ISSN: 0128-7680
Pertanika J. Sci. & Technol. **17 (1)**: 183 – 189 (2009)

Chemical Extraction and Separation from the Amoxicillin Plant's Waste-Stream

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ABSTRACT

In the production of amoxicillin for the activation of amoxicillin molecule, hydrolysis takes place at the final stage. In the hydrolysis, the side branch of the amoxicillin molecule, which is not activated, is debranched, and methyl aceto-acetate is easily separated from the amoxicillin molecule. It is highly desired to recycle methyl aceto-acetate as the chemical compound which is needed for the reformation of Dane salt. The main objective of this project was to carry out the liquid-liquid extraction for the recovery of methyl aceto-acetate from the waste stream. For this purpose, diluted ammonia solution was used as a solvent for the chemical extraction, and methyl aceto-acetate was extracted from the waste stream. The liquid-liquid extraction was carried out, while the pH was adjusted to 9.56 by adding ammonia solution. The extraction was repeated in three consecutive stages to enhance the yield in this process. The samples were taken from each stage of separation for GC analysis. The result obtained from the organic chemical extraction using ammonia in three consecutive stages of extraction with overall removal efficiency of 75%. The extraction process was accompanied with distillation for chemical recovery. Similarly, the undesired remaining organics were successfully extracted from the waste stream by distillation. The yield and partition coefficients of the extraction process were calculated based on the chemical analysis obtained from the GC results.

Keywords: Amoxicillin, distribution coefficients, extraction, methyl aceto- acetate, phase separation

INTRODUCTION

In the process of amoxicillin production, hydrolysis is applied at the final stage for the activation of antibiotic product. As a result of the reaction, methyl aceto-acetate is formed. In the waste stream, a number of solvents can easily be wasted along with other chemicals. Waste stream processing is needed for the recovery of fine chemicals for the prevention of environmental pollution and also improving economical feasibility of the production plant. Amoxicillin is one of the major ß-lactam antibiotics, which possesses a high spectrum of activity, high solubility, high rate of absorption, and chemical stability under acid conditions (Gonçalves *et al.*, 2005). Amoxicillin is a well-known antibiotic obtained after a sequence of reactions from 6-α-aminoacyl-penicillin (6-APA). It is a semi-synthetic penicillin antibiotic with a broad spectrum of bactericidal activity against a wide range of common gram-positive and gram-negative pathogens. Amoxicillin is usually the drug of choice within the class because it is easily absorbed, following oral administration, than other ß-lactam antibiotics. In specific, it has a low capacity for protein binding and is widely distributed in various tissues

Received: 23 January 2008
Accepted: 16 May 2008
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after absorption. Like all ß-lactam antibiotics, it prevents the formation of the bacterial cell wall by interfering with the final stage of peptidoglycan synthesis (Considine, 1974; Gonçalves *et al.*, 2002 & 2005; Katzung, 1998; Zayed and Abdallah, 2005; Jager *et al.*, 2007).

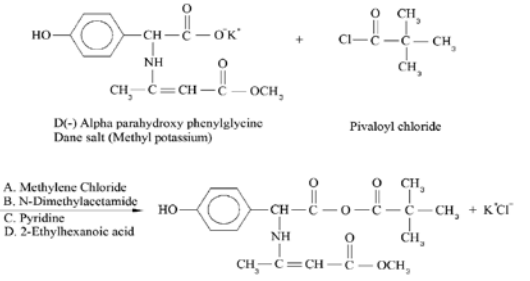
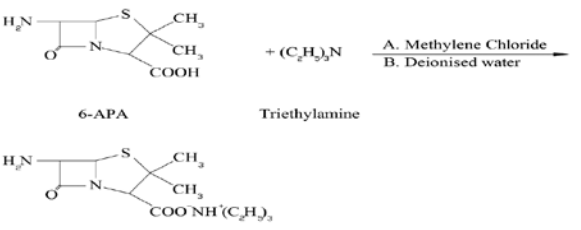
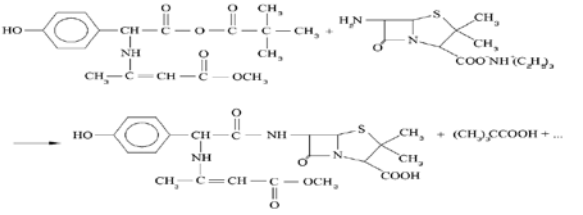
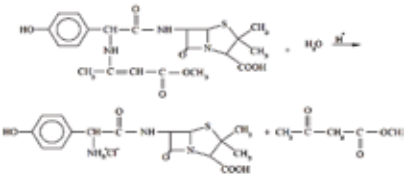
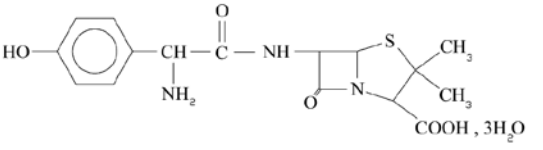
The preparation process of a 6-APA derivative comprises of a number of steps, which are very practical in antibiotic production (Diago and Ludescher, 1995 & 1998). The first step is the preparation of a mixed carboxylic acid anhydride by creating a reaction between Dane salt and an acylating agent, in a solvent which is water-immiscible or sparingly soluble in water such as methyl isobutyl-ketone, n-butyl acetate, isobutyl acetate and methylene chloride (Diago and Ludescher, 1995 & 1998; Centellas *et al.*, 1999). The first step of the reaction is shown in Table 1. For the preparation of Dane salt, the amine group of phenylglycine is temporarily protected by the formation of the corresponding enamine. From the reaction of phenylglycine with an alkyl aceto-acetate (such as methyl or ethyl aceto-acetate), Dane salt is formed in the presence of a base. The protected compound known as Dane salt has a general formula of R_1-$CHNHR_2COO^-$, where R_1 denotes an appropriate side chain (e.g. phenyl, 4-hydroxyphenyl or 1, 4-cyclohexadien-1-yl) and R_2 denotes alkyl, preferably C_{1-8} alkyl (Cabre *et al.*, 1999).

In the process of protecting 6- APA at the second stage, it is reacted with triethylamine, as shown in Table 1. The third step is the acylation reaction of the compound obtained from the first step with the protected 6-APA or a derivative of 6-APA in free acid or salt form (Diago and Ludescher, 1995 & 1998; Centellas *et al.*, 1999). A summary of the acylation reaction is also shown in Table 1. The ß-lactam ring in 6-APA is easily cleaved in aqueous phase; it is most preferable to carry out the reaction in a non-aqueous solvent system such as methylene chloride (Bender, 1979).

The fourth step involves the hydrolysis of enamine function, which was resulted from the product obtained from the previous acylation reaction. The hydrolysis was carried out with diluted solution of organic acid, or inorganic acid such as diluted aqueous hydrochloric acid, at a low temperature (Bender, 1979; Diago and Ludescher, 1995 & 1998). Methyl aceto-acetate is the by-product of the hydrolysis reaction. The diluted acid has created two phases, organic and aqueous phases. The librated methyl aceto-acetate is found in the organic phase, while the antibiotic remains in the aqueous phase. The final step is the crystallization of amoxicillin, obtained in the aqueous phase, with the use of chemical techniques (Bender, 1979). The chemical structure and action of hydrolysis and crystallization steps are shown in Table 1.

In the process of amoxicillin production, methyl aceto-acetate was formed from the acid hydrolysis of deactivated form of amoxicillin molecule (Bender, 1979). It is important to highlight that methyl aceto-acetate exists in the organic phase. The recovery of certain chemicals, such as methyl aceto-acetate from the organic phase, has an economical impact which makes the process economically feasible. Methyl aceto-acetate is one of the most consumable esters in the pharmaceutical industries and agricultural uses (Othmer, 1997; Boyd and Morrison, 2007). In addition, methyl aceto-acetate is also used in the preparation of "Dane Salt" which is used in the production of synthetic penicillin (Smith, 1963; Schweitzer, 1997). Therefore, the recovery of methyl aceto-acetate, in the production of antibiotic, is essential and makes the process more economical to reform Dane salt. Similarly, the recovery of methyl aceto-acetate also has an environmental impact. In particular, environmental pollution may occur if these chemicals are not recovered from the waste stream.

TABLE 1
The preparation process of Amoxicillin from 6-APA and Dane salt (Cabre *et al.*, 1999)

Preparation stages	Formulation
1. The formation of mixed anhydride	
2. Protection of 6-APA	
3. Acylation reaction of protected 6-APA and mixed anhydride	
4. Hydrolysis of enamine function	
5. Crystallization	

The objective of the present research work was to recover and reuse the downstream waste obtained from Antibiotic Company, Sari, in Iran. The samples were analyzed and the organic chemicals were successfully separated and recovered using the fractional separation. Finally, most of the methyl aceto-acetate was recovered by the use of the extraction process, which was followed by distillation.

MATERIALS AND METHODS

The standard chemicals used were supplied by Merck. The waste sample was obtained from the waste stream of amoxicillin plant, Antibiotic Company, Sari, Iran. The Gas Chromatograph (GC), was equipped with the capillary column TRB-G43 and FID detector, Varian, Model 3800 (USA) and Rotavapor Laborota Model 4001 Heidolph (Germany) for distillation, were used in each stage of the separation process.

The waste stream consists of methyl aceto-acetate, pivalic acid, N, N-dimethyl acetamid, 2-ethyl hexanoic acid, isopropyl alcohol and methylene chloride. In the first stage of the separation process, an alkaline solution, such as $NaHCO_3$ or ammonium solution was introduced to the mixture to extract acid compounds and amide from the waste stream. After separating the organic phase from the aqueous phase, methyl aceto-acetate was found in the organic phase.

Three consecutive stages of extraction were required to increase the efficiency of the process. At final stage, volatile chemical such as methylene chloride was recovered using a common distillation process.

Material balances were conducted at each stage of the separation process, and each product stream was analyzed using the GC, FID and a 30m capillary column (TRB-G43) for the identification and quantitative analysis of the chemical compositions. The detector and injector temperatures were set at 240°C and 220°C, respectively. For this purpose, the temperature programming was specifically used to run the GC. The column temperature was kept at 35°C for 5 minutes, then it was increased to 40°C, with a rate of 1°C/min. In the second stage of the temperature programming, the temperature was increased to 230°C, with a rate of 6°C/min . The carrier gas for the GC was nitrogen, with a flow rate of 30 ml/min. The GC was operated using the software known as star chromatography workstation. The run time for each sample was 41.67 min.

RESULTS AND DISCUSSION

The recovery of methyl aceto-acetate was carried out for the economical feasibility of the operating process. Meanwhile, methyl aceto-acetate needs to remain in the reaction and preferable from the environmental view point. As explained in the process description in the earlier section, the recovered methyl aceto-acetate could easily be converted into Dane salt, which is used in the preparation of the antibiotic. Furthermore, the recovery of methyl aceto-acetate is reduced in the effluent in order to eliminate the organic pollutants. The oily residues, which are retained after the solvent recovery, have to be disposed as schedule wastes or incinerated. In the recovery process of methyl aceto-acetate from the amoxicillin production plant, the waste stream mixture may contain a number of components. Both the waste sample and the extracted samples were analyzed using the GC. The boiling points and the chemical composition of the waste stream are shown in Table 2.

TABLE 2
The chemical composition of waste stream for amoxicillin plant

Chemical compound	Boiling point (°C)	Weight percent
Methylene chloride (MCH)	39.8	39.2271
N,N-Dimethyl acetamide (DMAC)	166	5.5092
Iso propyl alcohol (IPA)	82.3	4.6351
Pivalic acid (PIVA)	164	30.4195
2-Ethylhexanoic acid (2EHA)	226	0.6567
Methyl aceto-acetate (MAA)	164	19.5523

Since some of the compounds (DMAC, PIVA and MAA) in the waste stream have close boiling points, distillation is therefore not a suitable technique to separate all the chemicals which exist in the wastes. Hence, liquid extraction was implemented to separate all the components and recover methyl aceto-acetate.

Alkaline condition is required for the phase separation; Boyd and Morrison (2007) state that strong alkaline solution may cause cleavage of the methyl aceto-acetate to methanol and acetic acid. Ammonia was selected due to its mild alkaline condition and a suitable change in the pH of the solution. In addition, ammonia is found to be capable of extracting all the organic acids into aqueous phase. Ammonia solution was introduced as a chemical agent in extracting solvent, which was meant to extract acids and other undesired chemicals from the organic phase. Fresh feed of the organic waste from amoxicillin plant, one litre was mixed with 160 ml of 9 molar ammonia solutions, diluted with distilled water and the total volume was reached to 2 litres for equal volume of solvent to feed ratio. As a result, alkaline condition with pH of 9.56 was obtained. Aqueous and organic phases were distinctly separated after a few minutes of mixing and settling. The weight percentage of all the chemical species in the aqueous and organic phases, after the three stages of extraction using ammonia solution, are presented in Table 3.

In the first stage of extraction, the highest and lowest distribution coefficients were devoted to pivalic acid and methyl aceto-acetate. This means "most" of the pivalic acid was retained in the aqueous phase and the methyl aceto-acetate was kept in the organic phase along with methylene chloride. In the next stage of extraction, more methyl aceto-acetate was extracted into the organic phase, along with volatile solvent like methylene chloride. To extract all the acid residues, more amide and alcohol "extraction" was repeated for three consecutive stage of extraction. The overall process yield calculation was based on the mass ratio of the final to the initial methyl aceto-acetate. The process yield of 75% was obtained based on the analysis. The results showed that ammonia was very efficient for the extraction of most of the acids found in the organic phase as well as most of the amide and alcohol.

Finally, the low boiling point compounds such as methylene chloride were eliminated in a single stage distillation. Methyl aceto-acetate, with the purity of 93.82%, was obtained from the distillation process. The weight percentages of all the chemicals, in the distillate and residual phases after distillation, are shown in Table 4.

TABLE 3
The chemical analysis of the aqueous and organic phases, with ammonia extraction from amoxicillin waste

Chemical compound	Original sample	Aqueous phase	Organic phase	Distribution coefficient
	Weight percent	Weight percent	Weight percent	
The first stage of extraction with ammonia solution				
Isopropyl alcohol	4.635	9.136	4.125	2.214
Methylene chloride	39.226	4.083	57.975	0.07
Pivalic acid	30.420	77.815	0.566	137.216
Methyl aceto-acetate	19.552	1.366	31.775	0.043
N,N-dimethylacetamide	5.510	6.823	5.550	1.229
2-Ethylhexanoic acid	0.657	0.777	0.007	115.791
Sum.	100.000	100.000	100.000	
The second stage of extraction with ammonia solution				
Isopropyl alcohol	4.125	30.24	1.814	16.673
Methylene chloride	57.975	19.78	63.319	0.313
Pivalic acid	0.566	1.95	0.301	6.606
Methyl aceto-acetate	31.775	21.25	31.363	0.677
N,N-dimethylacetamide	5.550	26.78	3.147	8.511
2-Ethylhexanoic acid	0.007	0.000	0.056	0
The third stage of extraction with ammonia solution				
Isopropyl alcohol	1.814	25.230	0.836	30.168
Methylene chloride	63.319	30.022	62.442	0.481
Pivalic acid	0.301	4.245	0.027	157.211
Methyl aceto-acetate	31.363	25.888	34.618	0.748
N,N-dimethylacetamide	3.147	14.615	2.040	7.163
2-Ethylhexanoic acid	0.056	0.000	0.037	0
Sum.	100.000	100.000	100.000	

TABLE 4
Chemical analysis of the distillate and residue from distillation column

Chemical compound	Original sample Weight percent	Weight percent in distillate	Weight percent in residue
Isopropyl alcohol	0.836	2.468	0.017
Methylene chloride	62.442	93.481	0.658
Pivalic acid	0.027	0.000	0.000
Methyl aceto-acetate	34.618	3.806	93.818
N,N-dimethylacetamide	2.040	0.245	5.507
2-Ethylhexanoic acid	0.037	0.000	0.000
Sum.	100.000	100.000	100.000

CONCLUSIONS

From the waste stream processing of the amoxicillin production plant, the chemical compounds such as methyl aceto-acetate was recovered. The process set up was managed in a manner that part of the chemicals was extracted by adding ammonia solution. Distillation was also applied in the final stage. In this study, it was found that the overall process efficiency of 75 percent was achieved for the recovery of methyl aceto-acetate.

ACKNOWLEDGEMENTS

The work was made possible through the support of Faculty of Chemical Engineering, Biotechnology Centre and Nano-Bio Lab, Noushirvani University of Technology, Babol, Iran. The authors wish to acknowledge the research team, led by Mr. Safei at Antibiotic Company, Sari, Iran, for their cooperation and encouragement throughout the present research. Special thanks to Professor M. Tajbaksh, Faculty of Chemistry, University of Mazandaran, Babolsar, Iran, for his valuable assistant at the Organic Chemistry Lab.

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