SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF QUATERNARY AMMONIUM SALTS FROM AN EPOXIDE, ETHANOLAMINES AND A LONG CHAIN HALOALKANE

BY

ENYIA CHIBUIKE (B-Tech., FUTO)

REG. NO: 20134870978

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL

FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI,

IMO STATE

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

SEPTEMBER, 2021.

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CERTIFICATION

This is to certify that this research work **"SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF QUATERNARY AMMONIUM SALTS FROM AN EPOXIDE, ETHANOLAMINES AND A LONG CHAIN HALOALKANE"** was carried out by **ENYIA CHIBUIKE** with registration number **(20134870978)** in partial fulfillment of the requirements for the award of Master of Science (M.Sc.) degree in Organic Chemistry, Chemistry Department, Federal University of Technology,Owerri, Imo State.

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DEDICATION

This research work is dedicated to the Almighty God who is the author of all knowledge and wisdom.

ACKNOWLEDGEMENTS

My special thanks go to my supervisors Dr. F .C. Onwumere & Dr. C. O. Alisa for their ceaseless guidance and directives all through this study.

A very big thank you to Prof. M.O.C. Ogwuegbu, Prof. L.E.O. Agwaramgbo and Dr. (Mrs.) I. Duru for all the lectures, corrections and advice. I found them valuable.

I also appreciate the efforts of the post-graduate coordinator, Dr. C. K. Enenebeaku in facilitating my study.

I am indebted to the Head of Chemistry Department Prof. (Mrs). C. E Ogukwe and the entire staff of the Department whose constructive criticisms and corrections made this research work a success.

I am immeasurably grateful to my beloved parents Mr. & Mrs. Enyia for their unfailing support and encouragement throughout the course of this research work.

I wish to also thank my uncle, Mr. Bartholomew Enyia and colleagues, Chukwunonyerem Celestine C. and Amafili Kingsly C. for all their support. The good Lord bless you all immensely.

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ABSTRACT

Quaternary ammonium compounds were synthesized through epoxide ring opening with ethanolamines, and subsequent quaternization with cetylbromide. Fourier Transform Infrared (FTIR) spectrometric analyses of the two intermediates *N*-hydroxyethyl-*N*-methyl-2-hydroxy-3-phenoxypropylamine and *N*-hydroxyethyl –*N*,*N*-(bis)-[2-hydroxy-3-phenoxypropyl] amine and their corresponding starting materials showed a disappearance of the N-H stretch of the amines at 3291.2 cm^{-1} 3350.9 cm^{-1}, 3287.5 cm^{-1} and the appearance of a broad secondary O-H peaks at 3347.1 cm^{-1} and 3365.8 cm^{-1} from the ring opening of the epoxide group. Mass spectrometric analysis of the final product *N*-cetyl-*N*-hydroxyethyl–*N*-methyl-2-hydroxy-3-phenoxypropyl ammonium bromide showed the presence of the molecular ion (M^+) peak m/z = 451.0, while that of *N*-cetyl-*N*-hydroxyethyl-*N*,*N*-(bis)-[2-hydroxy-3-phenoxypropyl]ammonium bromide showed m/z = 587.0. The antibacterial activities of the intermediate and the final products were investigated, relative to ampicillin which was used as a reference, the synthesized quaternary ammonium salts exhibited good antibacterial activity on a number of bacteria strains especially against Gram-positive bacteria with the following zones of inhibition: *Staphylococcus aureus* (23 mm and 23 mm), *Lactobacillus spp* (24 mm and 25 mm), *Escherichia coli* (14 mm and 16 mm), *Salmonella enteric* (12 mm and 14 mm) and *Klebsiella spp* (14 mm and 14 mm).

Keywords: Quaternization, Mass Spectrometry, Infrared Spectrometry, Hydroxyl Functional Group, and Zone of Inhibition

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Quaternary ammonium compounds (QACs) are salts of quaternary ammonium cations with a counter anion. QACs are surfactants, and in 2004 the annual consumption was estimated at 500,000 tones (Tezel and Pevlostathis, 2011). The structure of these compounds is formed by a positively charged nitrogen (N) atom and four alkyl groups R_1 –R_4 (saturated, unsaturated, branched or straight, cyclic or acyclic), one of which is a long alkyl chain having different numbers of carbon atoms and a chloride or bromide anion (X^-) according to the structure

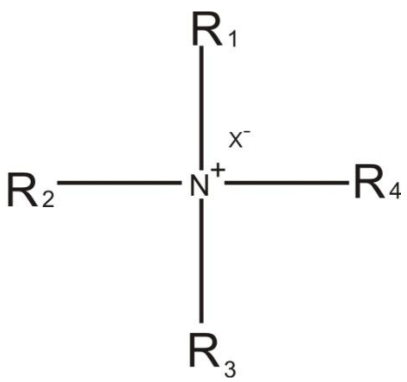


Figure 1.1. General structure of a QAC (R represents an alkyl group, X represents a counter-ion such as Cl^- or Br^-)

The alkyl chain is the hydrophobic part of the compound, and nitrogen with alkyl groups is the hydrophilic group. Positively charged complex ions affect the capability of reducing surface tension, which gives these compounds high surface activity. Compounds having 12-14 carbon atoms in the aliphatic chain show the highest surface activity. In turn negatively charged chlorine or bromine ions increase the biocidal activity (Oblak & Gamian, 2010; Lipinska-Ojrzanowska & Walasiuk –Skorupa, 2014).

Quaternary ammonium compounds are used as biocides due to their broad spectrum of activity and high thermal stability, among others. Examples of quaternary ammonium compounds are benzyldimethylhexadecy ammonium chloride and 1-hexadecylpyridinium chloride, among others.

Figure 1.2 Structure of benzyl dimethylhexadecylammonium chloride

Figure 1.3 Structure of I-Hexadecylpyridinium chloride

The antimicrobial effectiveness of QACs was described in the thirties of twentieth century, indicating their high activity against bacteria, mould and lipophilicviruses, at very low concentrations (Tischer, Pradel, Ohlsen & Holzgrabe, 2012). Their antimicrobial activity depends on the 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 & Guber, 1983). Most bacteria are classified into two broad categories: Gram-positive & Gram-negative. These categories are based on their cell wall composition and reaction to the Gram stain test. The Gram staining method developed by Hans Christian Gram, identifies bacteria based upon the reaction of their cell walls to certain dyes and chemicals.

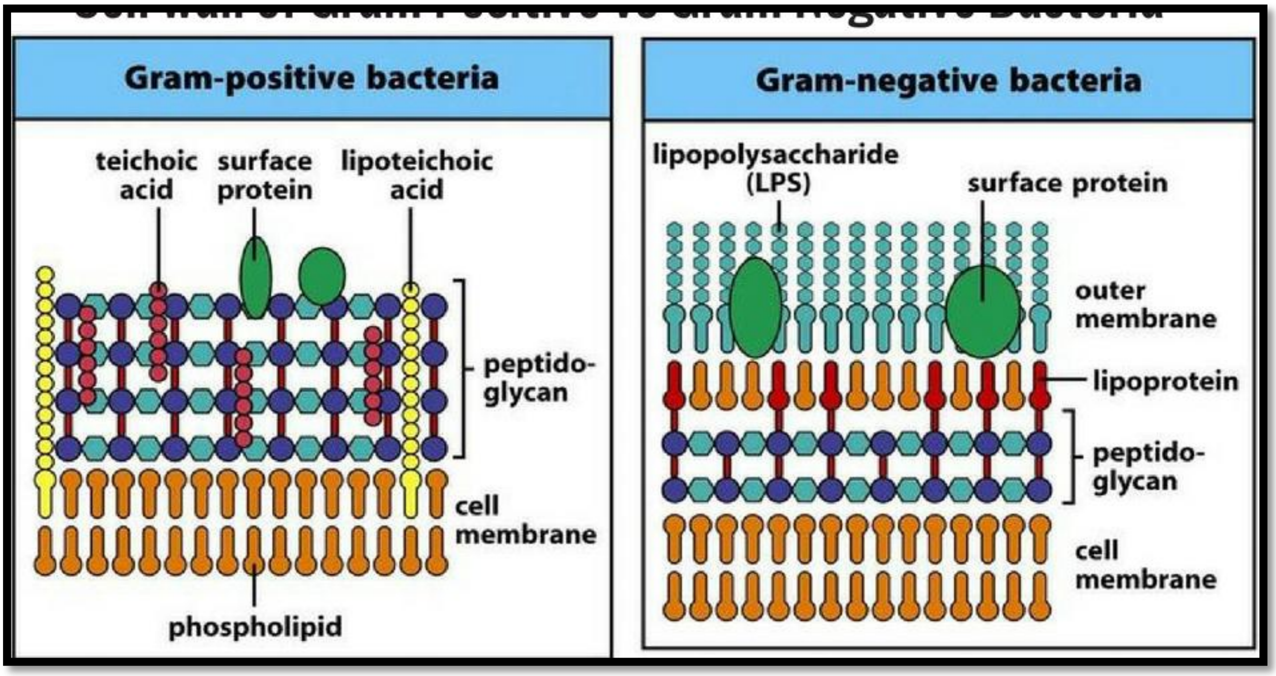


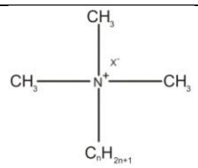
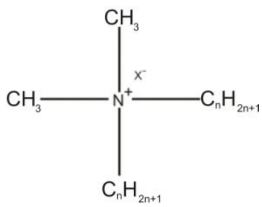
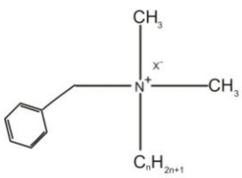
Figure 1.4 The cell wall of Gram-positive and Gram-negative Bacteria.

Gram-positive bacteria are those that stain purple on a gram stain test due to their thick peptidoglycan layer. Gram-positive bacteria take up the crystal violet dye used in the test, and then appear purple coloured when seen through a microscope. Gram-negative bacteria are those that appear pink under the microscope. They appear pink due to their thin peptidoglycan layer that takes up minimal crystal violet dye used in the Gram staining process (fig. 1.4). Some examples of Gram-positive bacteria are Staph. Aureus, Bacillus Anthracis, C. Titani and Viridans Mutans. On the other hand, Gram-negative bacteria include E. coli, H. Pylori, Cholera, Bordetella Pertussis and Enterobacter. It has been observed that for a QAC to have a high antimicrobial activity, at least one of the R groups must have a chain length in the range of C_8 –C_{18} (Viscardi *et al.,* 2007). The QACs do not kill bacterial spores, but they inhibit their growth (Brill*, Goroney-Bermes & Sand,* 2006).

Quaternary ammonium compounds are used in medicine and many industries (food, cosmetics and paper) as biocides and even Drugs (Raghavendra, 2002; Xiao, 2008). They are used as agents to combact biofilms formed by microorganism in cooling systems (Rucka, Oswiecimska & Witek, 1983). QACs are used in the restoration of historical buildings (Karbowska-Berent*, Kozielec, Jamilko & Brycki* 2011; Sterflinger & Pinar, 2013; Stupar*,* 2014), as ingredients of pesticides and wood preservatives (Dubois & Ruddick, 1998; Tezel & Pavlostathis, 2011), where they primarily act as antifungal compounds. They are also used as chemical corrosion inhibitors, plasticizers in the production of textiles & papers, or antistatic agents for material such as cotton or cellulose fibres (Oblak & Gamian, 2010; Lipinska- Ojrzanowska& Walasiuk-Skorupa, 2014). They are also used as disinfectants, surfactants, fabric softeners, plants growth retardants (Zhao & Sun, 2008). QACs are readily biodegradable, non-toxic to humans & animal, dissolve well in water & are active in a wide pH range (4-10)

Quaternary ammonium compounds can be classified in three major groups depending on the type of the functional groups: monoalkonium, dialkonium and benzalkonium halides (table 1.1)

Table 1.1 Representative QAC groups and their general structures

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

QACs are large molecules having molecular weight typically between 300 and 500 g/mole and are composed of two distinctly 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 affect QACs' 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 increase as alkyl chain length of the molecules decreases and vice versa (Prince, 2009).

QACs are prepared generally by the alkylation of the tertiary amines with a halocarbon. The modern chemists usually refer to it as quaternization (kuca, 2014). Examples are shown below:

Scheme1. Preparation of benzalkonium salt from a long chain alkyldimethylamine with benzyl chloride

$$CH_3(CH_2)_n\mathit{N}(CH_3)_2 + ClCH_2C_6H_5 \longrightarrow [CH_3(CH_2)_n\mathit{N}(CH_3)_2CH_2C_6H_5]^+Cl^-$$

(n represents alkyl chain length ranging from C_8 to C_{18})

Scheme2. Preparation of benzalkonium salt from *N*,*N*-dimethylbenzylamine with long chain n-alkylbromide

$$C_6H_5CH_2\mathit{N}(CH_3)_2 + R\text{-}Br \xrightarrow[\text{Reflux}]{\text{EtoH}} [C_6H_5CH_2\mathit{N}(CH_3)_2R]^+Br$$

1.2 STATEMENT OF PROBLEM

The challenge this research work seeks to address is the continued resistance of bacterial strains to existing anti-bacterial agent.

1.3 JUSTIFICATION OF STUDY

A number of long alkyl chain quaternary ammonium compounds have been identified through synthetic processes, and have been found to exhibit excellent antimicrobial properties which may help combact bacteria resistance to conventional antibacterial agents (Classissa *et al.,* 2007)

1.4 AIM OF STUDY

The aim of this research work is to synthesize quaternary ammonium compounds and to determine their antimicrobial activities.

1.5 OBJECTIVES OF STUDY

- To synthesize quaternary ammonium compounds using an epoxide, a primary and secondary amine and a long chain haloalkane
- To characterize the synthesized products using infrared and mass spectrometry
- To investigate the antimicrobial activities of the synthesized products.

1.6 SIGNIFICANCE OF STUDY

The significance of this research work is the need to develop new antibacterial agents that could effectively combat bacterial strains which are resistant to current anti-bacterial agents.

1.7 SCOPE OF STUDY

The scope of this research work is restricted to the synthesis of quaternary ammonium compounds using phenyl glycidylether, cetylbromide, *N*-methylethanolamine and monoethanolamine. The quaternized compounds would be characterized using FT-IR and MS spectrometry. The anti-microbial investigation is limited to bacterial strains.

CHAPTER TWO

LITERATURE REVIEW

2.1 Quaternary Ammonium Compounds (QACs)

Since the discovery in 1876 by Robert Koch that microorganisms cause infectious disease, physicians, microbiologists, and medicinal Chemists have been searching for drugs to prevent infections. As early as the 1930s quaternary ammonium compounds (QACs) were found to have antimicrobial activities, patents were immediately filed for this purpose (Domagk, 1935). Quaternary ammonium compounds are produced by a nucleophilic substitution reaction of alpha-olefins or fatty alcohol originated tertiary amines by an aryl halide or benzyl halide (Boethling, 1994; Dery, 2001).The quaternization reaction of tertiary amines results in an organic molecule that contains four functional group (R) covalently attached to a central nitrogen atom. The synthetic pathway of benzalkonium chlorides is given in Figure 2.1

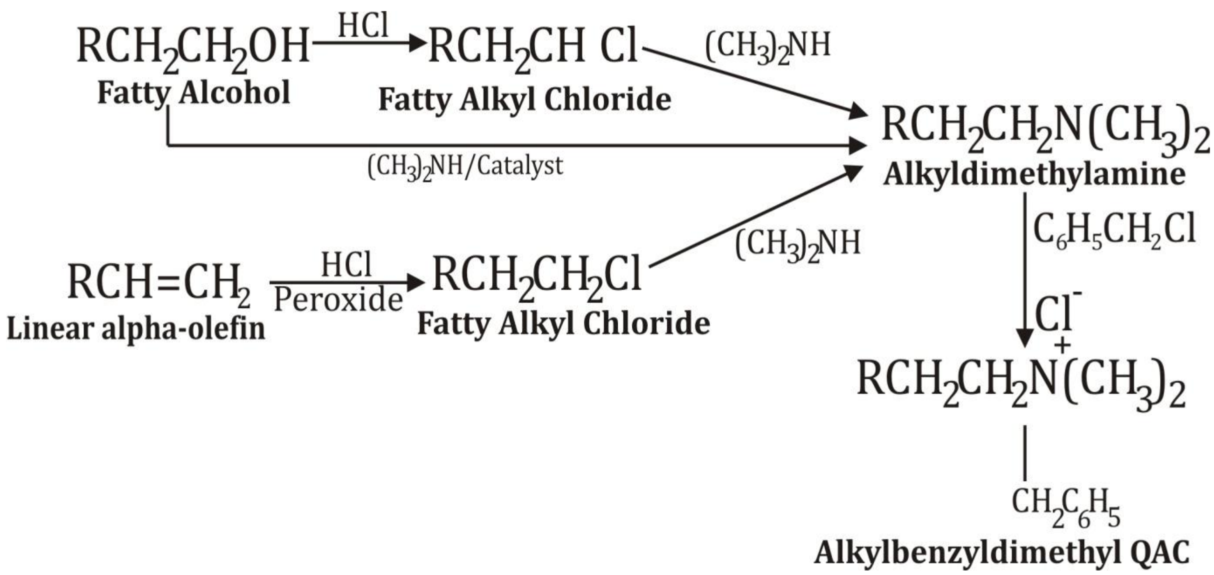


Figure 2.1 Synthetic pathway for alkylbenzyldimethylammonium chlorides (Dery, 2001).

Kivala, kula & Dohnal (2004) reported the synthesis of *N*-benzyl-*N*, *N*-dimethylalkylbromide from the reaction of *N*-benzyl-*N*,*N*-dimethylamine and long chain alkyl bromide. Kosswig (1977) also reported a typical synthesis of benzalkonium chloride from a long chain alkyldimethylamine and benzylchoride. He added that quaternary ammonium cations are unreactive towards even strong electrophiles, oxidants and acids but are very reactive and stable toward most nucleophiles.

QACs are usually odourless, nonstaining, non-corrosive and relatively non-toxic to users. They function well over a broad temperature range and a wide pH range, although activity is greater at warmer temperatures and in alkaline situations. While QACs tolerate light organic loads, heavy soil will decrease QAC activity significantly. Some QACs may not function adequately in hard water, but others are formulated with added chelating agents that allow such use (Schmidt, 2003). The most commonly used QACs are dialkonium and benzalkonium chlordes (Tiedink, 2001).

Table 2.1 Biocidal activity of alkyl (C_{12-16}) benzyldimethyl and didecyl (C_{10})dimethylammonium chloride

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

Quaternary ammonium compounds are interfacially active. They consist of a polar head group and a non-polar hydrocarbon chain. The polar part of the molecule can interact strongly with polar solvents, like water, and is therefore also called the hydrophilic part. The non-polar part, on the other hand, can form strong interactions with non-polar solvents, like oil, and is therefore also called lipophilic or hydrophobic part. QACs absorb preferably at interfaces where they find the energetically most favourable conditions due to their two-part structure. At a water surface, for example, QACs orient themselves in such a way that the head group resides in the water and the hydrocarbon chain points to the gaseous phase. Thus, QACs can mediate between two phases as they can from strong interactions with both of them. The interfacial tension consequently decreases. The addition of QACs hence facilitates the mixing of polar & non-polar phases, which is used in the detergent industry for example. The aqueous properties of quaternary salts reduce as alkyl chain length or hydrophobicity of the molecule rise, similarly, the critical micelle concentration (CMC) of the quaternary compounds, which affects the efficacy of the various surfactants decline with an increase in the alkyl chain length of the molecule. It has been observed that the

CMC of monoalkonium chlorides is significantly higher than those of benzalkonium chlorides with an identical alkyl chain length indicating that the addition of a benzyl group to the polar head of surfactants can decrease CMC, and the lesser the value of CMC, the more the efficacy of benzalkonium chloride against pathogenic microbes (Pernak & Mirska, 2001).

Boethling (1994) investigated the solubility of QACs and observed the solubility decreases as the alkyl chain length or hydrophobicity of the molecule increases. For instance, the aqueous solubility of DC_8DMA-CL, DC_{10}DMA-CL, DC_{12}DMA-CL, DC_{14}DMA-CL and DC_{18}DMA-CL are 8100, 700, 12 and 2.7mg/L, respectively.

2.2 Applications and Implications of QACs

QACs are used for a variety of clinical purposes such as preoperative disinfection of unbroken skin, application to mucous membranes, and disinfection of non-critical surfaces(McDonnell & Russell, 1999). QACs are high production–volume chemicals that constitute a large fraction of the cationic surfactant market. They are used as active agents in detergent formulations, fabric softener products, microbiodes, and personal care products, and they find application in a variety of industrial processes (Boethling & Iynch, 1992). The world- wide annual consumption rate of QACs was reported as 0.5 million metric tons in the 6th world surfactant congress held in Germany (CESIO, 2004), and this rate was expected to reach 0.7 million metric tons (Steichen, 2001). While 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 are the major ingredients of fabric softeners (20-30 wt%). Until the beginning of 1990s, di-tallow dimethylammonium chloride (DTDMAC) had represented the most important cationic softener which exhibit a poor biodegradability connected with a relatively high ecotoxicity in standard tests.

These facts triggered the development of alternative cationic surfactants exhibiting more favourable ecological characteristics. Today, the major active ingredient in softeners are esterquats, i.e., compounds with a quaternized (tetra-substituted) ammonium group (eg methyltriethanol ammonium, MTEA) and fatty acids esterified with the hydroxy group of the N-containing part of the molecule. Esterquat feature an excellent biodegradability and a considerably lower aquatic toxicity compared to DTDMAC. The various esterquat types used in fabric softeners have comparable ecotoxicological properties and have been recently scrutinized in an environmental risk assessment (Zachvieja, 2001).

Quaternary ammonium compounds are the raw materials for conditioners. They are high substantive to the hair via attraction to the anionic charges on the hair shafts providing reduction of combing forces, increased luster and improved antistatic properties (Benoit, 2001). Recently developed esterquats are widely used as hair conditioning agents. The diesterquat derived of dimethylamino- 1,2-propanediol is reported to be suitable for use in hair conditioning compositions. Esterquats compounds demonstrate excellent conditioning effect on all types of hairs. Other complex reaction mixtures of esterquats based triethanolamine with fatty acids has been suggested for use in hair and body care (Richmond, 1991).

Quaternary ammonium compounds find applications in froth floatation. QACs render hydrophobicity to the negatively charged mineral particles in an ore pulp and enable them to concentrate into a froth concentrate. Hu (2011) studied quantitative structure activity relationship between QAC collectors and bauxite (AL_2O_3) reverse flotation which show good flotation and adsorption behavior.

QACs are also used in paper processing to produce tissue paper or fluff pulp, which are used in diapers, toweling, napkins and facial and toilet 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 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 fibers against damage and reduce the defibration energy needed (Bergstrom, 2001).

Gowariker, Kalyani and sudha (2013) reported the use of QACs as plant growth retardants. In their report QACs reduce plants heights by inhibiting the production of gibberellins, the primary plant hormones responsible for cell elongation. QACs retard plant height physiologically without any formative effect. The use of plant growth retardants has been made for many years to manipulate the size, shape and overall quality of various crops. These are useful in the reduction of "lodging" in aerial crops, limiting size of fruit trees and in the treatment of decorative pot plants which are of great importance to horticulture

In organic synthesis, quaternary ammonium salts are employed as phase transfer catalysts (PTC). Phase transfer catalysts are often used in heterogeneous reaction mixtures to facilitate movement of a reactant from one phase to another. Phase transfer catalysts are known to sometimes accelerate reaction rates and minimize solvent waste, since the reactions tend to be heterogeneous. The highly reactive reagent dichlorocarbene is generated via phase transfer catalysts by reaction of chloroform and sodium hydroxide. Benzalkonium and monoalkonium salts are used extensively as phase transfer catalysts (Boethling, 1994).

QACs are used in agricultural formulations as biocides and adjuvants. The consumption of C_{12}-$_{16}$BDMA-Cl, DC_{10}DMA-Cl, DC_{8}-$_{10}$DMA-Cl and DC_{8}DMA-Cl as biocide in the state of California in 2003 were 3394, 1176, 157 and 79 kg, respectively (w.w.w.pesticideinfo.com). Many pesticides are insoluble in water and not active as they are applied individually, however QACs (as adjuvant) enhance the solubility rain fastness and penetration of pesticides as they are applied together with the pesticide. Typical QAC concentration in agrochemical tank-mixed sprays range from 0.05 to 0.50 % v/v (Gustavsson, 2001).

QACs are used in the production of organoclays. Organoclays are produced by the displacement of the inorganic cations on a clay mineral (i.e morillonite or hectorite) by organic cations. The organic cations used in the manufacturing of organoclays are QACs such as dialkonium and benzalkonium salt. 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 are able 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 organoclays, which typically contains 40 % by weight of QACs is around 16% of the QAC market. Oil-field applications of QACs include anti-swelling/clay stabilization, foaming, silt suspension, corrosion inhibition, biocides and demulsification (Witco Corporation, 1995, AKZO Chemicals Inc, 1998).

Quaternary ammonium salts are widely used for the control of bacterial growth in clinical and industrial environment (Torosyan, 2018), they have also been increasingly deployed in the treatment of bacterial infections. The antimicrobial activity of QACs 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-negative bacteria (Daoud, Dickinson & Guber, 1983).

2.3 QAC Related Antimicrobial Resistance Mechanisms as a Challenge to Human Health

Bacteria resistance to QACs has become a serious problem. Many aerobic & facultative microorganisms acquire resistance to QACs by changing the composition of their outer membrane proteins (Loughlin, Jones & Lambert, 2002; Tabata, Nagamune, Maeda, Murakami, Miyake & Kourai, 2003), fatty acids (increase in saturated fatty acids) (Guerin –Mechin*, Dubois-Brissonnet, Heyd & Leveau,* 1999; Guerin–mechin, *Dubois-Brissonnet, Heyd & Leveau,* 2000; Dubois-

Brissonnent, Malgrange, Guerin-Mechin, Heyd & Leveau, 2001) lipids (Loughlin *et al.*, 2002), lipopolysaccharide, cell wall surface hydrophobicity and the zeta-potential of the cell surface or the acquisition or hyper- expression of certain multi-drug efflux pumps (Poole, 2002). These QACs resistance mechanism are very similar to resistance mechanisms to hydrocarbons (Sikkema*, Debont & Poolman,* 1995; Van Hamme, Singh & Ward, 2003), metals and antibiotics (Poole, 2002; Poole, 2005).

The mode of QACs against bacteria 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 component out of the cell. Low concentration 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. Intermediate levels of QACs inhibit membrane-located processes such as respiration, solute transport, and cell wall biosynthesis. The high concentrations kill cells by disintegration of the membranes and release of cytoplasmic contents and coagulation of proteins and nucleic acids.

At the molecular level, action involves the association of the cationic quaternary nitrogen with the head groups of the acid-phospholipids with the membrane due to ionic interactions. The hydrophobic tail (alkyl group) 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 transition from fluid to liquid crystalline state and loses its osmoregulation 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 enzyme and the dissipation of proton motive force (PMF) which affect the microbial metabolism, active transport, oxidative physphorilation and ATP synthesis in bacteria (Knox, Auerbach, Zarudnaya & Spirtes, 1949; Maillard, 2002).

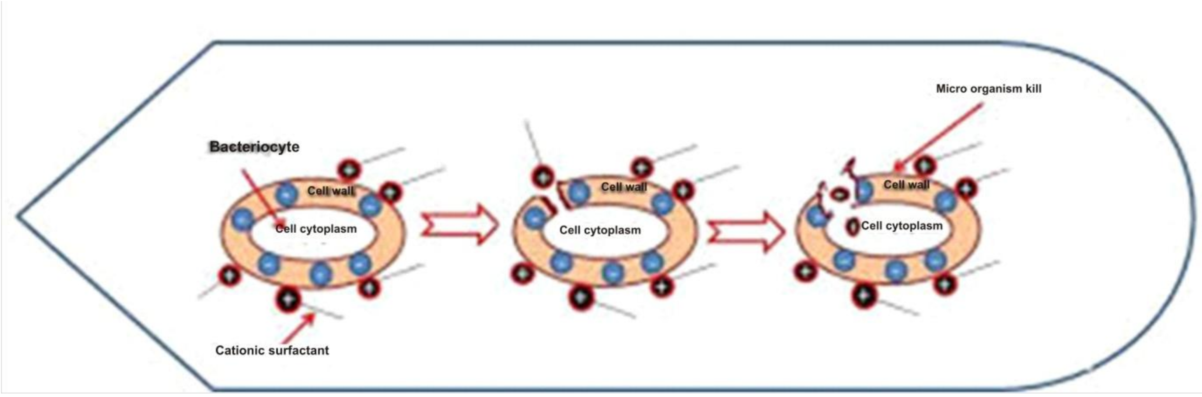


Figure 2.1. Schematic diagram for diffusion of QACs into the cell wall of micro-organism

2.4 Biotransformation of QACs

About 75% of the QACs consumed annually are released into the wastewater treatment system whereas the rest is directly discharged into the environment. The fate of QACs in aerobic biological treatment systems and receiving waters has been studied and the results of these studies have been reviewed extensively (Boethling, 1994; Van Ginkel, 1996). These studies indicated that QACs are degraded under aerobic conditions and up to 90% of QACs removal by means of biodegradation is reported in engineered and natural systems. In fact, the half-lives for aerobic ultimate degradation of QACs vary extensively from hours to months depending on the QAC concentration, structure, microbial acclimation and presence of QAC resistant / degrading microorganisms. The alkly chain length not only determines the physical/chemical properties of the QAC, but also may have a decisive role in the fate and effect of these compounds in the environment. Under aerobic condition, the biodegradability of QACs generally decreases with the number of alkyl groups as $R_4N^+ < R_3MeN^+ < R_2Me_2N^+ < RMe_3N^+ < Me_4N^+$. Moreover, substitution of a methyl group with a benzyl group can decrease biodegradability further (Ying, 2006). A comparison of the biodegradability rates of benzalkonium chloride and monoalkonium bromide 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 and C_{16}TMA-Brwas inversely related to the length of alkyl group (C_n) and substitution of benzyl group decreased the rate as well. Indeed, 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. Likewise, it was reported that the aerobic degradation of QACs was dependent on the length of the alkyl group; however, the number of alkyl group had a more pronounced effect on biodegradability. For example, dialkonium 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 xanthononas (Dean-Raymond & Alexander, 1997), Pseudomonas BI (Van Ginkel*, Vandijk & Kroon,* 1992), Pseudomonas fluorescens TN4 (Nishihara, Okamoto & Nishiyama, 2000), Aeromonas hydrophila sp.k (Patrauchan & Oriel, 2003), and Pseudomonas Spp. Strain 7-6 (Takenata, Tonoki, Taira, Murakami & Aoki, 2007) which were isolated from either sewage or soil.

Two biotransformation pathways, which are different from each other in terms of initial attack on alkyl chain, have been observed for monoalkonium, dialkonium & benzalkonium chlorides (figure 2.3): (1) hydroxylation of terminal C (w-hydroxylation) which is not adjacent to central N, followed by multiple β-oxidations, progressing toward the hydrophilic moiety, resulting in liberation of two carbons in each β-oxidation cycle from the alkyl chain of a QAC and (2) hydroxylation of C that is adjacent to central N (α-hydroxylation) followed by the central fission of the molecule resulting in separation of the hydrophobic form the hydrophilic moiety. The microbial attack to a QAC starts on the alkyl chain. Activation of the alkyl chain is commenced with NADH dependent hydroxylation of either w- or α-carbon of the alkyl group by a monooxygenase enzyme. Activation of the alkyl chain of a QAC is very similar to the activation of alkanes under aerobic conditions.

C

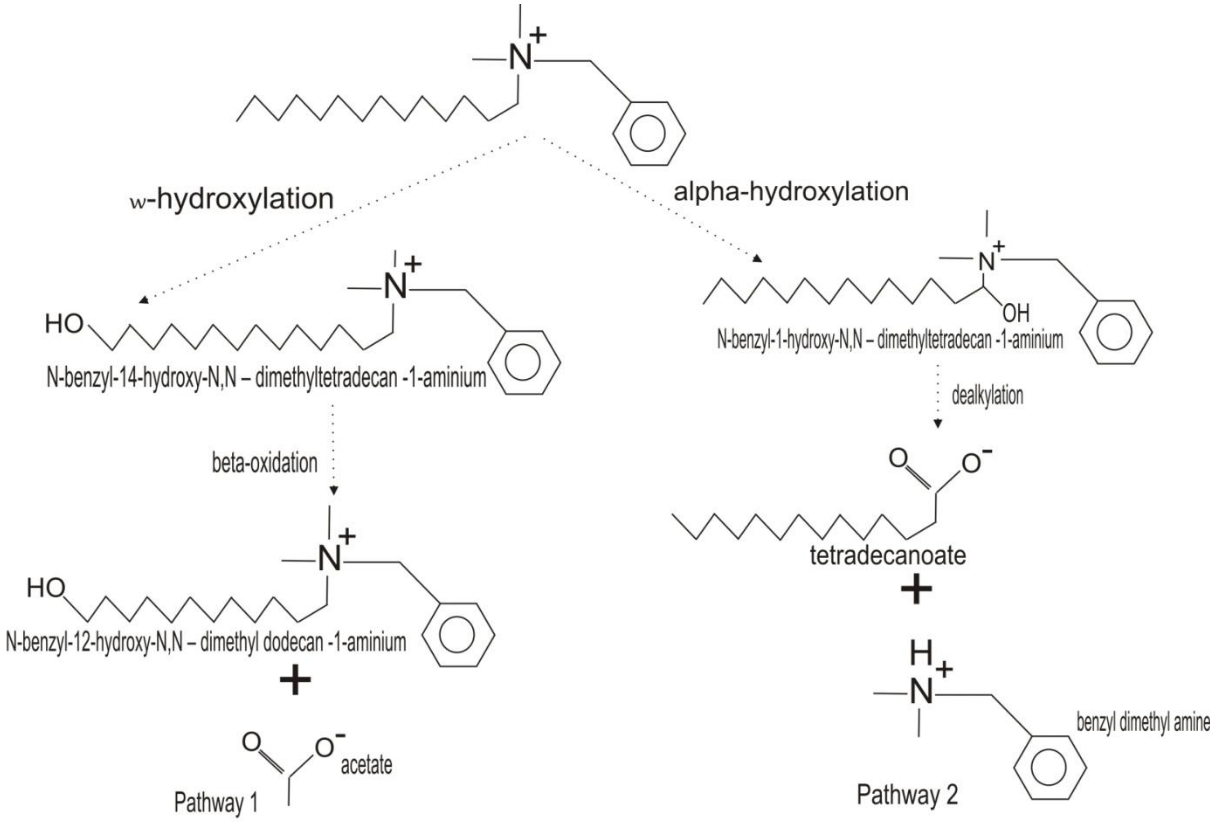


Figure 2.3: Two biotransformation pathways observed for tetradecyl benzyl dimethyl ammonium chloride under aerobic conditions

QACs are rapidly & strongly sorbed onto a wide variety of materials of environmental relevance such as biomass, sediment, clay, and minerals. Indeed, sorption generally outcompetes biodegradation in aerobic environments and therefore, QACs are transferred to anoxic/anaerobic compartments such as anaerobic digesters, as part of the primary and waste activated sludge, and aquatic sediments (Boethling, 1994). Under anaerobic conditions, there is no evidence of mineralization of QACs that contain alkyl or benzyl groups (Battersby & Wilson, 1989; Federle & Schwab, 1992; Garcia, Campos, Sanchez-Leal & Ribosa, 1999), 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, Campos, Sanchez-Leal & Ribosa, 1999; Tezel, Pierson & Pavlostathis, 2006).

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, Federie, Stalmans & Masscheleyn,* 1995). DEESSDMA-Cl differs structurally from dialkonium chlorides by the inclusion of two ester linkages between the ethyl and alkyl chains. These ester linkages allows 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 cholineand 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 bond (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.

QACs possess alkyl, benzyl and or methyl functional groups. As mentioned earlier, the initial step in QAC biotransformation is very similar to that of the hydrocarbons degradation under aerobic conditions. Moreover similar types of microorganism participate in the biotransformation of both types of compounds under aerobic conditions. Relatively recently it was discovered that both aliphatic & aromatic hydrocarbon can be degraded under anoxic/anaerobic conditions by the fumarate addition mechanism (Heider*, Spormann, Beller & Widdel,* 1998; Spormann & Widdel, 2000; Van Hamme*, Singh & Ward,* 2003; Suflita, Davidora, Gieg, Nanny & Prince, 2004; Davidova*, Gieg, Nanny, Kropp & Suflita,* 2005; Callaghan, Gieg, Kropp, Suflita & Young, 2006; Washer & Edwards, 2007; Winderl, Schaefer & Lueders, 2007; Callaghan*, Wawrik, Chadhain, Young & Zylstra,* 2008; Grundmann*, Behrends, Rabus, Amann, Halder, Heider & Widdel* 2008).

2.5 Distribution of QACs in the Environment

QACs have been extensively used in many industrial, domestic and agricultural applications over two centuries. Their production and consumption rates are increasing as they find new applications. On the other hand, QACs are inevitably released into the environment at the production stage or at the end of the consumption of the QAC-bearing product. QACs are, therefore, ubiquitous contaminants. Moreover, available data suggest that QACs are extensively accumulated in aquatic sediments, however, information on residues of QACs in aquatic sediments near sites of industrial or domestic effluent discharge are scarce. The concentration of QACs in domestic waste-water, effluent waste-water, sewage and surface water has been reported as 0.5, 0.05, 3000 and 0.04 ppm, respectively (Schmitt, 1994)

Random samples of sewage from treatment plants in Switzerland, which have various inputs from metallurgical processes or textile industry, had QAC levels ranging from 0.04 to 0.45 ppm (Michelsen, 1978). Huber (1979) and kupfer (1982) described monitoring studies in Germany, and Wee (1984) determined the levels of dialkoniumchlorides in untreated sewage and final effluent from a plant in the United States. The QAC concentration in the influent and effluent sewage ranged from 0.05 to 1.3 ppm and 0.01 to 0.2 ppm, respectively. The concentrations of monoalkonium chlorides were monitored in composite sewage samples in England and Germany. The total monoalkonium chloride concentration in the influent and effluent sewage was 0.13ppm and 0.03ppm, respectively. A recent study conducted in Austria to survey QAC concentrations in influents of five different wastewater treatment plants reported QAC concentration ranging from 1 to 170 ppb (Martinez–Carballo, Sitka, Gonzalez-Barreiro, Kreuzinger, Furhacker, Scharf & Gan, 2007). On the contrary, QAC concentrations would be higher in the effluents of specific industrial facilities, such as paper processing, textile & food processing (1-40ppm, based on the data obtained during a screening study for a poultry processing facility in Georgia), than the influents of municipal wastewater plants. Kummerer, Eitel, Braun, Hubner, Daschner, Mascart, Milandri, Reinthaler & Verheof (1997) analyzed benzalkonium chlorides in highly complex effluent samples from different sized European hospitals. The measure concentrations were between 0.05 and 6.03

ppm. Although the reported QAC concentration 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 100mg/L. Therefore, one would expect high QAC concentration in the wastewater treatment systems or surface waters receiving influents from industrial applications that use QACs extensively.

Levels of QACs in receiving waters are typically in the low microgram per liter range. Huber (1979) reported QAC concentration of 5 to 20 ppb in the Main River in Germany. Schneider and Levsen (1983) found that the concentration of dialkonium chlorides in sewage and surface water samples collected in Germany were 0.35 to 0.48ppm and 6 to 12 ppb, respectively. Likewise, 5 to 30 ppb of monoalkonium chloride concentration was reported in the random samples collected from several Rivers in the United States (Wee & Kennedy, 1982; Wee, 1984). Lewis and Wee (1983) subsequently conducted a follow-up study, in which samples were collected at various distances downstream from wastewater treatment facilities. 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 33 miles downstream from the wastewater treatment plants ranged from 191 to 100 ppb.

QACs adsorb strongly on suspended solids such as minerals, biomass, and inorganic particles and are transferred to anaerobic digesters or aquatic sediments. For instance, mean concentration of DC_nDMA-Cl's in anaerobically stabilized sludge samples from five different municipal sewage treatment plants in Switzerland were 3670, 960, 470, and 210ppm (mg/kg-dry) in 1991, 1992, 1993 and 1994, respectively (Fernandez, 1996), whereas, ppb levels were found in the sewage as discussed above. It was also reported that QAC concentration in anaerobic digesters may range from 4000 to 10,500 ppm (Mg/kg-dry). Lewis and Wee (1983) obtained sediment sample from Rapid Creek at distances from

0.8 to 88km downstream from a sewage outfall. DC_nDMA-Cl levels averaged 23ppm over 18 samples. Fernandez, Valls, Bayoma & Albaiges (1991) found that DC_nDMA-Cl was a ubiquitous contaminant in coastal sediments collected near Barcelona,Spain (Fernandez, 1991). Utsunomiya, Watanuki, Matsushita & Tomita (1989) reported the level of QACs in river water and sediments samples from Japan. Levels of QACs in influent sewage, river water & sediments were 0.10 to 0.15 and 6.2 to 69 ppm, respectively. Sun, Takata, Hata, Kasahara & Taguchi (2003) studied the fate of QACs in a river running through Toyama City, Japan. They found that the total influx of QACs into the river was 1.4g/min, and the concentration as between 0.01 to 0.02 ppm. The QACs in the sediments samples were 500 times higher than that found in the river water. In another study, it was reported that DC_nDMA-Cl was present at 0.63 ppm in surface water and 9.7 ppm (0 to 0.6m depth) and 7.4 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.

2.6 Tocixity and Inhibitory Effects of QACs in Biological Systems

As mentioned earlier, QACs are used extensively in domestic and industrial applications. About 75% of QACs consumed end up in wastewater treatment plants. The EC_{50} values for $C_{16}TMA$-Br and $C_{12}BDMA$-Cl obtained from a respirometricessay conducted with activated sludge ranged between 10 and 40 mg/L (Reynolds*, Blok, Demorsier, Gerike, Wellens & Bontinck,* 1987). The EC_{50} of $C_{14\text{-}18}TMA$-Cl for unacclimated sludge determined based on the inhibition of [^{14}C] glucose 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 20mg/L and the biofilm was totally eliminated at 160mg/L. Overall, these studies suggest that QACs are unlikely to manifest significant toxicity in wastewater treatment at the levels normally expected. However, sudden discharges of QACs resulting temporarily high levels in treatment plants could upset plant function. Microorganisms that have resistance to QACs and utilize them as the energy source at high concentration have been identified (eg. Pseudomonas Spp). On the other hand, many wastewater treatment plants practice biological nutrient removal and they use anaerobic biological units. A variety of physiologically different microorganism participate in the wastewater treatment process, therefore the response of each specie to QAC inhibition is expected to be different. For instance, QACs are particularly toxic to nitrifiers. Benzakonium chloride was inhibitory to a mixed nitrifying culture at 10 to 15 mg/L with a non-competitive inhibition coefficient equal to 1.5mg/L (Yang, 2007).

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 (Boethling, 1994). It was reported that QAC concentration may reach up to 50mg/L in anaerobic digesters of sewage treatment plants (ECETOC, 1993; Garcia*, Campos, Sanchez-Leal & Ribosa,* 1999).

QAC 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.,* 1999, 2000) most likely because of the highly reduced nature of these substituent groups. Moreover, QACs are inhibitory to anaerobic microbial processes such as methanognesis (Battersby and Wilson, 1989; Garcia *et al.*, 1999, 2000). Tezel, Pierson & Pavlostathis (2006) investigated the effect of four QACs – DC_8DMA –Cl, $DC_{8-10}DMA$-Cl, $DC_{10}DMA$-Cl and $C_{12-16}BDMA$-Cl- on a mixed mesophilic methanogenic culture. It was reported that all QACs tested in this study had short-or long-term inhibitory effect on the mixed methanogenic culture at 25mg/L and above. Methanogenesis was more sensitive to QAC inhibition than acidogenesis. The inhibitory impact of the individual QACs on the methanogenic activity decreased according to the following series: DC_8DMA-Cl > $DC_{8-10}DMA$-Cl > $C_{12-16}BDMA$ > $DC_{10}DMA$-Cl. Thus QACs with the shorter alkyl chain length are the most inhibitory QACs. Moreover, it was concluded that the inhibitory effect of QACs was inversely proportional to their adsorption affinity on the biomass or their hydrophobicity (Tezel *et al.,* 2006; Tezel*, Pierson & Pavlostathis,* 2007). Similar results were reported by Garcia *et al.* (1999).

About 25% of QACs consumed are discharged into the environment. The effect of monoalkonium and dialkonium QACs on the aquatic microbial communities in a lake ecosystem was investigated and the result showed that QACs elicit ecologically significant responses at concentrations below 1mg/L and mainly heterotrophic bacterial activity was affected (Ventullo & Larson, 1986). Tubbing and Admiraal (1991) examined the effect of $DC_{18}DMA$-Cl on natural populations of bacteria and phytoplankton from the lower River Rhine and reported significant decreases in the growth rate of bacteria plankton and in the photosynthetic rate of phytoplankton at a nominal $DC_{18}DMA$-Cl concentration 0.03 to 0.1 mg/L (Tubbing & Admiraal, 1991).

Nye *et al.* (1994) investigated the heterotrophic activity in a soil ecosystem treated with $C_{16}TMA$-Br. Addition of $C_{16}TMA$-Br to soil resulted in increased lag periods and decreased rates and extends of mineralization of ^{14}C-labeled organic compounds as a result of toxicity toward Gram-negative soil microorganisms (Nye*,* 1994).

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 weight ranging between 313.5 and 547.0 g/mole) were investigated in four bioessays, such as microtox, spirotox, protoxkit F and Artotoxkit M, comprising a bacterium (Vibrio fischeri), two ciliated protozoa (Spirostomum ambiguum and Tetrahymena thermophila), and an anostracean crustacean (Artemia franciscana). 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 430.25g/mole). The results indicated that QACs had high toxicity against the bioindicators tested & were toxic not only to bacteria, but also to non–target protozoa and crustacean. It was also stated that the toxicity of QACs decreases as the alkyl chain length increases resulting in low bioavailability and high partitioning with organic or negatively charged surface (Nalecz–Jawecki, 2003).

Algae represent a group of organisms which appears to be very sensitive to QACs. The EC_{50} values of C_nTMA-Br and C_nTMA-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 18mg/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 & larval growth and development (Lewis, 1991; Boethling, 1994; Utsunomiya *et al.,* 1997). The no 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 and co-workers (1997), the LC_{50} of C_nBDMA-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. (Kummerer *et al.,*1997). Data retrieved from the European Center for Ecotoxicology (ECOTOX) data bases using the OECD Application Tool Box (OECD, 2008) showed that monoalkonium, dialkonium & benzalkonium chlorides are toxic to aquatic organisms, i.e., bacteria, algae, fish, invertebrates, etc.

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 chromic (multiple-dose) studies, the toxic effect 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 QACs have been reported over the years. Xue, Hieda, Nomura, Fujihara, Takayama, Kimura & Takeshita (2004) investigated the distribution and disposition of C_nBDM-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 15mg/kg of C_nBDM-Cl were used for PO and intravascular administration, respectively. The fatal effects of C_nBDM-Cl appeared soon in JV-, FV – or JA-rats, but took hours in PO or FA-rats. No rat receiving benzalkonium chlorides into their lungs had some systemic symptoms and higher blood and tissue concentration of benzalkonium chloride. The blood benzalkonium chloride levels and kinetics were similar among the different route of intravascular administration, but the lung and kidney levels were higher in JV-rats. Pathological examination confirmed severe congestion and edema in the lungs and kidneys (Xue *et al.,* 2004).

QACs may be applied at concentrations varying from 100 ppm to 400 ppm as sanitizers, QACs are commonly applied at 200 ppm to food contact surfaces, and the solution is allowed to dry. Once dry, a residue of the QAC compounds remain and provides germicidal activity until degradation occurs.

2.7 Known or Suspected Health Effects of QACs

Association between human exposure to QACs and the potential for adverse health outcomes, such as skin irritation & respiratory effects, have been examined in a number of studies. Laboratory studies evaluated various QACs for additional effects, including reproductive outcomes, effects on the immune system, and altered cellular function. Selected studies reporting known or suspected health effects linked with exposure to QACs are described here.

2.7.1 Dermal effects

The US Environmental Protection Agency (US EPA) summarized guideline toxicology studies for alkyldimethylbenzyl ammonium chloride (US EPA, 2017b). These studies reported that alkyldimethylbenzyl ammonium chloride was dermal irritant, but not a dermal or photo sensitizer in acute toxicity studies. Skin irritaton was also observed in subchronic studies of rats and guinea pigs exposed to alkyldimethylbenzyl ammonium chloride (US EPA, 2017a). Didecyldimethyl ammonium chloride was corrosive in acute dermal irritation in rabbits, but was not a skin sensitizer in acute studies in guinea pigs. Skin irritation was observed in subchronic studies of rats & guinea pigs exposed to didecyldimethyl ammonium chloride (US EPA, 2017b). The European Chemical Agency (ECHA) has summarized guideline toxicology studies for akyl (C12-C16) dimethylbenzyl ammonium chloride (ECHA, 2015a) and didecyldimethyl ammonium chloride (ECHA, 2015b), both of which have registered biocide uses as wood preservative. ECHA (2015a; 2015b) reported that both of these compounds were corrosive in acute studies, and can be regarded as skin irritants, but not sensitizers.

The National Institute for Occupational Safety and Health (NIOSH) found that mice dermally exposed to didecyldimethyl ammonium chloride displayed both skin irritation and allergic sensitation (Anderson, Shane, Long & Lukomska, 2016)

Quaternium 15 was one of the most frequent allergens in a hand contact dermatitis study using data from the North American Contact Dermatitis Group (Warshaw, Ahmed, Belsito & DeLeo, 2007). In comparison, benzalkonium chloride was one of the less frequently found allergens in this same study. Perrenoud, Bircher, Hunziker & Suter (1994) reviewed the frequency of sensitization to preservatives in Swiss patients who had suspected allergic contact dermatitis. The percentage of positive skin sensitization reactions to benzalkonium chloride was 5.5%, one of the highest reported. For quaternium 15, the percentage was among the lowest reported

Cases of allergic contact dermatitis linked with some QAC exposures have been reported, including cetylpyridinium chloride in latex gloves (Steinkjer, 1998); quaternium 15 in a moisturizing lotion (Cahill and Nixon, 2005), rinse-off hair product (Tosti, Piraccini & Bardazzi, 1990), and electroencephalography skin preparation gel (Finch, Prais, & Foulds, 2001) and N,N-didecyl-N-methylpoly (oxyethyl) ammonium propionate in a dental clinic disinfectant (De Quintana Sancho, Raton & Eizaguirre, 2014). Quaternium 15 is a formaldehyde-releasing preservation used in cosmetics and other personal care products; allergic contact dermatitis is linked with exposure to quaternium 15, as well as to formaldehyde alone (de Groot, White, Flyvholm & Lensen 2010; Fasth, Ulrich & Johansen, 2018)

2.7.2 Respiratory Effects

Studies conducted among hospital staff (Gonzalez, Jegu, Kopferschmitt & Donnay, 2014) and staff in other health & social sectors (Paris, Ngatchou-Wandji, Luc & McNamee, 2012), and case reports (Bernstein, Stauder, Bernstein & Berstein, 1994; Burge & Richardson, 1994; Purohit, Kopferschmitt-Kubler, Moreauc & Popin, 2000) have found exposure to QAC-containing disinfectant & cleaning products to be linked with work-related asthma. Other potential triggers were examined, such as occupational use of chlorine/bleach and latex gloves (Gonzalez et al., 2014), and use of hairdressing products and non-QAC-containing cleaning products (Paris et al., 2012), and found to not be linked wth work-related asthma. A retrospective case series analysis concluded that a substantial proportion of participants who experienced asthma symtoms related to cleaning materials suffered from sensitizer induced occupational asthma, predominantly caused by QACs (Vandenplas, D'Alpaos, Evrard & Jamart 2013). Bellier, Barnig, Renaudin & Sbinne (2015) described a study in which patients were challenged with a QAC in water. The authors listed the compounds tested as didecyldimethyl ammonium chloride, alkyldimethylbenzyl ammonium chloride, didecyldimethyl ammonium propionate, or benzalkonium chloride. The most frequently QAC to induce a positive inhalation challenge was didecyldimethyl ammonium chloride. These authors suggested that QACs may induce work-related asthma through a specific immunologic response-sensitizing mechanism, and that irritation could also play a role

Lakind and Goodman (2019) reviewed human studies of asthmagenicity and occupational cleaning. They evaluated case reports linking occupational exposure to QACs with asthma and concluded that "Taken together, these case reports, particularly those that provided detailed information on specific inhalation challenge testing results, indicate that certain quats can act as asthmagens.' With regard to association studies between QAC exposure & asthma, the authors discussed methodological concerns, such as limited understanding of exposure pathways and an inability to quantify risk of new-onset asthma attributable to the exposure. They also noted data gaps in quantitative exposure measurements.

In a cohort of female nurses from the Nurses' Health Study II, exposure to disinfectants and cleaning products were evaluated based on job tasks (Dumas, Varraso, Boggs & Quinot, 2019). "High-level exposures" to multiple products, including QAC-containing disinfectants were significantly associated with increased risk of chronic obstructive pulmonary disease (COPD) incidence independent of asthma & smoking status. The study authors discussed that several exposures often occurred concurrently and that disentangling the role of each product was a challenge.

US EPA (2017b) summarized the result of a guideline subchronic inhalation toxicology study of didecyldimethyl ammonium chloride. Effects including ulceration of the nasal cavity and increased levels of lung inflammation markers were observed in rats administered the compound by nose-only exposure. A no-observed-adverse-effect concentration (NOAEC) was established in this study; the lowest concentration tested was 80μg/m^3 didecyldimethyl ammonium chloride.

Larsen, Verder & Nielson (2012) evaluated acute airway effects in mice after inhalation exposure to aerosols of selected QACs to generate information on toxicological mechanisms and to support risk assessment of occupational exposures. All QACs tested reduced tidal volume with concomitant increase in respiratory rate. The relative potencies for this effects were: benzalkonium chloride > hexadecyltrimethyl ammonium bromide > cetylpyridinium chloride > dioctadecyldimethyl ammonium bromide. Inhalation of benalkonium chloride and cetylpyridinium chloride gave rise to pulmonary inflammation.

Kwon, Kwon, Lim & Shim (2019) studied the inhalation toxicity in rats of benzalkonium chloride and triethylene glycol (TEG) aerosols, alone and in combination. They found that exposure to benzalkonium chloride aerosol induced pulmonary cell damage and inflammation. The combination of benzalkonium chloride and TEG induced significant ulceration and degenerative necrosis in the nasal cavities; pulmonary effects were not observed. The mass median aerodynamics diameter of the aerosol particles from aqueous solutions of benzalkonium chloride ranged from 1.3 micrometers (μm) (0.5 % solution) to 1.4 μm (2 % solution). When combined with TEG particle sizes were somewhat larger (1.3 -3.2 μm)

Dinis-oliveira (2008) reviewed the lung toxicity of paraquat dichloride, which selectively accumulated in lungs. This compound acts by a redox cycling mechanism of action inducing irreversible loss of lung function.

2.7.3 Nervous System Effects

ECHA reported that there was no evidence from guideline studies of neurotoxicity for either alkyldimethylbenzyl ammonium chloride (ECHA, 2015a) or didecyldimethyl ammonium chloride (ECHA, 2015b).

Diquat and paraquat exposure have been implicated in neurodegenerative diseases, such as Parkinson's disease (Magalhaes, Carvalho & Denis-Oliveira 2018; Zhang, Thompson & Xu, 2016).

2.7.4 Reproductive And Developmental Effects

In a summary of guideline studies, US EPA (2017a) stated that there was no evidence of developmental toxicity following prenatal alkyldimethylbenzyl ammonium chloride administration to rats or rabbits. Reduced pup body weight during lactation and post-weaning was observed in rats administered the highest dose of alkyldimethylbenzyl ammonium chloride no other reproduction or fertility effects were reported. (US EPA 2017a; US EPA 2017b) noted reduced pup body weight and weight gain in rats exposed to didecyldimethyl ammonium chloride. In their assessment of alkyldimethylbenzyl ammonium chloride (ECHA, 2015a) and didecyldimethyl ammonium chloride (ECHA, 2015b). ECHA stated that neither compound "affected reproduction or development at doses that were not toxic to the mother" in rodent studies. DPR (1996) reported no reproduction or teratogenic effects of didecyldimethyl ammonium chloride which has been designated by US EPA as the representative chemical for toxicology studies for all DADMACs used as pesticides, including dioctyldimethyl ammonium chloride, octyldecyldimethyl ammonium chloride, and octyldodecyldimethyl ammonium chloride.

Decreased fertility was observed in mice housed in facilities were a disinfectant containing a mixture of alkyl (60 % C14, 25 % C12, 15 % C16) dimethylbenzyl ammonium chloride was used (Melin, Potineni, Hunt & Griswold, 2014). Melin et al. (2014; 2016) further explored this observation in a series of experiments in which mice were exposed to the same disinfectant via the diet. These studies found that exposed mice had significantly reduced fertility and fecundity, and displayed reproductive effects including females progressing through fewer estrus cycles, and decreased sperm counts in males. Decreased sperm counts were also observed in male mice exposed only ambiently to the disinfectant used in the animal facility (Melin, Melin, Dessify & Nguyen, 2016). Hrubec, Melin, Shea & Ferguson (2017) conducted additional experiments in mice exposed to the same disinfectant via the diet, mice only garaged with a mixture of the pure QAC chemical constituent, and mice & rats exposed ambiently to the disinfectant used in the animal facility. This study reported developmental effects in both mice and rats exposed to these QACs via all these exposure routes. These effects manifested as neutral tube effects in early gestation & decreased pup size survivability in late gestation (Hrubec et al., 2017). The neutral tube effects persisted in mice for two generations after cessation of exposure alone was sufficient for observation of these effects (Hrubec et al., 2017). Hrubec and Hunt (2018) acknowledged that preventing ambient exposure to QAC-containing disinfectant in animal facility was a challenge in these studies.

Herron, Hines, Tomita & Seguin (2019) exposed mouse to benzyldodecyldimethyl ammonium chloride or benzylhexadecyldimethyl ammonium chloride via the diet and evaluated the effects on the neopatal brain tissues, indicating transfer across the placental and embryonic blood-brain barriers. The authors observed altered sterol and lipid homeostatis in the exposed neonatal pup brains. Some QACs have historically been used in the US as the active compound in fertility control. In an *in vitro* screening of organic compounds, Holzaepfel, Greenlee, Wyrant & Ellis (1959) identified some QA salts as having high spermicidal activity, including benzylhexadecyldimethyl ammonium chloride, n-octadecyldimethylbenzyl ammonium chloride (C 18), and alkyldimethyl ammonium chloride. A US patent from the 1970s describes QACs (including BACs, DADMACs, ATMACs, and QAC mixtures) as having the capability of controlling fertility if administered at the time of mating or within an effective period after mating (Dalgard and Coval, 1975). This patent described dog and rat studies that indicated QACs administered via the diet may be embryocidal, ovicidal, and/or spermicidal. Benzalkonium chloride is used as the active spermicidal ingredient in some sponges and vaginal creams and capsules currently sold in Europe (Aubeny, Colau & Nandeuil, 2000; Creatsas, 2001 Pharma GDD website, accessed 2020). Its spermicidal mechanism of action occurs through destruction of the sperm cell plasma membrane (Creatsas, 2001). Plasma membrane disruption is also the general mechanism of action by which QACs including benzalkonium chloride, are effective as preservatives, disinfectants & biocides (Gilbert & Moore, 2005; Wessels & Ingmer, 2013).

Magalhaes et al. (2018) reviewed the reproductive and developmetal effects of diquat in rodent studies, including intrauterine growth retardation in rats, delayed ossification in rabbits, and the role of redox cycling processes in decreased mouse litter size.

Exposure to either benzalkonium chloride or benzethonium chloride resulted in delayed hatching, embryonic mortality, and morphological malformations in zebrafish, and in germline toxicity in C. elegans (Sreevidya, Lenz, Svoboda & Ma 2017).

Rat studies of dermally applied distearyldimethyl ammonium chloride, benzylstearylmethyl ammonium chloride (C18), or stearyltrimethyl ammonium chloride identified no embryonic effects (Palmer, Bottomley, Edwards & Clark, 1983).

2.7.5 Immunological Effects

The NIOSH study (Anderson et al., 2016) mentioned earlier also linked markers of immune sensitization to the development of skin irritation and allergic sensitization observed in mice dermally exposed to didecyldimethyl ammonium chloride. McDonald (2017 dissertation) found that a mixture of alkyl (60 % C14, 25 % C12, 15 % C16) dimethylbenzyl ammonium chloride altered cytokine levels and phagocytic function *in vtro,* and affected antibody production and the gut microbiome in mice.

Sanidad, Yang, Wang & Ozay (2018) reported that benzalkonium chloride & benzethonium chloride increased inflammation in mouse model of induced colitis. These chemicals also caused a reduction in colon length, which is a biomarker of colitis. In another experiment using a mouse model of induced colon cancer, the authors found that treatment with benzalkonium chloride increased tumor size and increased the gene expression of several pro-tumorigenic genes in colon tumors. They also found that benzalkonium chloride increased activation of an innate immunity receptor (Toll-like receptor 4)

2.7.6 Altered Cellular Function And Effects on Metabolism

Benzalkonium chloride (Datta, Baudouin, Brignole-Baudouin & Denoyer, 2017a), cetylpyridinium chloride (Datta, He, Tomilov & Sahdeo, 2017b), and decyltrimethyl ammonium bromide (Inacio, Costa, Domingues & Santos, 2013) inhibited mitochondrial respiration *in vitro*. Levin, Han, Liu & Farmer (2007) reported that benzalkonium chloride's disruption of mitochondrial function inhibits steroidogenesis in leydig cells. Datta et al. (2017b) observed anti-ostrogenic activity of cetylpyridinium chloride *in vitro*, and hypothesized that this is mediated through effects on mitochondrial inhibition.

Decyltrimethyl ammonium bromide induced the generation of intracellular reactive oxygen species, particularly superoxide anion (Inacio et al., 2013}. Diquat & papaquat are also known to produce reactive oxygen & nitrogen species through redox cycling processes (Dinis-oliveira, 2008; Magalhaes et al., 2018)

Herron, Reese, Tallman & Narayanaswamy (2016) reported inhibition of cholesterol biosynthesis in *in vitro* studies of benzalkonium chlorides. The authors suggested that these effects decreased with increasing length of the alkyl chain; benzyldecyldimethyl ammonium chloride (C 10) was a more potent cholesterol biosynthesis inhibitor than benzylhexadecyldimethyl ammonium chloride (C 16). In additional *in vitro* studies, benzyldecyldimethyl ammonium chloride & benzylhexadecyldimethyl ammonium chloride differentially altered lipid homeostatis (Hines, Herron & Xu, 2017)

We searched US EPA's chemistry Dashboard (Williams, Grulke, Edwards & Eachran, 2017) for QAC bioactivity data in Toxcast/Tox21 assays of approximately 100 unique QAC CASRNS searched, 29 had ToxCast/Tox21 bioactivity information, 21 of these QACs were active in at least 100 essays. Some examples of the diversity of QAC bioactivities reported at subcytotoxic concentrations include: dodecyltrimethyl ammonium chloride's effect on receptor binding (e.g. G protein-coupled receptor) & altered cell proliferation, N,N,N-trimethyloctadecan-1-amminium chloride's impact on altered gene expression, and ethylhexadecyldimethyl ammonium bromide's effect on enzyme activity (e.g., increased enzymatic activity of selected cytochrome P45OS), gene expression, and cell morphology.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Materials

3.1.1 Preparation of the Quaternary Ammonium Salts.

3.1.2 Reagents used: Phenyl glycidylether, cetylbromide, ethanol, monoethanolamine and N-methylethanolamine. All reagents used were of analytical grade.

3.1.3 Solvents used; Water, dichloromethane, hexane and isopropanol.

3.1.4 Apparatus used: Two–neck round bottom flask, magnetic stirrer, electric heater, 250 ml beakers, thermometer, condenser, separating funnel, weighing balance, retort stand, syringes (10 ml and 20 ml), test tubes and cotton wool

3.1.5 Equipment used; Fourier Transform Infrared spectrophotometer and mass spectrometer.

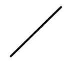
3.1.6 Methodology: Generally, quaternary ammonium compounds are prepared from the reaction of tertiary amines with alkyl or benzyl halides (Kivala *et al.,* 2004).

3.1.7 Synthetic Procedure for N-cetyl-N-hydroxyethyl-N-methyl-2-hydroxy-3-phenoxypropylammonium bromide

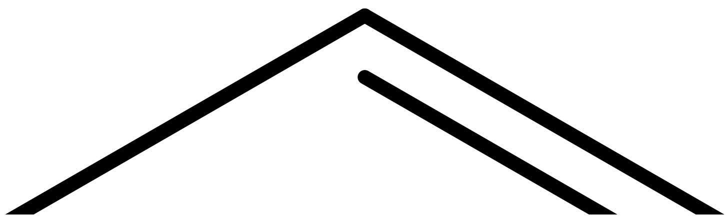
About 3.2 ml (3.5 g, 0.02 mol) of phenyl glycidyl ether and 1.8 ml (1.7 g, 0.02mol) of N-methylethanolamine were mixed in 35 ml of ethanol and the mixture was refluxed for 24 hours at 78 ^{o}C. After the completion of the reaction, the product obtained was washed with water. Thereafter, 3.8 g (0.017 mol) of the product obtained was mixed with 6.1 ml (6.1 g, 0.02 mol) of cetylbromide in 35 ml of ethanol and the resulting mixture was refluxed for further 24 hours at 78 ^{o}C. Finally, the product obtained was washed with dichloromethane. See illustration below;

3.1.8 Synthetic procedure for N-cetyl-N-hydroxyethyl-N,N-(bis)-[2-hydroxy-3-phenoxypropyl]ammonium bromide

About 3.2 ml (3.5 g, 0.020 mol) of phenyl glycidyl ether and 1.4 ml (1.4 g, 0.020 mol) of monoethanolamine were mixed in 35 ml of ethanol and the mixture was refluxed for 24 hours at 78^{o}C. After the completion of the reaction, the product obtained was washed with water. Thereafter 3.2 g (0.009 mol) of the product obtained was mixed with 6.11 ml (6.1 g, 0.020 mol) of cetylbromide in 35 ml of ethanol and the resulting mixture was refluxed for further 24 hours at 78 ^{o}C. Finally, the product was washed with dichloromethane. See illustration below;



monoethanolamine



3.2 TEST FOR ANTIMICROBIAL ACTIVITY

3.2.1 Collection of Test Organisms

The pure bacteria strains (*Staphylococcus aureus, Escherichia coli, Salmonella enteric, Lactobacillus spp. and Klebsiella spp.*) used for this analysis were collected from Microbiology Laboratory, University of Jos, Plateau State.

3.2.2 Preparation of Macfarland'sTurbidity Standard

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

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

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 prepared test standard. A sterile swab stick was dipped into the inoculums used to streak the surface of the agar. A sterile cork borer was then used to produce wells of 8 mm allowing 30 mm between adjacent wells and the pertri dish.

Sterile syringes were used to introduce fixed volume of test compounds into the wells. The plates were incubated at 30 ^oC 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

RESULTS AND DISCUSSIONS

4.1 RESULTS:

The results obtained from the synthesis, characterization and antimicrobial evaluation of the quaternary ammonium salts are shown in tables & figures below:

4.1.1 The Percentage Yield of N-cetyl-N-hydroxyethyl-N-methyl-2-hydroxy-3-phenoxypropylammonium bromide

The percentage yield is calculated as follows:

Mass of phenyl glycidyl ether = 3.5 g

Mass of N-methylethanolamine = 1.7 g

Mass of the intermediate = 3.8 g

Mass of Cetylbromide = 6.1 g

Mass of QAC = 7.6 g

The percentage yield of intermediate (N-hydroxyethyl-N-methyl-2-hydroxy-3-phenoxypropylamine);

$$\frac{3.8}{\{3.5 + 1.7\}} \times \frac{100}{1} = 73.07\%$$

The percentage yield of QAC (N-cetyl-N-hydroxyethyl-N-methyl-2-hydroxy-3-phenoxypropylammonium bromide);

$$\frac{7.6}{(3.8 + 6.1)} \times \frac{100}{1} = 76.76\%$$

4.1.2 The Percentage Yield of N-cetyl-N-hydroxyethyl-N,N-(bis)-[2-hydroxy-3-phenoxypropyl]ammonium bromide

The percentage yield is calculated as follows:

Mass of phenyl glycidyl ether = 3.5 g

Mass of monoethanolamine = 1.4 g

Mass of intermediate = 3.2 g

Mass of cetylbromide = 6.1 g

Mass of QAC = 6.4 g

The percentage yield of intermediate (N-hydroxyethyl-N,N-(bis)-[2-hydroxy-3-phenoxypropyl]amine);

$$\frac{3.2}{(3.5 + 1.4)} \times \frac{100}{1} = 65.30\ \%$$

The percentage yield of QAC (N-cetyl-N-hydroxyethyl-N,N-(bis)-[2-hydroxy-3-phenoxypropyl]ammonium bromide;

$$\frac{6.4}{(3.2 + 6.1)} \times \frac{100}{1} = 68.81\ \%$$

Table 4.1: Solubility Test for the Compounds

Products	Isopropanol	Ethanol	Water	Hexane	Dichloromethane
1.	+	+	+	-	-
2.	+	+	+	-	-

Key

1. =*N*-Cetyl-*N*-hydroxyethyl-*N*-methyl-2-hydroxy-3-phenoxypropylammonium bromide
2. =*N*-cetyl–*N*-hydroxyethyl-*N*,*N*-(bis)-[2-hydroxy-3-phenoxypropyl]ammomium bromide

\+ Soluble

\- Not 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 (10mg)	Lactobacillus spp.	Staphylococcus aureus	Escherichia coil	Salmonella enteric	Klebsiella spp.
1.	3	4	2	2	3
2.	4	4	2	2	4
3.	5	4	1	2	2
4.	24	23	14	12	14
5.	25	23	16	14	14
Ampicillin (10mg)	19	18	20	20	20

Key:

1. = Cetylbromide
2. =*N*-hydroxyethyl-*N*-methyl-2-hydroxy-3-phenoxypropylamine
3. =*N*-hydroxyethyl –*N*,*N*-(bis)-[2-hydroxy-3-phenoxypropyl] amine
4. =*N*-Cetyl-*N*-hydroxyethyl-*N*-methyl-2-hydroxy-3-phenoxypropylammonium bromide
5. =*N*-cetyl–*N*-hydroxyethyl-*N*,*N*-(bis)-[2-hydroxy-3-phenoxypropyl]ammomium bromide.

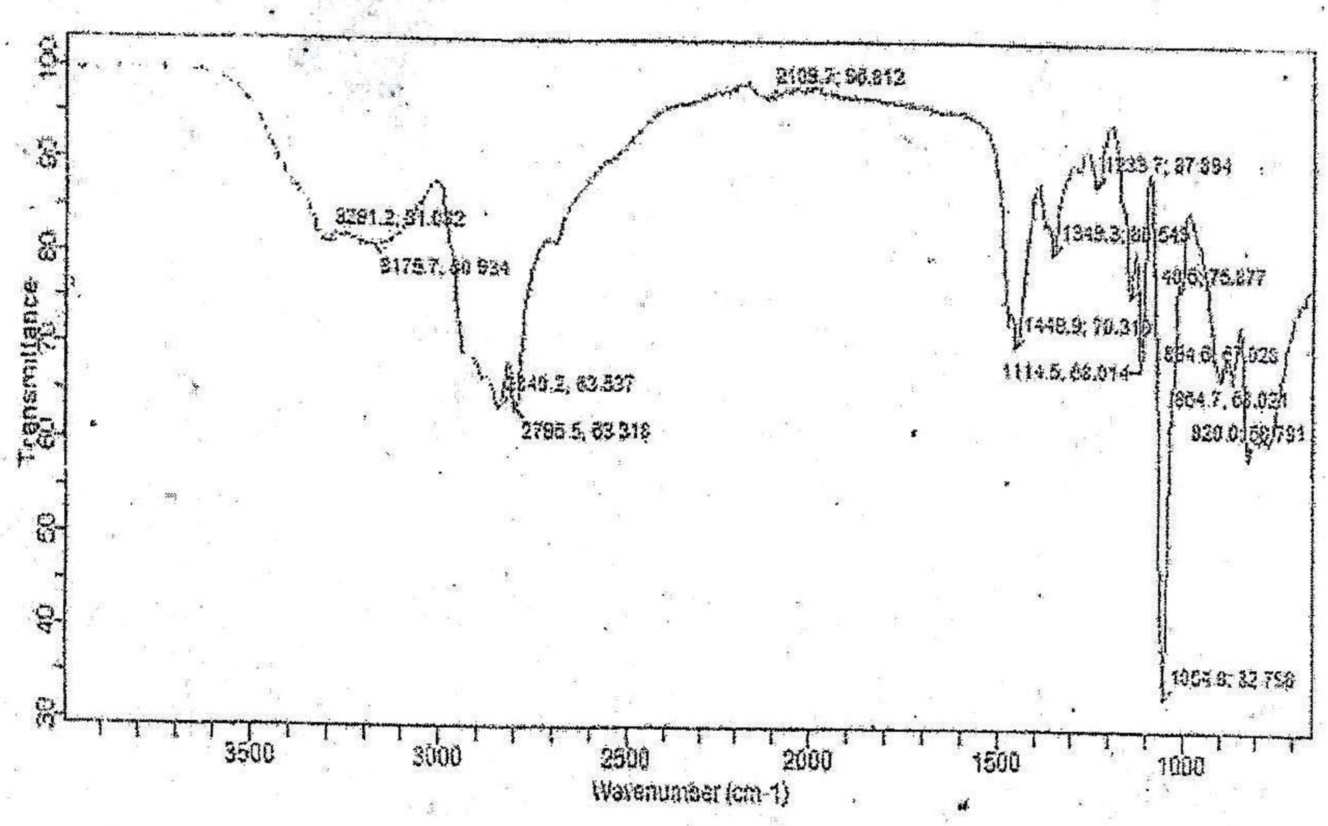


Figure 4.1: Infrared spectrum of *N*-methylethanolamine

Table 4.3: Results of FTIR obtained for *N*-methylethanol amine

Peaks (cm^{-1})	Inference	Remarks
3291.2	N-H	The N-H stretch of secondary amine
3175.7	O-H	The O-H of alcohol
2785.5	C-H	C-H stretch of methyl group ($-CH_3$) of aliphatic hydrocarbon
2840.2	C-H	C-H stretch of methylene group ($-CH_2$) of aliphatic hydrocarbon
1233.7	C-N	C-N stretch of amine

The Infrared spectrum of figure 4.1 (N-methylethanolamine) shows the presence of the functional groups of interest.

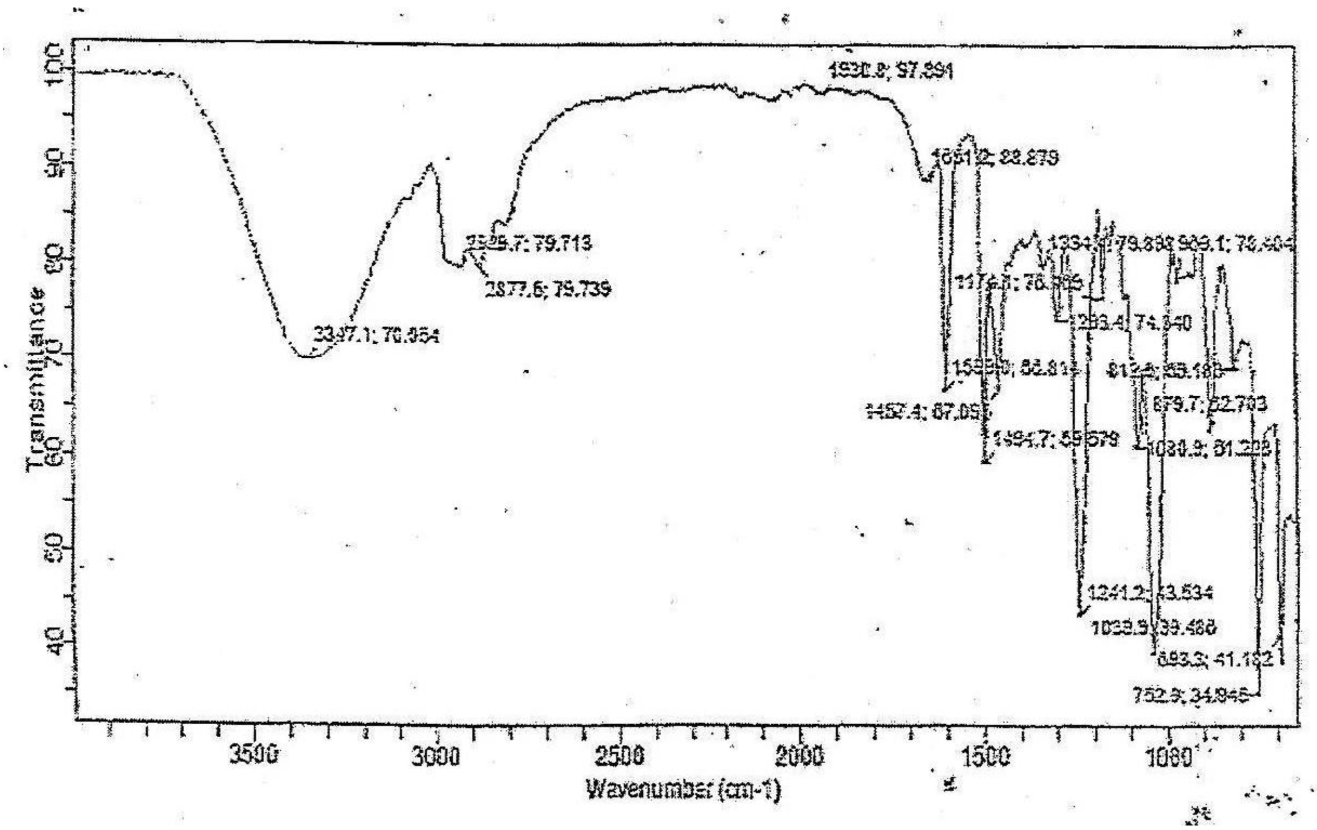


Figure 4.2: Infrared Spectrum of *N*-hydroxyethyl-*N*-methyl-2-hydroxy-3-phenoxypropylamine

Table 4.4: Results of FTIR obtained for *N*-hydroxyethyl-*N*-methyl-2-hydroxy-3-phenoxypropylamine

Peaks (cm^{-1})	**Inference**	**Remarks**
3347.1	O-H	The O-H stretch of alcohol
1599.0	-C=C-	The –C=C- of aromatic alkenes
1179.7	C-O	The C-O stretch of ether
1233.4	C-N	The C-N stretch of amine

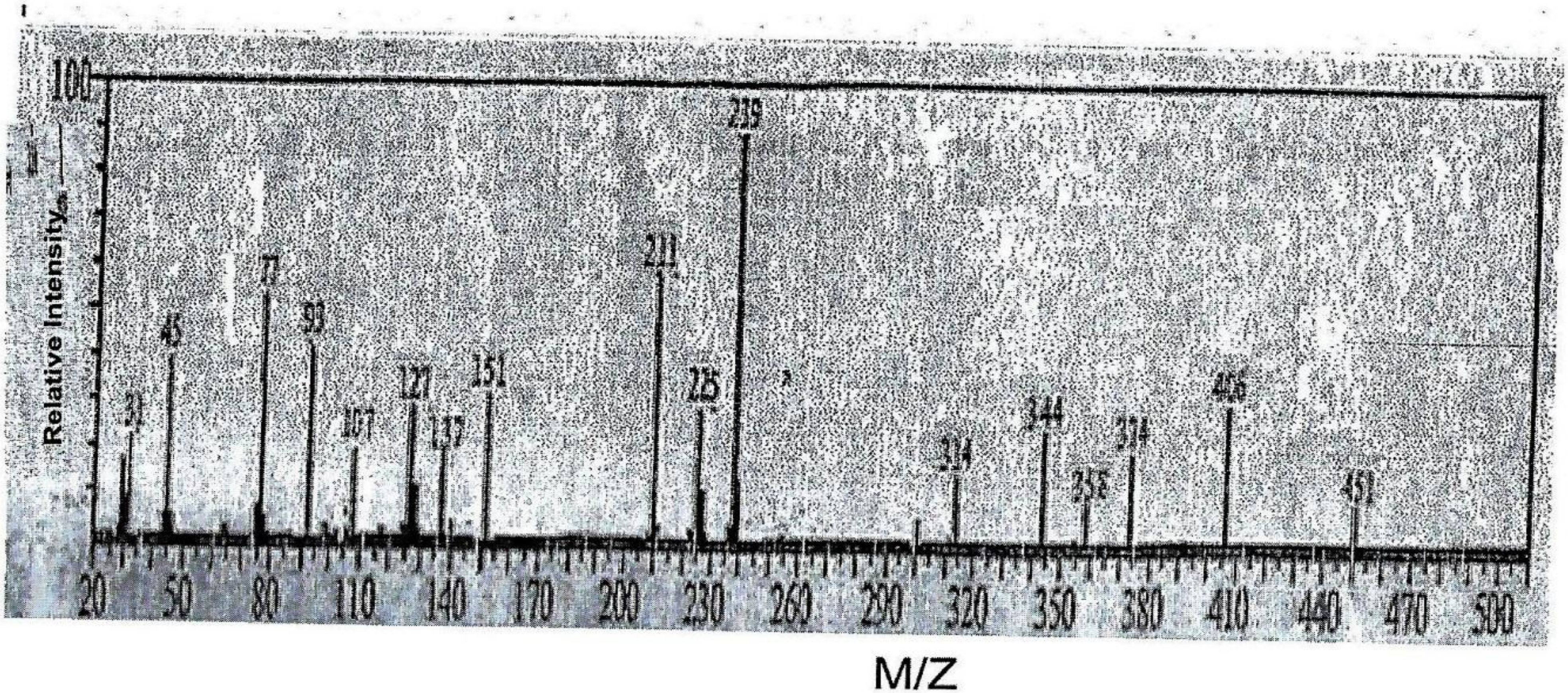


Figure 4.3: Mass spectrum of *N*-cetyl-*N*-hydroxyethyl-*N*-methyl-2-hydroxy-3-phenoxypropylamonium bromide

Table 4.5: Mass spectrum results of *N*-cetyl-*N*-hydroxyethyl-*N*-methyl-2-hydroxy-3-phenoxypropylamonium bromide

M/Z	Fragments
451	$[C_6H_5OCH_2(OH)CH_2N(CH_3)(CH_2CH_2OH)CH_2(CH_2)_{14}CH_3]^+$
225	$[C_6H_5OCH_2(OH)CH_2N(CH_3)(CH_2CH_2OH)]^+$
239	$[C_6H_5OCH_2(OH)CH_2N(CH_3)(CH_2CH_2OH)CH_2]^+$
93	$[C_6H_5O]^+$
45	$[OHCH_2CH_2]^+$
77	$[C_6H_5]^+$
31	$[OHCH_2]^+$

The mass spectrum of the salt showed the molecular ion peak at M/Z = 451.0.

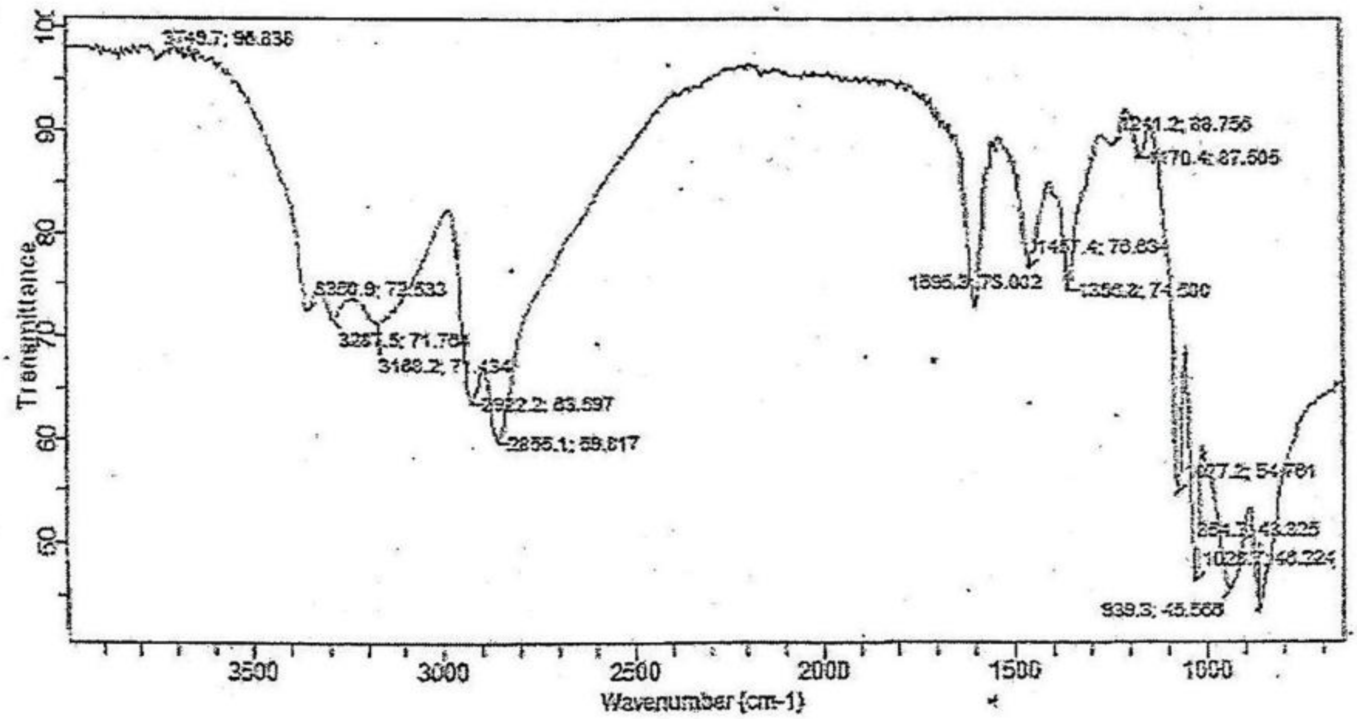


Figure 4.4: Infrared spectrum of monoethanolamine

Table 4.6: Results of FTIR obtained for monoethanolamine

Peaks (cm^{-1})	Inference	Remarks
3350.9	N-H	Antisymmetric stretch of primary amine
3287.5	N-H	Symmetric stretch of primary amine
3168.2	O-H	The O-H of alcohol
2922.2	C-H	C-H stretch of methylene group (-CH_2) of aliphatic hydrocarbon
2858.1	C-H	C-H stretch of methyl group (-CH_3) of aliphatic hydrocarbon
1241.2	C-N	C-N stretch of amines

The infrared spectrum of figure 4.4 (monoethanolamine) shows the functional groups of interest.

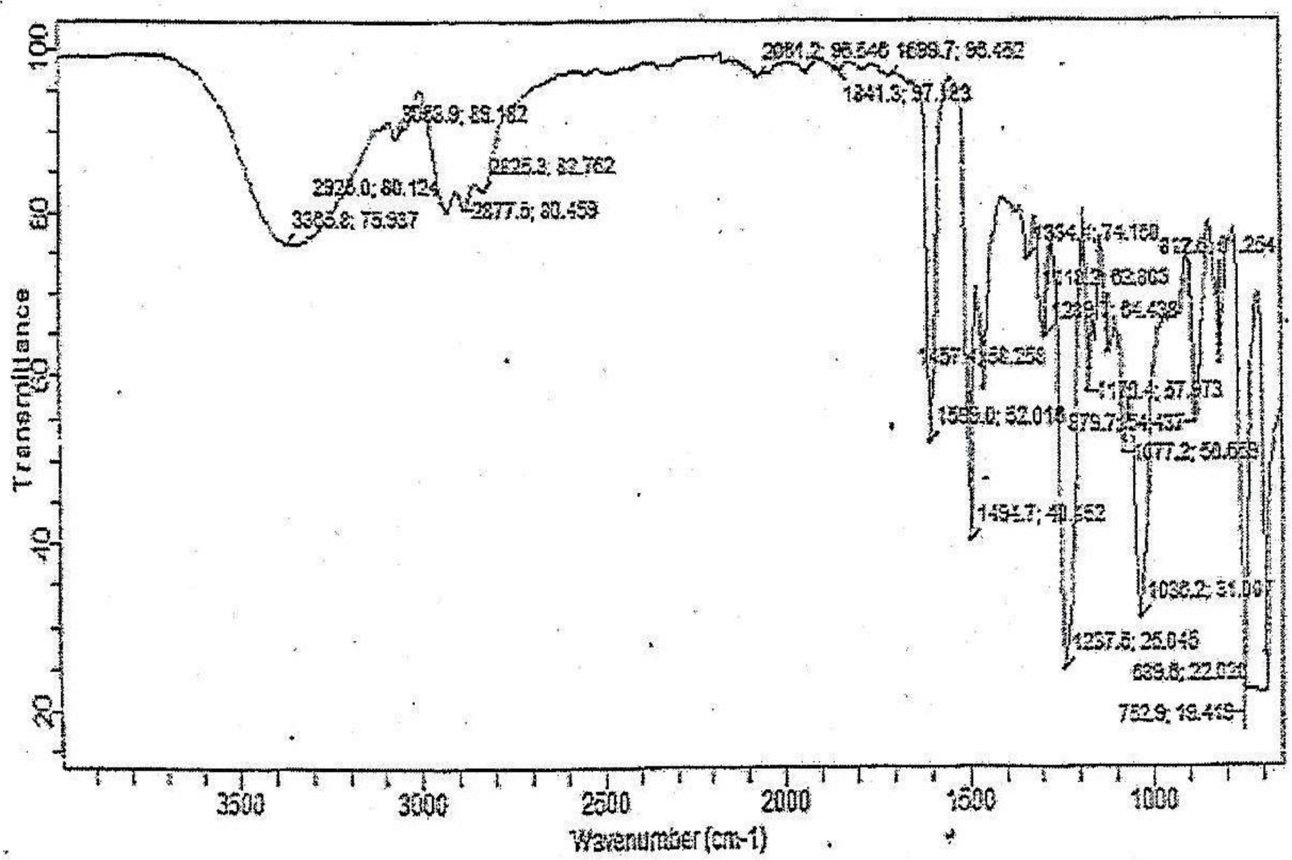


Figure 4.5: The infrared spectrum of *N*-hydroxyethyl-*N*,*N*-(bis)-[2-hydroxy-3-phenoxypropyl]amine

Table 4.7: Results of FTIR obtained for *N*-hydroxyethyl-*N*,*N*-(bis)-[2-hydroxy-3-phenoxypropyl]amine

Peaks (cm^{-1})	**Inference**	**Remarks**
3365.8	O-H	The O-H stretch of alcohol
1599.0	-C=C-	The –C=C- of aromatic alkenes
1171.4	C-O	The C-O stretch of ether
1239.1	C-N	The C-N stretch of amine

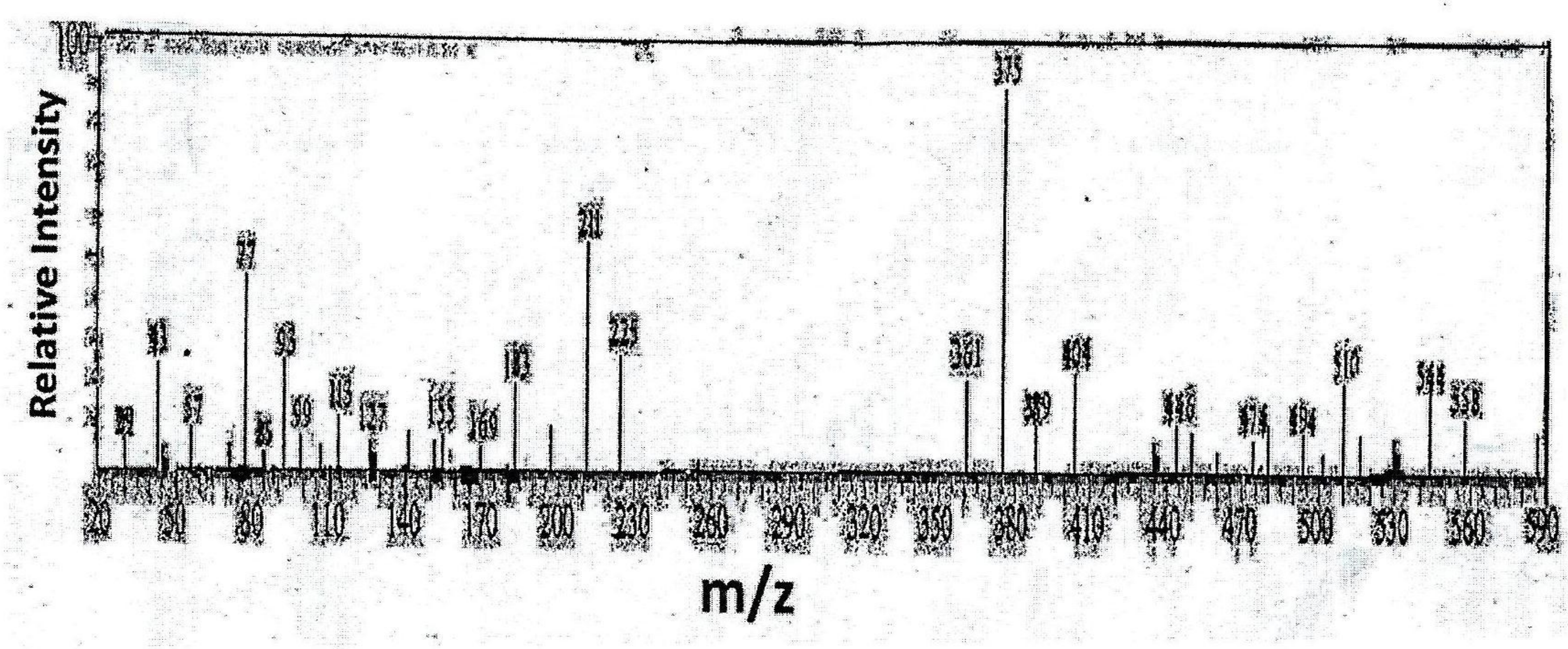


Figure 4.6: Mass spectrum of *N*-cetyl-*N*-hydroxyethyl-*N*,*N*-(bis)-{2-hydroxy-3-phenoxypropyl}ammonium bromide

Table 4.8: Mass spectrum results of *N*-cetyl-*N*-hydroxyethyl-*N*, *N*-(bis)-[2-hydroxy-3- phenoxypropyl]ammonium bromide

M/Z	Fragments
587	$[C_6H_5OCH_2CH(OH)CH_2)_2N(CH_2CH_2OH)(CH_2(CH_2)_{14}CH_3)]^+$
211	$[CH_3(CH_2)_{13}CH_2]^+$
375	$[C_6H_5OCH_2CH(OH)CH_2)_2N(CH_2CH_2OH)(CH_2)]^+$
93	$[C_6H_5O]^+$
43	$[CH_3CH_2CH_2]^+$
77	$[C_6H_5]^+$
29	$[CH_3CH_2]^+$

The mass spectrum of the salt showed the molecular ion peak at M/Z = 587.0.

4.2 DISCUSSION

The percentage yields of *N*-cetyl-*N*-hydroxyethyl-*N*-methyl-2-hydroxy-3-phenoxypropylammonium bromide and *N*-cetyl-*N*-hydroxyethyl-*N*,*N*-(bis)-[2-hydroxy-3-phenoxypropyl]ammonium bromide formed were calculated, and they gave 76.76 % and 68.81 % respectively.

Solubility tests were conducted on the quaternary compounds. Expectedly, results indicate that the products foam and are only soluble in polar solvents (water, ethanol and isopropanol) as shown in table 4.1.

Table 4.2 shows the diameter of zones of inhibition of the synthesized salts against various micro-organisms. The synthesized quaternary ammonium salts exhibit excellent bacterial activity, particularly against Gram-positive bacteria. This is attributable to the long alkyl chain length of the compounds, which is the major determinant of anti-microbial activity (Viscardi, Quagliotto, Barolo, Savarino, Barni & Fisicaro, 2007). The quaternizing agent (cetyl bromide) and the intermediates (*N*-hydroxyethyl-*N*-methyl-2-hydroxy-3-phenoxypropyl amine and *N*-hydroxyethyl-*N*,*N*-(bis)-[2-hydroxy-3-phenoxypropyl]amine) show very insignificant bacterial activity against the selected micro-organisms. Relative to the standard antibiotic (Ampicillin), the products are less effective against Gram-negative bacteria, but more effective against Gram-positive bacteria.

Infrared spectrum of figure 4.1 (*N*-methylethanolamine) shows a peak at 3291.2 cm^{-1} due to the N-H stretch of secondary amine. Within the same region, there is also a smooth broad peak at 3175.7 cm^{-1} due to the presence of O-H stretch of alcohol. The sharp peak at 2785.5 cm^{-1} is due to the C-H stretch of methyl group ($-CH_3-$) of aliphatic hydrocarbon. Peak at 2840.2 cm^{-1} indicates the presence of the C-H stretch of methylene group ($-CH_2-$) of aliphatic hydrocarbon. Peak at 1233.7 cm^{-1} represents the C-N stretch of amine.

The infrared spectrum of figure 4.2 (*N*-hydroxyethyl-*N*-methyl-2-hydroxy-3-phenoxypropylamine) indicates that there is a disappearance of the N-H stretch of the amine found at 3291.2cm^{-1} due to the formation of a hydroxyl group (OH). A new hydroxyl group is formed after the reaction of phenyl glycidyl ether with *N*-methylethanolamine. The O-H absorption is found at 3347.1 cm^{-1}. The aromatic ring C=C appeared at 1599.0 cm^{-1}. The peak at 1179.7 cm^{-1} is due to the C-O stretch of ether. The C-N stretch of amine appeared at 1233.4 cm^{-1}.

The mass spectrum of figure 4.3 (*N*-cetyl-*N*-hydroxyethyl-*N*-methyl-2-hydroxy-3-phenoxypropylammonium bromide) shows the molecular ion peak at M/Z = 451.0 ($[C_6H_5OCH_2(OH)CH_2N(CH_3)(CH_2CH_2OH)CH_2(CH_2)_{14}CH_3]^+$). There are also some other identifiable stable ion fragments which are part of the synthesized compound. These are: m/z = 239$[C_6H_5OCH_2CH(OH)CH_2N(CH_3)(CH_2CH_2OH)CH_2]^+$, m/z =77$[C_6H_5]^+$, m/z= 225$[C_6H_5OCH_2(OH)CH_2N(CH_3)(CH_2CH_2OH)]^+$, m/z =31$[OHCH_2]^+$, m/z=93$[C_6H_5O]^+$, etc

The infrared spectrum of figure 4.4 (monoethanolamine) shows peaks at 3350.9 cm^{-1} and 3287.5 cm^{-1} due to the antisymmetric and symmetric N-H stretches of the primary amine respectively. There is also a smooth broad peak at 3168.2 cm^{-1} due to the presence of O-H stretch of alcohol. The C-H stretch of methylene group ($-CH_2-$) of aliphatic hydrocarbon appeared at 2922.2 cm^{-1}. The C-H absorption of methyl group ($-CH_3-$) of aliphatic hydrocarbon is found at 2858.1 cm^{-1}. The peak at 1241.2 represents the C-N stretch of amine.

The infrared spectrum of figure 4.5 (*N*-hydroxyethyl-*N*,*N*-(bis)-[2-hydroxy-3-phenoxypropyl]amine) reveals that there is a disappearance of the antisymmetric and symmetric N-H stretches of the primary amine found at 3350.9 cm^{-1} & 3287.5 cm^{-1} due to the formation of two new hydroxyl groups. The new hydroxyl groups are formed after the reaction of phenyl glycidyl ether with monoethanolamine. The O-H absorption appeared at 3365.8 cm^{-1}. The C=C of aromatic ring appeared at 1599.0 cm^{-1}. The absorption at 1239.1 cm^{-1} is due to the C-N stretch of amine. The peak at 1171.4 cm^{-1} represents the C-O stretch of ether.

The mass spectrum of figure 4.6 (*N*-cetyl-*N*-hydroxyethyl-*N*,*N*-(bis)-[2-hydroxy-3-phenoxypropyl]ammonium bromide) shows the molecular ion peak at m/z = 587.0 ($[C_6H_5OCH_2CH(OH)CH_2)_2N(CH_2CH_2OH)(CH_2(CH_2)_{14}CH_3)]^+$). There are also some other identifiable stable ion fragments which are part of the synthesized quaternary compound. These are; m/z = 29$[CH_3CH_2]^+$, m/z= 43$[CH_3CH_2CH_2]^+$, m/z=77$[C_6H_5]^+$, m/z=93$[C_6H_5O]^+$, m/z=211$[CH_3(CH_2)_{13}CH_2]^+$, m/z = 375

$[C_6H_5OCH_2CH(OH)CH_2)_2N(CH_2CH_2OH)(CH_2)]^+$ etc

CHAPTER FIVE

5.1 CONCLUSION

The synthesis, spectrometric characterization and antibacterial evaluation of *N*-cetyl-*N*-hydroxyethyl-*N*-methyl-2-hydroxy-3-phenoxypropylammonium bromide and *N*-cetyl-*N*-hydoxyethyl-*N*,*N*-(bis)-[2-hydroxy-3-phenoxypropyl]ammonium bromide were described. The presence of the molecular ions (m/z) 451.0 & 587.0 respectively, in the mass spectra of these compounds confirms that they were indeed synthesized. The results of investigation of antibacterial activities showed that these salts have bactericidal properties towards a number of bacteria strains especially against Gram-positive bacteria with the following zones of inhibition for the two products respectively: *Staphylococcus aureus* (23 mm and 23 mm), *Lactobacillus spp* (24 mm and 25 mm), *Escherichia coli* (14 mm and 16 mm), *Salmonella enteric* (12 mm and 14 mm) and *Klebsiella spp* (14 mm and 14 mm).

5.2 RECOMMENDATIONS

A quaternary salt having a long chain or halo-substituted epoxide as starting material can be synthesized to see if the bactericidal properties of the salt can be enhanced. The synthesized compounds showed very strong bactericidal activities against *Staphylococcus aureus* and *Lactobacillus spp*. Therefore, these salts should be subjected to clinical studies.

5.3 CONTRIBUTION TO KNOWLEDGE

The synthesis of the quaternary ammonium salts led to the formation of the hydroxyl (OH) functional group through epoxide ring opening. Therefore, the OH functional group can successfully be incorporated into the molecular framework of quaternary ammonium salts via an epoxide.

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