SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITIES OF QUATERNIZED LONG CHAIN AMIDES

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AMAFILI, KINGSLEY CHUKWUKULITEM

(B.Tech., FUTO)

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI, IMO STATE

SEPTEMBER, 2021

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

SEPTEMBER, 2021

CERTIFICATION

This is to certify that this research work **"SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITIES OF QUATERNIZED LONG CHAIN AMIDES"** was carried out by **AMAFILI KINGSLEY CHUKWUKULITEM** with registration number **(20134870918)** 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 my parents for their love, financial and moral support to me throughout this program.

ACKNOWLEDGEMENT

May all praise, exaltation and thanksgiving be ascribed unto the Almighty God for his unconditional guidance, protection, support, sustenance and for making my research work a success.

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TABLE OF CONTENTS

CHAPTER ONE

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The synthesis, characterization and antimicrobial activities of quarternized fatty amide having an amide functional group differing in the length of an alkylating chain (C_8 and C_{12}) were carried out. Their solubility in water, FTIR analysis of the starting materials, intermediate and final products were conducted and results showed the formation of amide functional group (-C=0 at 1643 cm^{-1}, 1640 cm^{-1} and N-H at 3295 cm^{-1} and 3403 cm^{-1}) which resulted from the removal of the ester group (-C=0 at 1740 cm^{-1}, 1744cm^{-1} and C-0 at 1170 cm^{-1}) respectively. The mass spectrometric analysis of the final products were also conducted and results showed the presence of Molecular ion peak (M/Z = 334 and 390) which is the Molecular weight of the synthesized quats. Antimicrobial activity of the starting materials, the intermediate and the final products were evaluated and the results obtained show that N-benzyl-N, N-diethylaminoethyloctylamidium chloride is effective against ***Klebsiella spp.*** (20 mm) and ***Salmonella enterica(19*** mm). Whereas *N*-benzyl-*N*,*N*-diethylaminoethyllaurylamidium chloride were effective against ***Echerichia coli*** (29 mm) and ***Salmonella enterica*** (22 mm) which is higher than the activity of the control on the organisms mentioned above.

Keywords: synthesis, characterization, antimicrobial, quarternized fatty

CHAPTER ONE

1. INTRODUCTION:

Quaternary ammonium salts refers to any class of salt derived from ammonium in which the nitrogen atom is attached to four organic groups covalently, as in benzalkonium chloride (R_4N^+).

These salts are cationic surface active compounds. Quaternary ammonium salts are among the high production volume chemicals (i.e chemicals produced or imported in amount equal to or greater than one million pounds per year) as seen on the lists of United State Environmental Protection Agency and Organization for Economic Co-operation and Development.

Quaternary ammonium salts are positively charged polyatomic ions of the structure R_4N^+X with R being an alkyl or Aryl group (Smith, Michael; March Jerry, 2001) and X represents a halo group.

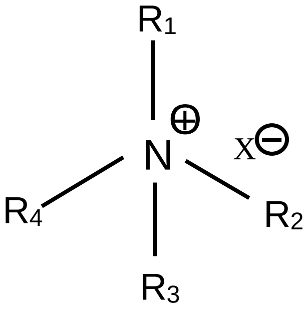


Figure 1.1 General molecular structure of a quaternary ammonium compound.

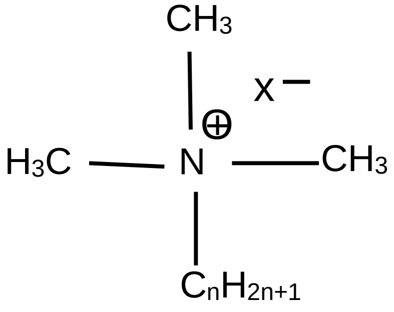
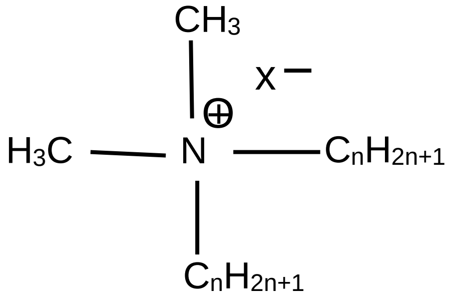
These compounds are extensively used in domestic, agricultural and health care settings. In industries they are used as surfactants, emulsifiers fabric softeners, disinfectants, pesticides, corrosion inhibitors and antiseptics

(Garcia, Campos Sanchez – Leal and Commelles, 2006). In 2004, the annual consumption of quaternary ammonium compounds worldwide was reported as 500,000 tons (CESIO, 2004) and was expected to exceed 700,000 tons (Steichen 2001). Unlike the ammonium ion (NH_4^+) and the primary, secondary or tertiary ammonium cations, the quaternary ammonium cations are permanently charged over a wide range of pH of their solutions.

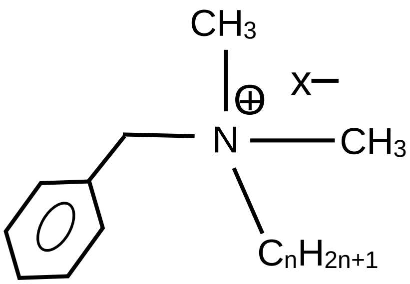
Quaternary ammonium compounds are salts of quaternary ammonium cations with a counter anion. In oilfield parlance, quaternary ammonium compounds are called quaternary amines. Quaternary ammonium compounds including benzalkonium salts constitute an economically important class of industrial chemicals that are widely distributed among a diverse array of products and users from an industrial to the household sector. Quats are stable with a long shelf life (Smiths & March, 2001)

Quaternary ammonium compounds can be classified in three major groups depending on the type of functional groups;

Table 1:1 Quaternary Ammonium Compounds, groups and their general structure

Quaternary ammonium Compound group	**Molecular Structure**
Monoalkonium halides	 $(CH_3)_3N^{\oplus}C_nH_{2n+1}\ X^-$
Dialkonium halides	 $(CH_3)_2N^{\oplus}(C_nH_{2n+1})_2\ X^-$

Benzalkonium halides



Where n = length of alkyl chain

X = halide.

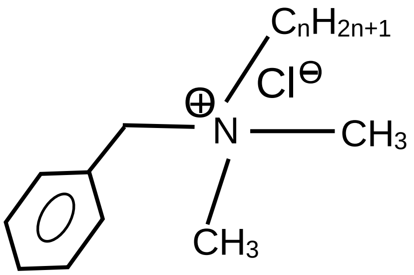
Benzalkonium chloride as an example of a quaternary ammonium salts are a mixture of alkyl benzyldiethyl ammonium chloride in which the alkyl group has various even numbered alkyl chain lengths. Depending on purity, benzalkonium chloride ranges from colourless to a pale yellow (impure) Quaternary ammonium salts are readily soluble in ethanol and acetone. Aqueous solutions of quaternary ammonium salts are neutral to slightly alkaline. Quaternary ammonium solution foams when shaken. Concentrated solutions of Quaternary ammonium compounds have bitter taste and a faint almond-like odour. To prevent increase in viscosity or gel formation under low temperature condition, modifiers such as alcohol or glycols are incorporated into quaternary ammonium compound.

In Quaternary ammonium compound the organic radical is the cation and the halo group is usually the anion (chloride or bromine). Quaternary ammonium compounds are commonly called **“quats**” and have two different groups; **hydrophobic** alkyl group and a **hydrophilic** positively charged nitrogen atom which retains its cationic character at a very wide range of pH values. Quaternary ammonium may be freely soluble or insoluble water. The aqueous solubility of quaternary ammonium compounds decreases as the alkyl chain length increases (Prince *et al.*, 1999).

Quats are large organic molecules having molecular weight typically between 300- 500 g/mole. The hydrophobic alkyl groups and the hydrophilic positively charged central nitrogen atom group confer distinct physical and chemical properties to quaternary ammonium compounds.

Examples of Quaternary ammonium compound include.

(a)



Where n = 8,10,12,14,16,18

Figure 1.2 Molecular Structure of Benzalkonium Chloride

(b)

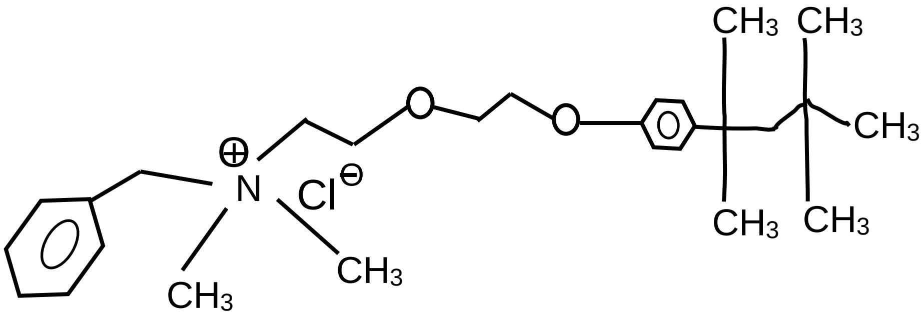


Figure 1.3 Structure of Benzethonium Chloride

(c)

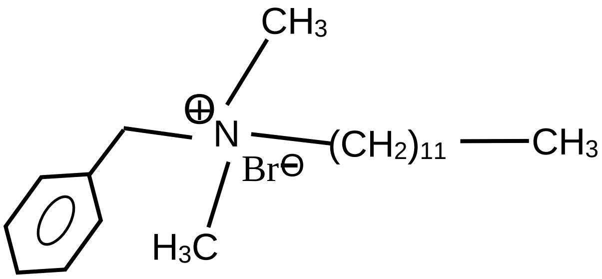


Figure 1.4 Structure of Benzododecinium Ammonium Bromide

(d)

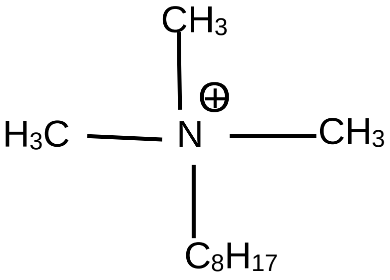


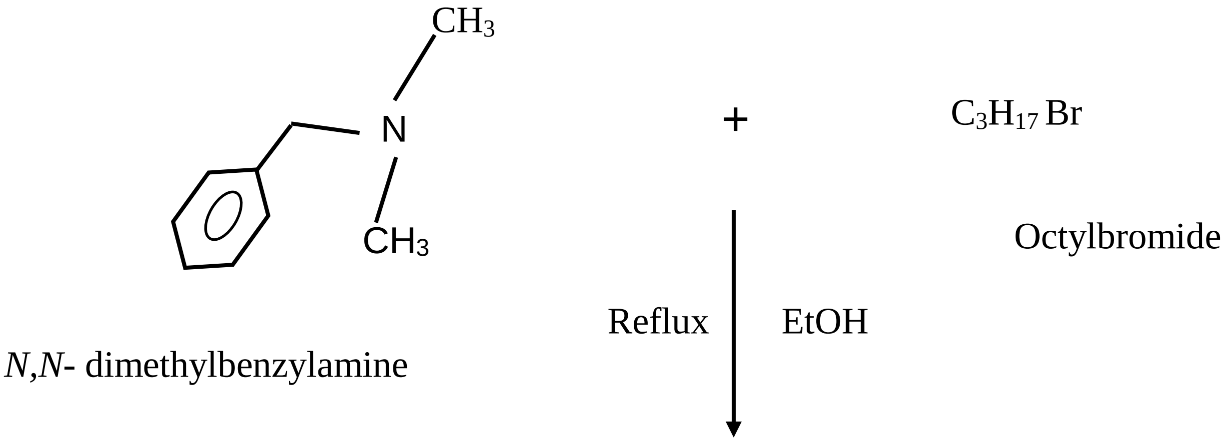
Figure 1.5 Structure of *N*-Octyl-*N,N,N*-trimethyl Ammonium Chloride

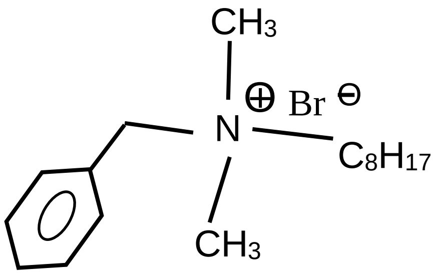
1.1 SYNTHESIS OF QUATERNARY AMMONIUM COMPOUNDS

Quats are prepared by alkylation of tertiary amines with a halo carbon. This process by which a tertiary amine is alkylated with a halo carbon is called quaternization by modern chemist. (Kuca, *et al.*,2004). Typically one of the alkyl groups on the amine is longer than the other (Kosswig, K., 2002) when making cationic surfactants.

Two common methods of preparation of quaternary ammonium compounds are:

1.1.1 Preparation of Benzalkonium salt from *N,N*–dimethylbenzylamine and long chain n-alkylbromide as shown below (Debreceni, G, 2007)





N–benzyl –*N*,*N*–dimethyloctylammonium chloride

Figure 1.6 Preparation of Benzalkonium Bromide

1.1.2. Preparation of Quaternary Ammonium Salt from a Long-chain Alkyl dimethylamine and Benzyl Chloride

$CH_3\ (CH_2)_nN(CH_3)_2$ + $CLCH_2C_6H_5$

Alkyldimethylamine benzyl chloride

Reflux ↓ EtOH

$\left[CH_3(CH_2)_nN(CH_3)_2CH_2C_6H_5\right]^{+}Cl$

Benzalkonium chloride

Figure 1.7 Preparation of Benzalkonium Chloride

1.2 STATEMENT OF PROBLEM

The insufficiency of new antibacterial agents and increasing resistance of bacteria species to convention antibacterial agents are very challenging issues which call for urgent attention.

1.3 JUSTIFICATION OF THE STUDY

Quaternary ammonium salts with long alkyl chain length have been identified through synthetic process and have been reported to exhibit effective antimicrobial activity which may eliminate bacteria species resistance of the already available antimicrobial agents. (Classissa, Singhal, Widmer, Wright, Sorrel and Jollifer, 2007)

1.4 AIM OF THIS STUDY

The present research work is aimed at synthesis, characterization and antimicrobial activities of quarternized fatty amides

1.5 OBJECTIVES OF THE STUDY

- To synthesize amide tertiary amines from the reactions of methyl ester groups and fatty amine (Amidation)
- To synthesize quaternary ammonium salts containing amide functional group from the reaction of amide tertiary amine (adduct) and a halocarbon (quaternization).
- Fourier Transformed Infrared characterization of the synthesized compounds
- Mass spectrometric analysis of the synthesized salts
- To determine their antimicrobial activities.

1.6 SIGNIFICANCE OF THE STUDY

The amidation of the fatty esters generates an amide functional group as the molecules framework of the quaternary ammonium compound which breaks the long alkyl chain and positive nitrogen center may eliminate the bacteria resistance to conventional antimicrobial agents.

1.7 SCOPE OF THE STUDY

The scope of this work is limited to the amidation of the fatty ester groups with a tertiary amine to form an amide tertiary amines (intermediates). Quaternizing the amide tertiary amine with benzyl chloride. FTIR characterization of the quarternized compounds, mass spectrometric analysis of the quarternized fatty amides. The antimicrobial activity is limited to bacteria.

1.8 REACTIONS OF QUATERNARY AMMONIUM SALTS

Quaternary ammonium cations are unreactive towards strong electrophiles and acids. They also stable towards most nucleophiles. Because of these, most unusual anions have been isolated as the quaternary ammonium salts. Permanganate can be solubilized in organic solvents when deployed as its NBu_4^+salt (Pine, Stanley H., 2011)

They can undergo Steven's rearrangement as well as deakylation under harsh conditions. Quaternary ammonium cations containing N-C-C-H units can also undergo the Hoffmann elimination, that is quaternary ammonium salts with beta hydrogen can undergo elimination (Brasen, W.R. *et al.*, 1963). Quaternary ammonium compounds have also been shown to have antimicrobial activity (Thorsteinsson, Masson, Hilmarsson and Loftsson, 2008). Their antimicrobial activity depends on a changing length of the side n– alkyl chain. It is already established that the C_{12}–homolog is most effective against yeast and fungi, C_{14} – homolog is effective against gram – negative bacteria (Daoud, Dickinson and Gilbert 1953).

Gram – negative bacteria refer to those bacteria 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 whereas the Gram – positive bacteria are those that appear purple under microscope due to their thick peptidoglycan layer. For a quaternary ammonium compound to have a high antimicrobial property, at least one of thee alkyl groups must have a chain length in the range C_8 to C_{18} (Viscardi, Quagliotto, Barolo, Savarino, Barni and Fisicaro, 2007). Quarts can inhibit the growth of bacteria spores (Brill Goroncy – Bermes and Sand, 2006).

1.9 APPLICATION OF QUATERNARY AMMONIUM SALTS

Quaternary ammonium salts found application in the following ways;

As Antimicrobials: Certain quaternary ammonium salts containing long alkyl chains are used as antimicrobials and disinfectants. Examples are benzalkonium chloride, cetalalkonium chloride, tetraethylammonium chloride. It is believed that quaternary ammonium compounds act by disrupting the cell membrane. They are effective mostly in soft water at temperature up to 100°C and concentration of about 200ppm.

Plant Growth Retardant: Chlormequet chloride (cycocel) reduces plant height by inhibiting the production of primary plant hormone responsible for cell elongation (gibberllins)

Osmolytes: Quats are present in Osmolytes, specifically glycine butane which stabilizes osmotic pressure in cells (Sleator, Roy, 2001)

As Fabric Softener: Example is Distearyldimethylammonium chloride with low biodegradability now has been phased out. Diethylester dimethylammonium chloride (Zhishem et al, 2001).

Phase Transfer Catalyst: These are those catalysts that accelerate reactions between reagents dissolved in immiscible solvents. Dichlorocarbene is generated via phase transfer catalytic reaction of chloroform and aqueous sodium hydroxide

$$CHCl_3 + NaOH \longrightarrow Cl–C–Cl + NaCl + H_2O$$

Figure 1.8 Preparation of Dichlorocarbene from chloroform and aqueous sodium hydroxide

1.10 HEALTH EFFECTS OF QUATERNARY AMMONIUM COMPOUNDS

Quaternary ammonium salts can display the following range of health effects:

- Respiratory Irritation (Bello, Anila *et al.*, 2009) and severe caustic burns on skin.
- Quaternium – 15 salts causes allergic contact dermatitis of the hands.
- Quaternary ammonium based disinfectants such as Virex and Quatricide are the most probable cause of jumps in the birth defects and fertility problems in caged laboratory mice (Melin V.E. *et al,* 2104).

  Usually quaternary ammonium compounds are not generally used as a single component but rather as a mixture composed of two or three benzalkonium members differing only in length of alkyl chain (Labranche L.P. 2007). Such mixtures are produced on a large scale in industries. However, due to specificity of each of these salts against different pathogen, a target synthesis of a pure individual was carried out as our interest.

CHAPTER TWO

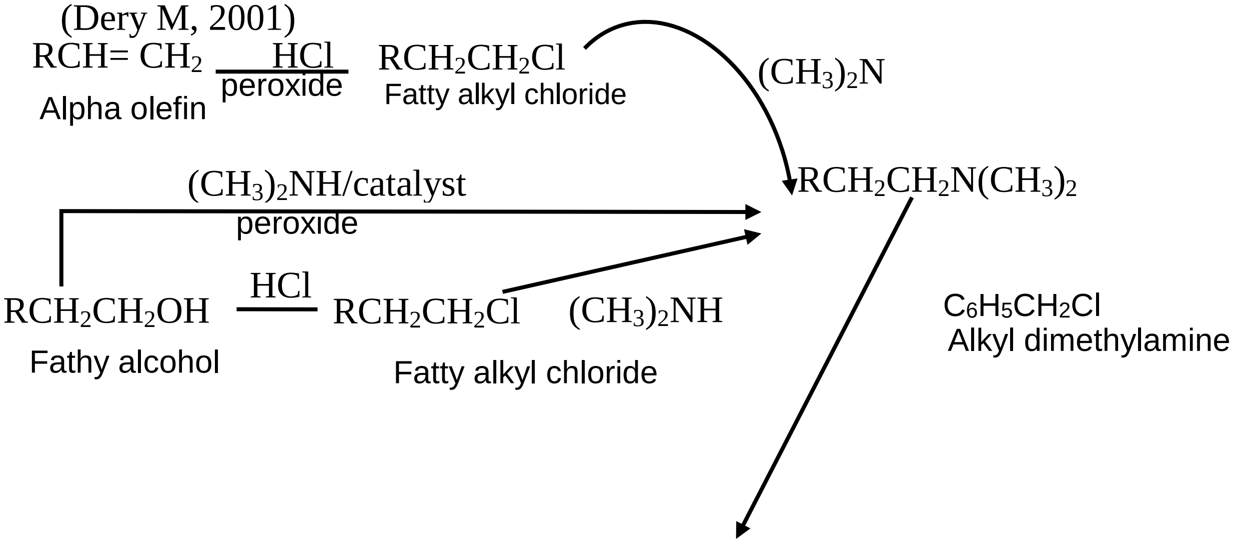
2.0 LITERATURE REVIEW

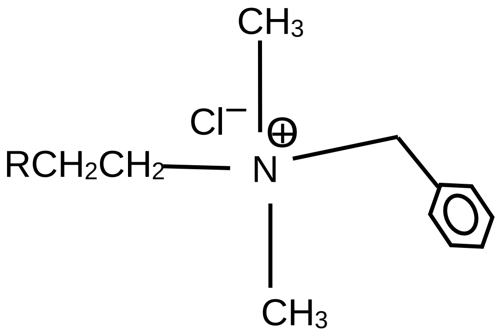
Quaternary ammonium salts are usually prepared by the alkylation of a tertiary amine and a halocarbon in a process called *MENSHUTKIN* reaction by old literature and quaternization by modern chemist (Smith, Michael; March, Jerry, 2001). The use of these quaternary ammonium compounds as an antimicrobial has been an acceptable practice due to their effectiveness when administered.

Large numbers of quaternary ammonium compounds are being studied to investigate their antimicrobial properties as well as their cat ionic surface activities. But most of these studies revealed that they have the above listed properties while others are still in progress.

Nucleophilic substitution reaction of alpha – olephines or fatty alcohol originated tertiary amine by an alkyl halide or benzyl chloride results to the formation of an organic compound that contains four functional groups covalently attached to a central nitrogren atom called "*QUAT*". Below is the synthetic pathway for the synthesis of benzylalkonium chloride

(Dery M, 2001)





Benzalkonium chloride

Figure 2.1 Synthetic pathway of *N*-benzyl-*N,N*-dimethylalkyl ammonium chloride.

However, various methods of synthesizing quaternary ammonium salts and their applications have been reported by several authors as been reviewed below;

Kamil Kuca *et al.*, (2007) Investigated the preparation of benzalkonium salts differing in the length of a side alkyl chain by reacting *N,N*–dimethylbenzylamine and an alkyl bromide. They reported that the cationic surface activity of these salts determine their potential to act as a biocides on both target and non-target organism.

Kosswig Arthur (1997) reported a typical synthesis of benzalkonium chloride from a long chain alkyldimethylamine and benzyl chloride. He added that quaternary ammonium cations are unreactive towards strong electrophiles, oxidants and acids but are very reactive and stable towards most nuclophiles.

Kivala, Kuca and Dohnal (2004) also investigated the synthesis of *N*–benzyl–*N,N*-diethylakylbromide from the reaction of *N*–benzyl–*N,N* dimethylamine and a long chain alkyl bromide under reflux with ethanol.

The solvent was evaporated and adequate yield of the quaternary ammonium salts was obtained. They reported that the synthesized salt was effective against *E.coli* and *Samonella typhimurium*. Hence, it can be used as a biocide.

Daoud N. *et al*., (1983) investigated an important feature of quaternary ammonium salts which are their bactericidal and antimicrobial activities. They reported that the antimicrobial activity of quats depends on changing the length of the side n – alkyl chain. It is well known that those quaternary ammonium salts with C_{12} –homolog are effective against yeast and fungi, those with C_{14} – homolog are effective against gram–positive bacteria while those with C_{16} –homolog are effective against gram–negative bacteria. They also reported that quaternary ammonium compounds can be used as preservatives in ophthalmic nasal and parental products. Also, it can be used as disinfectants and antiseptics for medical equipment.

Galletti, J.G *et al.*, (2012) reported that simultaneous ocular exposure to aninert antigen and benzalkonium chloride leads to a significant change in the system immune response to the administered antigen in mice, thus suggesting that the preservative effect could be more profound than the local disruption of the epithetical barrier integrity and raising the question of whether benzalkonium chloride has a role in the induction of allergy.

Brignole – Baudouin, *et al*.,(2012) studied the penetration by benzalkoniun chloride of the deeper ocular structures in rabbit eyes. It was shown to penetrate healthy eyes even after a short duration and was not only detected on the ocular surface structures, but also in deeper tissues especially in sensitive areas involved in glaucoma pathophysiology such as the trabecular meshwork and the optic nerve areas. A 2012 case study describes the process of ocular surface reconstruction following exposure to 10% benzalkonium chloride (Shigeyasu C. *et al.*, 2102)

Grillitsch, Gans, Frenzinger, Scharfis, Feuhacker and ScharfUhl (2006) further analysed 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 reagents, dichlorocarbene, CCl_2 is generated via phase transfer catalyst by reaction of chloroform and aqueous sodium hydroxide.

Gowariker, Kalyaniparanjape and Sudha (2013) studied the use of quaternary salts as plant growth retardants. In their reports, quaternary ammonium salts reduce plant's height by inhibiting the production of gibberlins, the primary plant hormone responsible for cell elongation. They concluded that the effects are primarily on the stem, petiole and flower stalk tissues.

Frank T. Sanders (2006) studied the toxicology of quaternary ammonium compounds and reported that quats are highly toxic to fish (LC_{50} = 280 μgai/L) very highly toxic the aquatic invertebrates (LC_{50}= 5.9 μgai/L) moderately toxic to birds (LD_{50} = 136mg/kg–bw) and slightly toxic to mammals (LD_{50} = 430 mg/kgbw). From his findings, highly dilute solution of quaternary ammonium salts are for human use whereas concentrated solution are toxic to humans, causing corrosion or irritation to the skin and eye. It can lead to death if taken internally in over dosage. Contact lens solutions typically contain about (0.002% to 0.01%) concentration of quaternary ammonium salts for effective preservative actions (US patent, 2006).

Spiller Henry A. (2014) studied the detailing fatal ingestion of up to 240 ml of 10% benzalkonium chloride in an aged male. Two infants were found to be poisoned due to incorrect pharmacy dilution of benzalkonium chloride. Benzalkonium chloride is effective at exceptionally low concentrations.

Swan K.C. (2005) studied the reactivity of theocular tissues to wetting agents. He found that repeated use of quaternary ammonium salts at 10 – fold higher concentrations of 1:500(0.02%) or stronger can denature corneal protein and cause damage to the eye.

De Saint Jean M. *et al.,* (1999) studied the effect of quaternary ammonium salt on growth and survival of change conjuctival cells and reported that the effect of benzalkonium chloride are cumulative and dose dependent. They also reported that benzalkonium chloride triggers an apoptotic mechanism at low temperature and a necrotic process at high concentration.

Walter(2014) studied the use of a mixture of coconut oil derivatives as a bactericide in the operation room. He identified that concentration of less than 0.1% benzalkonium chloride instilled into human eyes produced no adverse sensations whereas concentration of 0.1% or more produced burning and stringing reactions also the maximum non–irritating concentration of benzalkonium chloride in rabbit eyes is 0.05%.

Labbe A. *et al.*, (2006) studied the comparison of toxicological profiles of benzalkonium chloride and polyquaterium–1. They reported that quaternary ammonium salts have been found to consistently and dramatically alter the corneo conjuctival surface as evaluated by slit – lamp examination, the fluorescent test, impression cytology, invivo confocal microscopy and histology, in addition to decreasing their production. Intraocular benzalkonium chloride is very toxic to corneal endothelium in concentration’s commonly used extraocularly. When administered via the anterior chamber or instilled at 0.1% concentration benzalkonium chloride destroys the endothelium and causes irreversible corneal edema.

Benzalkonium chloride totally destroys the anterior segment of an experimental animal in less than a week when administered at 1–2% concentration.

Pernak, Rogoza and Mirska (2001) reported that quaternary ammonium compounds can be hydrophobic and hydrophilic. They added that the aqueous properties of the quaternary ammonium salts reduce as the alkyl chain length increases or its hydrophobicity. Similarly, the critical micelle concentration of the quaternary ammonium compounds which affects the efficacy of various surfactants associated applications decline with an increase in the alkyl chain length of the molecule. They added further that critical micelle concentration of mono–alkoniumchlorides is significantly higher than those of benzalkonium chlorides with an identical alkyl chain length indicating that the addition of benzyl group to the polar head can also reduce the critical micelle concentration and the lesser the value of critical micelle concentration, the more effective the benzalkonium chloride against pathogenic microbes.

Stepanenko B. *et al.*, (1974) investigated the synthesis of some quaternary salts ammonium compounds. They reported adequate yield of the compound as well as their bactericidal activities on fungi, gram – positive bacteria and gram – negative bacteria. Brief toxicological studies of these salts were also reported.

Stephen T. (2008) in his lecture, outlined “Some Special Antimicrobials” reported that quaternary ammonium compounds in contrast with phenolics are not very effective in the presence of organic compounds. Yet they are very effective in combination with phenols.

They further stated that quaternary ammonium compounds are deactivated by soaps, other anionic agents and cotton fibres. Also they are not recommended for use in hard water. But are effective at temperatures up to 100^{o}C (212^{o}F). They later reported that quats can be used in the food service industry as sanitizing agents.

Hunt, P. (2008) investigated how some laboratory disinfectant affects the fertility of mouse. He reported that quaternary ammonium–based disinfectants such as virex, and quatricide were tentatively identified as the most probable cause of jumps in the birth defects and fertility problems in caged laboratory mice.

Beule (2010) in an extensive review of literature in 2010 supports the nasal toxicity of quaternary ammonium salts and its role in drug induced rhinitis. He also noted the trend towards substitution of benzalkonium chloride with other preservatives in nasal decongestant sprays in line with similar trends in ophthalmic and eye care preparations.

David Dyer (1998) in the testing of a new alcohol–free land sanitizer to combat infections reported that the hand sanitizers based on quaternary ammonium compounds had showed better residual activity and lesser irritant than alcohol gels. Also an added advantage to quaternary ammonium salts not shared by ethanol – based antiseptics or hydrogen peroxide antiseptics is that quaternary ammonium salts – bases antiseptics do not cause a burning sensation when applied to a broken skin.

Sleator Roy D. *et al.*, (2001) Analyzed the role of OpuC, an osmolyte transport system, in salt tolerance, and virulence potential of listeria monocytogenes and found out that quaternary ammonium compounds are, present in osmolytes specifically glycine betaine which stabilizes osmotic pressure in cells.

Garcia *et al.*,(2006) reported that critical micelle concentration of quaternary ammonium compound affects the efficiency of many surfactant related applications decreases as the alkyl chain length of the molecule increase. For instance, the critical micelle concentration of C_{12} BDMA-Cl, C_{14} BDMA-Cland C_6BDMA-Cl are 3, 2 and 0.5 mM respectively. Quaternary ammonium salts has be referred to been the ultimate work-horse of the surfactant industry. The worldwide annual consumption rate of quaternary ammonium compounds was reported as 500,000 metric tons in the 6th world surfactant congress held in Germany (CESIO, 2004) and is even expected to be up to 700,00 metric tons (Steichen, 2001). They are on the high volume chemical list of the United State Environmental protection Agency (US-EPA). Quaternary ammonium compounds possess surface active properties, self assembly characteristics, detergency and antimicrobial properties (Keen and Montforts, 2011). Due to their uniqueness in physical and chemical properties, they have found variety of uses and a high level of population in domestic and industrial applications as surfactants, fabric softners, emulsifiers, pesticides, phase transfer catalyst and corrosion inhibitors. (Boethling,1994). The most commercially variable softener active ingredients in today's market place are dialkonium salts, diethylenetriamide compounds and ester quaternary salts. The dialkonium salts has the highest demand in the market however, ester quaternary salts are their good substitutes since they are ready biodegradable and less toxic than the dialkonium salts. Quaternary ammonium salts are used in soil removal purposes in laundry detergents. Those laundry detergents that provide fabric softening utilizes simple quaternary ammonium compounds such monoalkonium salts with an alkyl chain length in the range C_{12} to C_{18} (Zachvieja, 2001).

Quaternary ammonium compounds reduce the surface and interfacial tension by sorbing to a surface or an interface such as hair and skin. The adsorption ability of quats onto organic surfaces makes the use of quaternary ammonium compounds extremely important in the personal care industry (Rutala and Weber, 2008).

Hair conditioners and skin care products contain monoalkonium, dialkonium and trialkonium salts, ethoxylated salts as well as ester quaternary ammonium compounds in their formulations (Tang, 2001). Quats are also used in paper industry to produce tissue paper or fluf pulp, which are used in diapers, toweling, napkins and facial and toilet tissue products. Quaternary ammonium compounds such as dialkonium salts are effective chemical debonding agents in paper industry. Quats interact with neutral fibre–to–fibre bonding that occurs during the process of paper productions. The hydrophobic and hydrophilic groups of the quaternary ammonium compounds interact with the fibre surface, reduce the inter–fibre bonding and form a thin lubricant layer. This reduction of the inter-fibre bonding together with the lubricating effect gives the paper a soft feel. Also in the mechanical fluf pulp process, Quats protects the fibres against damage and reduces the defibration energy needed (Bergstorm, 2001).

Quats exhibit a broad spectrum of antimicrobial activity over a wide range of pH and are used in domestic, agricultural, industrial and medical applications. They are also used in pesticides, fungicides, wood preservatives, sanitizers and hard- surface cleaners (May, 2012).Quaternary ammonium compounds are used either as a single component or in combination with other biocides such as bactericides, fungicides or insecticides. The most common among all are the dialkonium and benzalkonium chlorides (Tiedink, 2001). The applied concentration of quaternary ammonium compounds is typically between 400-450ppm and always below 1000ppm, such as 0.1% w/v in Lysol®.One of the applications of quaternary ammonium compounds is in phase transfer catalysis. Numerous organic synthesis are carried out in which one reactant is dissolved in an aqueous solution and the other in a hydrophobic phase. Quats as a phase transfer catalyst 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.

They are believed to show better selectivity, greater rate increase and cost effective when compared to other phase transfer catalysts. Monoalkonium and benzalkonium salts are major ones used extensively as phase transfer catalysts (Boethling,1994).The organic cations used in the manufacture of organoclays are quaternary ammonium compounds such as dialkonium salts. Organoclays are produced by the displacement reaction of inorganic cations in a clay mineral (iehectorite or morillonite) by organic cations. Organoclays are used in oil based drilling fluids, printing inks, latex polymers, oil based paints and nail polishers (Hoey, 2001). Organoclays absorb organic molecules from both aqueous system and air system and air system.

They are used land fill liners, air filters and ground water remediation. The annual demand for organophilic clays which contain 40% by weight of quaternary ammonium compounds is about 16% of the quaternary ammonium compound market. Quats are used in oil field in the areas of foaming, silt suspension, biocides anti-swelling/clay stabilization, demulsification and anti corrosion process (Witco Corporation, 1995; Also chemicals incorporated, 1980).Quats as an adjuvant in pesticides enhances the solubility, rain fastness and penetration of pesticides when applied in combination with pesticides. A thesis titled plant growth retardants presented in Wagenian University of Agriculture in Netherland reported that quaternary ammonium compounds such as chlormequat chloride reduces plant height by inhibiting the plant homorne called gibberllins that is primarily responsible for cell elongation.

Therefore there effects are primarily on stem, petiole and flower stalk tissues. Lesser effects are seen in reductions of leaf expansion resulting in thicker leaves with darker green colour.

Pine Stanley, (2011) investigated the base-promoted rearrangement reactions of quaternary ammonium salts and reported that quaternary ammonium compounds undergo Sommelet-Hauser rearrangements and Steven's rearrangements as well as dealkylation under harsh conditions. He also reported quaternary ammonium cations can undergo Hoffman elimination and Emde degradation if they possess hydrogen beta to the nitrogen center.

Schmitt, (1994) reported the concentration of Quats in domestic wastewater, effluent wastewater, sewage sludge and surface water as 0.5, 0.05, 3000 and 0.04 ppm respectively random samples of sewage from treatment plant in Switzerland has quaternary ammonium compounds levels ranging from 0.04 to 0.45 ppm due to various inputs from metallurgical processes or textile industry (Michelson, 1978). Moreover, available data suggest that quats are extensively accumulated in aquatic sediments. However, information or residues of quats in aquatic sediments near sites of domestic or industrial effluent discharge are scarce. The use of quaternary ammonium compounds increases as they find newer applications.

Wee (1984) determined the level of dialkonium chlorides in untreated sewage and final effluent from a plant in United State of America. He reported that the quaternary ammonium compounds 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 studied in composite sewage sample in Germany and England. The total monoalkonium chloride concentration in the influent and effluent sewage was 0.13ppm and 0.03ppm respectively.
Martinez–Carballo, Sitka, Gonzalez – Barreiro, Kreuzinger, Furhacker, Scharf and Gans,(2007) in a study conducted in Austria to monitor Quaternary Ammonium compound concentrations in influent of five different waste-water treatment plants reported concentration of quats ranging from 1- 170ppb. On the contrary, the quaternary ammonium compounds concentration would be higher in effluents of specific industrial facilities such as textile, paper process and food processing (1 –-40ppm based on a data obtained during a screening study for a poultry processing facility) than the influents of municipal waste water treatment plants.

Kummerer *et al.*, (1997) analyzed benzalkonium chlorides in highly complex effluent samples from different European hospitals, the measured concentrations were between 0.05ppm to 6.03ppm. Although the reported concentrations in waste-water is low, many studies delineating the effect of and biodegradability of quaternary ammonium compounds in waste-water treatment systems have worked out at concentrations ranged from 10 – 100 mg/L. However, one would expect higher quaternary ammonium concentration in the waste-water treatment systems or surface water receiving influents from industries that use quats extensively.

Levels of quaternary ammonium compounds in receiving waters are typically in low microgram per liter.

Huber (1979) reported quaternary ammonium compounds concentration of 5 – 20 ppb in the main River in Germany.

Lewis and Wee (1983) subsequently conducted a follow-up study whereby samples were collected at various distances downstream from the wastewater treatment plants. Mean dialkonium chlorides levels were < 2, 24, 17 and 33 ppb for millers River, offer River, Blackstone River and Rapid Creek respectively. The concentration of Quaternary ammonium compound in the samples collected at distances from 44 to 53 miles downstream from the wastewater treatment plants ranged from 191 – 100 ppb.

Quaternary ammonium compounds absorb strongly on suspended solids such as minerals, biomass and inorganic particles and inorganic particles and are transferred to anaerobic digesters or aquatic sediments for example, the mean concentration of quaternary ammonium compounds in an aerobically stabilized sludge samples from five different municipal sewage treatment plants in Switzerland were 3670, 960,470 and 210 ppm in 1991, 1992, 1993 and 1994 respectively. (Fernandez *et al.*, 1996). It is also reported that the quaternary ammonium compound concentration in anaerobic digesters may range from 4000 to 10,500ppm.

Sun, Takata, Hata, Kachara and Taguchi (2003) studied the fate of quaternary ammonium compounds in a river running through Toyama city, in Japan. The reported that the total influx of quaternary ammonium compounds into the river was 1.4gmin^{-1}and the concentration was between 0.01 and 0.02 ppm. The quats in the sediment samples were 500 times higher than that found in the river water.

Lewis and wee (1983) obtained major samples at a distance 0.8 – 88km downstream from a sewage outfall from a Rapid Creek. The quaternary ammonium compound levels averaged 23ppm over twenty samples.

It was also reported in 1997 that the level of quaternary ammonium compounds in influent sewage, river water and sediment from Japan were 0.10 to 0.15, 0.05 and 6.2 – 69ppm respectively (Utsunomiya, Watanuki, Matsushita and Tomita, 1997) studied the effect of four quaternary ammonium compounds on a mixed mesophobic methanogenic culture.

They reported that all the four quats tested short or long term inhibitory effects on the mixed methanogenic culture at 25mg/L and above. The inhibitory effects of the individual quaternary ammonium compounds on the methanogenic activity decreases as their alkyl chain length increases. It was concluded that the inhibitory effects of quats was inversely proportional to the adsorption affinity of the biomass or their hydrophobicity.

Jia, Zhisen *et al.*, (2001) studied the synthesis and antimicrobial activities of quaternary ammonium salts of chotisan. They reported that certain quaternary ammonium compounds especially those containing long alkyl chains have antimicrobial activity. Examples are benzethonium chloride, cetalkonium chloride, domiphen bromide etc, They are good against fungi, amoeba and envelop viruses. They are believed to act by disrupting the cell membrane. Furthermore, quaternary ammonium salts are lethal to a wide variety of organisms except endospores, mycobacterium, tuberculosis and non-enveloped viruses.

Advancement in the quality and efficacy of quaternary ammonium compounds such as benzalkonium chloride in current non–alcohol hand sanitizers has addressed the CDC concerns regarding gram–negative bacteria; with the leading products being equal if not more effective against gram– negative particularly New Delhi metallo–beta– lactomase-1 and steer antibiotic resistant bacteria.

Peter, Whams *et al*., (1998) reported that Newer formulations using benzalkonium blended with various quaternary ammonium derivatives can be used to extend the biocidal spectrum and enhance the efficacy of quaternary ammonium salts based disinfectants products. This formulation technique has been used to a greater effect in enhancing the virucidal activity of quaternary ammonium based disinfectants such as virucide-100 to typical health care infection hazards such as hepatitis and HIV. They reported that the use of appropriate excipients can greatly enhance the spectrum performance and detergency and also under use conditions. Also formulation can help minimize deactivation of benzalkonium solutions in the presence of organic and inorganic contaminants.

Ying (2006) reported that the alkyl chain length not only determines the physical/ chemical properties of the quaternary ammonium compounds but also may have a decisive role in the fate and effects of these compounds in the environment. Under aerobic conditions, the biodegradability of quaternary ammonium compounds generally decreases with the number of alkyl groups as $R_4N^+ < R_3M_eN^+ < R_2\ Me_2N^+ < RMe_3N^+ < Me_4N^+$

Also substitution of methyl group with benzyl group can decrease biodegradability further. A comparism of the degradation rates of benzyl chloride and monoalkonium bromides under aerobic conditions was undertaken. The rate of degradation of benzalkonium chlorides and monoalkonium bromides was inversely related to the length of alkyl groups and substitution of benzyl group decreased the rate. Indeed the benzalkonium chloride with the longest alkyl chain length was found to be more recalcitrant of the tested compounds with a plateau at only 30% degradation after 9 days. Also it was reported that the aerobic degradation of quaternary ammonium compounds was dependent on the length of alkyl group. However the number of alkyl group had more effect on biodegradability for instance dialkonium quats were degraded five times more slowly than the monoalkonium quats. Certain microorganisms that are capable of quats degradation can be isolated (Van Ginkel and Kolvenbach, 1991). Those micro organisms that can degrade quaternary ammonium compound includes; pseudomonas B1, Xanthomonas, Aeromonas hydrophila Sp. K, Pseudomonas florescens TN4 and pseudomonas spp. Strain 7– 6 (Takenaka, Tonoki, Taira, Aoki and Murakami, 2007) which were isolated from either soil or sewage. Quaternary ammonium compounds are strongly and rapidly solved onto a wide variety of materials of environmental relevance for instance biomass, clay, sediment and even minerals. Indeed, sorption outcompetes biodegradation generally and therefore, quaternary ammonium compounds are transferred to anoxic/anaerobic environments, there is no established facts of mineralization of quaternary ammonium compounds that contain alkyl or benzyl groups.

(Wilson and Battersby 1981;Federle and Schwab,1992, Garcia, campus Sanchez- Leal and Ribosa, 2000) because of the highly reduced nature of these substituent groups. Moreover, quaternary ammonium compounds are inhibitors to anaerobic microbial processes for example in methanogenes is (Tezel, Pierson and Pavlostathis, 2007).

Giolando, Rapaport, Larson, Ferderle, Stalmans and Masscheleyn, (1995) reported that diethylesterdimethylammonium chlorides a recent analogue of diaklonium chlorides were completely degraded by anaerobic digester sludge in a standard test based on biogas formation. Diethyl ester dimethyl ammonium chloride differs from diakoium chlorides by the inclusion of two ester linkages between ethyl and alkyl chains. These ester linkages allow diethylester dimethyl ammonium chloride to be rapidly and completely degraded in a 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 18days.Likewise, naturally occurring quats such as chlorine & betain has been known to be ultimately degraded under anaerobic /anoxic environments. (Grimme and Dawson, 1978; King, 1984) However, quaternary ammonium compounds in which the hydrophobic groups are linked to the head groups with ester bonds(ester quats) betaine, chlorine (natural quats) and those in which alkyl chains are linked directly to N^+ have a different fate under anoxic environment.

The natural quats are always recalcitrant under the anoxic/ anaerobic environment.

Mcdonell G. *et al.,* (1998) in his study of antiseptics and Disinfectants, Activity, Action and Resistance reported the minimum inhibitory concentration of benzalkonium chlorides. (C_{12}–C_{16}) and dialkonium chlorides (C_{10}) for clinically important bacteria as shown below:

Microorganisms	**Minimum Inhibitory Concentration.(mg/L)**	
	$C_{12} – C_{16}$	**DC_{10}**
Gram – Negative		
Echericha coli	100	25
Pseudomonas aeruginosa	700	250
Salmonella typhmurium	150	40
Proteus mirabilis	300	200
Legionella pneumophila	80	30
Campylobacter jejuni	45	4
Gram Positive		
Staphylococus aureus	40	10
Listeria monocytogenes	25	5
Enterococcus faecium	30	10

The present research work investigates the synthesis of amide tertiary amine from the reaction of methyl ester groups and fatty amine. To synthesize quaternary ammonium salts containing amide group from the amide tertiary amine and a halocarbon (benzyl chloride). Also to characterize the synthesized compounds with Fourier transformed infrared spectroscopic equipments. Furthermore to analyse the products with mars spectrometric machines as well as to determine the antimicrobial activities of the synthesized products starting materials as well as the intermediate products.

Methyl ester groups such as methyl capylate and methyl laurate can be isolated from coconut oil. Coconut oil is the chief source of methyl laurate, methyl octanaote and even methyl hexadecanaote.

Coconut oil can be sourced locally here in Nigeria. Using coconut oil which is a mixture of esters as starting material and fatty amine will yield a mixture of amide tertiary amines and glycerol. The amide tertiary amide on reaction with benzyl chloride will yield mixtures composed of two or more quaternary ammonium salts differing only in the length of the alkyl chains (Cabal, J. *et al,* 2003) which are produced on a large scale in industries. But our interest is to synthesize specific individuals because of the specificity of each salt against different pathogens. Also of interest is the inclusion of amide functional group which breaks the link between the fatty hydrocarbon and the cat ionic nitrogen cater which may reduce or enhance the antimicrobial and other properties of these compounds.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1.1 APPARATUS AND EQUIPMENTS

The following apparatus and equipments were used in the process:

Standard volumetric flask, pipette, beakers (250 ml), three neck round–bottom flask, condenser, oil bath, thermometer, cover lid, stop watch, magnetic stirrer hot plate, retort stand, rubber cork, rubber hoses, calcium chloride tube, cotton wool, swab stick. FTIR spectrometer (Agilant cary 630FTIR) Bijou bottles, syringes (10 ml), weighing balance.

3.1.2 REAGENTS: The following reagents were used in this research work, Methyl octanoate, methyl laurate, Benzyl chloride, tetrahydrofuran, *N,N*–diethylethylenediamine, Calcium chloride, paraffin oil, sulphuric acid, Barium chloride, Ampicillin. All the reagents used are of analytical grade.

3.2 SYNTHETIC PROCEDURE

Generally quaternary ammonium compounds are prepared from the reaction of tertiary amines with alkyl or aryl halides (Kwala m. *et.al.*, 2004)

For this research work, quaternary ammonium compounds were produced from methyl octanoate, methyl laurate, *N,N*–diethylethylenediamine, benzyl chloride and tetrahydrofuran as described below.

3.2.1 Preparation of *N,N*–diethylaminoethyloctylamide

About 5.4 ml (4.74 g, 0.03 mole) of methyl octanoate and 4.2 ml (3.48 g, 0.03 mole) of *N,N*–diethylethylenediamine were added separately into a three neck round bottom flask. To avoid moisture interference in the reaction flask, a calcium chloride tube was filled with calcium chloride and inserted into the neck of the condenser.

Thermometer was inserted from one neck of the flask and the other neck closed. The mixture was refluxed in an oil bath for 10hours at 120-130^{O}C to form an amide tertiary amine (An intermediate)

Equation for the reaction.

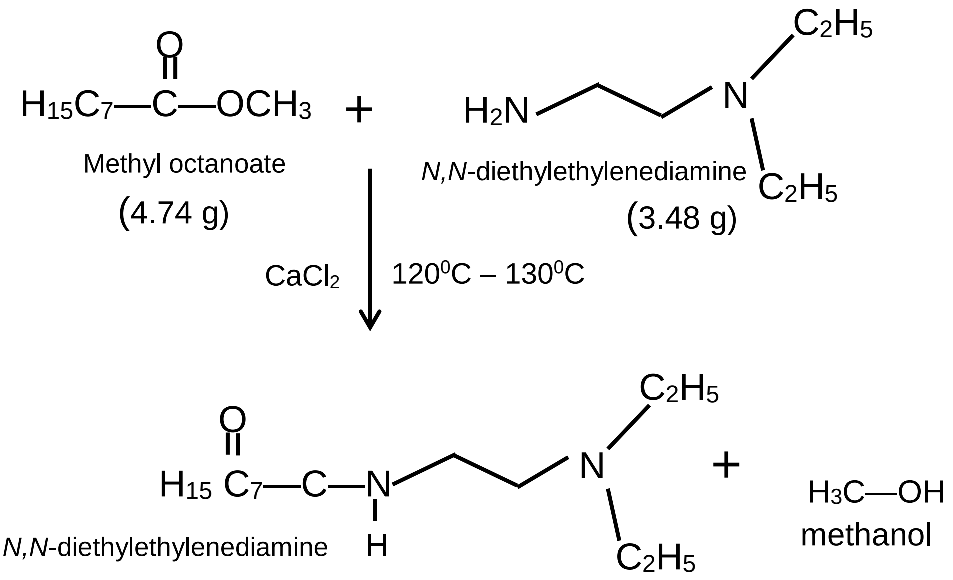


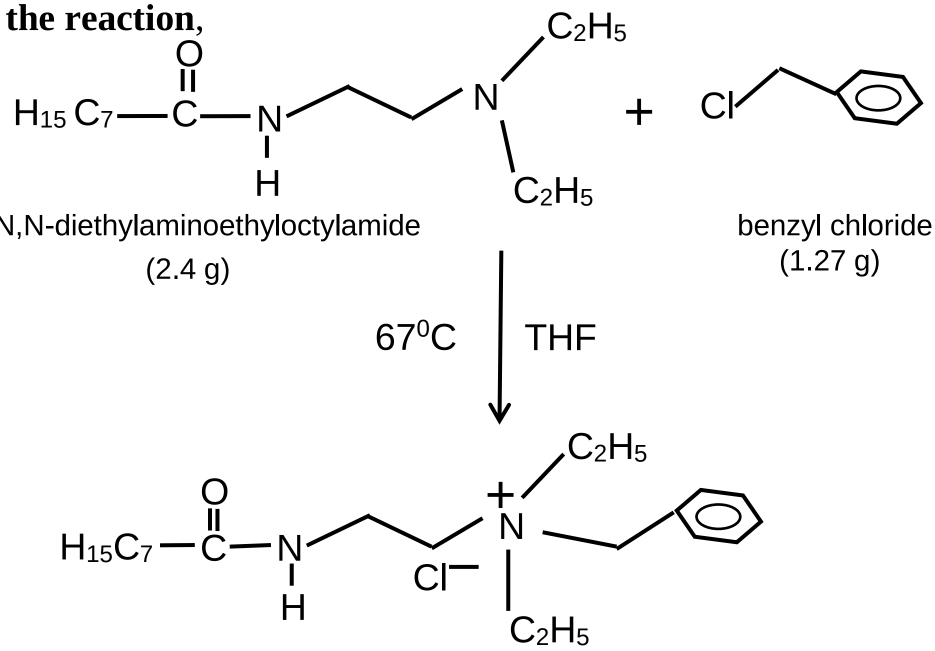
Figure 3.1 Preparation of *N,N*–diethylaminoethyloctylamide

The intermediate was washed with distilled water to remove the unreacted amine and methanol leaving behind the amide tertiary amine as a viscous liquid.

3.2.2 Preparation of *N*–benzyl–*N,N*–diethylaminoethyloctylamidium chloride

About 2.4 g (0.01 mole) of the amide formed (*N,N*-diethylaminoethyloctylamide), 1.27 g,(1.3 ml, 0.01 mole) of benzyl chloride and 14 ml of tetrahydrofuran were added in another three neck round bottom flask. The mixture was refluxed for 12 hours at 67^{O}C. After the reaction, solvent was evaporated and a dark brown viscous liquid was left as the product (quaternary ammonium compound)

Equation of the reaction,



N- benzyl–*N*,*N*-diethylaminoethyloctylamidium chloride

Figure 3.2 Preparation of *N*–benzyl–*N*,*N*–diethylaminooctaunidium chloride.

3.2.3 Preparation of *N*,*N*–diethylaminoethyllaurylamide

About 5 ml (4.3 g, 0.02 mole) of methyl laurate and 6 ml (4.7 g, 0.04 mole) of *N*,*N*-diethylethylenediamine were added into the reaction flask. To avoid moisture interference in the reaction flask, a calcium chloride tube with calcium chloride was inserted into the neck of a condenser. Thermometer was inserted from one of neck of the flask and the other neck closed. The mixture in flask was refluxed on continuous stirring over an oil bath for 10 hours at 120^0C to form an *N,N*-diethylaminoethyllaurylamide.

Equation of the reaction,

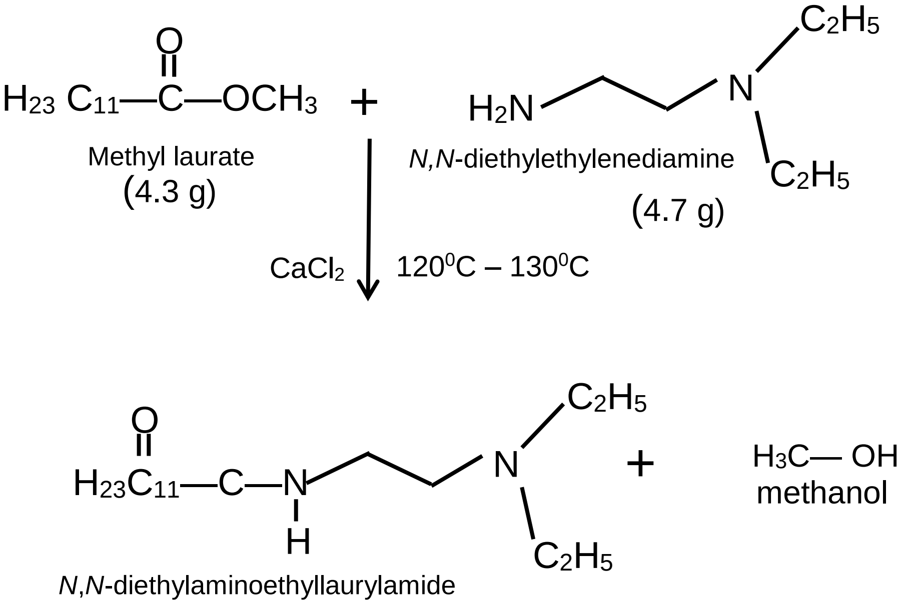


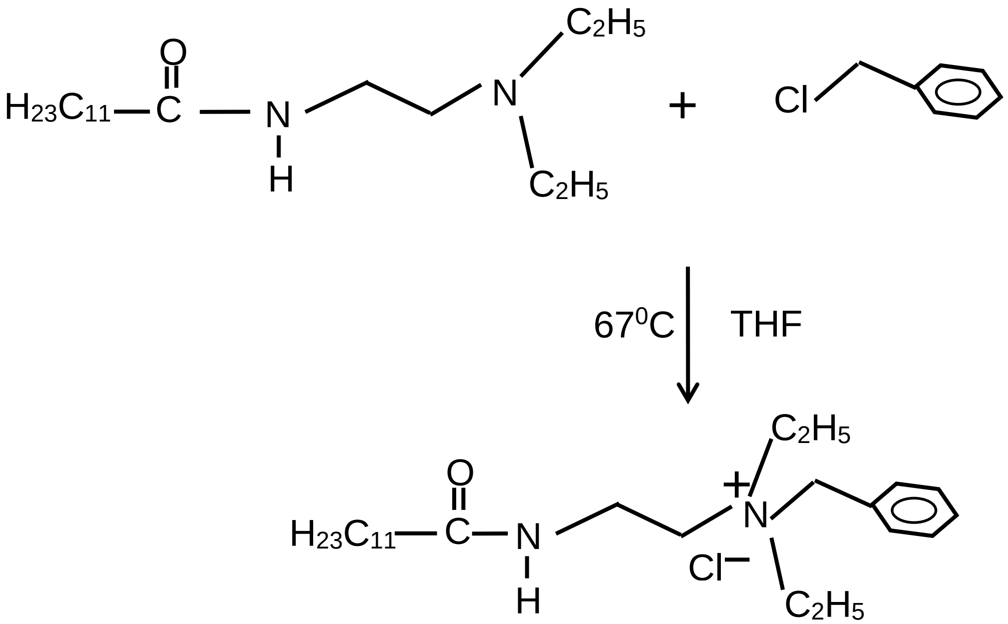
Figure 3.3: Preparation of *N,N*-diethylaminoethylaurylamide

The amide was washed with distilled water to remove the unreacted excess amine and methanol leaving behind an amide tertiary amine.
(*N,N*- diethylaminoethylaurylamide)

3.2.4 Preparation of *N*-benzyl-*N,N*-diethylaminoethylaurylamidium chloride.

About 2.98 g (0.01 mole) of the *N,N*-diethylaminoethylaurylamide, 1.2 ml (1.27 g, 0.01mole) of benzyl chloride and 14.4ml of tetrahydrofuran were added to another round bottom flask, the mixture in the flask was placed in an oil bath and refluxed for 12 hours at 67^0C on continuous stirring.

Equation of the reaction



N-benzyl-*N*,*N*-diethylaminoethyllaurylamidium chloride

Figure 3.4: Preparation of *N*-benzyl-*N*,*N*-diethylaminoethyllaurylamidium Chloride.

After the reaction, the solvent was evaporated and a dark brown viscous liquid was left as the product.

3.3 ANTIBACTERIAL TEST

3.3.1. Collection of Test Samples

The pure bacterial stains such as *salmonella enterica, Echerichia coli, staphylococcus aureus, klebsiella spp. and lactobacillium spp*. used for this analysis were collected from the Microbiology Laboratory Federal University of Technology Owerri, Imo State.

3.3.2. Preparation of Macfarland's Turbidity Standard.

A 1% v/v solution of sulphuric acid was prepared with a 1% v/v solution of dehydrated barium chloride. 0.6 ml of barium chloride solution was added to 99.4 ml of the prepared sulphuric acid solution with mixing.

A quantity of the turbic solution formed was transferred into a capped tube of the same type used for preparing the test and control inoculums. The bacterial population used in this test was standardized using 0.5 Macfarland's standard.

Equation of reaction

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

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

3.3.3. Antibacterial Studies

The bacterial 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 test 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 8 mm allowing 30mm between adjacent wells and the petri dish. Sterile syringes were used to introduce fixed volumes of test compounds into the wells. The plates were incubated at 30^0C for 24 hours. After the period of incubation, the diameter of the zones of inhibition were measured in mm (Chestbrough, M., 2000). Ampicillin 10 mg/L was used as control

3.4 INSTRUMENTATION

3.4.1 Fourier Transform-Infrared Spectroscopy

The samples were dissolved in 98% dimethysulfoxide (1 mL). The samples were molded into pellets. The source emits infrared radiation spanning a wide frequency range. A wavelength selector permits only a particular wavelength of radiation transmitted by the sample to fall on a detector which measures the intensity of radiation & a plot of this intensity for each wavelength is traced on paper by a recorder.

3.4.2 Mass Spectroscopy

The samples were vaporized & introduced into an evacuated chamber, where it was bombard with high-energy electrons (70 ev, 1600 kcal/mol) that strip an electron from the molecule. The resulting positive ions were attracted to & accelerated by a magnetic field perpendicular to the plane of the paper. Varying the strength of the magnetic field changed the radius of the curvature of the different ions of different molecular weight going through the chamber, so that the spectrum of ions was gradually swept past the detector slit. The spectrum was recorded on graph paper as a plot of mass (actually mass/charge ratio) versus intensity. Neutral molecules were not detected & were removed from the system by several vacuum pumps.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

The results obtained from the synthesis, characterization and antimicrobial activities of quarternized fatty amides are shown in tables and figures below;

Table 4.1: Supporting Evidence for Quaternary Ammonium Salts Formation

Compounds	Appearance	Solubility in water
Methyl Octanoate $C_9H_{18}O_2$	Colourless liquid	Not soluble
Methyl Laurate $C_{13}H_{26}O_2$	Colourless liquid	Not soluble
N,*N*–diethylethylene diamine ($C_6H_{26}O_2$)	Colourless liquid	Soluble
N,*N*–diethylaminoethyl Octylamide ($C_{14}H_{30}N_2O$)	Light brown Viscous liquid (oily)	Not Soluble
N,*N* –diethylaminoethyl Laurylamide ($C_{18}H_{38}N_20$)	Light brown Viscous liquid (oily)	Not soluble
Benzyl Chloride (C_7H_7Cl)	Colourless Liquid	Not Soluble
N–benzyl–*N*,*N*–diethyl aminoethyloctylamidium Chloride ($C_{21}H_{37}N_2OCl$)	Dark brown Viscous liquid	Soluble and foaming
N–benzyl–*N*,*N*–diethyl aminoethylluarylamidium Chloride ($C_{25}H_{45}N_2OCl$)	Dark brown Viscous liquid	Soluble and foaming

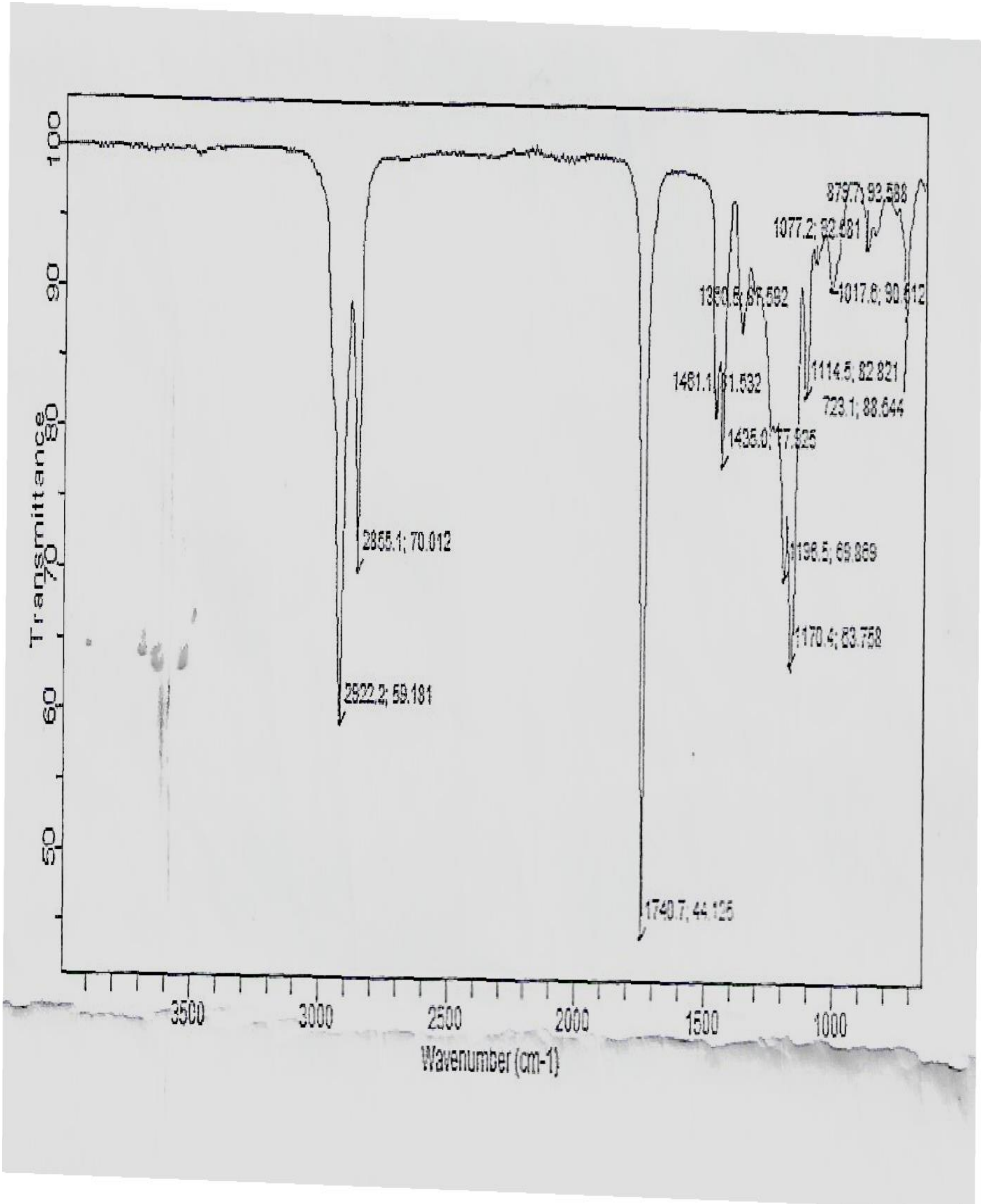


Figure 4. 1 Infrared Spectrum of Methyl Octanoate

Table 4.2: FTIR values obtained in methyl octanoate

Peak Value (cm^{-1})	Functional group	Remark
1740	C=O	Stretch for ester
1170	C-O	Stretch of ester
2855	-CH	Stretch of methylene group
2922	-CH	Stretch of methyl group

Table 4.2 showed the wave numbers of the functional groups present in methyl octanoate. An intense peak at 1740 cm^{-1} showed the stretch for the carbonyl functional group of esters (C=O). Also sharp peak at 1170 cm^{-1} indicates the absorption of –C-O stretch of alkyl ether.

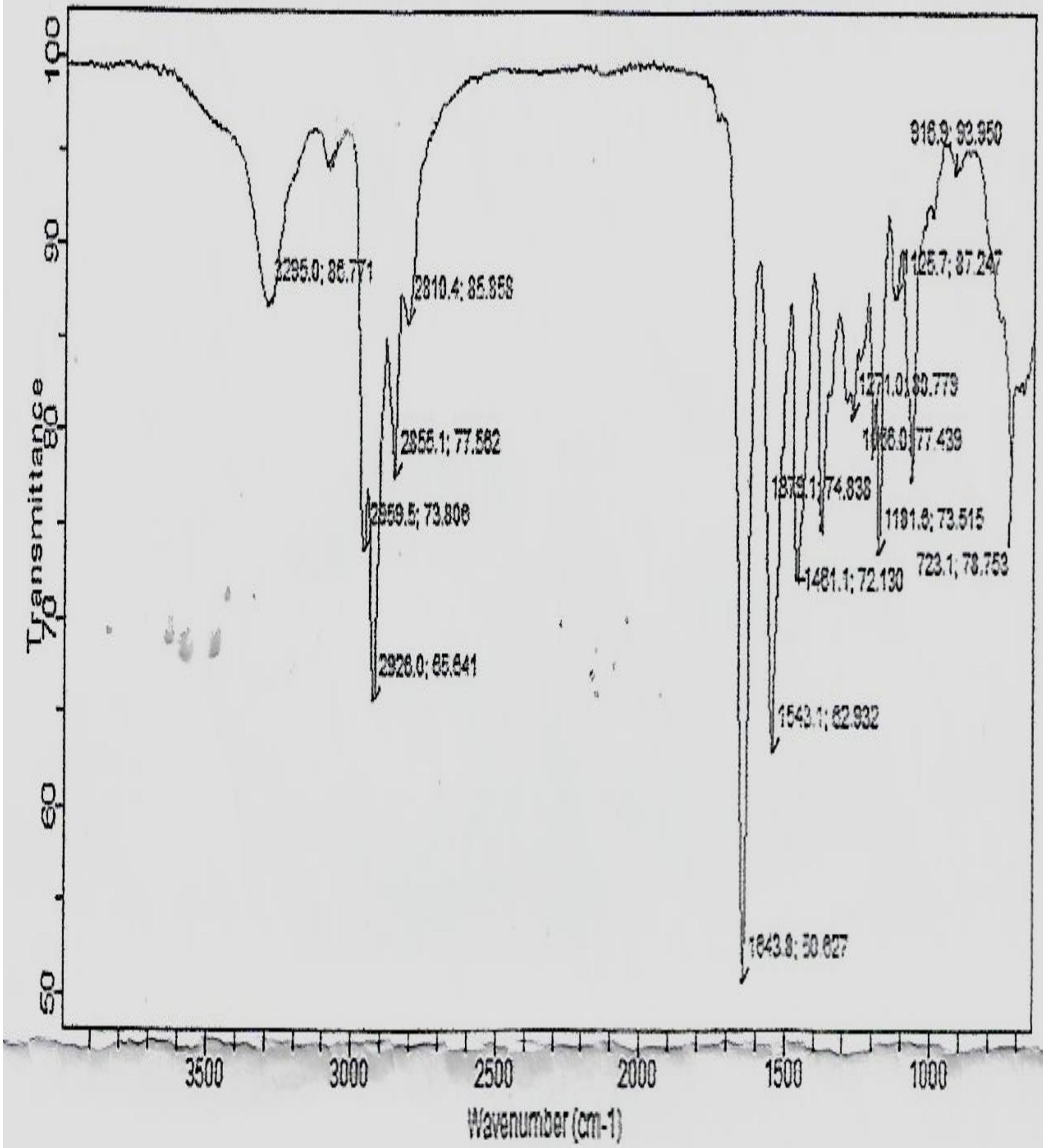


Figure 4.2 Infrared Spectrum of *N,N*-diethylaminoethyloctylamide

Table 4.3: FTIR values obtained in *N*, *N*- diethylaminoethyloctylamide

Peak value (cm^{-1})	Functional group	Remark
1643	C=O	Stretch of amide
3295	N-H	Stretch of amide
2925	-CH_3	Stretch for methyl group

Table 4.3 showed the peak values obtained in FTIR analysis of *N*,*N*-diethylaminoethyloctylamide. An intense sharp peak at 1643 cm^{-1} indicates absorption of C=O of an amide. A singlet peak of 3295 cm^{-1} indicates the N-H peak of an amide.

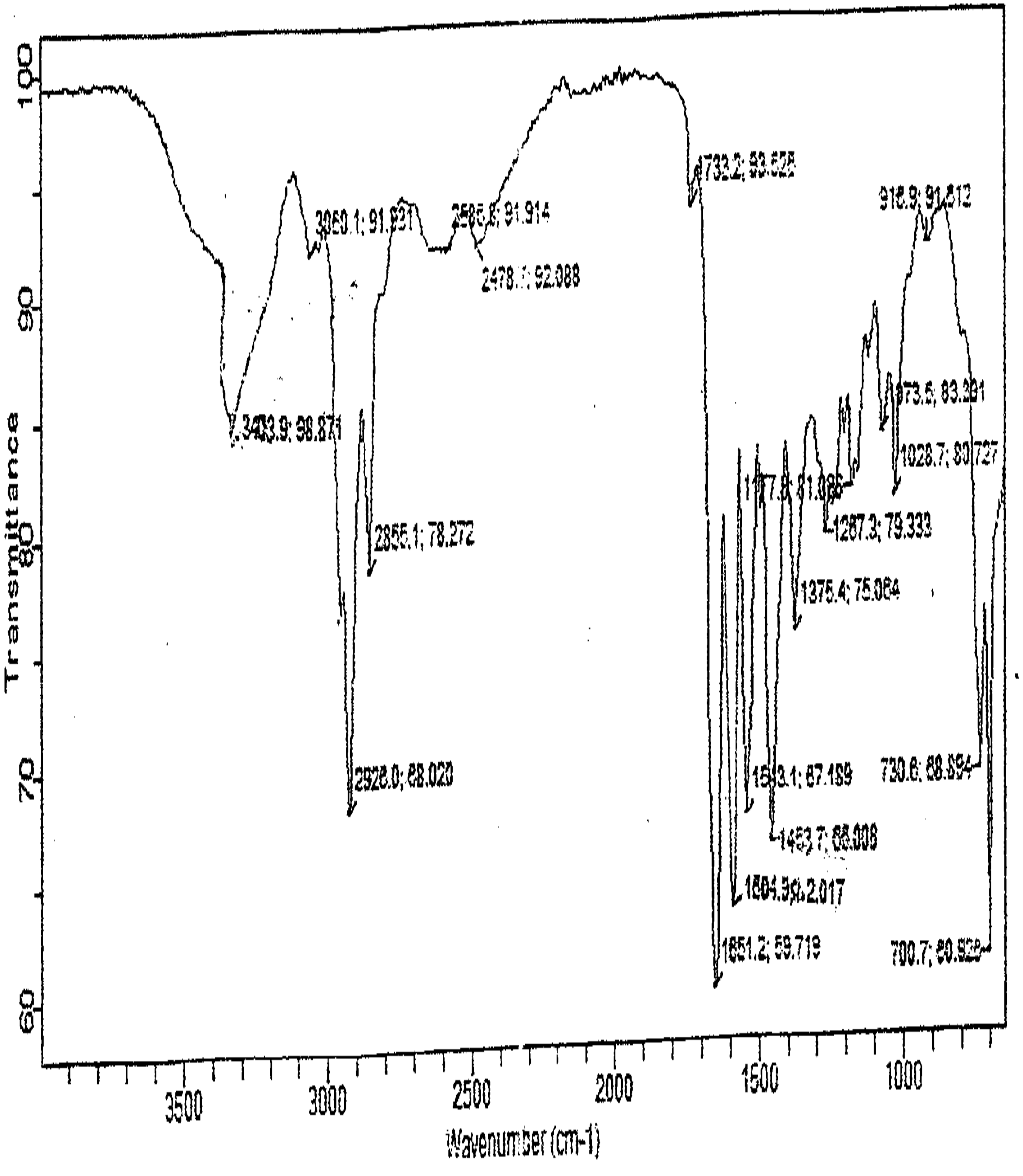


Figure 4.3 Infrared Spectrum of N-benzyl-*N,N*-diethylaminoethyloctylamidium Chloride

Table 4.4: FTIR Values Obtained In *N*- benzyl-*N*, *N*- diethylaminoethyl Octylamidium Chloride

Peak value (cm^{-1})	Functional group	Remark
1651	C=O	Carbonyl of an amide
3403	N-H	Stretch for an amide
1604	C=C	Aromatic peak (benzene group)

Table 4.4: showed the values of peaks obtained in FTIR analysis of N- benzyl-*N*, *N*-diethylaminoethyloctylamidium chloride. An intense strong peak of 1651cm^{-1} indicates the C=O stretch of an amide. A singlet peak at 3403 cm^{-1} is the absorption band for N-H of an amide. An intense strong peak at 1604 cm^{-1} is the absorption band for C=C stretch of benzene ring.

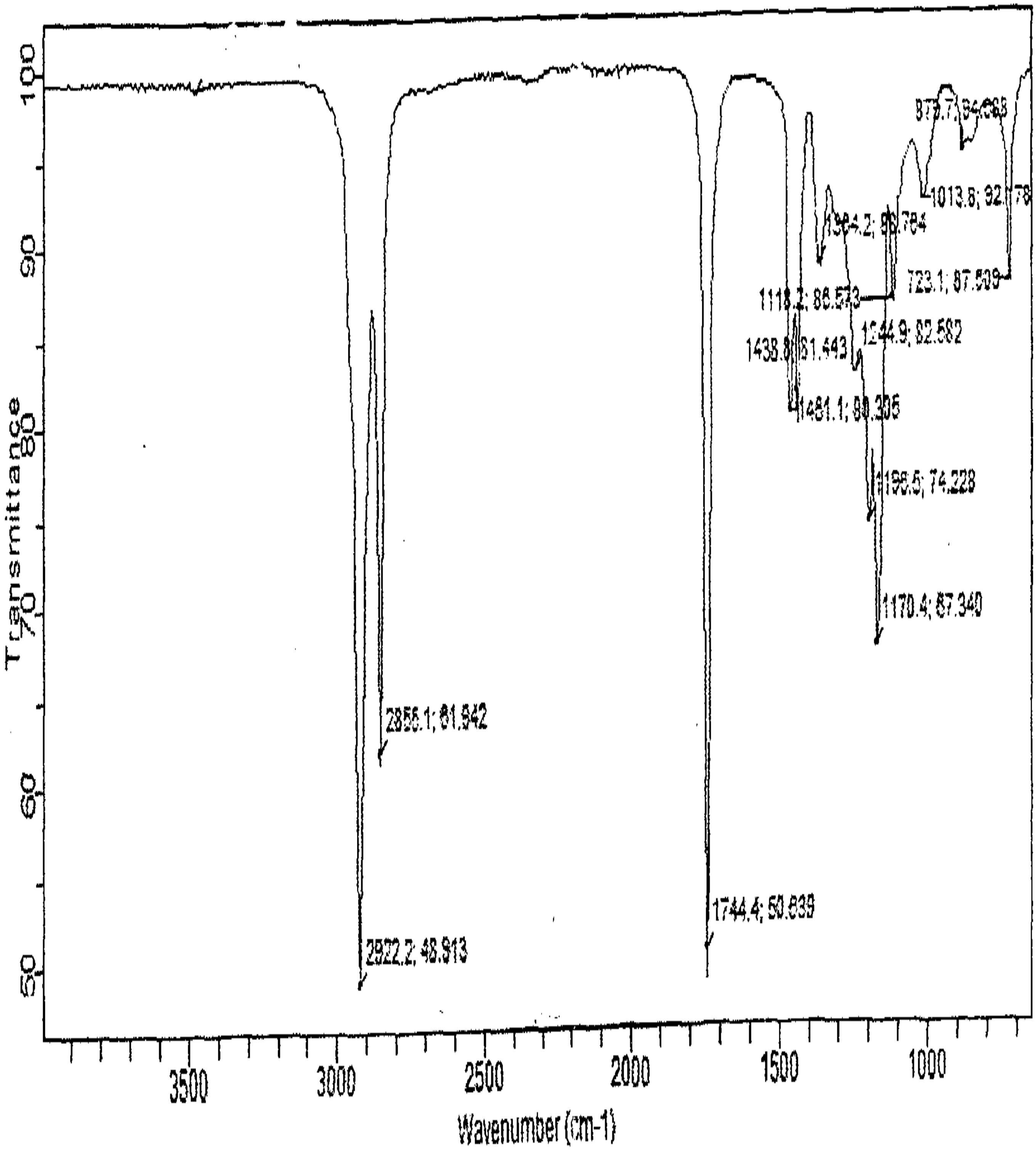


Figure 4.4 Infrared Spectrum of Methyl Laurate

Table 4.5: FTIR values obtained in methyl laurate

Peak value (cm^{-1})	Functional group	Remark
1744	C=O	Carbonyl stretch of ester
1170	C-O	Stretch for ester
2855	-CH	Stretch of methylene group

Table 4.5 showed the values of peaks obtained in FTIR analysis of methyl laurate. An intense sharp peak at 1744 cm^{-1} showed the absorption band of C=O group of an ester. Sharp peak at 2855 cm^{-1} is the absorption band for methyl group (-CH) of an aliphatic hydrocarbon. Also sharp peak at 1170 cm^{-1} is the C-O stretch of an alkyl ether.

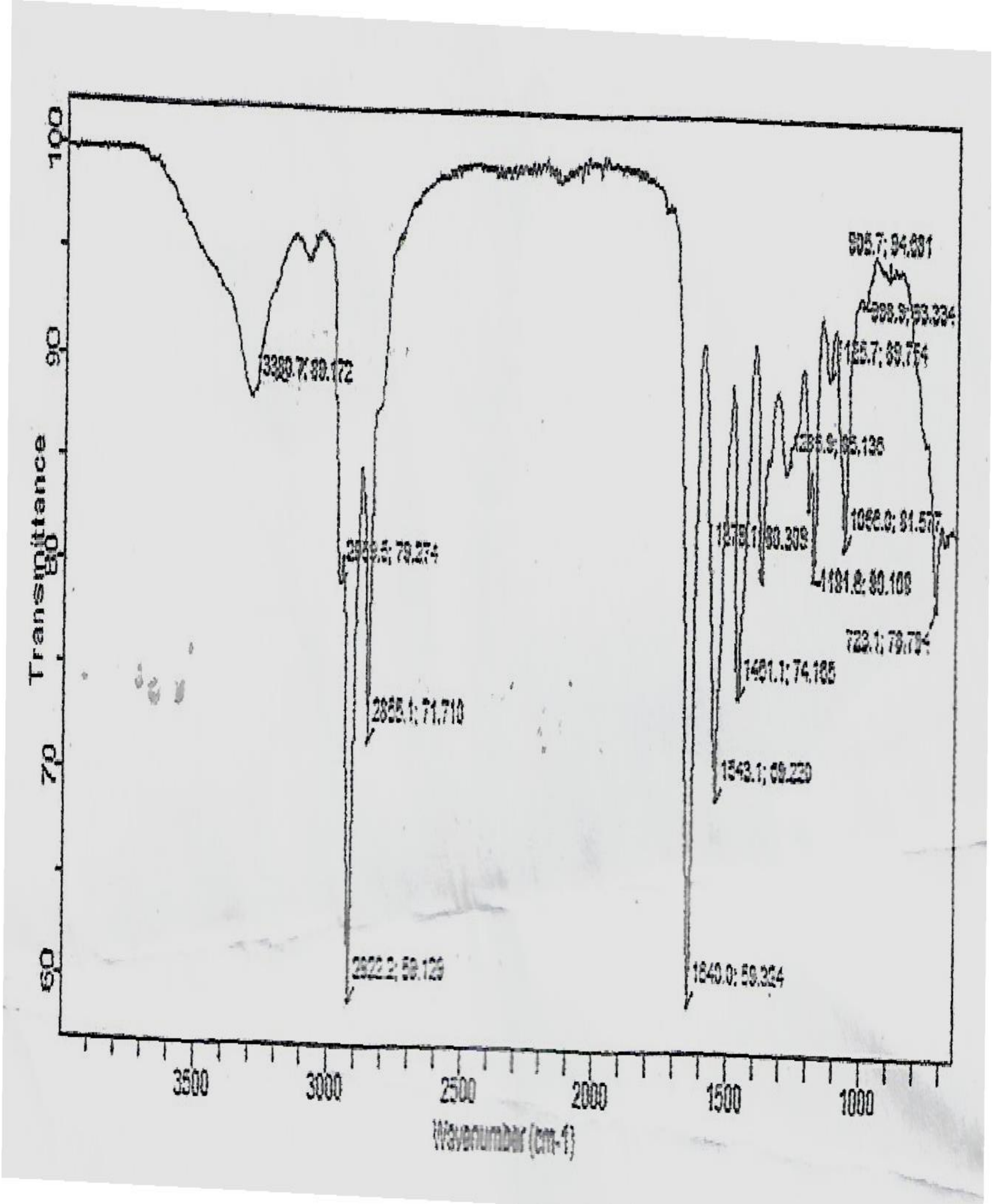


Figure 4.5 Infrared Spectrum of *N,N*-diethylaminoethyllaurylamide

Table 4.6: FTIR values obtained in *N*, *N*- diethylaminoethyllaurylamide

Peak value (cm^{-1})	Functional group	Remark
1640	C=O	Carbonyl stretch of an amide
3380	C-O	Stretch of an amide
2922	$-CH_3$	Stretch of methyl group of aliphatic hydrocarbon

Table 4.6 showed the values of peak obtained in FTIR analysis of *N*,*N*-diethylaminoethyllaurylamide. An intense strong sharp peak at 1640 cm^{-1} showed the carbonyl (C=O) stretch of an ester. A singlet strong peak at 3380 cm^{-1} is N-H absorption band of an amide. Sharp peak at 2922 cm^{-1} is the stretch of the methyl group of an aliphatic hydrocarbon.

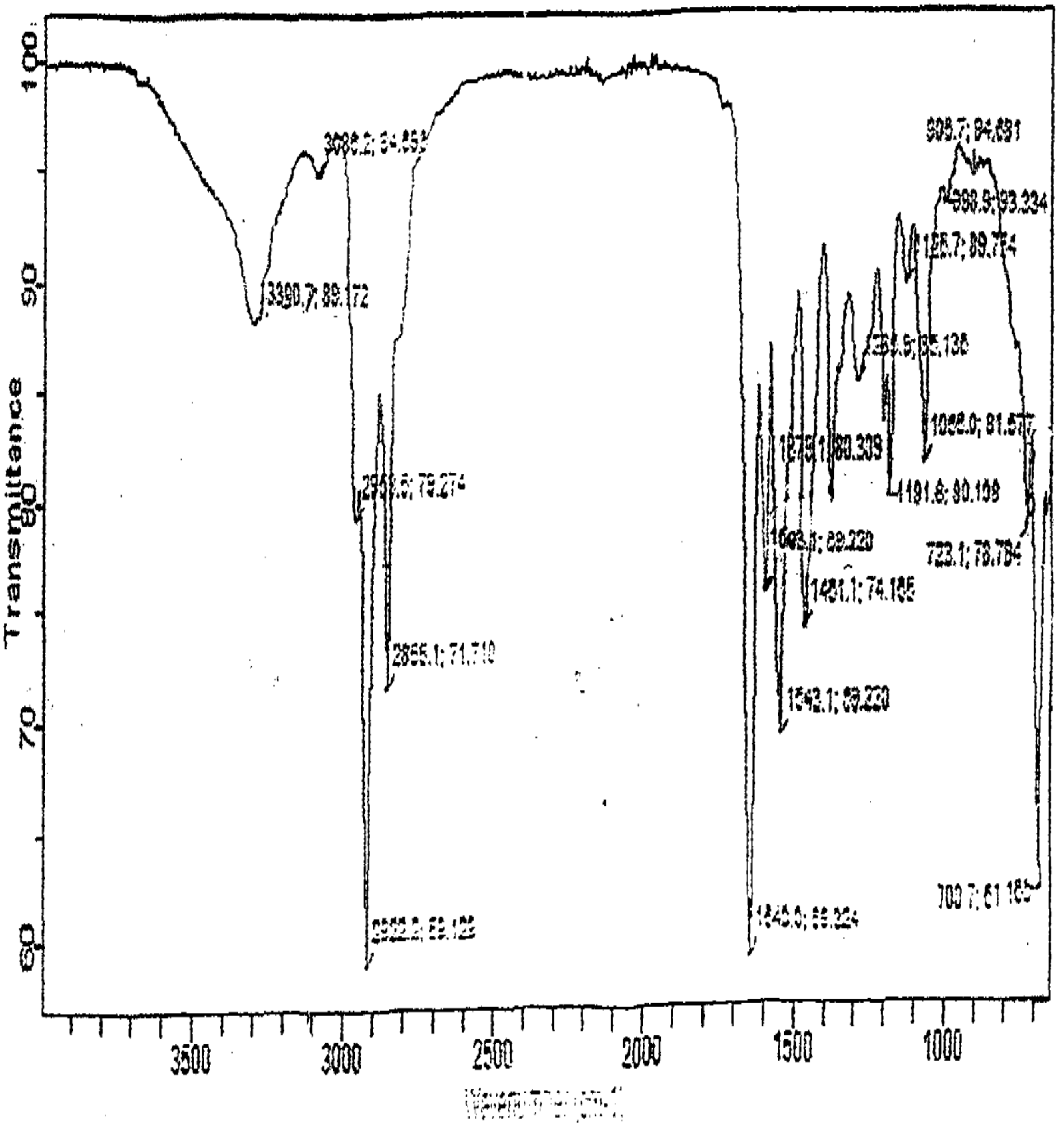


Figure 4.6 Infrared Spectrum of *N*-benzyl,*N*,*N*-diethylaminoethyllaurylamidium Chloride

Table 4.7: FTIR values obtained in *N*-benzyl-*N*,*N* diethylaminoethyllaurylamidium chloride

Peak value (cm^{-1})	Functional group	Remark
1640	C=O	Carbonyl stretch of an amide
3390	N-H	Stretch of an amide
1603	C=C	Stretch of a benzene ring
2922	-CH	Stretch of methylene group

Table 4.7: showed the values of peaks obtained in FTIR analysis of *N*-benzyl-*N*, *N*-diethylaminoethyllaurylamidium chloride. A strong sharp peak at 1640 cm^{-1} showed the N-H absorption band of an amide. Also an intense strong sharp peak at 1603 cm^{-1} indicates the absorption of C=O of benzene group.

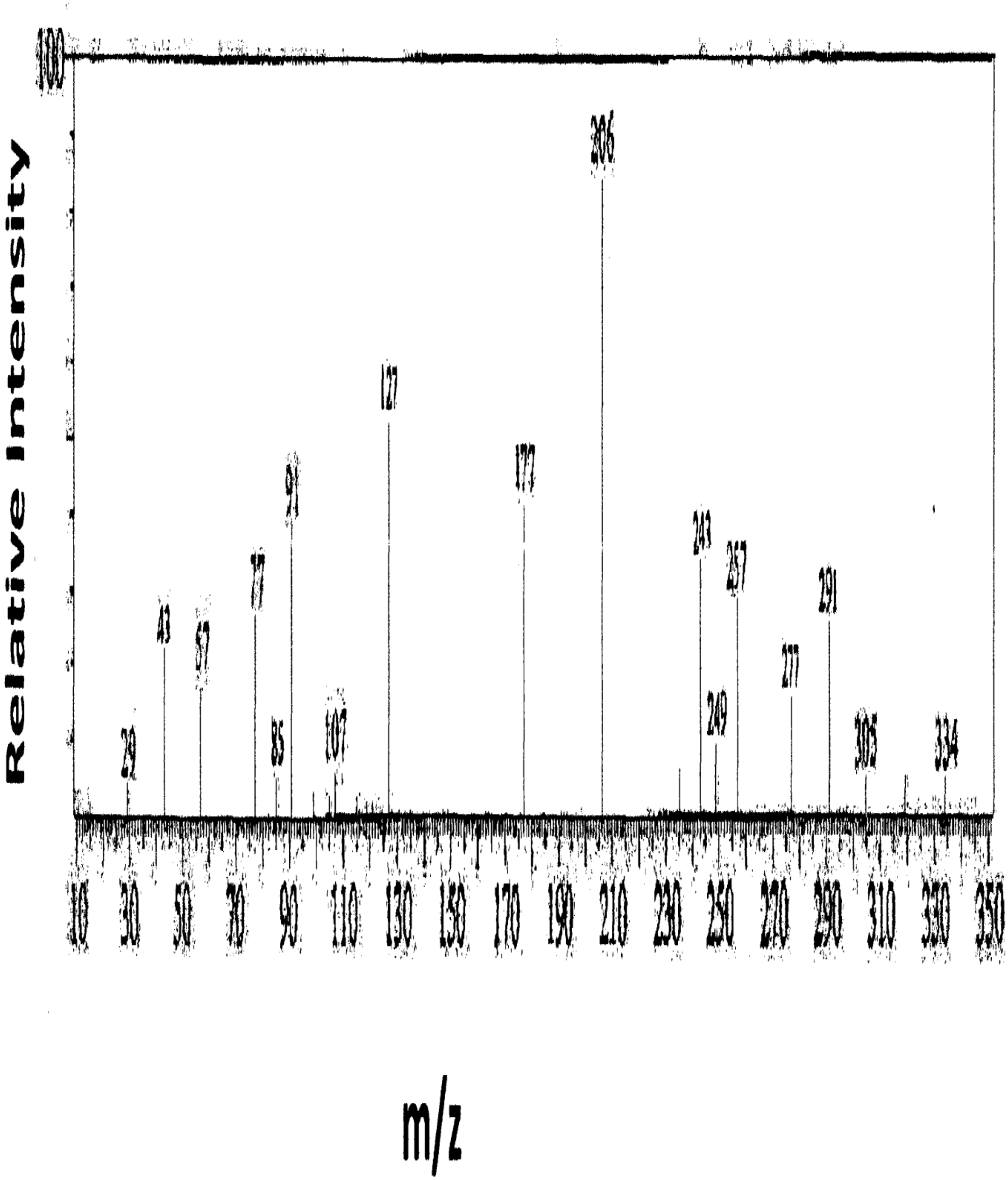


Figure 4.7 Mass Spectrum of *N*-benzyl- *N,N*-diethylaminoethyloctylamidium Chloride

Table 4.8: M/Z values obtained in mass spectrometric analysis of N-benzyl-*N*,*N*-diethylaminoethyl octyl amidium chloride

M/Z values	Fragment	Remark
334	M^+	Molecular ion peak
77	$C_6H_5^+$	Phenyl Cation
43	$^+C_3H_7$	Ethyl cation
91	$C_6H_5CH_2^+$	Benzyl cation
15	$^+CH_3$	Methyl cation
99	$^+C_7H_{15}$	Heptyl cation
206	$C_6H_5CH_2\overset{+}{N}(C_2H_5)_2CH_2CH_2\text{-}NH$	Base peak
57	$^+C_4H_9$	Butyl cation
27	$C_7H_{15}\overset{\|}{C}^+$	Hexaoxyl cation

Table 4.8: showed various fragments obtained in mass spectrometric analysis of *N*-benzyl-*N*,*N*-diethylaminoethyloctylamidium chloride. Peak with M/Z value 91 is the benzyl cation fragment. Peak at 77 is phenyl cation, also M/Z value 43 is ethyl cation. M/Z = 99 is heptyl cation. M/Z = 206 is the fragment with highest stability usually called the base peak. The molecular ion fragment peak (M^+) was found having M/Z value at 334.

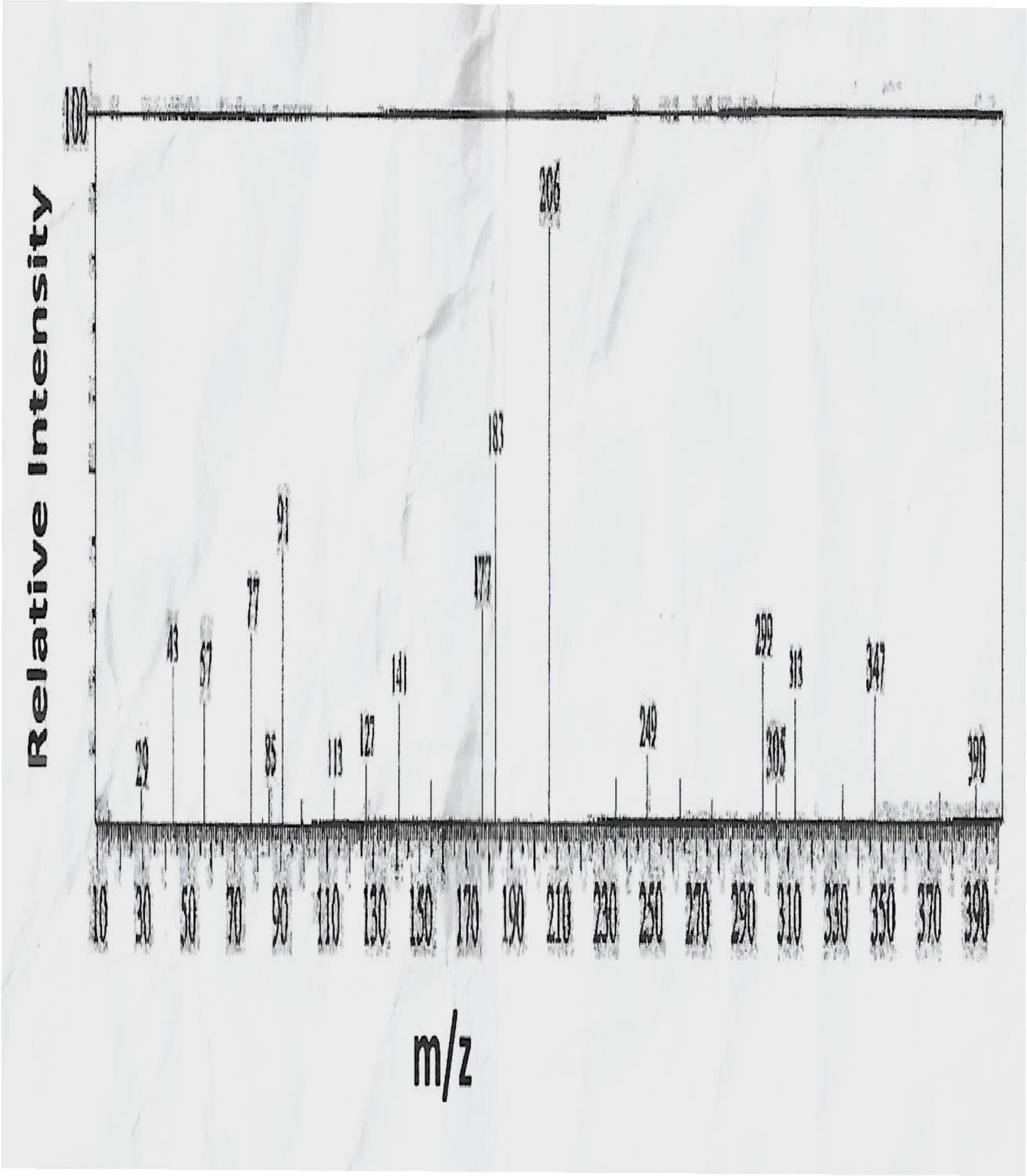


Figure 4.7 Mass Spectrum of *N*-benzyl-*N*,*N*-diethylaminoethyllaurylamidium Chloride

Table4.9: Mass-to-charge values obtained during the mass spectrometric analysis of *N*-benzyl-*N,N*-diethylaminoethyllaurylamidium chloride

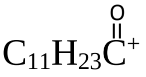
M/Z value	Fragment	Remark
113	$C_8H_{17}^+$	Heptyl cation
29	$C_2H_5^+$	Ethyl cation
43	$C_3H_7^+$	Propyl cation
77	$C_6H_5^+$	Phenyl cation
91	$^+CH_2C_6H_5$	Benzyl cation
127	$C_9H_{19}^+$	Nonyl cation
57	$C_4H_9^+$	Butyl cation
390	M^+	Molecular ion peak
206	$C_6H_5CH_2\overset{+}{N}(C_2H_5)\ CH_2CH_2NH$	Base peak
15	$^+CH_3$	Methyl cation
283	 $C_{11}H_{23}\overset{\overset{O}{\|}}{C}{}^+$	Dodecanoxyl cation

Table 4.9 showed various fragments obtained in mass spectrometric analysis of *N*-benzyl-*N,N*-diethylaminoethyllauryamidium chloride. Peak with M/Z, 113 is heptyl cations fragment. M/Z, 91 is the benzyl cation. M/Z 43 is the ethyl cation. M/Z, 57 is the butyl cation. M/Z, 15 is the methyl cation. M/Z, 283 is the fragment for dodecanoxyl cation. M/Z, 127 is nonyl cation. The M/Z value of 206 is the base peak. The molecular ion fragment has the M/Z value of 390

Table 4.10 Diameters of Zones of Inhibition of the test samples on the microbes

	Diameters of zones of inhibition (mm)				
Samples (10mg)	***Lactobacillus spp.***	***Salmonella entrica***	***Kiebsiella spp.***	***Echricha coli***	***Staphylococcus aureus***
Methyl octanoate	—	—	—	—	—
Methyl laurate	—	—	—	—	—
N–*N* diethylethylenediamine	—	—	—	—	—
Tetrahydrofuran	—	—	—		
N,*N*–diethylaminoethyl octylamide	—	—	4	—	2
N,*N*–diethylaminoethyl laurylamide	2	8	—	—	—
N–benzyl–*N*,*N*–diethyl aminoethyloctylamidium chloride	16	19	20	—	18
N–benzyl–*N*,*N*–diethyl aminoethyllauramidium chloride	17	22	20	29	19
Ampicillin (10 mg)	19	20	20	20	18

4.2 DISCUSSIONS

Solubility tests were conducted on the starting materials, intermediates and the final products. Result in **Table 4.1** showed that *N*,*N*-diethylethylene diamine (fatty amine) is soluble in water, methyl octanoate and methyl laurate are insoluble in water. The intermediates which includes,*N*,*N*-diethylaminoethyloctylamide and *N*,*N*-diethylaminoethyllaurylamide are also insoluble in water. *N*-benzyl-*N*,*N*-diethylaminoethyloctylamidium chloride and *N*-benzyl-*N*,*N*-diethylaminoethyllaurylamidium chloride which are the synthesized quaternary ammonium compounds are readily soluble as well as foaming in water which is a physical evidence/confirmatory test for the formation of quaternary ammonium salts. Also the quaternary ammonium compounds synthesized are more viscous than there intermediates (amides fatty amine) respectively.

Infrared Spectrum on Figure 4.1 showed an intense sharp peak at

1740 cm^{-1} indicating the absorption of carbonyl group (-C=O) of an ester. Peaks at 2855 cm^{-1} and 2922 cm^{-1} are for –CH stretch of methylene group of an aliphatic hydrocarbon, sharp peak at 1170 cm^{-1} indicates the absorption of –C-O stretch of an alkyl ether.

Figure 4.2; showed the infrared spectrum of *N*,*N*-diethylaminoethyl octylamide. Strong intense sharp peak at 1643 cm^{-1} indicates the absorption of carbonyl group (C=O) of an amide. Peak at 2959 cm^{-1} showed C-H stretch of a methylene group (-CH_3) of an aliphatic hydrocarbon, sharp peak at 2855 cm^{-1} indicates C-H stretch of methylene group (-CH_3) of an aliphatic hydrocarbon. A singlet sharp peak at 3295 cm^{-1} indicates N-H peak of an amide. Absence of peak at 1740 cm^{-1}and 1170 cm^{-1} indicates that C=O of an ester and C-O of an alkyl ether were totally converted to C=O of an amide at 1643 cm^{-1} and 3295 cm^{-1} for N-H stretch of an amide respectively.

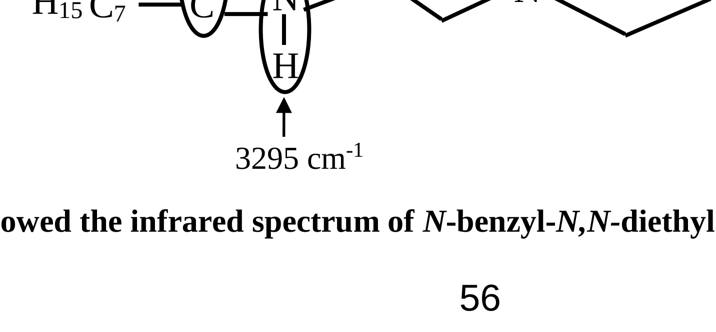
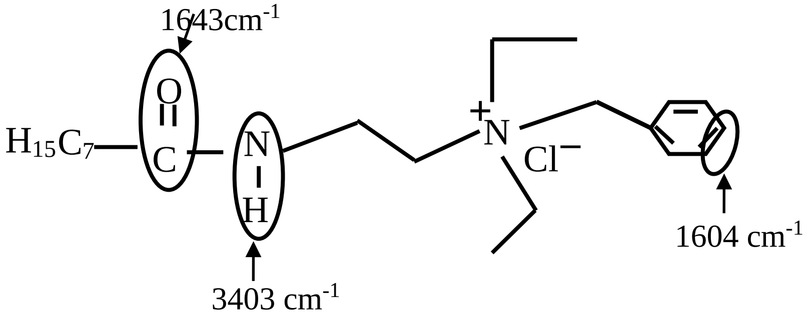


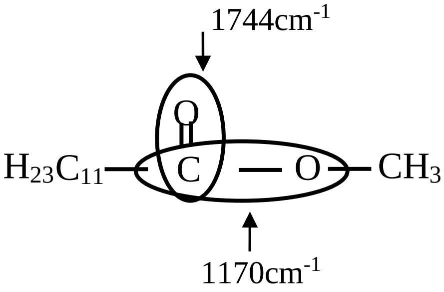
Figure 4.3 showed the infrared spectrum of *N*-benzyl-*N,N*-diethylaminoethyloctylamidium chloride.

An intense strong sharp peak at 1651 cm^{-1} indicates an absorption of the carbonyl group (C=O) of an amide. A singlet sharp peak at 3403 cm^{-1} showed an absorption of N–H band of an amide. Peak at 2926 cm^{-1} indicates C-H stretch of methylene group (-CH_2) of an aliphatic hydrocarbon. Sharp peak at 3060 cm^{-1} is a peak for C-H stretch of an aromatic compound. Intense sharp peak at 1604 cm^{-1} and 1543 cm^{-1}are peaks for absorption of C=C of an aromatic compound. A sharp peak at 2855cm^{-1} is for (-CH_3) of an aliphatic hydrocarbon.



Peaks at 730 cm^{-1} and 700 cm^{-1} are peaks for monosubstituted nature of the benzene ring. Peak at 2478 cm^{-1} indicates C-N absorption in quaternary fatty amine, peak at 1267 cm^{-1} is for absorption of C-N band of an amine. Sharp peak at 1395 cm^{-1} is for –CH_3 band of aliphatic hydrocarbon.

Figure 4.4; showed the infrared spectrum of methyl laurate (fatty ester). An intense sharp peak at 1744 cm^{-1} showed the absorption of carbonyl group (C=O) of an ester. Sharp peak at 2855 cm^{-1} and 2922 cm^{-1} are peaks for absorption of methyl group (-CH_2) of an aliphatic hydrocarbon. Also sharp peak at 1170 cm^{-1} and 1196 cm^{-1} indicates the absorption of C-O stretch of an alkyl ether.



$$H_{23}C_{11}-\overset{\overset{O}{\|}}{C}-O-CH_3$$

Figure 4.5, showed the Infrared spectrum of *N*,*N*-diethyl aminoethyllaurylamide (amide tertiary amine). An intense strong sharp peak at 1640 cm^{-1} showed the absorption of carbonyl group of an amide (C=O). Intense sharp peak at 2922 cm^{-1} showed the absorption of methylene group ($-CH_2$) of an aliphatic hydrocarbon. Also singlet sharp peak at 3380 cm^{-1} showed the peak for the absorption of N-H group of an amide. Peaks at 2855 cm^{-1} indicates C-H stretch of methyl group ($-CH_3$) of an aliphatic hydrocarbon. Absence of intense sharp peaks at 1744 cm^{-1} clearly shown that the whole carbonyl group of an ester were converted to the carbonyl group of an amide (C=O) at 1640 cm^{-1} and 3380 cm^{-1} respectively.

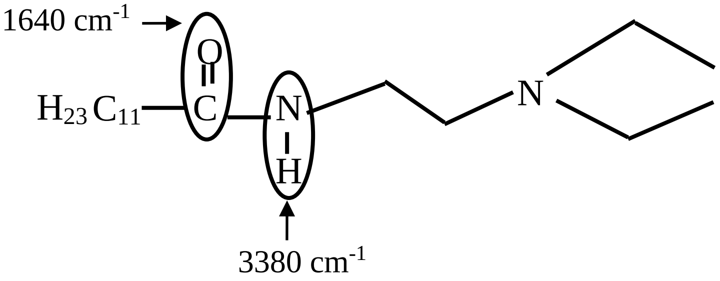


Figure 4.6; showed the Infrared spectrum of *N*-benzyl-*N*,*N*-diethylaminoethyllaurylamiduim chloride. An intense strong sharp peak at 1640 cm^{-1} showed the absorption of carbonyl group (C=O) of an amide. A singlet strong peak at 3390 cm^{-1} showed absorption of N-H band of an amide. Peak at 2922 cm^{-1} indicates C-H stretch of methylene group of an aliphatic hydrocarbon. Peak at 3016 cm^{-1} is a peak for C-H stretch of methyl group of an aromatic hydrocarbon. A sharp

peak at 2855cm^{-1} showed a C-H stretch of methyl group of an aliphatic hydrocarbon (- CH_3). Also an intense strong sharp peak at 1603 cm^{-1} and 1543 cm^{-1} indicates the absorption of C=C of an aromatic hydrocarbon. Peaks at 723 cm^{-1} and 700 cm^{-1} showed the substitution pattern on the benzene ring. It clearly shown that it is mono- substituted.

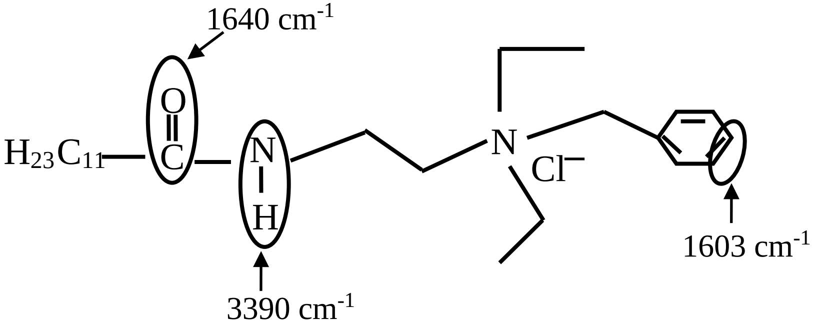
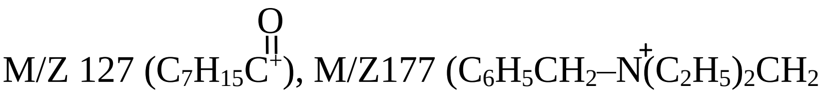


Figure 4.7; showed the result of mass spectrometric analysis of the *N*–benzyl–*N*,*N*– diethylaminoethyloctylamidium chloride.

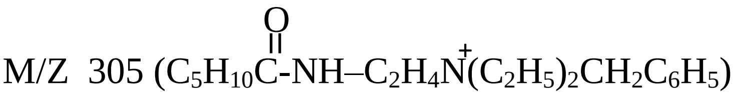
The following M/Z values for different fragment were observed.

M/Z334 (M^+) which is the molecular ion peak. M/Z 77 ($C_6H_5^+$), M/Z 43 ($^+C_3H_7$) M/Z57 ($^+C_4H_9$) ,M/Z 99 ($C_7H_{15}^+$), M/Z 91 ($C_6H_5CH_2^+$),M/Z 15 ($^+CH_3$)

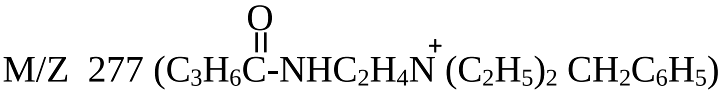


M/Z 127 $(C_7H_{15}\overset{\overset{O}{\|}}{C}{}^{+})$, M/Z177 $(C_6H_5CH_2\text{–}\overset{+}{N}(C_2H_5)_2CH_2$

M/Z 319 $(C_6H_{12}\overset{\overset{O}{\|}}{C}\text{-}NHCH_2CH_2CH_2\overset{+}{N}(C_2H_5)_2CH_2C_6H_5$



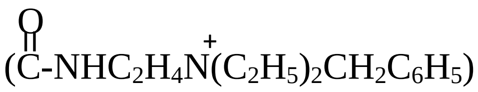
M/Z 305 $(C_5H_{10}\overset{\overset{O}{\|}}{C}\text{-}NH\text{–}C_2H_4\overset{+}{N}(C_2H_5)_2CH_2C_6H_5)$



M/Z 277 $(C_3H_6\overset{\overset{O}{\|}}{C}\text{-}NHC_2H_4\overset{+}{N}\,(C_2H_5)_2\,CH_2C_6H_5)$

M/Z 257 $(C_7H_{15}\overset{\overset{O}{\|}}{C}\text{–}NHC_2H_4\overset{+}{N}(C_2H_5)_2CH_2)$

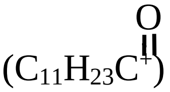
M/Z 243 
$(C_7H_{15}\overset{\overset{O}{\|}}{C}\text{-}NHC_2H_4\overset{+}{N}(C_2H_5)_2)$

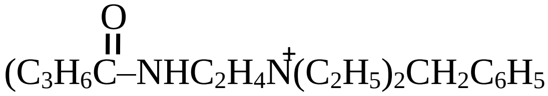
M/Z 235 
$(\overset{\overset{O}{\|}}{C}\text{-}NHC_2H_4\overset{+}{N}(C_2H_5)_2CH_2C_6H_5)$

M/Z 206 $(C_6H_5CH_2\overset{+}{N}(C_2H_5)_2CH_2CH_2\text{–}NH)$ which is the base peak.

Figure 4.8 showed the result of mass spectrometric analysis of *N*-benzyl–*N*,*N*- diethylaminoethyllaurylamidium chloride. The following M/Z values for different fragmenst were observed;

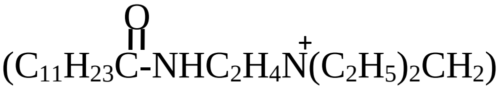
M/Z 113 $(C_8H_{17}^+)$,	M/Z 43 $(C_3H_7^+)$
M/Z 29$(C_2H_5^+)$	M/Z 57 $(C_4H_9^+)$
M/Z 77 $(C_6H_5^+)$	M/Z 91 $(^+CH_2C_6H_5)$
M/Z 85 $(^+CH_2CH_2CH_2CH_2CH_2CH_3)$	
M/Z 127 $(C_9H_{19}^+)$,	M/Z 141 $(^+C_{10}H_{21})$

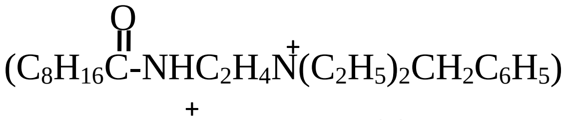
M/Z 177 $(C_6H_5CH_2\ \text{–}\overset{+}{N}(C_2H_5)_2CH_2)$M/Z 183 
$(C_{11}H_{23}\overset{\overset{O}{\|}}{C^+})$

M/Z 277 
$(C_3H_6\overset{\overset{O}{\|}}{C}\text{–}NHC_2H_4\overset{+}{N}(C_2H_5)_2CH_2C_6H_5$

M/Z 299 
$(C_{11}H_{23}\overset{\overset{O}{\|}}{C} – NHC_2H_4\overset{+}{N}(C_2H_5)_2)$

M/Z 305 
$(C_5H_{10}\overset{\overset{O}{\|}}{C}\text{-}NHC_2H_4\overset{+}{N}(C_2H_5)_2CH_2C_6H_5)$

M/Z 313 
$(C_{11}H_{23}\overset{\overset{O}{\|}}{C}\text{-}NHC_2H_4\overset{+}{N}(C_2H_5)_2CH_2)$

M/Z 347 
$(C_8H_{16}\overset{\overset{O}{\|}}{C}\text{-}NHC_2H_4\overset{+}{N}(C_2H_5)_2CH_2C_6H_5)$

M/Z 206 ($C_6H_5CH_2$-N($_6CH_5$)$_2CH_2CH_2$NH) Which is the base peak.
M/Z 390 (M^+), this is the molecular ion peak.

Table 4.10 Clearly shown the diameters of zones of inhibition of the test samples on various micro-organisms. From the Table, it can be clearly stated that methyl octanoate and methyl laurate which are fatty esters has no anti-microbial activity on the tested micro-organisms. Then *N*,*N*-diethylethylenediamine has no anti microbial effect on *lactobacillus spp.*, *Salmonella enterica, staphylococcus aureus*. However, it has a minor effect on *Echerichia coli and Kiebsiella spp*. which can be categorized as insignificant effect. Tetrahydrofuran which was also a reagent used in the reaction process from **Table 4.10** showed clearly that this solvent has no antimicrobial effect on the tested micro- organisms. *N*,*N*-diethylaminoethyloctylamide (amide tertiary amine) in the **Table 4.10** showed entirely no antimicrobial effect on *Salmonella enterica, lactobacillus spp. Echerichia coli*, however, it showed insignificant antimicrobial activity on *staphylococcus aureus and Klebsiella spp.*

Also *N*,*N*-diethylaminoethyllaurylamide showed no significant antimicrobial activity on the tested micro-organisms except on *Salmonella enterica* where it showed zone of inhibition up to 8mm.

N-benzyl-*N*,*N*-diethylaminoethyloctylamidium chloride which is one of the synthesized quaternary ammonium compounds showed an excellent anti-bacteria activity on both gram-positive and gram-negative bacteria. This quaternary ammonium salt showed highest efficacy/antimicrobial activity on *Klebsiella spp*. with diameter of zone of inhibition of 20 mm equivalent to the diameter of zone of inhibition of the control. Also this quat has entirely no effect on *Echerichia coli*. Also *N*-benzyl-*N*,*N*-diethylaminoethyllaurylamidium chloride exhibits excellent antibacterial activity to both the gram-positive and gram-negative bacteria. The effectiveness of *N*- benzyl-*N*,*N*-diethylaminoethyllaurylamidium chloride is much more profound in *Echerichia coli and Salmonella enterica* with diameters of zones of inhibition of 28 mm and 22 mm respectively, which is higher to the diameters of zone of inhibition of the control on those bacteria.

Also the diameters of zone of inhibition obtained from the antibacterial studies of *N*-benzyl-*N*,*N*-diethylaminoethyllaurylamidium chloride is greater to those of the *N*-benzyl-*N*,*N*-diethylaminoethyloctylamidium chloride. This may be attributed to the longer side alkyl chain length of this quaternary ammonium salt which allows it to adsorb over a wider area of the cell membrane thereby disrupting the cell activity of the bacterial which eventually inhibit the bacterial growth. From the result on **Table 4.10.** The synthesized quaternary ammonium salts have much more effective antibacterial activity on all the tested micro organisms when compared to the standard antibiotic (control)

CHAPTER FIVE

5.1 CONCLUSION

Quaternary ammonium compounds were synthesized from fatty ester/fatty amine reaction. FTIR analysis was conducted on the starting materials, intermediates (amide tertiary amine) and the final products. Results obtained showed the disappearance of C=O and C–O peaks of an ester during aminolysis. Other vital functional groups such as C=C of aromatic compound, C–N for quaternary fatty amine and -CH_3 of an aromatic hydrocarbon were observed in the products. Mass spectrometric analysis of the final products were conducted and results showed the Molecular Ion peak (M+) which is the molecular weights of the products. Vital fragments such as **base peaks** were observed for both quats, other fragmented radical cations were observed.

Anti-microbial studies of the starting materials, the intermediate and the final products were evaluated. Based on the results obtained, we have been able to establish that *N*-benzyl-*N*-diethylaminoethyloctylamidium chloride and *N*-benzyl-*N*,*N*-diethylaminoethyllauryamidium chloride exhibit excellent anti-bacterial activity on various tested micro–organisms. It also showed that *N*-benzyl-*N*,*N*-diethylaminoethyllauryamidium chloride which is the quat with a longer side alkyl chain length has much higher anti-microbial potential towards gram–positive and gram–negative bacteria which goes in line with the fact that quaternary ammonium salts with a larger side n–alkyl chain exhibits higher anti-microbial potential. This increase in anti-microbial potential may be attributed to the ability of the quat to adsorb over a larger part of the bacterial cell membrane thereby disrupting the cell activity hence a greater antimicrobial activity.

Finally, inclusion of amide group which breaks the long side alkyl chain length and the positive nitrogen center did not deactivate the anti-microbial potentials of these quaternary ammonium compounds.

5.2 RECOMMENDATION

Quaternary ammonium compounds should be synthesized using natural occurring oils due to their cost effectiveness. The anti-microbial activities of the quats synthesized from these natural oils should be compared to determine the effect of variation in the alkyl chain length on some selected micro–organisms

5.3 CONTRIBUTION TO KNOWLEDGE

This research work has revealed that the inclusion of the amide functional group as part of the molecular framework of the quaternary ammonium salts enhanced the elimination of bacteria resistance to conventional quaternary ammonium compounds.

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