Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (19):215-222
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

New Generation Separation and Identification Methods for Erythromycin

Sing Chuong Chuo[a], Siti Hamidah Mohd-Setapar[a,b*], Akil Ahmad[b,c] and David Lokhat[c]

[a]Faculty of Chemical Engineering, UniversitiTeknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia
[b]Centre of Lipids Engineering and Applied Research (CLEAR), UniversitiTeknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia
[c]Department of Chemical Engineering, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Durban-4041, South Africa

---

ABSTRACT

Erythromycin is an antibiotic which can effectively deal with a broad spectrum of Gram-positive bacterial pathogens and is usually used by people who have allergy to penicillin. During production of erythromycin, downstream processing contributes to a high portion of the production costs due to the nature of the broth. Conventional liquid-liquid extraction method has the problems of having limited choice of solvents and formation of emulsion which greatly hinders the separation process. Recently, various separation and purification alternatives can be found in literature such as pre-dispersed solvent extraction, ionic liquids, membrane filtration, liquid membrane, adsorption, and reverse micelle extraction. The improvements of downstream processing for antibiotics are necessary not only to enhance the quantity and quality of antibiotic products but also to improve the process itself to be more sustainable and environmental friendly.

Keywords: Erythromycin, separation, membrane filtration, adsorption, reverse micelle

---

INTRODUCTION

Erythromycin is a type of macrolide antibiotic that can slow the growth of bacteria by limiting their access to protein and then kill the bacteria. It is commonly used to treat diseases caused by Gram-positive bacterial pathogens especially those related to respiratory system and skin [1]. Semi-synthetically modified derivatives of erythromycin such as azithromycin, roxitromycin, and clarithromycin are also used for treating various infectious diseases.

Erythromycin is generally produced through fermentation of Saccharopolysporaerythraea[2-4]. During the fermentation process, several variants of erythromycin can be found as the products: erythromycin A (EryA), erythromycin B (EryB), erythromycin C (EryC), and Erythromycin D (EryD)[1,3,5]. The schematic structures of the different variants of erythromycin are shown in Figure 1.

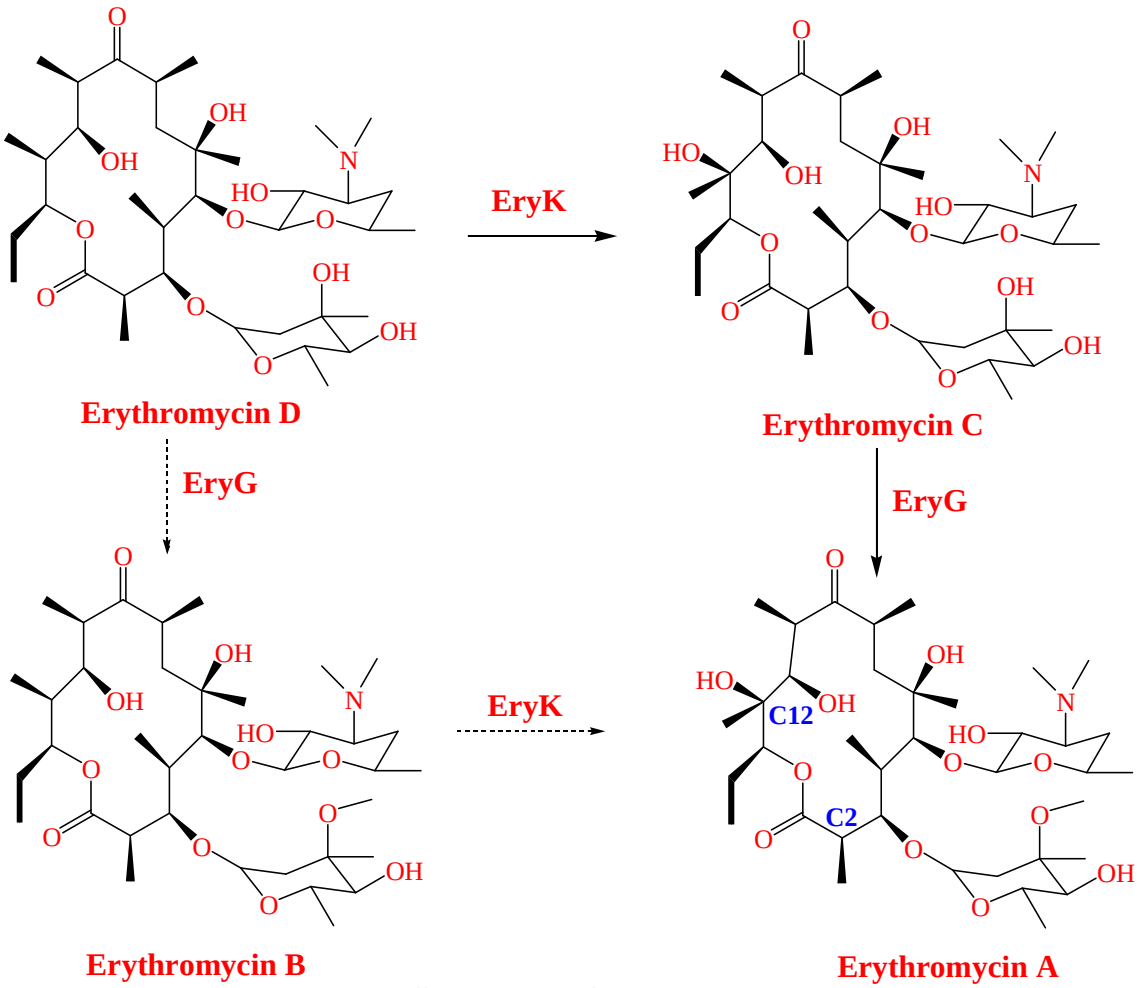

Figure 1: Different structures of erythromycin variants [1]

After the fermentation process, the product stream needs to undergo series of processes to separate the desired erythromycin from other impurities. This downstream processing stage contributes to a large portion of total erythromycin production costs due to the fermentation broth being a much diluted stream[6]. The purpose of this paper is to give a brief review on the separation methods applied for downstream processing of erythromycin.

2.0 Conventional Liquid-liquid Extraction

Conventional liquid-liquid extraction involves simple process where the desired products are solubilized into appropriate solvent and then recovered. Liquid-liquid extraction is claimed to give high product purity and have relatively shorter production time compared to other separation methods such as adsorption and filtration [7]. Butyl acetate is most commonly used for liquid-liquid extraction of antibiotics because it is biodegradable and has relatively low toxicity. However, it also has high boiling point which will make subsequent processing becomes more costly [8] (Manic *et al.*, 2011). The potentials of other solvents such as methyl-iso-butyl ketone [9] (Bosnjakovic, 1984), non-ionic alkyl phenol ethoxylate[10] (Müller *et al.*, 1989), tri-iso-butyl phosphate [11], and mixtures of solvents (pentanol-chloroform, butanol-chloroform) [12] were investigated for the extraction of erythromycin. The effects of important parameters such as solution pH, erythromycin concentration, and temperature on the liquid-liquid extraction of erythromycinwere also been studied [13, 14, 15]. Although many solvents had been investigated, only few of them were actually being used in industry because most of themhave undesirable properties such as high solubility in water or high toxicity[16].

The main problem occurred during liquid-liquid extraction of antibiotics is formation of stable emulsion. The cell and finely dispersed impuritiessuch as protein and polysaccharides in the product stream are the main substances causing the formation of emulsion [6]. The stable emulsion form during extraction process will hinder the phase separation process. It will also causelow product yield andhigh solvent consumption. An easy solution to this problem is by adding de-emulsifier during the liquid-liquid extraction process. However, application of de-emulsifier willfurther increase the production costs and the capacity of centrifugal extractors needed [6]. Direct release of de-emulsifier with the waste stream will also causes negative impacts to the environmental. Filtering the

broth before conducting liquid-liquid extraction is another method to reduce emulsion but the emulsion problem will still persists in conventional broth extraction.

3.0 Advanced/Modified Liquid-liquid Extraction

Various modifications were conducted on liquid-liquid extraction by researchers to overcome the limitations of conventional liquid-liquid extraction and improve the product yield. A novel phase transition extraction to extract erythromycin from fermentation broth was reported by Le *et al.*[7]. During the extraction process proposed by the authors, the fermentation broth is first mixed with an organic solvent. Then, an inorganic salt is added to induce phase separation process. Finally, vacuum distillation and crystallization are conducted to obtain purified erythromycin. The organic solvent chosen for the extraction must be soluble with fermentation broth prior to the addition of salt. The chosen must also be able to reduce the mutual solubility of the fermentation broth and organic solvent mixture so that they can be separated into two distinct phases at the end of the liquid-liquid extraction process. Several combinations of organic solvents and inorganic saltswere explored by the authors and they found that acetonitrile with NaCl is the most effective for extraction of erythromycin. This combination is also able to achieve phase separation in short period. The authors mentioned that the organic solvent chosen needs be easily recovered and the combination of organic solvent/inorganic salt used should be in noxiousto erythromycin besides having good extraction efficiency. The main affecting parameters of this liquid-liquid extraction method are the extraction pH and solvent volume ratio (acetonitrile/broth). The extraction process is found to be relatively insensitive to changes in extraction temperature. The authors showed that 98.5% extraction efficiency can be achieved with only single stage extraction. Phase separation was achieved within a few minutes indicating that it is a very fast process compared to conventional liquid-liquid extraction which will takes 12hr to35hr. Other advantagesof this extraction method are no emulsion formed during the process andeasier subsequent purifying processes due to low boiling point of acetonitrile.

Pre-dispersed solvent extraction (PDSE) technique using colloidal liquid aphrons (CLAs) for separation of erythromycin from fermentation broth was studied by Lye and Stuckey [17]. PDSE techniquehad been reported to be able to extract several bio-molecules [18-20].The structure of single CLA is illustrated in Figure 2. These CLAs are micron sized solvent droplets enclosed by thin aqueous film. The droplets are stabilized by non-ionic and ionic surfactant. The importance of surfactants used were described by Lye and Stuckey [17]. Surfactants must be selected carefully for the formation of CLAs because the nature of surfactants and phase contact processes will greatly affect the mass transfer of erythromycin. Further information regarding the stability of CLAs can be found in the work by Sebba [21]. The CLAs studied by Lye and Stuckey were formed using organic solvent decanol, non-ionic surfactant Softanol 120, and ionic surfactant SDS [17]. The extraction is found to be driven by favorable partitioning of erythromycin between the aqueous phase and solvent cores of CLAs. Erythromycin can be extracted within short period due to large total interfacial area available for mass transfer.

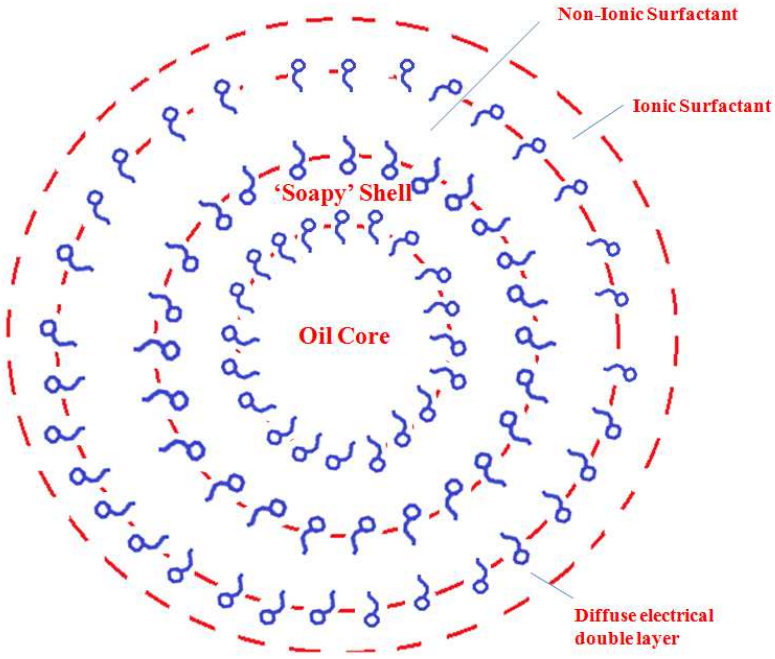

Figure 2: Structure of CLA [22]

The important parameters affecting PDSE using CLAs formed by decanol, Softanol 120, and SDS for the extraction of erythromycin were reported by Lye and Stuckey [22]. Extraction pH was identified as the most dominant parameter during the extraction. The authors reported that when pH is higher than pK_a of erythromycin (8.6), extraction efficiency is enhancedbecause most erythromycin molecules present in non-polar form at that pH range. On the other hand, stripping process of erythromycin is enhanced at solution pH lower than pK_a of erythromycin since most erythromycin molecules present in positively charged form. The authors also suggest usinglow CLAs to feed ratio so that the amount of erythromycin remained in aqueous phase at the end ofthe extraction can be reduced, thus providing higher purification fold. Another method to enhance the extraction process is to use multi-stage processing at the price of higher equipment and maintenance costs. In the studies of Lye and Stuckey [22] and Lye and Stuckey [17],the impacts of SDS during the stripping process is found to be more significant compared to those of non-ionic surfactant used. Increasing SDS concentration caused the stripping efficiency to decrease. The reduced erythromycin recovery may be the result of several undesirable processes such as the formation of reverse micelles, microscopic interfacial turbulence, andirreversible interactions between SDS and erythromycin at the interface.

Conventional liquid-liquid extraction is criticized to be relatively insensitive by Kamarei *et al.*[23] because impurities in fermentation broth might be extracted together with the desire products. Therefore, the authors suggested a liquid phase extraction with back extraction (LPE-BE) for higher quality separation of erythromycin from fermentation broth. This methodis claimed to bemore sensitive,simple to operate, rapid process, and cheaper compared to conventional liquid-liquid extraction. The LPE-BE was conducted by first converting the erythromycin into non-ionic form through pH adjustment to enable the solubilization of erythromycin into the organic solvent with mixing. The requirements for an organic solvent to be fit for the extraction purpose are: high affinity to erythromycin, immiscible with broth, lower density than water, andlow volatility. After that, erythromycin are transformed into ionic form by mixing with suitable acceptor solution and finally recovered. The authors in their experiment adjusted the broth pH to 10 and then mix it with n-butylacetateto extract the erythromycin. Then, the n-butylacetatesolution containing erythromycin was mixed with an acidic acceptor solution (pH 5) for back extraction. The effects of important parametersincluding the solutions pH, salt concentration, and types of organic solvent were illustrated by the authors. Solution pH is the most crucial parameter because it controlsthe conversion of erythromycin molecules between ionic andnon-ionic form. On the other hand,salt concentration showed insignificant impacts on the extraction efficiency and can be omitted from the extraction of erythromycin to reduce costs. The overall recovery efficiency achievable can be more than 99%. The author also showed that by incorporating the LPE-BE with high performance liquid chromatography-diode array detector (HPLC-DAD), it can be abetteralternative to analyzefermentation broth containingEryA, EryB, and EryC.

Besides conventional organic solvents, ionic liquids can be used to extract bio-molecules [24-26]. Room temperature ionic liquids (RTIL) are proposed for extraction of antibiotics because they arestable over wide range of operating temperature, non-flammable, nontoxic, havinglow volatility, and environmentally friendlier than organic solvents [25]. The extraction processes involvefirst mixing the feed phase containing antibiotics with a suitable RTIL at room temperature. After that, the liquid two phase system is left until equilibrium is achieved and then phase separation is conducted. RTIL 1-methyl-3-butylimidazolium hexafluorophosphate $[C_4mim][PF_6]$ is shown to be able to extract EryA from an aqueous solution by Cull *et al.*[27]. Manic *et al.*also successfully extracted erythromycin from an aqueous solution using RTIL 1-butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl) imide [BMPyrrol][Tf_2N][8]. The most significant parameter during the extraction process is identified to be pH of solution. This indicates that electrostatic interactions between erythromycin molecules and ionic liquid are the main driving forces during the extraction. However, erythromycin is found to be degraded at both strong acidic and alkaline conditions.

The characteristics of ionic liquids that can be designed to meet specific purposeindicate that this extraction method can be highly selective. However, the biggest hindrance to the wide application of ionic liquids is the difficulty of product recovery from the ionic liquid [8]. In order to recover erythromycin from ionic liquid after extraction, Manic *et al.*proposed the use of high-pressure $CO_2$to strip the erythromycin from ionic liquid [8]. Then, the high pressure CO_2 containing erythromycin was depressurized. After that, the precipitates of erythromycin were formed at low temperature. Since ionic liquid is insoluble in CO_2, theserecovery procedures can prevent any ionic liquid frombeing potential impurities in the final erythromycin product. CO_2 itself is also depressurized and not been found in the final erythromycin product. Therefore, high purity of erythromycin can be achieved through this separation method. Increasing the stripping pressure can enhance the extraction yield but it will increase the operating costs considerably. Up to 97% product yield were reported by the authors. Although extraction using ionic liquids and recovery using high-pressure CO_2 can give high quality erythromycin product, there are still several uncertainties waiting to be solved. Some researchers had reported significant solubilization of ionic liquids in CO_2which hinder the recovery process [28, 29]. Significant reduction of CO_2 solubility in ionic liquid when water is presentwas also reported[30]. Further investigations are necessary before this method can be practically used in industries.

4.0 Membrane Filtration

Membrane filtration is used to separate substances through the size exclusion principle. Rejection and permeate flux of the process are usually used to evaluate the performances of membranes. Commonly studied membrane filtration techniques are microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO),each having their own range of pore sizes. Conventionally, filter press is used to filter the fermentation broth before liquid-liquid extraction process[31]. Active substances such as protein and polysaccharides must be removed to avoid formation of emulsion during liquid-liquid extraction.Recently, many researchers are studying membrane filtration techniques as replacements to the conventional filter press in downstream processing of bio-products.

Li *et al.*reported a study to improve theliquid-liquid extraction of various antibiotics including erythromycinby applying UF to the antibiotics broths [6]. The UF membranes used by the authors were made from polyvinylidene fluoride. These UF membranes have molecular weight cut-off (MWCO) ranging from 5 kDa to 50kDa. Emulsion was formed during the extraction of erythromycin from broth not treated withUF membrane. The emulsion prevented phase separation of the broth and organic solvent. The UF membranes with MWCO of 50 and 20kDa are found to be unsatisfactory in dealing with formation of emulsion. On the other hand, no emulsion was formedwhen 5kDa UF membrane was used to treat the brothand good phase separation was obtained. Similar improvements were reported for other antibiotics tested by the authors. This indicates that the UF membrane with MWCO 5kDa is capable of removing surface active substances in broth effectively, thus preventing the formation of emulsion during the liquid-liquid extraction of antibiotics. The production costs of erythromycin can be reduced by pre-treating fermentation broth using the UF membranes because expansive de-emulsifier and high speed centrifugal extractor will not be necessary for downstream processing of the antibiotic.

Most studies reported for membrane filtration in downstream processing of antibioticsfocus on only either one of MF/UF or NF [31]. Although UF is very useful in removing impurities from the fermentation broth, the need of diafiltration (DF) in order to obtain good filtration causes the process stream after the filtration to have very large volume. This will increases the amount of solvent and time needed during subsequent antibiotic extraction process [32]. Furthermore, the production and wastewater treatment costswill also be increased. Filtration through a combination of UF-NF membrane system was proposed by He *et al.*[31] as the pre-treatment for erythromycin fermentation broth. The purpose of NF membrane was to concentrate the process streamcoming from UF membranes. The membrane pairs selected must be able to prevent the formation of emulsion during subsequent liquid-liquid extraction besides giving high permeate flux during the filtration process. The UF membranestested were made from polysulphoneand had MWCO ranging from 10kDa to 100kDa. The authors reported that 30kDa UF membrane gave the best filtration performances. Subsequent liquid-liquid extraction of erythromycin using n-butylacetate was successfully conducted without formation of emulsion. The study showed that pH of UF permeate has significant impacts on NF. Alkaline UF permeate resulted inunsatisfactory NF performance. The authors explained this observation as limited solubility of erythromycin in alkaline solution and formation of cationhydroxides at the membranethat caused membrane fouling. Nevertheless, adjusting pH of UF permeate to near neutral had significantly improved the NF performance. Up to 99% filtration yield can be obtained using this UF-NF system. The authors also showed that consumptions ofn-butylacetateand pure water can be reduced by using this method as pretreatment for erythromycin fermentation broth.

5.0 Liquid Membrane

Instead of using solid membrane sheets,liquid membrane technique uses immiscible liquid layer as membrane between feed phase and stripping phase. Liquid membrane technique combinesboth extraction step and stripping step in one operation and it operates using safer solvents [33]. 1-decanol was used by Kawasaki *et al.*[16] to form liquid membrane for the extraction of erythromycin from buffered aqueous solution. The liquid membrane was supported by a porous material. At the same time, an acidic aqueous solution was used as stripping phase to recover the erythromycin. A two compartment mass transfer cell was selected by the authors to conduct the extraction process. The most significant parameters identified during the extraction process were pH of both feed phase and stripping phase. The authors reported faster mass transfer of erythromycin when the pH of feed solution was increased from 8.5 to 10.5. The mass transfer remained constant at feed solution pH higher than 10.5. On the other hand, high stripping phase pH reduced the recovery of erythromycin. The authors explained these observations as the results of erythromycin molecules existing at ionic or non-ionic forms at different pH ranges. This separation technique for erythromycin is claimed to be easily controllable through adjustment of solution pH, a safe process for utilizing non-toxic carrier, and havehigh antibiotic yield.

Separation of erythromycin from broth contents using emulsion liquid membrane (ELM) has several disadvantages such aslow separation performance andlow flux. Habaki *et al.*carried out a study to improve the performances of W/O/W ELM technique for separation of erythromycin [34]. The authors used a solution of Span 80 in heptane to form the liquid membrane. According to their simulation study,the greatest hindrance to the separation process was

unfavorableerythromycin concentration profile. The suggestion given by the authorsto improve the separation efficiency was selecting an appropriate phase contacting method. The authors themselves tested a spray column where feed phase is dispersed in continuous organic phase for the separation of erythromycin from aqueous phase. Better separation performancesthan conventional contacting method were reported.

6.0 Adsorption

Adsorption is a popular technique in separation processes. During adsorption process, erythromycin molecules are transferred from feed phase to the surface of adsorbents thus separated from impurities. Desorption process is needed to recover the erythromycin product afterward. The adsorption process is mainly controlled by thermodynamic equilibrium of erythromycin concentration. The adsorption capacity determines amount of erythromycin that can be adsorbed per unit mass adsorbent. Generally, adsorption method gives high separation efficiency and regeneration of some adsorbents allows for multiple usages.

Several neutral polymeric sorbents and ion exchange sorbents are availablefor the adsorption of products from fermentation broth. Some commonly used sorbents include copolymer of styrene and divinylbenzenecan be found in industrial processes [35]. The performances of several anionic resin, cationic resin, and neutral resinfor separation of erythromycin from fermentation broth were studied by Ribeiro and Ribeiro [35]. Good erythromycin adsorption was reported for both neutral resin and cationic resin. On the other hand, poor erythromycin adsorption was reported with anionic resindue to strong electrostatic repulsive forces between anionic resin andcharged erythromycin molecules. More erythromycin can be adsorbed onto the resins at higher operating temperature. The adsorption equilibriums wereachieved within 3 hr for all resins tested by the authors. They also mentioned several important aspects that need to be investigatedduring adsorption studies such as rates of adsorption, effects of operating temperature, interactions between antibiotic and adsorbents,shapes of the isotherms, andsignificance of plateau in the isotherms. However, recovery of erythromycin from the resins after adsorption was not reported in that study.

When there more than one variant of erythromycin exist in fermentation broth, it is usually desirable to recover as much EryA as possible in the final product. Non-polar resins are reported to have higher selectively extracting EryA over EryC[36]. The potential of a macroporous non-polar polystyrene resin for separation of erythromycin variants from fermentation broth was investigated by Zheng *et al.*[37]. Studies showed that hydrophobic forces are the dominant driving forces of the adsorption process. Therefore,higher erythromycin concentration in the feed phase led to bettererythromycin adsorption. After adsorption process, the resin was washed and erythromycin was recovered throughdesorption of resin using butyl acetate. More than 96% erythromycin can be recovered into butyl acetate. However, the adsorption-desorption process could not separate the erythromycin variants effectively. After that, the organic solvent containing erythromycin was mixed with a neutral buffer solution. Subsequently, all butyl acetate was removed through azeotropic distillation. Finally, crystallization was conducted to obtain the final erythromycin product. EryA and EryCvariants can be separated during crystallization step by adjusting the operating pH. The authors found that crystallization of EryC can be limitedat operating pH between 9 and 10. The final product yields reported were92.3% and 41.9% for EryA and EryC respectively. An advantage of this method is the reduced consumption of organic solvent compared to conventional liquid-liquid extraction. However, the authors reminded that their method is not suitable for separation of broth with high EryB concentration because EryBwill crystallize easier than EryA and will become the main impurity in the final product.Therefore, it is suggested to limit the formation of EryB and EryC during the fermentation process rather than trying to separate them from EryA during downstream processing the antibiotic.

7.0 Reverse Micelle Extraction

Reverse micelle extraction is one alternative which gained growing interests from researchers for the extraction of antibiotics in recent years[38-41]. This separation technique utilizes reverse micelles formed by appropriate surfactants tothen extract antibioticsfrom broth. The structure of reverse micelle is shown in Figure 3. Advantages of reverse micelle extraction include easy operations, mild operating conditions, andhigh selectivity. It also has the potential forsolvent recycling, continuous operation, andscale up. This technique is better than conventional liquid-liquid extraction because it is able to limit the formation of emulsion, preserve activities of antibiotics been extracted, achieve separation within short time, and allow the use of safer solvents[42].

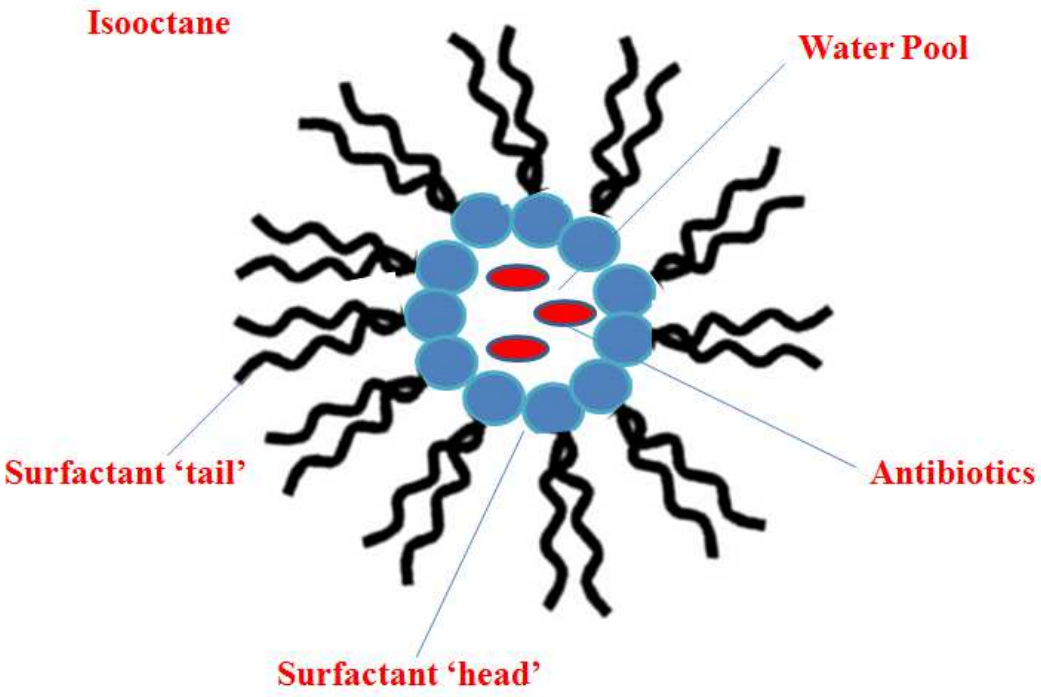

Figure 3: Structure of reverse micelle [43]

Solubilization of penicillin G into bis(2-ethylhexyl) sulfosuccinate (AOT) reverse micelle in isooctane was reported by Mohd-Setapar *et al.*[43]. The effects of important parameters such as aqueous phase pH, surfactant concentration, salt concentration, and antibiotic concentration for the separation of penicillin G using reverse micelle extraction were studied by Mohd-Setapar *et al.*[44]. Optimum separation of antibiotics can be achieved by adjusting these parameters. Reverse micelle extraction are also successfully applied for the separation of amoxicillin from aqueous solutions [45, 46]. This separation technique has the potential to beused for separation of erythromycin.

8.0 Concluding Remarks

Downstream processing of erythromycin is commonly conducted through liquid-liquid extraction method. However, the limitations of conventional liquid-liquid extraction method pushed researchers to find alternatives for separation of erythromycin. This led to various innovative extraction methods being proposed. Continuous improvements of downstream processing methods for erythromycin and other antibiotics will help to reduce the production costs and minimize the impacts of the processes to the environment.

Acknowledgment

The authors thank the financial supports from Research Management Centre (RMC), Research University Grant (14H37), UniversitiTeknologi Malaysia (UTM).

REFERENCES

[1] H Zhang, Y Wang, J Wu, K Skalina, B APfeifer, *Chem Biol*,**2010**, 17, 1232-40.
[2] W Schönfeld, H A Kirst, *Macrolide Antibiotics*. Basel: Birkhäuser**2002**.
[3] X Zou, H F Hang, J Chu, Y P Zhuang, S LZhang, *Bioresour Technol*,**2009**, 100, 3358-65.
[4] X Zou, H F Hang, J Chu, Y P Zhuang, S LZhang, *Bioresour Technol*,**2009**, 100, 1406-12.
[5] J Tan, J Chu, Y Hao, Y Wang, S Yao, Y Zhuang, S Zhang, *J Taiwan Inst Chem Eng*,**2013**, 44, 538-544.
[6] S ZLi, X YLi, Z FCui, D ZWang, *Sep Purif Technol*,**2004,**34, 115-123.
[7] Q Le, L Shong, Y Shi, *Sep Purif Technol*,**2001**, 24, 85-91.
[8] MSManic, M Nda Ponte, VNajdanovic-Visak, *Chem Eng J*,**2011**, 171, 904-911.
[9] A A Bosnjakovic, Extraction of erythromycin from fermentation broth. *Kern Industry*. **1984,** 29, 173.
[10] U Müller, U Merrettig, M Träger, U Onken, In-situ Extraction of Secondary Metabolites. *Dechema biotechnol conference*,**1989**, 3, 1089.
[11] ZLu, *Chinese Pattern*. **1989**, 1,037,343/22.
[12] N LEgutkin, V VMaidanov, Y ENikitin, *Pharm Chem J*,**1984,**18, 196-197.
[13] A KCharykov, L IShirokova, *Zhurnal Fizicheskoi Khimii*. **1987,**99.
[14] V V Russin, A L Solodov, S A Zhukovskaya, V L Pebalk, *Khimii Farmatsevticheskii Zhurnal*. **1976**, 10, 114.

[15] V V Russin, S A Zhukovskaya, V L Pebalk, *Antibiotiki*. **1975**, 20, 222.
[16] J Kawasaki, R Egashira, T Kawai, H Hara, L Boyadzhiev,. *J Membr Sci*,**1996,**112, 209-217.
[17] G JLye, D CStuckey, *Chem Eng Sci*,**2001**, 56, 97-108.
[18] G JLye, L VPoutiainen, D CStuckey, *Proceedings Biotechnol* **1994,***94*,25-27.
[19] G JLye, D CStuckey, Extraction of macrolide antibiotics using colloidal liquid aphrons (CLAs). In D. L. Pyle (Ed.) *Separations for biotechnology* **1994**, *3* (pp. 280-286). Cambridge: Royal Society of Chemistry.
[20] D A Wallis, D L Michelsen, F Sebba, J K Carpenter, D Houle, *Biotechnol Bioeng Symposium Series*. **1985**, 15, 399-408.
[21] FSebba, *Foams and biliquid foams aphrons*. New York: Wiley**1987**.
[22] G JLye, D CStuckey, *J Chem Technol Biotechnol*,**2000**, 75, 339-347.
[23] F Kamarei, H Attar, S Nikjah, M Goodarzi, *Arabian J Chem*, **2014**, 7, 292-296.
[24] Y Jiang, H Xia, J Yu, C Guo, H Liu, *Chem Eng J*,**2009**, 147, 22-26.
[25] A Soto, A Arce, M K Khoshkbarchi, *Sep Purif Technol*,**2005**, 44, 242-246.
[26] J Wang, Y Pei, Y Zhao, Z Hu, *Green Chem*,**2005**, 7, 196-202.
[27] S GCull, J DHolbrey, VVargas-Mora, K RSeddon, G JLye, *Biotechnol Bioeng*,**2000,**69, 227-233.
[28] J W Hutchings, K L Fuller, M P Heitz, M M Hoffmann, *Green Chem*,**2005**, 7, 475-478.
[29] W Wu, J Zhang, B Han, J Chen, Z Liu, T Jiang, J He, W Li, *Chem Comm*,**2003,** 1412-1413.
[30] DFu, XSun, JPu, SZhao, *J Chem Eng Data*. **2006,**51, 371-375.
[31] Y He, G Chen, Z Ji, S Li, *Sep Purif Technol*,**2009,**66, 390-396.
[32] A I C Morão, A M B Alves, M C Costa, J P Cardoso, *Chem Eng Sci*,**2006**, 61, 2418-2427.
[33] L Boyadzhiev, Z Lazarova, Liquid membranes (liquid pertraction). In Richard, D. N., and Stern, S. A. (Ed.) *Membrane Science and Technology*. **1995,**(pp. 283-352) Elsevier.
[34] HHabaki, REgashira, G WStevens, J Kawasaki, *J Membr Sci*,**2002,**208, 89-103.
[35] M H L Ribeiro, I A C Ribeiro, *Sep Purif Technol*,**2005**, 45, 232-239.
[36] Z Sheng, Z Jia-Wen, C Kui, *Chem Eng Comm*,**2011**, 198, 1206-1217.
[37] W Zheng, K Chen, J Zhu, L Ji, *SepPurif Technol*,**2013**, 116, 398-404.
[38] S CChuo, AAhmad, S HMohd-Setapar, S NMohamad-Aziz, *Der Pharma Chemica*,**2014,**6, 37-44.
[39] S HMohd-Setapar, HMat, S NMohamad-Aziz, *J Taiwan Inst Chem Eng*,**2012**, 43, 685-695.
[40] S H Mohd-Setapar, S N Mohamad-Aziz, C S Chuong, MA Che Yunus, M A A Zaini, M J Kamaruddin, *Chem Eng Comm*, **2014**, 201, 11, 1664-1685.
[41] S H Mohd-Setapar, S N Mohamad-Aziz, N H Harun, S H Hussin, *Adv Mat Res*,**2012**, 545, 240-244.
[42] S HMohd-Setapar, S NMohamad-Aziz, *Adv Sci Lett*,**2013**, 19, 3688-3694.
[43] S HMohd-Setapar, R JWakeman, E STarleton, *Chem Eng Res Desig*,**2009**, 87, 833-842.
[44] S HMohd-Setapar, SNMohamad-Aziz, N HHarun, C Y Mohd-Azizi, *APCBEE Procedia*. **2012**, 3, 78-83.
[45] S N Mohamad-Aziz, S H Mohd-Setapar, R A Rahman, *J Bionanosci*,**2013**, 7, 195-201.
[46] S CChuo, S HMohd-Setapar, SNMoha*mad-Aziz, V MStarov, Colloids Surf A**Physi**cochem Eng As**p**,* **2014,**460, 137-144.