Antibacterial Activity of Quaternary Ammonium Salt from Diethylaminoethyl Methacrylate

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Abstract: A quaternary ammonium salt was synthesized from diethylaminoethyl methacrylate (DEAEMA) by quaternization with hexadecyl bromide. The resultant compound (**Am-h**) was characterized by FTIR and NMR spectroscopy. Its bactericidal activity was evaluated by determining minimum inhibitory concentration (MIC) values and inhibitory zone diameter against gram positive bacteria (*Streptococcus sp.*) and gram negative bacteria such as *Acenito baumannii*, *Klebsiella pneumoniae* and *Proteus sp.* respectively. The results showed that the MIC values of the synthesised compound (**Am-h**) were 2 µg/mL against *Acenito baumannii*, *Klebsiella pneumoniae*, *Proteus sp.* and *Streptococcus sp.*

Keywords: Quaternary ammonium salts, DEAEMA, Antibacterial activity, MIC.

Introduction

Recently, serious infections of microbe have become a social problem¹. Therefore, safe examination of microbe is very important to human health care at the present time, the most commonly used antimicrobial reagent include cationic active molecules such as quaternary ammonium salts. They attack microorganisms by reacting with specific cellular components or disturbing their metabolism. Their mode of action is selective and independent of pH. The quaternary ammoniums, endowed with anti-microbial properties belong to the family of cationic surfactants². They are used in the hygiene field, made of hydrophobic cationic part

(the functional of the molecule consisting of nitrogen atom connected to four aliphatic or aromatic groups) and an anion (consisting of either inorganic anion of low molecular weight or an organic anion of high molecular weight) part. These products are stable in acidic and basic medium and are usually soluble in water and alcohols. Their derivatives are bactericidal to a wide variety of bacteria, especially gram positive they often have fungicides, algacides and virucides properties³. Their fields of application are wide; we can cite for example, disinfection of water, surfaces and instruments, antiseptic of healthy skin and injured skin and disinfection of the mucosa, curator of pharmaceutical preparations⁴. There are many other uses such as agriculture, veterinary medicine, cosmology⁵, food, textile and leather industry. We have prepared the quaternary ammonium salt using a tertiary amine diethylaminoethyl methacrylate (DEAEMA) and halogen derivative of hexadecyl bromide.

Experimental

DEAEMA (Aldrich, 99%) posses the following characteristics: B.p. 80 °C/10 mm Hg, d 0.922 g/mL, 25 °C, n_D^{20} 1.444 and hexadecyl bromide (Aldrich, 97 %) has the following characteristics: B.p 190 °C/11 mm Hg, d 0.999 g/mL, 25 °C, n_D^{20} 1.4609.

Synthesis of quaternary ammonium salt

In a 100 mL flask equipped with a magnetic stirrer, a cooler and a thermometer, 10 mL of DEAEMA, an expected amount of hexadecyl bromide (hexadecyl bromide / DEAEMA (1.1/1 mol)) and a small amount of hydroquinone were charged. Then 29.2-36.4 mL of acetonitrile was added as solvent. After that, the mixture was stirred with constant interval at 40-45 °C. Finally, white needle crystals were obtained by cooling and filtering the reaction solution and then washed with dry ether several times and dried under vacuum at room temperature⁶. The yield of the reaction is about 73% and the structure of the quaternary ammonium salt (Am-h) obtained is showed in Scheme 1.

Scheme. 1. Structure of quaternary ammonium salt

Characterizations

IR spectra of synthesized product was recorded from 400 to 4000 cm⁻¹ on Shimadzu 2401 FTIR spectrophotometer using KBr pellets with 32 scanning. ¹H NMR and ¹³C NMR spectra were collected on a FT-NMR (BRUKER-300 MHz) spectrometer using CDCl₃ as solvent. The ¹H NMR and ¹³C NMR spectrum of ammonium salt (**Am-h**) obtained are shown in the Figures 2 and 3 respectively.

Minimum inhibitory concentration (MIC) and the inhibition zone diameter test

In this work we used the bacteria that have been provided us by the microbiology laboratory of the academic hospital center of Oran (31000) in Algeria. The origin of every strain is shown in the Table 1. They are descended of the human biologic liquids. The substance was

received as solid. A starting solution was prepared to a concentration of 0.1 mg/mL from 0.03 g of product. Dilutions were done in Eppendorf tubes in the following concentrations: 2, 4, 8 and 16 µg/mL; this witnessed the solvent used for the solubility of the product. After the preparation of the concentrations, discs made of paper were introduced in every tube and then sterilized to the autoclave to 120 °C during 20 minutes. After sterilization, the tubes were introduced in the oven so that the solvent evaporates.

Table. 1 Bacterial strains used and origin

Bacterial strain	Origin
<i>Klebsiella pneumoniae</i>	Pus
<i>Acinetobacter baumannii</i>	Urine
<i>Proteus sp.</i>	Blood culture
<i>Streptococcus sp.</i>	Endotracheal tube

Preparation of the growth medium

Plates were poured into Petri dishes to a thickness of 4 mm and left to dry.

Subculture on nutrient agar

Bacterial strains were inoculated on GN medium. For *Streptococcus sp.*, bacterial strain was inoculated on GN medium; boxes were made in an oven at 37 °C for 24 h to obtain a pure culture.

Phase planting

In the laboratory of the Hospital of Oran, the sensitivity was done by diffusion method. Discs were impregnated with antibacterial solution and measurement of diameter was made using Kirby Bauer method.

Sowing

Initially, the Petri dishes must contain the MH medium (4mm thick). For *Streptococcus sp.* using the MH medium supplemented with blood.

Application of the discs

To test our product, towards the four bacterial strains, we used the discs to the required concentration with a sterilized clip.

Incubation

For the *Streptococcus sp.* the incubation was done in a jar at 35 °C for 24 h. Then we measured inhibitory zone diameters (in millimetre) with a calliper to evaluate the efficiency of our product to test.

Results and Discussion

Spectral data

FT IR : 2865-2979 cm⁻¹ (saturated C-H stretching vibration), 1720.40 cm⁻¹ (C=O absorption), 1641.30 cm⁻¹ (C=C stretching vibration), 730 cm⁻¹ (CH₂)_n n ≥ 2 rocking vibration.

(Am-h), **¹H NMR** (CDCl₃, δ) : 6.10 (1H, a), 5.64 (1H, b), 1.92 (3H, c), 4.65 (2H, d), 3.95 (2H, e), 3.63 (2H, f), 3.37 (2H, g), 1.83 (2H, h), 0.83-1.44 (35H, j-k)⁷. The yield of the synthesized product was 70%.

(Am-h), **¹³C NMR** (CDCl₃, δ) : 127.25 (C, 1), 135.16 (C, 2), 18.20 (C, 3), 166.43 (C, 4), 57.89 (C, 5), 59.02 (C, 6), 55.02 (C, 7), 8.30 (C, 8), 56.65 (C, 9), 22.63 (C, 10), 26.43 – 33.96 (C, 11 - C, 12)⁷.

Figure 2. ^1H NMR spectra of (Am-h)

Scheme 2. Structure of quaternary ammonium salt (Am-h)

Figure 3. ^{13}C NMR spectra of (Am-h)

Scheme 3. *N,N*-diethyl-*N*-(2-(methacryloyloxy)ethyl)hexadecan-1-ammonium

According to the spectroscopic data, we propose the structure of the ammonium salt in the Scheme 1. After having passed the stage of the purification of the bacterial strain and their conservation for a possible use; the Mueller-Hinton medium has been prepared for the test of the bacterial strains sensitivity. The used method was made by test disc paper, 0.1 mg/mL of the dilute substance. The results of the bacteria sensitivity are presented in the Table 2.

Table 2. Evaluation of the minimum inhibitory concentration (MIC) and the inhibition zone diameter of (Am-h) compound against the bacteria (*Klebsiella pneumoniae*, *Proteus sp.*, *Acinetobacter baumannii*, *Streptococcus sp.*).

Bacteria	<i>Klebsiella pneumoniae</i>		<i>Proteus sp.</i>		<i>Acinetobacter baumannii</i>		<i>Streptococcus sp.</i>	
	MIC	Diam	MIC	Diam	MIC	Diam	MIC	Diam
Am-h	2 µg/mL	7 mm	2 µg/mL	6 mm	2 µg/mL	6 mm	2 µg/mL	7 mm

The chemical substances synthesized in our laboratory, has been tested to enhance its antibacterial power against gram negative bacteria (*Klebsiella pneumoniae*, *Proteus sp.*, *Acinetobacter baumannii*) and gram positive bacteria (*Streptococcus sp.*). The detection of the sensitivity of these pathogenic bacteria against the tested molecule is made by the realization of an antibiogram. The results show that the product (Am-h) was more efficient for the inhibition of all the bacteria tested. In previous studies the treatment of the bacteria *E. Coli* and *S. aureus* by the dimethylaminoethyl methacrylate (DMAEMA) quaternized by hexadecyl bromide showed that the resulting ammonium salt possessed a minimum bactericidal concentration (MBC) values of 12 µg/mL and inhibitory zone diameters of 13.4 and 14.5 mm respectively⁶.

The high affinity of cationic agents for the membrane suggests a strong interaction with the negatively charged polar head group of phospholipids, an affinity moderated by alkyl chain length in quaternary ammonium salts⁸. Changes in phospholipids packing and phase separation arise from electrostatic interactions⁹. For the quaternary ammonium salts a successive interplay of polar and hydrophobic interactions with the membrane may determine the progress of antibacterial events¹⁰.

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