{Ex}]^{n} = \frac{\left[\overline{Amox^{2-}Ex_n}\right]}{[Amox^{2-}]} \quad (D.4)$$

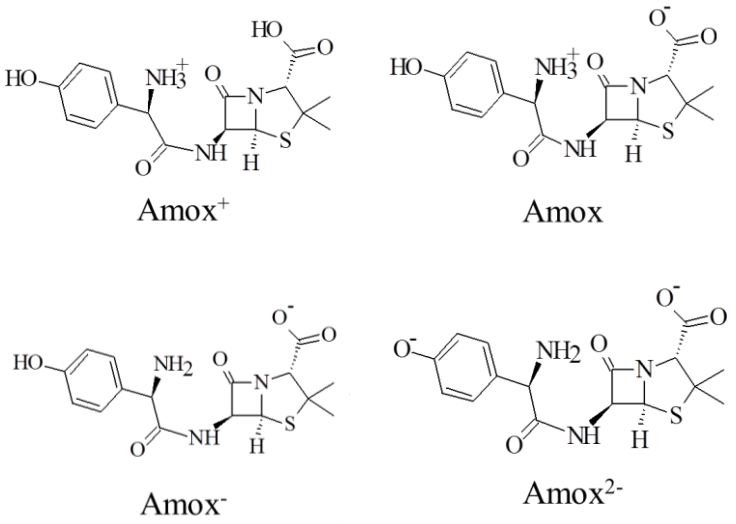

Figure D.1 Various forms of amoxicillin in aqueous solution and their nomenclature

There is no precise analytical method to measure the concentration of chemical complexes in the organic phase and $Amox^{2-}$ in the aqueous phase, so these two terms are derived into the detectable variables by using three acid dissociation equilibriums:

According to $Amox^{+} \leftrightarrow Amox + H^{+}$, (D.5)

$$K_{a1} = \frac{a_{Amox} a_{H^+}}{a_{Amox^+}} = \frac{\gamma_{Amox}\gamma_{H^+}}{\gamma_{Amox^+}} \times \frac{[Amox^+]^{\emptyset}}{[Amox]^{\emptyset}[H^+]^{\emptyset}} \times \frac{[Amox][H^+]}{[Amox^+]} \quad (D.6)$$

$$K_{a1} = \frac{[Amox][H^+]}{[Amox^+]} \quad (D.7)$$

According to $Amox \leftrightarrow Amox^{-} + H^{+}$, (D.8)

$$K_{a2} = \frac{a_{Amox^-} a_{H^+}}{a_{Amox}} = \frac{\gamma_{Amox^-}\gamma_{H^+}}{\gamma_{Amox}} \times \frac{[Amox]^{\emptyset}}{[Amox^-]^{\emptyset}[H^+]^{\emptyset}} \times \frac{[Amox^-][H^+]}{[Amox]} \quad (D.9)$$

$$K_{a2} = \frac{\left[Amox^{-}\right][H^{+}]}{[Amox]} \quad (D.10)$$

According to $Amox^{-} \leftrightarrow Amox^{2-} + H^{+}$, (D.11)

$$K_{a3} = \frac{a_{Amox^{2-}} a_{H^{+}}}{a_{Amox^{-}}} = \frac{\gamma_{Amox^{2-}} \gamma_{H^{+}}}{\gamma_{Amox^{-}}} \times \frac{[Amox^{-}]^{\emptyset}}{\left[Amox^{2-}\right]^{\emptyset}[H^{+}]^{\emptyset}} \times \frac{\left[Amox^{2-}\right][H^{+}]}{[Amox^{-}]} \quad (D.12)$$

$$K_{a3} = \frac{\left[Amox^{2-}\right][H^{+}]}{[Amox^{-}]} \quad (D.13)$$

Based on the definition of distribution ratio of amoxicillin (*D*),

$$D = \frac{\left[\overline{Amox^{2-}Ex_n}\right]}{\left[Amox^{+}\right]+[Amox]+\left[Amox^{-}\right]+[Amox^{2-}]} \quad (D.14)$$

Sub Eq. D.7, D.10 and D.13 into Eq. D.14:

$$D = \frac{\left[\overline{Amox^{2-}Ex_n}\right]}{\frac{[H^{+}]^{3}[Amox^{2-}]}{K_{a1}K_{a2}K_{a3}}+\frac{[H^{+}]^{2}[Amox^{2-}]}{K_{a2}K_{a3}}+\frac{[H^{+}][Amox^{2-}]}{K_{a3}}+[Amox^{2-}]} \quad (D.15)$$

$$D = \frac{\left[\overline{Amox^{2-}Ex_n}\right]}{[Amox^{2-}]\left(\frac{[H^{+}]^{3}}{K_{a1}K_{a2}K_{a3}}+\frac{[H^{+}]^{2}}{K_{a2}K_{a3}}+\frac{[H^{+}]}{K_{a3}}+1\right)} \quad (D.16)$$

Sub Eq. D.4 into Eq. D.16:

$$D = \frac{K_E[\overline{Ex}]^n}{\frac{[H^+]^3}{K_{a1}K_{a2}K_{a3}}+\frac{[H^+]^2}{K_{a2}K_{a3}}+\frac{[H^+]}{K_{a3}}+1} \quad \text{(D.17)}$$

Taking the log of both sides:

$$\log\left(D\times\left(\frac{[H^+]^3}{K_{a1}K_{a2}K_{a3}}+\frac{[H^+]^2}{K_{a2}K_{a3}}+\frac{[H^+]}{K_{a3}}+1\right)\right) = n\log([\overline{Ex}])+\log(K_E) \quad \text{(D.18)}$$

Let $\alpha = D\times\left(\frac{[H^+]^3}{K_{a1}K_{a2}K_{a3}}+\frac{[H^+]^2}{K_{a2}K_{a3}}+\frac{[H^+]}{K_{a3}}+1\right)$, Eq. D.18 becomes:

$$\log(\alpha) = n\log([\overline{Ex}]) + \log(K_E) \quad \text{(D.19)}$$

According to the experimental data (Table C.2), the calculated values of both log (α) and log($[\overline{Ex}]$) for each single extractant are shown in Table D.1. Plots of log (α) versus log($[\overline{Ex}]$) yielded straight lines well fitted to the experimental data for Aliquat 336, D2EHPA and Alamine 336 (Fig. D.2). The slope signified the number of extractants involving chemical complexes or the exponent on concentration of extractants (Eq. D.3).

Table D.1 The value of log(α) in Equation D.19 at different value of log($[\overline{Ex}]$)

log($[\overline{Ex}]$)	log(α)			
	Aliquat 336	D2EHPA	TBP	Alamine 336
-2.699	-0.115	-0.378	-1.113	-0.602
-2.523	0.061	-0.388	-1.145	-0.590
-2.398	0.181	-0.348	-1.007	-0.552
-2.301	0.237	-0.329	-1.188	-0.609
-2.222	0.485	-0.204	-1.072	-0.521
-2.097	0.595	-0.166	-0.994	-0.516
-2.046	0.634	-0.064	-1.086	-0.483
-2.000	0.918	-0.056	-1.106	-0.426
-1.921	0.950	-0.035	-1.041	-0.328
-1.824	1.036	-0.064	-1.065	-0.394

Note: [Ex] is concentration of extractant (M) and overbar refers to the species in the organic phase.

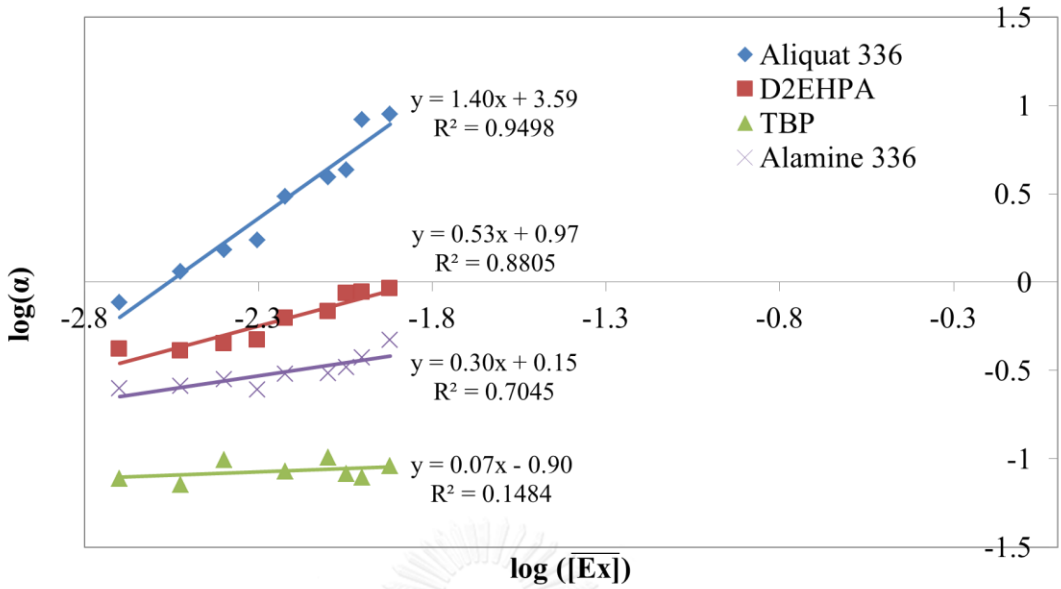

Figure D.2 Plots of log (α) versus log([$\overline{Ex}$]) by using different single extractant systems when the initial pH of amoxicillin solution was maintained at 10

VITA

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