# SYNTHESIS OF A QUATERNARY AMMONIUM SALT FROM EPOXIDE AND FATTY AMINE AND EVALUATION OF ITS ANTIMICROBIAL PROPERTIES

**BY**

**CHUKWUNONYEREM, CELESTINE C. (B.Tech., FUTO)**

**REG NO: 20134870968**

**A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL,**

**FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI,**

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**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE(M.Sc.) IN ORGANIC CHEMISTRY**

**MAY, 2019.**

## CERTIFICATION

This is to certify that this work "Synthesis of a Quaternary Ammonium Salt from Epoxide and Fatty Amine and Evaluation of its Antimicrobial Properties" was carried out by CHUKWUNONYEREM, CELESTINE C. (Reg. No. **20134870968**) in partial fulfillment of the requirements for the award of the degree of Masters of Science (M.Sc.) in Organic Chemistry in the Department of Chemistry of Federal University of Technology, Owerri.

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## DEDICATION

This work is wholeheartedly dedicated to God Almighty in whom are hidden all the treasures of wisdom and knowledge, and to my parents for their care, love, financial and moral supports to me.

## AKNOWLEDGEMENTS

My first but unalloyed thanks go to God Almighty. He is my hope and strength, and it is with this enabling strength he bestowed on me that I undertook to do this tasking work.

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“Without Socrates drinking the helmlock and his death, the Westhern Philosophy would not have been what it is today”. I owe unpayable debts to my parents, Late LivinusOnukuba C. and Mrs. Eunice Onukuba C. for their care, love, concern and supports to my affairs, in spite of all odds and intricacies here and there.

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Once again, I thank and remain grateful to you all. May God Almighty bless you all abundantly. Amen.

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## ABSTRACT

Quaternary ammonium compound was synthesized from epoxy and fatty amine reaction. Fourier Transform Infrared(FTIR) spectrometric analysis of the starting materials, and the final product(N-benzyl-N,N-dioctyl-2-hydroxy-3 phenoxypropylammonium chloride) were conducted, and results showed a disappearance of sharp N-H peak of $2^{o}$ amine, and appearance of broad O-H peak of alcohol in the spectrum of the final product. Mass spectrometric(MS)analysis of the final product was also conducted, and results showed the presence of molecular ion ($M^{+}$)peak withM/Z 483, which stands for the molecular weight of the compound.Antimicrobial activity of the starting materials, the intermediate, and the final product were evaluated, and results showed that only the Quaternary ammonium salt exhibited excellent antibacterial activity on a variety of bacteria including Gram-positive bacteria and Gram-negative bacteria with the following zones of inhibition: *Salmonella Enteric* (20 mm), *Escherichia Coli* (25 mm), *Staphylococcus Aureus* (15 mm), *Lactobacillus Spp.* (12 mm).



Keywords: Synthesis, Fourier Transform Infrared (FTIR) Characterization, Mass Spectrometric Analysis, Antimicrobial Analysis.

# CHAPTER ONE

## 1. INTRODUCTION

Quaternary ammonium salts are organic compounds in which four groups(usually benzene and/or alkyl)are covalently attached to a central positively charged nitrogen atom($R_4N^+$).Quaternary ammonium compounds are among the High Production VolumeChemicals (HPVCs, i.e., chemicals producedor imported in amounts equal to or greater than one million pounds per year)found on the lists of both the United State Environmental Protection Agency (USEPA) and the Organization for Economic Co-operation and Development (Dean-Raymond & Alexander, 1977). QACs are used extensively in agricultural, healthcare,domestic and industrial applications as emulsifiers, fabric softeners, disinfectants, pesticides, surfactants,corrosion inhibitors and personal care products (Garcia, Campos, Sanchez-Leal, & Comelles, 2006; Steichen, 2001; Patrauchan& Oriel, 2003). The 2004 world-wide annual consumption of QACs was reported as 500,000 tons (CESIO, 2004) and was expected to reach or exceed 700,000 tons (Steichen, 2001).

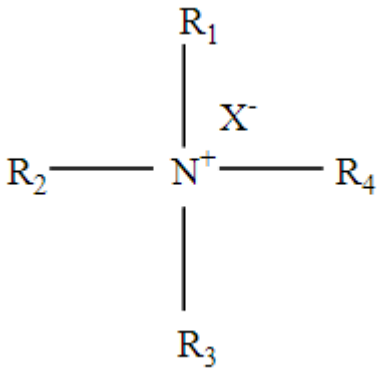
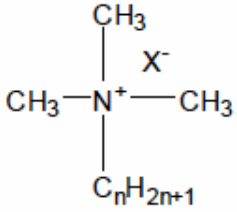
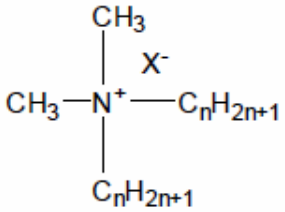
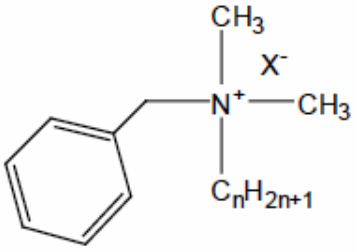



**Figure 1.1**. General molecular structure of a QAC (R represents a functional group, X-represents a counter ion such as $Cl^-$, $Br^-$)

In quaternary ammonium compounds, the organic radical is the cation, and chlorine or bromine is usually the anion. Unlike the ammonium ion ($NH_4^+$) and the primary, secondary, or tertiary ammonium cations, the quaternary ammonium cations are permanently charged, independent of the pH of their solution and they are stable with a long shelf life (Smith & March, 2001).

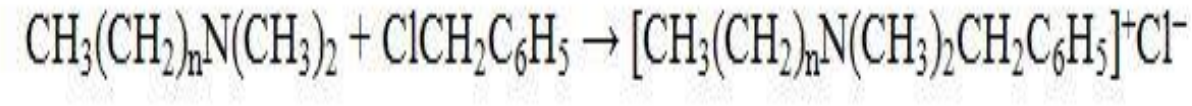
QACs can be classified into three major groups: monoalkonium, dialkonium and benzalkonium halides(Boethling, 1994) as shown in Table 1.1.

**Table 1.1.** General structures of three major classes of quarternary ammonium compound (X is a halide counter-ion, n represents length of alkyl chain, C8-C18)

| QAC Group | Molecular Structure | Abbreviation |
|---|---|---|
| Monoalkonium halides |  | $C_nTMA$-X |
| Dialkonium halides |  | $DC_nDMA$-X |
| Benzalkonium halides |  | $C_nBDMA$-X |

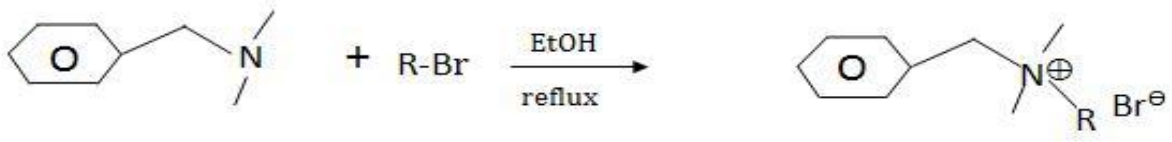
Quaternary ammonium compounds are molecules that have molecular weights ranging between 300 and 500 g/mole and are composed of two noticeably different moieties: hydrophobic alkyl groups and a hydrophilic, positively charged central N atom, which retains its cationic character at all pH values. The two moieties of QACs affect their physical and chemical properties (Boethling, 1994).

QACs have distinct physical/chemical properties, which are conferred by their substituents, mainly the alkyl chain length. QACs may be freely soluble or insoluble in water. The aqueous solubility of QACs increases as alkyl chain length of the molecule decreases and vice versa(Prince, McLaury, Allen &McLaury, 2009). Quaternary ammonium compounds are prepared generally by the alkylation of tertiary amines with a halocarbon. The modern chemists usually refer to it simply as **quaternization**(Kuca, Kivala & Dohnal, 2004). Examples are shown below:



**Scheme 1**.Preparation of benzalkonium salt from a long-chain alkyldimethylamine with benzyl chloride.

(**n** represents alkyl chain length ranges from C8 to C18).




**Scheme 2.**Preparation of benzalkonium salt from N,N-dimethylbenzylamine with long chain n-alkylbromides

Quaternary ammonium compounds have also been shown to have antimicrobial activity (Thorsteinsson*et al.*, 2008). Their antimicrobial activity depends on changing length of the side n-alkyl chain. It is well known that the $C_{12}$ –homolog is most effective against yeast and fungi, $C_{14}$-homolog against gram-positive bacteria and $C_{16}$-homolog against gram-negative bacteria (Daoud, Dickinson, & Gubert, 1983). Gram-positive bacteria are those that appear purple under microscope due to their thick peptidoglycan layer, whereas Gram-negative bacteria are those that appear pink under the microscope due to their thin peptidoglycan layer that takes up minimal crystal violet dye used in the gram staining process (Ghuysen& Hakenbeck, 1994) as shown in **Figure 1.2.**

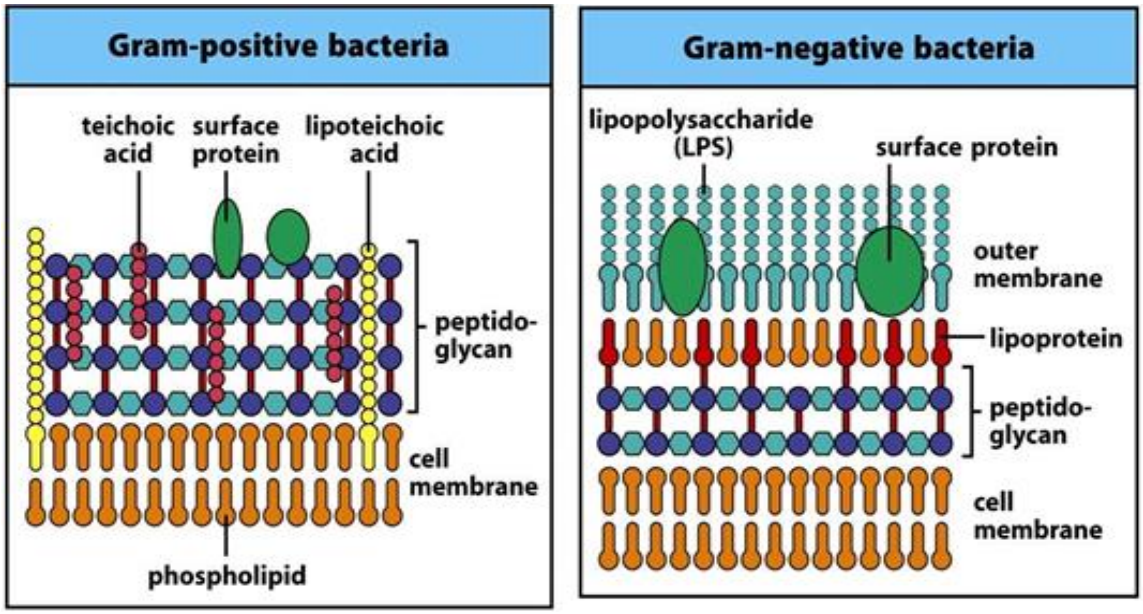



**Figure 1.2.** The cell wall of Gram-positive and Gram-negative bacteria.

For a QAC to have a high antimicrobial activity, at least one of the R groups must have a chain length in the range C8 to C18 (Viscardi *et al*., 2007). The quats do not kill bacterial spores but can inhibit their growth (Brill, Goroncy-Bermes &Sand, 2006). Quaternary ammonium salts are used as disinfectants, surfactants, fabric softeners, plant growth retardants, antistatic agents (Zhao & Sun, 2008).

## 1.1STATEMENTS OF THE PROBLEM

The shortage of new antibacterial agents and increasing resistance of bacteria to conventional antimicrobial agents are very challenging issues needed to be addressed.

## 1.2JUSTIFICATION OF THE STUDY

To find out if the presence oflong alkyl chain length and hydroxyl functional group in the molecular framework of quaternary ammonium compound will reduce or eliminate the bacteria resistance to conventional antimicrobial agents.

## 1.3 AIM OF THE STUDY

To synthesize, characterize, and evaluate the antimicrobial activity of quaternary ammonium compound from the epoxy and fatty amine reaction.

## 1.4OBJECTIVES OF THE STUDY

- To synthesize tertiary amine compound from the reaction of epoxy group and fatty amine.

- To synthesize quaternary ammonium compound from the opened epoxide tertiary amine (adduct).
- To characterize the synthesized compound using Fourier Transform Infrared (FT-IR) spectrometer
- To analyze the synthesized compound using Mass Spectrometer (MS)
- To determine the anti-microbial activities of the synthesizedcompound.

## 1.5SIGNIFICANCE OF THE STUDY

The opening of epoxide ring generates hydroxyl functional group as part of molecular framework of quaternary ammonium compound which may eliminate the bacterial resistance to conventional antimicrobial agents.

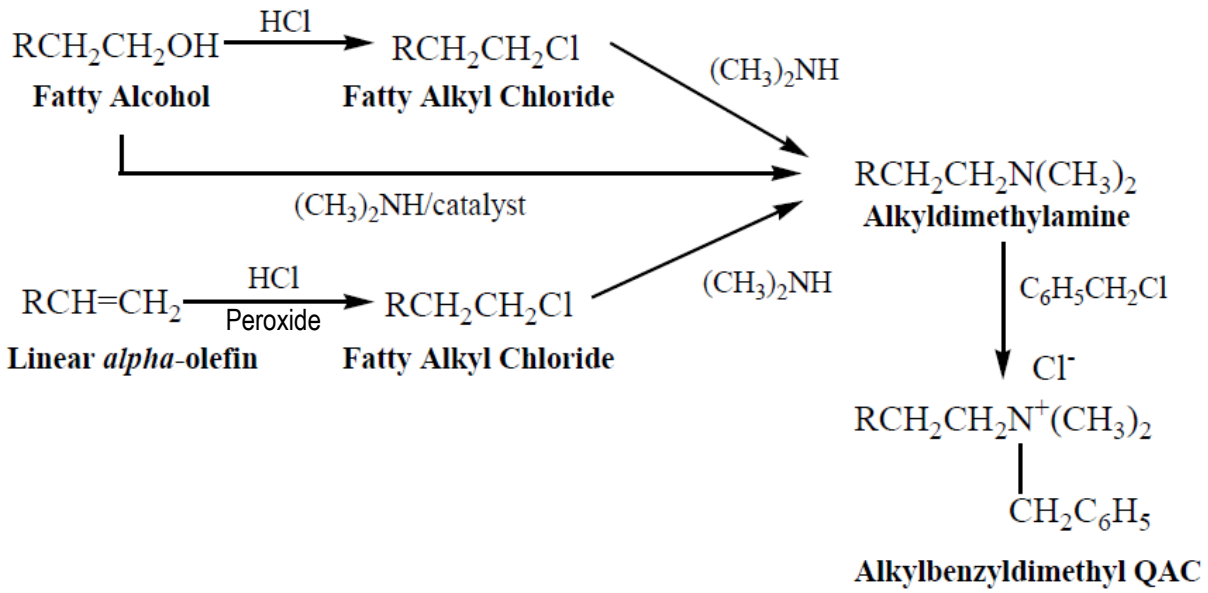
## 1.6SCOPE OF STUDY

This research study focuses on the opening of epoxide ring to form tertiary amine(the intermediate), quaternizing the tertiary amine with benzyl chloride, FT-IR characterization of the quaternized compound, MS analysis of the quaternized compound, and anti-microbial evaluation of the synthesized compound.

# CHAPTER TWO

## 2. LITERATURE REVIEW

QACs are produced by a nucleophilic substitution reaction of alpha-olephines or fatty alcohol originated tertiary amines by an alkyl halide or benzyl halide (Boethling, 1994; Dery, 2001). The synthesis pathway of benzalkoniumchlorides is given in Scheme 2.1.




**Scheme 2.1**Synthetic pathway of alkylbenzyldimethylammonium chloride (Dery, 2001).

Kuca, KivalaandDohnal (2004) reported the synthesis of N-benzyl-N,N-dimethylalkylbromide from the reaction of N-benzyl-N,N-dimethylamine and long chain alkyl bromide.They added that quaternary ammonium cations are unreactive

towards even strong electrophiles, oxidants, and acids but are very reactive and stable towards most nucleophiles.

Grillitsch*et al.*(2006) reported the use of quaternary ammonium salts (benzalkonium chlorides) as biocides, detergents, and phase transfer catalysts or agents because of their strong cationic surface activity.

Daoud*et al.* (1983) also investigated a very important features of quaternary ammonium salts which are their bactericidal and antimicrobial properties, and reported that their antimicrobial activity depends on changing length of side n-alkyl chain. It is well known that the $C_{12}$-homolog is most effective against yeast and fungi, the $C_{14}$-homolog is most effective against gram-positive bacteria, and $C_{16}$-homolog is most effective against gram-negative bacteria(Daoud*et al*., 1983).Pernak, Rogoża and Mirska(2001) reported that quaternary ammonium salts can be hydrophobic or hydrophilic. He added that the aqueous properties ofthe quaternary salts reduce as the alkyl chain length or hydrophobicity of the molecule rises, similarly, the critical micelle concentration (CMC) of the quaternary compounds, which affects the efficacy of various surfactants associated applications declines with an increase in the alkyl chain length of the molecule. Quaternary ammonium salts are widely used as preservatives for ophthalmic, nasal and parenteral products, and are also used as topical antiseptics and disinfectants for medical equipments(Debrecem & Mestyan, 2007).

Grillitsch*et al.* (2006) further analyzed the use of quaternary ammonium halides as phase transfer catalysts and reported that such catalysts accelerate reactions between reagents dissolved in immiscible solvents, and the highly reactive reagent, dichlorocarbene, $CCl_2$ is generated via phase transfer catalyst (PTC) by reaction of chloroform and aqueous sodium hydroxide.

Gowariker, Kalyaniparanjape and Sudha(2013) reported the use of quats as plant growth retardants. In their reports, quats reduce plants height by inhibiting the

production of gibberellins, the primary plant hormones responsible for cell elongation. They concluded that their effects are primarily on stem, petiole and flower stalk tissues.

Cooper(1988) studied the toxicology of quaternary ammonium salts and found that they are highly toxic to fish ($LC_{50}$ = 280 µg ai/L), very highly toxic to aquatic invertebrates ($LC_{50}$ = 5.9 µg ai/L), moderately toxic to birds ($LD_{50}$ = 136mg/kg-bw), and slightly toxic to mammals ($LD_{50}$ = 430 mg/kg-bw). Based on his findings, highly dilute solution ofquats are for human use whereas concentrated solutions are toxic to humans, causing corrosion/irritation to the skin and mucosa, and death if taken internally in sufficient volumes.Quaternary ammonium compounds are effective at exceptionally low concentrations; Contact lens solutions typically contain about (0.002% to 0.01%) concentrations of quaternary ammonium compound for effective preservatives actions (US patent, 2006).

Swan(2005) found that repeated use of quaternary ammonium salts at 10 folds higher concentrations of 1:50(0.02%) or stronger can denature corneal proteins and cause damage to the eye.

Walter(2001) identified that concentrations of less than 0.1% quaternary ammonium compound instilled into human eyes, produced no adverse sensations, whereas concentrations of 0.1% or greater, produced “burning and stinging reactions.

Whitehill(2008) determined that the maximum non-irritating concentration of quats in rabbit eyes is 0.05%.QACs may be freely soluble or insoluble in water. The aqueous solubility of QACs decreases as the hydrophobicity or the alkyl chain length of the molecule increases. For instance, the aqueous solubility of N,N-dioctyl-N,N-dimethylammonium chloride ($DC_8$DMA-Cl), N,N-didecyl-N,N-dimethylammonium chloride($DC_{10}$DMA-Cl), N,N-didodecyl-N,N-dimethylammonium chloride($DC_{12}$DMA-Cl), N,N-ditetradecyl-N,N-

dimethylammonium chloride ($DC_{14}DMA$-Cl), N,N-dioctadecyl-N,N-dimethylammonium chloride($DC_{18}DMA$-Cl) are 8100, 700, 77, 12 and 2.7 mg/L, respectively (Boethling,1994).

Garcia *et al*(2006) also discovered that the critical micelle concentration of quaternary ammonium compounds, which affects the effectiveness of many surfactant-related applications, decrease as the alkyl chain length of the molecule increases. For example, the CMC of N-benzyl-N-dodecyl-N,N-dimethylammonium chloride ($C_{12}BDMA$-Cl), N-benzyl-N-tetradecyl-N,N-dimethylammonium chloride($C_{14}BDMA$-Cl), N-benzyl-N-hexadecyl-N,N-dimethylammonium chloride($C_{16}BDMA$-Cl) are 3, 2 and 0.5mM, respectively (Garcia *et al*., 2006). QACs are designated as the ultimate work-horse of the surfactant industry. They are on the High Production Volume Chemicals list of the USEPA. The world-wide annual consumption rate of QACs was reported as 0.5 million metric tons in the 6$^{th}$World Surfactants Congress held in Germany (CESIO, 2004) and this rate was expected to reach 0.7 million metric tons (Steichen, 2001). The demand for QACs is usually found to be 10% of the total surfactant demand, they nevertheless represent an irreplaceable category of surfactants for over two centuries (Steichen, 2001). QACs have surface-active, detergency and antimicrobial propertiesand self-assembly characteristics(Keen& Montforts, 2011). The uniqueness in physical and chemical properties of QACs have resulted in a variety of uses as surfactants, emulsifiers, fabric softeners, disinfectants, pesticides, phase-transfer catalysts and corrosion inhibitors (Figure 2.2) (Boethling, 1994).

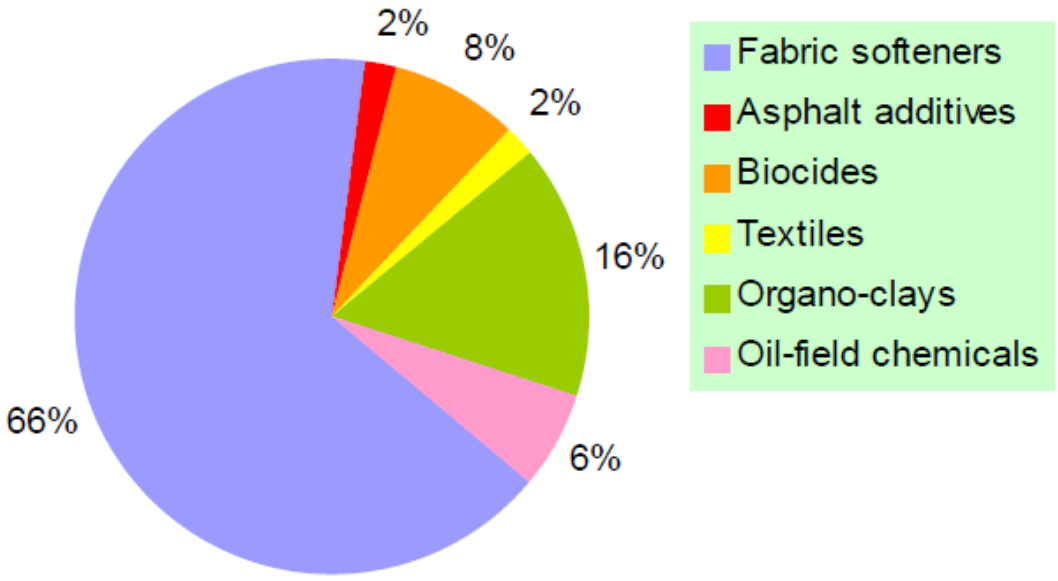



**Figure 2.2.**Distribution of QAC use in the market (Boethling, 1994).

QACs are the major active ingredient of fabric softeners (20-30 wt %). The most common softener active ingredients that are commercially available and viable in the marketplace aredialkonium salts, diethylenetriamide compounds and ester quaternary salts. The first group has the highest demand in the market. Nevertheless, ester quaternary salts are good substitutes for the dialkonium salts since they are readily biodegradable and less toxic than the dialkonium salts(Tezel, Pierson& Pavlostathis, 2006). QACs are also utilized for soil removal purposes in laundry detergents. Laundry detergents that also provide fabric softening utilize simple QACs such as monoalkonium salts with an alkyl chain length of 12 to 18 carbons (Zachvieja, 2001). QACs reduce surface and interfacial tension by sorbing to a surface or an interface such as hair and skin. The adsorption ability of QACs onto organic surfaces makes the use of QACs extremely important in the personal care industry(Rutala & Weber, 2008). Skin care products and hair conditioners contain mainly alkyl QACs (including mono-, di- and tri- alkonium salts), ethoxylated and ester QACs in their formulations (Tang, 2001). QACs also are used in processing paper used for the production of tissue paper or fluff pulp, which are used in diapers, toweling, napkins and facial and toilette tissue products. It is known that QACs, such as dialkonium salts are effective chemical debonding agents in paper. QACs interact with the natural fiber-to-fiber bonding that occurs during the paper-making process. The hydrophobic and hydrophilic moieties of the QACs interact with the fiber surface, reduce the inter-fiber bonding, and form a thin lubricant layer. This reduction of the inter-fiber bonding, together with the lubricating effect, gives a soft feel to the paper. In the mechanical fluff pulp process, QACs protect the fibers against damage and reduce the defibration energy needed (Bergstrom, 2001). QACs exhibit a broad spectrum of antimicrobial activity over a wide range of pH and are used extensively in domestic, industrial, agricultural and medical applications as wood preservatives, pesticides, fungicides,

sanitizers/disinfectants, and hard-surface cleansers(Meng, 2012). The applied concentration ofquarternary ammonium compounds when they are used as disinfectantsis typically between 400 and 500 ppm and almost always below 1000 ppm (e.g., 0.1% w/v in Lysol®) (Tiedink, 2001). The use of QACs as biocides in wood preservation formulations is a common application. QACs are used either by themselves or in combination with other modern biocides, such as fungicides, bactericides or insecticides. The most commonly used QACs are dialkonium and benzalkonium chlorides (Tiedink, 2001).

**Table 2.1**.Biocidal activity of alkyl ($C_{12-16}$)benzyldimethyl and didecyl ($C_{10}$)dimethylammonium chlorides.

| Microorganism | Minimum Inhibitory Concentration (ppm) | |
|---|---|---|
| | $C_{12-16}$BDMA-Cl | $DC_{10}$DMA-Cl |
| *Enterococcus faecium* | 30 | 10 |
| *Staphylococcus aureus* | 40 | 10 |
| *Escherichia coli* | 100 | 25 |
| *Pseudomonas aeruginosa* | 700 | 250 |
| *Salmonella typhimurium* | 150 | 40 |
| *Proteis mirabilis* | 300 | 200 |
| *Campylobacter jejuni* | 45 | 4 |
| *Legionella pneumophila* | 80 | 30 |
| *Listeria monocytogenes* | 25 | 5 |

QACs are used in agricultural formulations as biocides and adjuvants. The consumption of $C_{12\text{-}16}$BDMA-Cl, $DC_{10}$DMA-Cl, $DC_{8\text{-}10}$DMA-Cl and $DC_8$DMA-Cl as biocides in the State of California in 2003 were 3394, 1176, 157 and 79 kg, respectively. Many pesticides are insoluble in water and not active as they applied individually, however QACs (as adjuvants) enhance the solubility, rain fastness and penetration of pesticides as they are applied together with the pesticides. Typical QAC concentrations in agrochemical tank-mixed sprays range from 0.05 to 0.5% v/v (Gustavsson, 2001). QACs such asdialkonium and benzalkonium salts are used in the production of organoclay and these organoclays are used in a number of different formulations such as oil-based drilling fluids, printing inks, oil based paints, latex polymers and nail polishers (Hoey, 2001). Organoclays have the ability to absorb organic molecules from both aqueous systems and air and are used in landfill liners, groundwater remediation and in air filters (Boyd, Lee &Mortland, 1988). The annual demand for organophilic clays which contain about 40% by weight of QACs is around 16% of the QAC market (Figure 2.2). Oil-field applications of QACs include anti-swelling/clay stabilization, foaming, silt suspension, corrosion inhibition, biocides and demulsification (Black, 1996). One of the new applications for QACs is phase-transfer catalysis. Many organic syntheses are carried out in which one reactant is dissolved in an aqueous solution and the other in a hydrophobic organic phase. QACs act as phase-transfer catalysts and mediate the reaction at the interface between the two phases or after one of the solutes has passed through the interface and entered the other phase. QACs are believed to show better selectivity, greater rate increase, and cost less than other types of phase transfer catalysts. Benzalkonium and monoalkonium salts are used extensively as phase transfer catalysts (Boethling, 1994). Quaternary ammonium compounds have been used extensively in many domestic, agricultural and industrialapplications over two centuries. Their production and consumption rates

are increasing asnew applications are found(Tezel *et al.,* 2007). The concentration of quaternary ammonium compounds in effluent wastewater, sewage sludge, domestic wastewater and surface water has been reported as 0.05, 3000,0.5 and 0.04 ppm, respectively (Schmitt, 1994).

Wee (1984) found the levels of dialkonium chlorides in untreated sewage and final effluent from a plant in the United States. The QAC concentrations in the effluent and influent sewage ranged from 0.01 to 0.2 ppm and 0.05 to 1.3 ppm respectively. The concentrations of monoalkonium chlorides were monitored in composite sewage samples in England and Germany. The total monoalkonium chloride concentration in the effluent and influent sewage was 0.03 ppm and 0.13 ppm, respectively. A recent study conducted in Austria to survey QAC concentrations in influents of five different wastewater treatment plants reviewed quaternary ammonium compound concentrations ranging from 1 to 170 ppb (Martinez-Carballo*et all.*, 2007).

On the contrary, the QAC concentrations would be higher in the effluents of specific industrial facilities such as paper processing, textile and food processing (1-40 ppm, based on the data obtained during a screening study for a poultry processing facility in Georgia), than the influents of municipal wastewater treatment plants (Tezel *et al.*, 2006).

Kummerer *et al.* (1997) studied benzalkonium chlorides in highly complex effluent samples from different sized European hospitals and found that the measured concentrations were between 0.05 and 6.03 ppm. Although the reported quaternary ammonium compound (QAC) concentrations are low in the wastewater, many studies delineating the effect and biodegradability of QACs in wastewater treatment systems have worked at concentrations ranging from 10 to 100 mg/L.

Therefore, one would expect high QAC concentrations in the wastewater treatment systems or surface waters receiving influents from industrial applications that use QACs extensively.

Wang-Hsien Ding and Ying-Hsiao Liao(2001) discovered that the concentrations of dialkonium chlorides in sewage and surface water samples collected in Germany were 0.35 to 0.48 ppm and 6 to 12 ppb, respectively.

Lewis (1991) subsequently carried out a follow-up study, in which samples were collected at various distances downstream from wastewater treatment facilities, and found that the mean dialkonium chloride levels were <2, 24, 17, and 33 ppb for Millers River (MA), Otter River (MA), Blackstone River (MA) and Rapid Creek (SD). The concentration of $DC_nDMA$-Cl in the samples collected at distances from 4.4 to 55 miles downstream from the wastewater treatment plants ranged from 191 to 100 ppb. Quaternary ammonium compounds (QACs) adsorb strongly on suspended solids such as biomass, minerals and inorganic particles and

are transferred to anaerobic digesters or aquatic sediments. For instance, mean concentrations of $DC_nDMA$-Cl's in anaerobically stabilized sludge samples from five different municipal sewage treatment plants in Switzerland were 3670, 960, 470, and 210 ppm (mg/kg-dry) in 1991, 1992, 1993, and 1994 respectively (Tezel *et al.,* 2006). It was also reported that QAC concentrations in anaerobic digesters may range from 4000 to 10,500 ppm (mg/kg-dry solids).

Fernandez *et al.*(1996) discovered that dialkyl dimethylammonium chloride ( $DC_nDMA$-Cl) was a ubiquitous contaminant in coastal sediments collected near Barcelona, Spain.

Utsunomiya, Watanuki, Matsushita and Tomita (1997) studied the levels of QACS in river water and sediment samples from Japan and reported that the levels of QACs in influent sewage, river water and sediment were 0.10 to 0.15, 0.05 and 6.2 to 69 ppm, respectively.

Sun *et all.*(2003) and Tezel *et al.* (2006) studied the fate of QACs in a river running through Toyama City, Japan. They found that total influx of QACs into the river was 1.4 g/min, and the concentration was between 0.01 and 0.02 ppm. The quaternary ammonium compounds in the sediment samples were 500 times higher than those found in the river water. In another study, it was reported that $DC_nDMA$-Cl was present at 0.63 ppm in surface water and7.4 ppm (0.6 to 1.2 m depth) in the sediment at a pond that had been receiving untreated wastewater from a laundromat since 1962 (Federle& Schwab, 1992). Dialkonium chlorides were also detected in drinking water samples derived from river water and groundwater in England. About 75% of QACs consumed end up in wastewater treatment plan (Tezel *et al.,* 2006). The $EC_{50}$values for N-hexadecyl-N,N,N-trimethylammonium bromide ($C_{16}TMA$-Br) and N-benzyl-N-dodecyl-N,N-dimethylammonium chloride($C_{12}BDMA$-Cl) obtained from a respirometric assay conducted with activated sludge ranged between 10 and 40 mg/L (Reynolds *et all*., 1997).

The $EC_{50}$of $C_{14\text{-}18}$TMA-Cl for unacclimated sludge determined based on the inhibition ofglucose uptake was 28 mg/L (Larson & Schaeffer, 1982). Another study showed that $DC_{10}$DMA-Cl inhibited the COD removal in a rotating biological contactor at concentrations above 20 mg/L and the biofilm was totally eliminated at 160 mg/L. QACs have high affinity to adsorb onto (bio)solids. Generally, adsorption outcompetes biodegradation in aerobic biological treatment systems and therefore QACs are transferred to anaerobic digesters as part of the primary and waste activated sludge(Tezel *et al.*, 2006). It was reported that QAC concentrations may reach up to 50 mg/L in anaerobic digesters of sewage treatment plants (Garcia *et al*., 2006). Quaternary ammonium compound concentrations may exceed these levels in biological treatment systems of industrial facilities, such as food processing, that extensively use QACs. Under anaerobic conditions, there is no evidence of mineralization of QACs that contain alkyl or benzyl groups (Battersby& Wilson, 1989; Federle& Schwab, 1992; Garcia *et al*., 2006), most likely because of the highly reduced nature of these substituent groups. Moreover, QACs are inhibitory to anaerobic microbial processes such as methanogenesis (Battersby& Wilson, 1989; Garcia *et al*., 2006).

Tezel, Piersonand Pavlostathis (2006) studied the effect of four QACs: $DC_8$DMA-Cl, $DC_{8\text{-}10}$DMA-Cl, $DC_{10}$DMA-Cl and $C_{12\text{-}16}$BDMA-Cl on a mixed mesophilicmethanogenic culture. It was reported that all QACs tested in this study had short- or long-term inhibitory effects on the mixed methanogenic culture at 25 mg/L and above. The inhibitory impact of the individual QACs on the methanogenic activity decreased according to the following series: $DC_8$DMA-Cl> $DC_{8\text{-}10}$DMA-Cl> $C_{12\text{-}16}$BDMA-Cl> $DC_{10}$DMA-Cl. Thus, QACs with the shorter alkyl chain length are the most inhibitory QACs (Tezel *et al.*, 2006). QACs are toxic at ppm levels and lower to aquatic organisms including algae, fish, mollusks, barnacles, rotifers, starfish, shrimp, and others. Toxicity of 15 QACs (with

molecular weights ranging between 313.5 and 547.0 g/mole) were investigated in four bioassays, such as *Microtox*, *Spirotox*, *Protoxkit F* and *Artotoxkit M*, comprising a bacterium (*Vibrio fischeri*), two ciliated protozoa (*Spirostomumambiguum*and *Tetrahymenathermophila*), and an anostracean crustacean (*Artemiafranciscana*). The Microtox® assay acute toxicity $EC_{50}$values for tested QACs ranged between 0.6 to 50 μM (0.24 to 21.5 mg/L at the average QAC molecular weight of 430.25 g/mole). The results indicated that QACs had high toxicity against the bioindicators tested and were toxic not only to bacteria, but also to non-target protozoa and crustacean (Garcia *et al.,* 2006). It was also reported that the toxicity of QACs decreases as the alkyl chain length increases, since the hydrophobicity of QACs with longer alkyl chain length increases resulting in low bioavailability and high partitioning with organic or negative charged surfaces (Nalecz-Jawecki, Grabinska-Sota,&Narkiewicz, 2003). Algae represent a group of organisms which appears to be very sensitive to QACs. The $EC_{50}$values of $C_n$TMA-Br and $C_n$TMA-Cl for algae range between 0.03 and 0.38 mg/L. On the other hand, $EC_{50}$values for dialkonium QACs range between 0.05 and 18 mg/L, therefore the toxicity of dialkonium QACs is less than the toxicity of monoalkonium QACs (Lewis, 1991; Utsunomiya *et al*., 1997). Benzalkonium QACs are toxic to aquatic organisms below 1 mg/L. The toxicity of QACs to fish and invertebrates has also been studied. It was reported that all QACs are acutely toxic to aquatic invertebrates and fish as indicated by $EC/LC_{50}$values below 1 mg/L by affecting the reproduction and larval growth and development (Lewis, 1991; Boethling, 1994; Utsunomiya *et al*., 1997). The none observed effect concentration (NOEC) for *Daphnia* exposed to $DC_{18}$DMA-Cl and $C_{12}$TMA-Cl in river water was 0.38 and 0.065 mg/L, respectively (Lewis, 1991). According to Kummerer*et al*.(1997) the $LC_{50}$of $C_n$BDMA-Cl to fish is between 0.5 and 5.0 ppm, and the toxicity to daphnids is even higher, with an $LC_{50}$from 0.1 to 1.0 ppm. Data

retrieved from the European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC) and U.S. EPA Ecotoxicology (ECOTOX) databases using the OECD Application ToolBox showed that monoalkonium, dialkonium and benzalkonium chlorides are toxic to aquatic organisms such as, bacteria, algae, fish, invertebrates, etc. The minimum, maximum and median of reported $EC_{50}$values are 37 pg/L, 58 mg/L and 0.5 mg/L, respectively. QACs are widely distributed in the environment and are detected in drinking water. They are the main active ingredients of many household products and cosmetics. As a result of these widespread uses, humans are exposed to them with almost all body surfaces and cavities and QACs have the potential to be adsorbed, inhaled, and ingested. In general, the acute (single-dose) toxicity of QACs is characterized, at lethal doses, by peripheral paralysis and central nervous system stimulant-like effects. In chronic (multiple-dose) studies, the toxic effects of QACs commonly consist of adverse effects on body weight or growth, reduced food consumption, dehydration, and increased mortality (Drobeck, 1994). Several human fatalities due to exposure to benzalkonium chlorides have been reported over the years.

Xue*et all*.(2004) investigated the distribution and disposition of $C_n$BDM-Cl following oral administration (PO) and intravascular jugular vein (W), femoral artery (FA), femoral vein (FV) and jugular artery (JA) administration in rats along with pathological examinations. In this study, toxic doses of 250 and 15 mg/kg of $C_n$BDM-Cl were used for PO and intravascular administration, respectively. The fatal effects of $C_n$BDM-Cl appeared soon in JV-, FV- or JA-rats, but took hours in PO or FA-rats. No rat receiving benzalkonium chloride via FA survived longer than 1 day. The PO-rats that aspirated benzalkonium chlorides into their lungs had some systemic symptoms and higher blood and tissue concentrations of benzalkonium chloride. The blood benzalkonium chloride levels and kinetics were similar among the different routes of intravascular administration, but the lung and

kidney levels were higher in JV-rats. Pathological examinations confirmed severe congestion and edema in the lungs and kidneys (Xue*et al*., 2004). QACs have been actively deployed as antimicrobial agents since the 1930s in many clinical, industrial and domestic applications. The mode of action of QACs against bacterial cells involves perturbation of lipid bilayer of the bacterial cytoplasmic membrane and the outer membrane of Gram-negative bacteria. Such action leads to a progressive leakage of cytoplasmic components out of the cell (Tezel *et al*., 2007). Low concentrations of QACs bind to anionic sites found on the membrane surface, cause cells both to lose osmotic regulation capability and to leak potassium ions and protons whereas the high concentrations kill cells by disintegration of the membranes and release of cytoplasmic contents and coagulation of proteins and nucleic acids (Garcia *et al.*, 2006). . At the molecular level, action involves the association of the cationic quaternary nitrogen with the head groups of the acidic-phospholipids within the membrane due to ionic interactions. The hydrophobic tail (alkyl groups) then integrates into the lipid core with hydrophobic interactions. Such interactions increase the surface pressure in the exposed layer of the membrane and decrease membrane fluidity. The membrane undergoes a transition from fluid to liquid crystalline state and loses its osmoregulatory and physiological functions. As a result, QACs penetrate into the cell and reach their target cites of action (Maillard, 2002; Gilbert & Moore, 2005). QACs are also involved in the inhibition of respiratory enzymes and the dissipation of proton motive force (PMF) which affects the microbial metabolism, active transport, oxidative phosphorilation and ATP synthesis in bacteria (Knox, Auerbach, Zarudnaya & Spirtes, 1999; Maillard, 2002). Recently, several bacterial species including *Pseudomonas fluorescens*, *pseudomonas* spp., *Aeromonashydrophila*, *Enterobacteriaceae*, *Serratiaproteamaculans*and *Serratia*spp that contain *qacEΔ1* have been isolated from a quaternary ammonium compound polluted environment near a textile mill.

This study is an important example that indicates antibiotic resistance may not reside in only clinical species but spread in the environmental systems and QACs seems to be the major facilitator of antibiotic resistance in the environment (Ceccarelli *et all*., 2006).Most uses of QACs lead to their release into wastewater treatment systems or directly into the environment. The fate of QACs in aerobic biological treatment systemand receiving waters has been studied and the results of these studies have been reviewedextensively (Tezel *et al*., 2006). These studies showed hat QACs are degraded under aerobic conditions and up to 90% of QAC removal by means of biodegradation is reported in engineered and natural systems. In fact, the half-livaerobic ultimate degradation of QACs vary extensively from hours to months dependingon the QAC concentration, structure, microbial acclimation and presence of QAC resistant/degrading microorganisms(Boethling, 1994; Van Ginkel, 1996). The alkyl chain length not only determines thephysical/chemical properties of the QACs, but also may have a decisive role in the fate and effects of these compounds in the environment. Under aerobic conditions, the biodegradability of QACs generally decreases with the number of alkyl groups as $R_4N^+ < R_3MeN^+ < R_2Me_2N^+ < RMe_3N^+ < Me_4N^+$. Furthermore, substitution of a methyl group with a benzyl group can decrease biodegradability further (Ying, 2006). A comparison of the degradation rates of benzalkonium chlorides and monoalkonium bromides under aerobic conditions was undertaken. The rate of degradation of $C_{12}$BDMA-Cl, $C_{14}$BDMA-Cl and $C_{16}$BDMA-Cl, and $C_{12}$TMA-Br, $C_{14}$TMA-Br and $C_{16}$TMA-Br was inversely related to the lengthof alkyl group($C_n$) and substitution of benzyl group decreased the rate as welI. In fact, $C_{16}$BDMA-Cl was found to be the most recalcitrant of the tested compounds, with a plateau at only 30% degradation after 10 days (Tezel *et al*., 2007). Likewise, it was reported that the aerobic degradation of QACs was dependent on the length of

the alkyl group; however the number alkyl groups had a more pronounced effect on biodegradability. For exampledialkonium QACs were degraded five times more slowly than monoalkonium QACs (Van Ginkel & Kolvenbach, 1991). Certain microorganisms that are capable of QAC degradation have been isolated.

These microorganisms are *Xanthomonas* (Dean-Raymond & Alexander, 1977), *Pseudomonas* B1 (VanGinkel, Vandijk & Kroon, 1992), *Pseudomonas fluorescens* TN4 (Nishihara, Okamoto & Nishiyama, 2000), *Aeromonas hydrophila* sp.K (Patrauchan & Oriel, 2003), and *Pseudomonas* spp. strain 7-6 (Takenaka, Tonoki, Taira, Murakami & Aoki, 2007) which were isolated from either sewage or soil.QACs are rapidly and strongly sorbed onto a wide variety of materials of environmental relevance such as biomass, sediment, clay, and minerals. In fact, sorption generally outcompetes biodegradation in aerobic environments and therefore, quaternary ammonium compounds are transferred to anoxic/anaerobic compartments such as anaerobic digesters, as part of the primary and waste activated sludge, and aquatic sediments (Tezel *et al*., 2007). Under anaerobic conditions, there is no evidence of mineralization of QACs that contain alkyl or benzyl groups most likely because of the highly reduced nature of these substituent groups (Tezel *et al*., 2007).

On the other hand, diethylester dimethyl ammonium chloride (DEEDMA-Cl), a recent analog of dialkonium chlorides was completely degraded by anaerobic digester sludge in a standard test based on biogas formation (Giolando, Rapaport, Larson, Federle, Stalmans & Masscheleyn, 1995). DEEDMA-Cl differs structurally from dialkonium chlorides by the inclusion of two ester linkages between the ethyl and alkyl chains. These ester linkages allow DEEDMA-Cl to be rapidly and completely degraded in standard laboratory screening tests and a range of environmental media such as sludge, soil and river water with half-lives ranging

from 0.8 to 18 days. Likewise, it is known that natural QACs such as choline and betaine can be ultimately degraded under anoxic/anaerobic conditions (Neill, Grime & Dawson,1978; King, 1984). As a result, QACs in which the hydrophobic moieties are linked to the head group with ester bonds (esterquats), choline, betaine (natural QACs), and those in which alkyl chains are linked directly to $N^+$have a different fate under anoxic/anaerobic conditions. The latter are recalcitrant under these conditions.

# CHAPTER THREE

## 3. MATERIALS AND METHOD

### 3.1 Preparation of theQuaternary Ammonium Salt

**3.1.1 Reagents Used:** 1,2-epoxy-3-phenoxypropane (phenyl glycidyl ether), benzyl chloride, N,N-dioctylamine, tetrahydrofuran and calcium chloride (Sigma-Aldrich Co LLC).

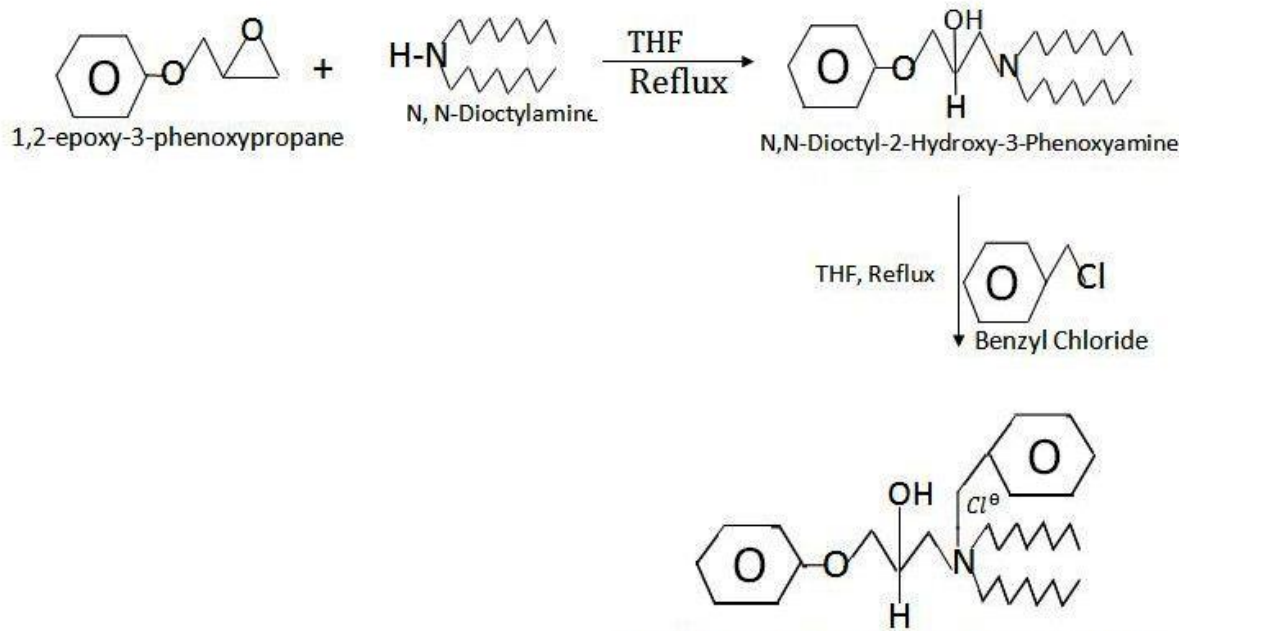
**3.1.2 Apparatus Used:** Condenser, two neck round bottom flask, 250ml beakers, magnetic stirrer, electric heater, thermometer, weighing balance, retort stand, syringes(10ml and 20ml), drying tube, cotton wool, Fourier Transfom Infrared spectrometer (Agilent cary 630 FTIR), Mass spectrometer.

**3.1.3 Methodology:** Generally, quaternary ammonium compounds are prepared from the reaction of tertiary amines with alkyl or benzyl halides (Kivala*et al*., 2004).

In our interest, quaternary ammonium compound was produced using 1,2-epoxy-3-phenoxypropane (phenyl glycidyl ether), dioctylamine, benzyl chloride and tetrahydrofuran. Reaction procedure is described as follows:

Phenyl glycidyl ether 3.4ml (3.8g, 0.025mol), N,N-dioctylamine 7.5ml (6.0g, 0.025mol), and 33 ml of tetrahydrofuran(THF) were added into two neck round bottom flask. The mixture was refluxed for 8 hours at temperature of 67 $^{o}$C to form a tertiary amine compound (the intermediate). Then, 0.02mol (6.5g) of the intermediate, 0.02mol (2.1g, 1.9ml) of benzyl chloride, and 29ml of tetrahydrofuran(THF) were added into another two neck round bottom flask. The

mixture was refluxed for 12 hours at temperature of 67°C. After the reaction, solvent was evaporated by distillation, and the dark brown, viscous liquid was left as the final product. Illustration is shown in scheme 3.




**Scheme 3:** Synthesis of a quaternary ammonium salt from epoxide and fatty amine reaction

## 3.2 TEST FOR ANTIMICROBIAL ACTIVITY

### 3.2.1 Collection of Test Organism

The pure bacteria strains(*Staphylococcusaureus,Escherichia coli, Salmonella enteric, Lactobacillus spp.*, and *Klebsiella spp.*) used for this analysis were

collected from Microbiology Laboratory, Federal University of Technology, Owerri.

### 3.2.2 Preparation of Macfarland's Turbidity Standard

$BaCl_2$(1%)was preparedby dissolving 1g of $BaCl_2$ in99ml of distilled water. Also, 1% of $H_2SO_4$was prepared by dissolving 1ml of Conc. $H_2SO_4$ in 99ml of distilled water. The bacterial population used in this study was standardized using 0.5 Macfarland's standard prepared by reacting 0.6ml of 1% $BaCl_2$ and 99.4ml of 1% $H_2SO_4$ to form a $BaSO_4$ precipitate.

$$BaCl_{2(aq)} + H_2SO_{4(aq)} \longrightarrow BaSO_{4(s)}\downarrow + 2HCl_{(aq)}$$

A bacterial population equal to the turbidity of the 0.5 Macfarland's standard was used for the study.

### 3.2.3 Antibacterial Studies

The bacteria colonies were picked using a sterile wire loop to make a suspension of the test organism in a sterile Bijou bottle. The turbidity of the suspension was compared against the turbidity of the prepared test standard. A sterile swab stick was dipped into the inoculums and used to streak the surface of the agar. A sterile cork borer was then used to produce wells of 8mm allowing 30mm between adjacent wells and the perti dish.

Sterile syringeswere used to introduce fixed volumes of test compounds into the wells. The plates were incubated at 30$^{o}$C for 24 hours. After the period of incubation, the diameter of the zones of inhibition was measured in millimeter(mm). Ampicillin 10mg/ml was used as control (Cheesbroug, 2000).

# CHAPTER FOUR

## 4. RESULTS AND DISCUSSION

### 4.1 RESULTS

The results obtained from the synthesis, characterization, and antimicrobialevaluations of the quaternary ammonium salt are shown in Tables and Figures below;

**Table 4.1:** Solubility/froth test on the quaternary ammonium salt

| COMPOUNDS | APPEARANCE | SOLUBILITY IN WATER |
|---|---|---|
| 1,2-epoxy-3-phenoxypropane ($C_9H_{10}O_2$) | Colourless Liquid | Not soluble |
| N,N-dioctylamine ($C_{16}H_{35}N$) | Colourless liquid | Not soluble |
| N,N,-dioctyl-2-hydroxy-3-phenoxyamine ($C_{25}H_{45}NO_2$) | Yellowish liquid (oily) | Not soluble |
| Benzylcholride ($C_9H_9Cl$) | Colourless liquid | Not soluble |
| N-benzyl-N,N-dioctyl-2-hydroxy-3-phenoxyammonium chloride ($C_{32}H_{52}NO_2Cl$) | Dark brown, foamy liquid | Soluble |

**Table 4.2:** Antimicrobial test results showing the zones of inhibition (mm) of the samples and standard antibiotic (**Ampicillin**)

| Diameter of Zones of Inhibition (mm) | | | | | |
|---|---|---|---|---|---|
| Samples **(10 mg)** | *Lactobacillius Spp.* | *Salmonella enteric* | *Klebsiella spp.* | *Escherichia Coli* | *Staphylococcus aureas* |
| 1 | 12 | 20 | 22 | 25 | 15 |
| 2 | 2 | 4 | 4 | 6 | 2 |
| 3 | 2 | 2 | 4 | 4 | 2 |
| 4 | - | - | - | - | - |
| 5 | - | - | - | - | - |
| Ampicillin (10mg) | 19 | 20 | 20 | 20 | 18 |

**Key:**

1 = Quaternary Ammonium Salt

2 = Tertiary Amine (The Intermediate)

3 = Benzyl Chloride

4 = 1,2-epoxy-3-phenoxypropane (phenyl glycidyl ether)

5 =N,N-dioctylamine

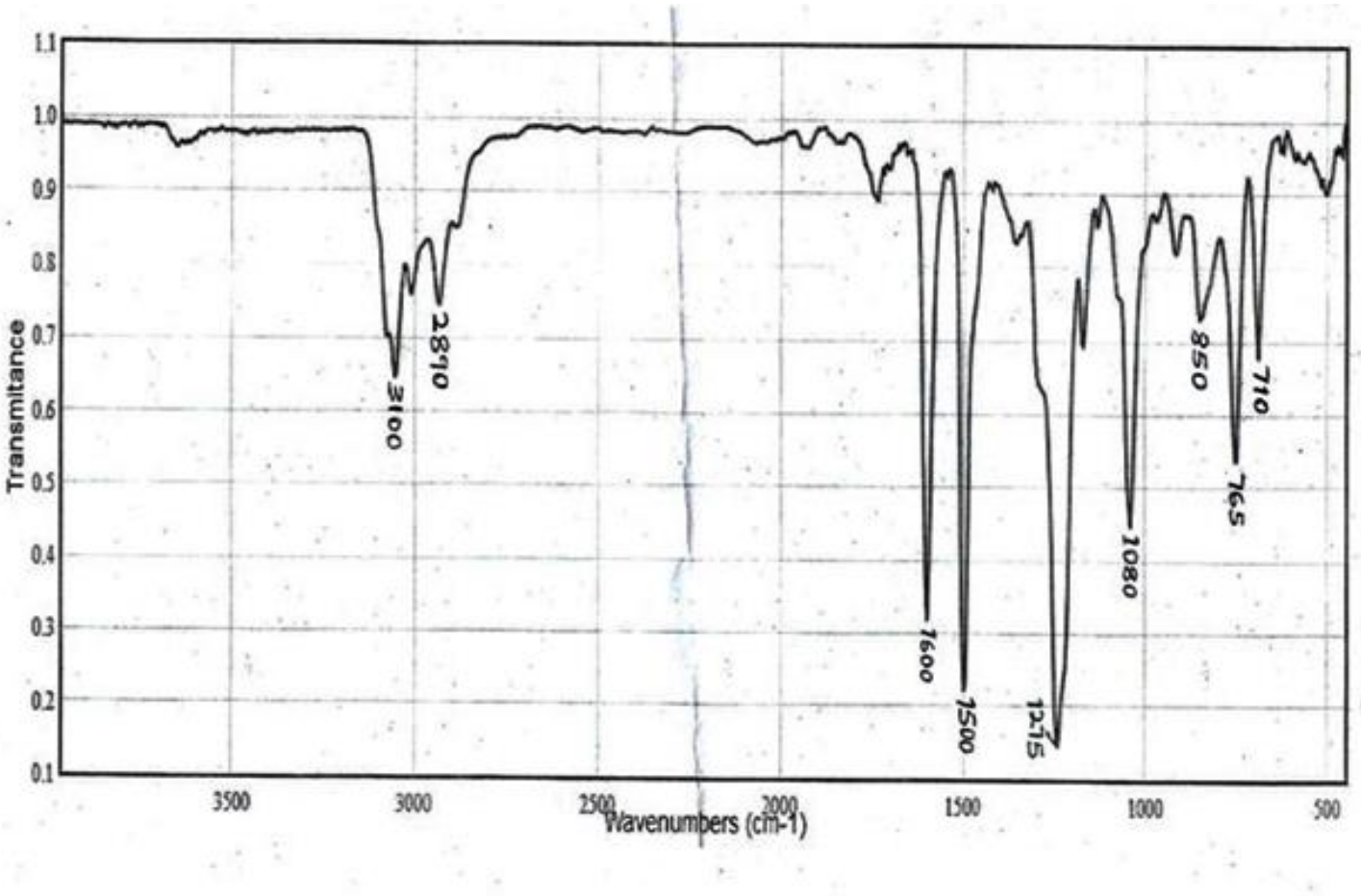



**Figure 4.1:** Infrared Spectrum of 1,2-epoxy-3-phenoxypropane (phenyl glycidyl ether)

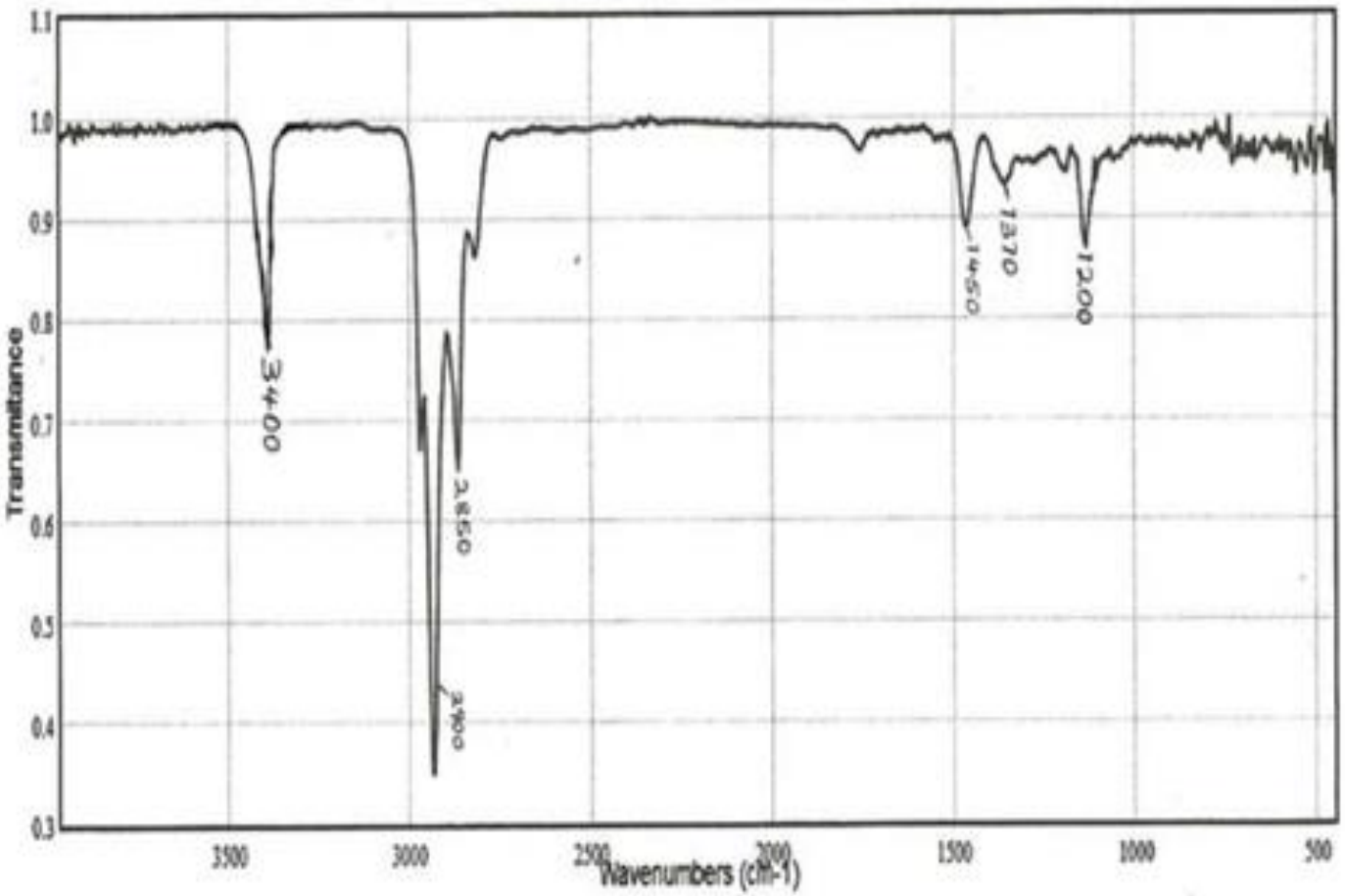



**Figure 4.2:** Infrared Spectrum of N,N-dioctylamine

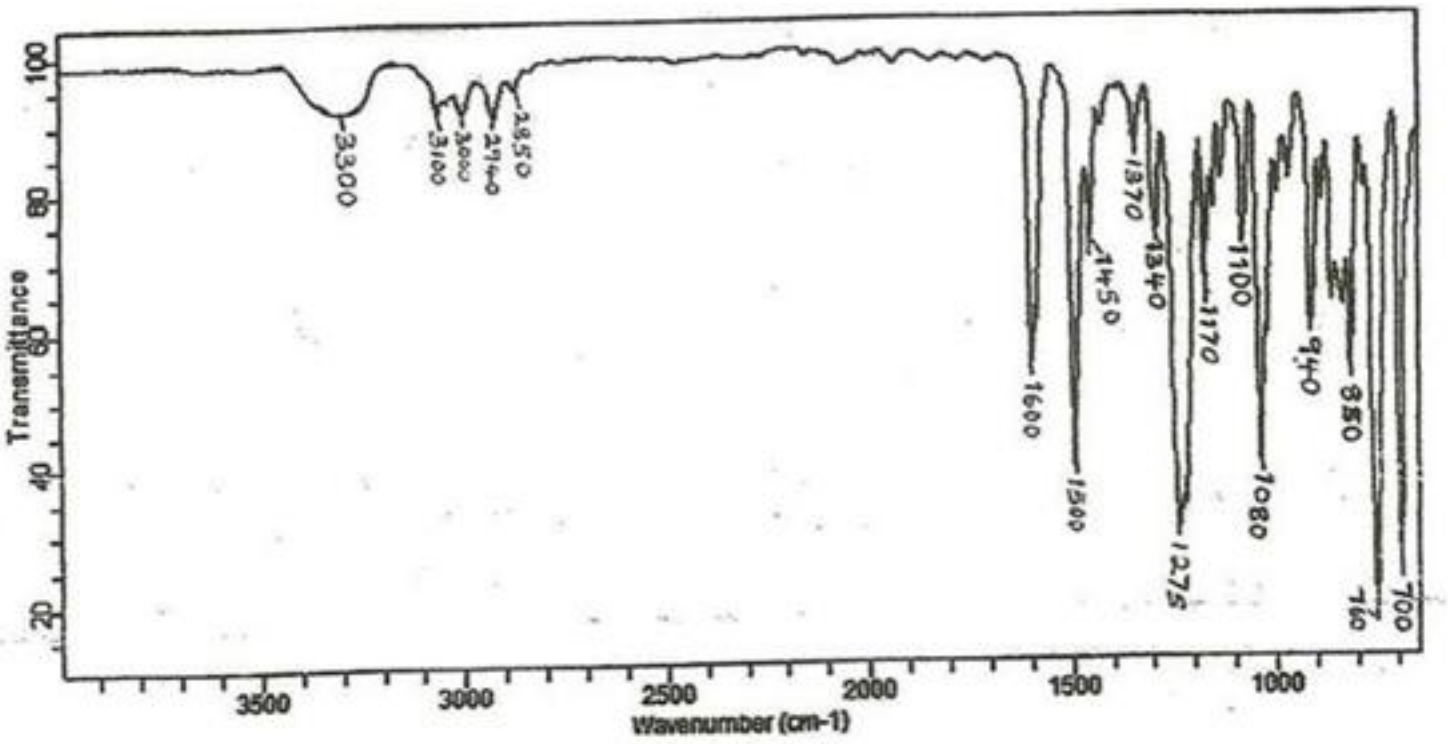



**Figure 4.3:** Infrared Spectrum of N-benzyl-N,N-dioctyl-2-hydroxy-3-phenoxypropylammonium chloride (final product)

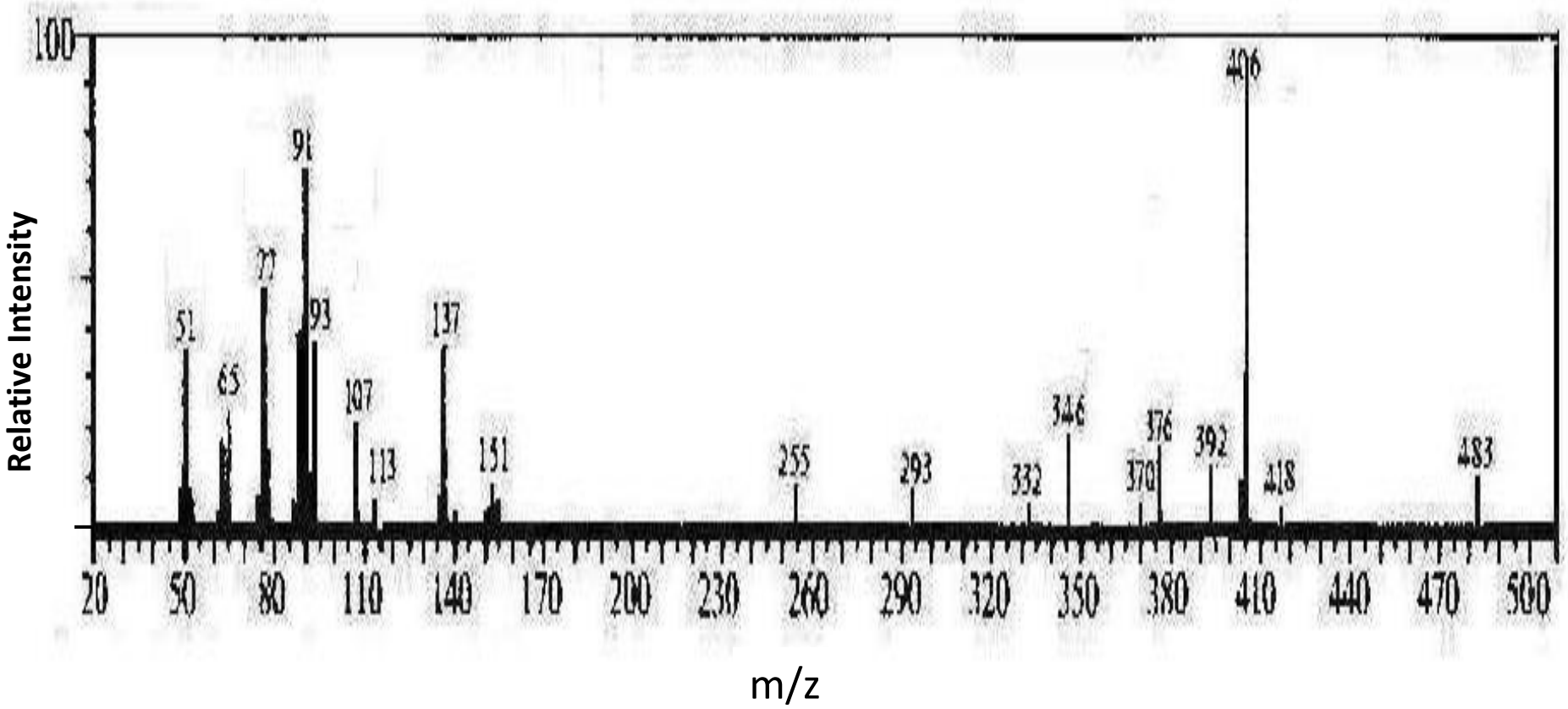



**Figure 4.4:** Mass Spectrum of the Final Product

## 4.2: DISCUSSION

Solubility/Froth tests were conducted on the starting materials, intermediate, and the final product. Results obtained show that only the final product is foamy and soluble in water, as shown in**Table 4.1**, which is a physical evidence that a desired product, quaternary ammonium salt formed.

**Table 4.2**showed that only quaternary ammonium compound exhibits an excellent anti-bacteria activity, especially on gram-negative bacteria. This is because of the alkyl chain length of the compound. Also comparing the product with the standard antibiotic(Ampicillin), the product is more effective on gram-negative bacteria, but less effective on the gram-positive bacteria.

**Infrared spectrum ofFigure 4.1** showed a peak at 3100$cm^{-1}$ indicating absorption of C-H stretch of an aromatic compound, peak at 2890$cm^{-1}$ indicates C-H stretch of methylene group(-$CH_2$) of aliphatic hydrocarbon, sharp peaks at 1500$cm^{-1}$ and 1600$cm^{-1}$ indicate absorption of C=C stretch of aromatic compound, peak at 1275$cm^{-1}$ indicates absorption of C-O stretch of aryl ether, peak at 1080$cm^{-1}$ indicates absorption of C-O stretch of alkyl ether, and peaks at 710$cm^{-1}$ and 765 $cm^{-1}$ indicate monosubstituted aromatic compound.

**Infrared spectrum of Figure 4.2** showed a sharp peak at 3400$cm^{-1}$ indicating absorption of N-H stretch of $2^o$ amine, peak at 2900$cm^{-1}$ indicates C-H stretch of methylene group(-$CH_2$) of aliphatic hydrocarbon, peak at 2850$cm^{-1}$ indicates C-H stretch of methyl group(-$CH_3$) of aliphatic hydrocarbon, and peak at 1200$cm^{-1}$ indicates absorption of C-N stretch of amine.

**Infrared spectrum of Figure 4.3** showed a broad, smooth peak at 3300$cm^{-1}$ indicating absorption of O-H stretch of alcohol which resulted from the ring opening of

epoxide, peaks at 3000cm$^{-1}$ and 3100cm$^{-1}$ indicate absorption of C-H stretch of aromatic compounds, peaks at 2940cm$^{-1}$ and 2850cm$^{-1}$ indicate absorption of C-H stretch of -$CH_2$ and -$CH_3$ groups of aliphatic hydrocarbon, sharp peaks at 1600cm$^{-1}$ and 1500cm$^{-1}$ indicate absorption of C=C stretch of aromatic compounds, peak at 1340cm$^{-1}$ indicates absorption of C-N of ammonium compound, peak at 1275cm$^{-1}$ indicates absorption of C-O stretch of aryl ether, peak at 1100cm$^{-1}$ indicates absorption of C-O stretch of 2$^{o}$ alcohol, peak at 1080cm$^{-1}$ indicates absorption of C-O stretch of alkyl ether, and peaks at 700cm$^{-1}$ and 760cm$^{-1}$ indicate monosubstituted aromatic compounds.

**Figure 4.4** showed the fragmentation pattern of the final product andthe following M/Z valueswere observed: M/Z 51, M/Z 65, M/Z 77, M/Z 91, M/Z 93, M/Z 107, M/Z 113, M/Z 137, M/Z 151, M/Z 255, M/Z 293, M/Z 332(M-151), M/Z 346(M-137), M/Z 376(M-107), M/Z 370(M-113), M/Z 392(M-91), M/Z 418(M-65), M/Z 432(M-51), M/Z 406(M-77) which is the base peak, and M/Z 483($M^+$) which is the molecular weight of the product.

# CHAPTER FIVE

## 5.1 CONCLUSION

Quaternary ammonium compound was synthesized from epoxy/fatty amine reaction. Fourier transform infrared (FTIR) and mass spectroscopic analysis were conducted and results obtained was an indication for the formation of the quaternary ammonium salt. Also antimicrobial analysis was conducted and result obtained show that only the final product exhibited an excellent anti-bacteria activity with the following zones of inhibition: *Salmonella Enteric* (20 mm), *Escherichia Coli* (25 mm), *Klebsiella Spp*. (22 mm), *Staphylococcus Aureus* (15 mm), and *Lactobacillus Spp*. (12 mm).

## 5.2 RECOMMENDATION

Synthesis and antimicrobial evaluation of quaternary ammonium salt should be carried out using different fatty amines to open up the epoxide ring. This is to compare the different products that will be formed and know the effect of variation in alkyl chain length on a selected micro-organisms.

## 5.3 CONTRIBUTION TO KNOWLEDGE

This work revealed that the opening of epoxide ring generates hydroxyl functional group as part of molecular frame work of quaternary ammonium

compound whichenhances the elimination of the bacteria resistance to conventional quaternary ammonium compound.

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