# Synthesis of oligoguanidine - Based on polycondensation and compare their Antimicrobial Activities with Chloramine B

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***Abstract—*** *Oligoguanidine with chain extension were synthesized by condensation and cross-linking polymerizations in an attempt to increase molecular weight and charge density of the anti microbial oligoguanidine. The reactions are procedure at 170 $^oC$ for 5h with ratio molar of HMDA: GHC = 1: 1.1, refined by vacuum filter at 60 $^oC$. The Oligoguanidine OHMG.HCl synthesized has molecular weight equal 521 g/mol, chain structure and good water solubility. The comparison results of the antimicrobial activities of oligoguanidine with chloramine B indicated that MIC index of OHMG.HCl is 5ppm during MIC index of chloramine B is 50 ppm; MBC of OHMG.HCl is 12 minutes and MBC of chloramine B is 11 minutes; The SEM, TEM imagines exhibited clear biocide, low residue microorganism of OHMG.HCl. The LC-MS spectrum indicates the presence of haloacetic acid following the bactericidal action of chloramphen B, while OHGM.HCl is not available. This result confirms the environmental friendliness of oligoguanidine.*

***Keywords— Oligoguanidine, Oligohexamethylene guanidine hydrochloride, antimicrobial polymer.***

## I. INTRODUCTION

Antimicrobial polymer is a range of agents with bacteria or fungi inhibition ability, which has been used widely in plastic, textile, water purification, and so on. Most of antimicrobial polymers bear cationic groups along polymer chains, thus facilitating the adsorption of the polymer onto bacteria surfaces and further inhibiting the growth of bacteria via various mechanisms. As one of the cationic antimicrobial polymers, the guanidine-based polymer has attracted substantial interests due to its wide spectrum antimicrobial activity, excellent biocide efficiency and nontoxicity. Oligoguanidine is usually synthesized by polycondensation between guanidinium and diamine, The chain cross-linking would occur if prolonging reaction time and increasing reaction temperature. At low molecular weight constrains the guanidine polymer as antimicrobial agent would be used [6],[12] Guanidine polymer inhibits bacterial growth by attacking them through electrostatic attraction between cationic guanidio-groups and anionic groups on the cell surface of bacteria. After attaching to bacteria cells, guanidine polymer induces bacterial membrane collapsed and intracellular components leaked. Therefore positive charge density, molecular weight and molecular structure of guanidine polymer would influence adsorption on bacterial surface and inhibition efficiency [4], [7], [10]. At present, antibacterial substances such as atomic chlorine, chloramine B are used in practice. However these antibacterial substances are often used in large quantities, especially when reducing, especially after in water disinfected, there are a residue and by-product of chlorine compounds that are directly harmful to the health of humans and organisms [1]. In this article, a method synthesizing oligohexamethyleneguanidine hydrochloride (OHMG.HCl) synthesis, and evaluating its disinfection efficacy over chloramine B are presented in detail.

## II. EXPERIMENTAL

### 2.1 Materials

Guanidine hydrochloride (GHC), purity ≥ 99.0% (Merck, Germany). Hexamethylene diamine (HMDA), purity ≥ 99.0% (Merck, Germany). Chloramine B, purity ≥ 99.0% (Pharmacy Center 2 Company).

Escherichia coli (E. coli) were obtained from the National Institute of Hygiene and Epidemiology; and the E. coli was cultured overnight at 37 $^oC$ in LB Broth prior to use.

### 2.2 Synthesis method of Oligoguanidine

Oligo guanidine was synthesized by a two-step polymerization. The poligo was obtained by polycondensation from amine and guanidine hydrochloride. The detailed procedures are as follows: HMDA: GHC = 1: 1.1 ratio molar ratio of chemicals containing guanidine groups and amino groups were added into a 500 mL three-neck flask, the reagents were stirred mechanically and reaction temperature was increased step by step, first kept at 100$^oC$ for 1 hr, then 170 $^oC$ for 5 hrs, until the stirrer could continue for the high viscosity of polymer. The reactant was further diluted with de-ionized water to 20% (wt) and temperature was increased to 60 $^oC$ for 6 hrs; then the reaction was stopped and the modified guanidine-based polymer was obtained. De-ionized water was used as a solvent for the polymer [3], [9].

The obtained oligoguanidine by polycondensation between hexamethylene diamine and guanidine hydrochloride is as following scheme 1 [8], [11].

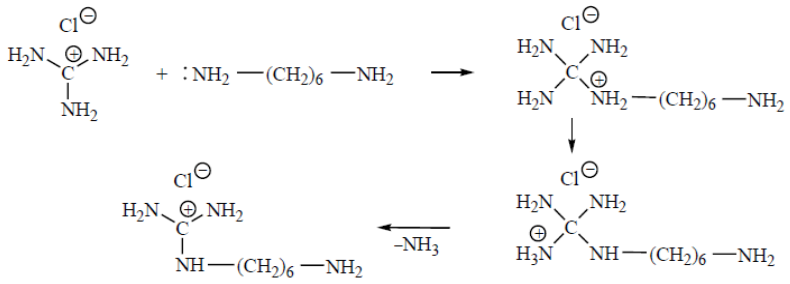


**FIGURE 1. SCHEME OF OLIGOGUANIDINE SYNTHESIS**

The oligoguanidine synthesis from GHC and HMDA can be carried out by nucleophilic mechanisms. The reaction between the monomers is implemented by bonding the free electron pairing of the nitrogen atom in the HMDA molecule into the positive charge of the carbon atom in the GHC (with the proton transfer from the amino group HMDA into the amine group of GHC). Under the reaction medium, the polymer chain continues to grow and will be limited by the solubility of the oligoguanidine. Therefore, the experimental conditions of this method are very important, which affect the quality and the characteristics of OHMG.HCl.

### 2.3 Characterization of Oligoguanidine.

GPC (Pump: Waters 600E System Controller; Detector: Waters 410 Differential Refractometer; Colums: Ultrahydrogel 250 and 500) on Shimadzu CLASS-VIP V6.14SP1 gel electrophoresis equipment, at the Faculty of Chemistry, Hanoi National University, was used to measure molecular weight and $^{1}$H-NMR, COSY, HSQC spectra was used to determine structure of of Oligoguanidine.

### 2.4 Minimum Inhibition Concentration (MIC).

The minimum inhibitory concentration (MIC) is the lowest concentration of a chemical which prevents visible growth of a bacterrium. A serial dilution method was used to determine MIC of Oligoguanidine against *E. coli*. Fresh cultured *E. coli* was diluted with LB broth to 106CFU/ml which was reckoned by an OD600-Con standard curve. Oligomers dissolved in sterile deionized water were serially diluted in LB broth, the same amount of E. coli were put into polymer broth solution. Both seeded tubes and growth controls were incubated at 37 $^{o}$C for 18h. MIC was the lowest concentration inhibiting visible growth of bacteria compared with control samples.

### 2.5 The time for killing bacteria by minimum bacteria concentration (MBC).

The minimum bactericidal concentration (MBC) is the lowest concentration of an antibacterial agent equired to kill a particular bacterrium. The minimum antibacterial concentration index was tested on Ecoli bacteria with a concentration of 1 to 100 ppm of product in aqueous solvents. Initiate bacterial culture is implemented with 5 mL of product solution in test tubes, then the tubes are being shaken before incubation at 37 ° C for 48 hours. To test the growth of bacteria in growth medium with and without bacterial growth by determining the growth of the organism together with the turbidity in the tubes.

### 2.6 Study of microstructure of oligome by SEM, TEM

Scanning electron microscopy (SEM) to evaluate the surface structure of the object by magnification to tens of thousands. he images obtained from this method, combined with the results of other methods, can give an estimate of the surface structure of the sample about size, distribution…

Transmission electron microscopy (TEM) is a device for the study of sample microstructure, using high energy electron beams, penetrating through thin sample specimens and using magnetic lenses. The TEM method can produce solid-state images with high contrast and resolution, and at the same time provide easy information about the structure of the material. TEM gives the real picture of the structure inside a solid, so it gives more information.

**Experimental:** The SEM image, TEM image used in this thesis was recorded on JEOL 1010 (Japan) at National Institute of Hygiene and Epidemiology.

## III. RESULTS AND DISCUSSION

### 3.1 Result of synthesis and Characterization of Oligoguanidine.

#### 3.1.1 The molecular weight (Mw) of Oligoguanidine was presented in Table 1.

**TABLE 1**
**RESULT OF THE MOLECULAR WEIGHT, CHARGE DENSITY**

| Value | pre-oligomer | Oligomer |
|---|---|---|
| Mw (g/mol) | 160 ± 10 | 521 ± 20 |
| Electric charge density (meq/g) | 2.45 ± 0.11 | 3.60 ± 0.13 |

#### 3.1.2 The results of $^{1}$H-NMR

Nuclear magnetic resonance spectra ($^{1}$H-NMR) at 500 MHz of Oligoguanidine (OHMG.HCl) in the synthesized product showed in Figure 2 including peak at 7.490 ppm - corresponding to the group - NH-C (NH) -NH-; peak at 1,809 ppm - corresponding to the group (- $CH_2$-)n.

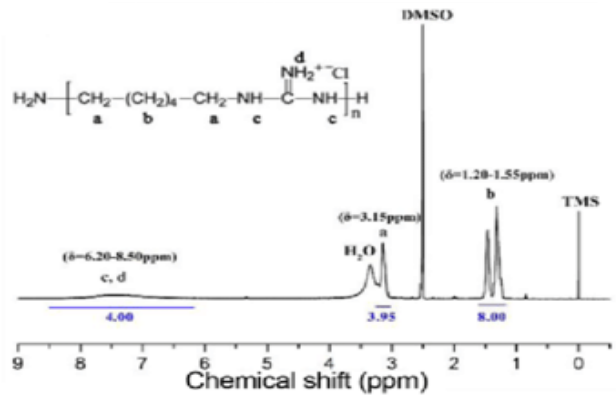



**FIGURE 2. H-NMR SPECTRUM OF TH SYNTHESIZED OHMG.HCL**

The results of this study have shown that OHMG.HCl can be synthesized from guanidine hydrochloride and hexamethylene diamine by melting molten condensation. Figure 2 confirmed the synthesized product having a chain structure.

### 3.2 The result of antimicrobial Effects of oligoguanidine OHMG.HCl and chloramine B in the sample

The antibacterial effects of OHMG and were compared to chloramine B baesd on MIC, MBC index, antibacterial time SEM and TEM spectra

#### 3.2.1 Results of MIC index

The results of determining MIC are given in Table 2. This is shown that, for the concentrations of OHMG.HCl > 5 ppm there is no growth of the E. coli strain in, while for chloramine B is 50 ppm.

**TABLE 2**
**MIC VALUE OF OHMG.HCL AND CHLORAMINE B**

| Concentration (ppm) | MIC value | |
|---|---|---|
| | OHMG.HCl | Chloramine B |
| 100 | - | - |
| 50 | - | - |
| 10 | - | + |
| 5 | - | + |
| 3 | + | + |
| 1 | + | + |

***(-): no growth of bacteria.***
***(+): there is the development of bacteria***

### 3.2.2 The time for killing bacteria (MBC)

The results are shown in Table 3. It was shown that the MBC index of OHMG.HCl was 12 minutes and that of Chloramine B was 11 minutes.

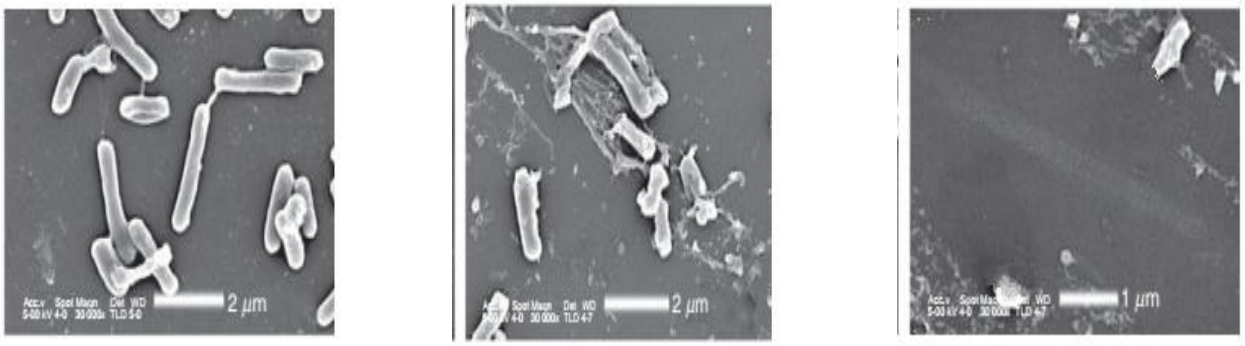
**TABLE 3**
**MBC RESULT OF OHMG.HCL AND CHLORAMINE B**

| Time (minute) | MBC Value | |
|---|---|---|
| | OHMG.HCl (5 ppm) | Chloramine B (50 ppm) |
| 1 | + | + |
| 2 | + | + |
| 4 | + | + |
| 6 | + | + |
| 8 | + | + |
| 10 | + | + |
| 11 | + | - |
| 12 | - | - |
| 13 | - | - |
| 14 | - | - |
| 15 | - | - |

***(-): no growth of bacteria.***
***(+): there is the development of bacteria***

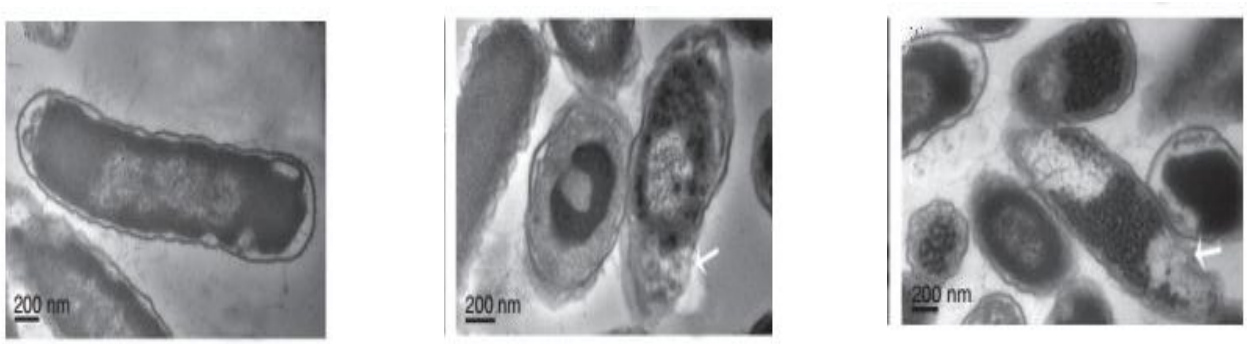
### 3.2.3 Results of SEM and TEM imagines

The SEM and TEM measurements of antimicrobial Effects of samples showed in Figure 3 and 4




**(a) Control sample** **(b) Chloramine B** **(c) OHMG.HCl**

**FIGURE 3. SEM IMAGINE OF THE RESIDUE OF E.COLI AFTER DISINFECTION**




**(a) Sample with 5 ppm OHMG.HCl after 1 minute** **(b) Sample with 5ppm OHMG.HCl after 5 minute** **(c) Sample with 5ppm OHMG.HCl after 12 minute**

**FIGURE 4. TEM IMAGINE SAMPLE OF E.COLI USING 5PPM OHMG.HCL**

SEM images showed the extent of the damage of the cells after exposured to different doses of OHMG.HCl, and only high concentrations of OHMG.HCl could result in concave collapses in most of the treated cells (Fig. 3c). In accordance with the SEM results, the TEM results showed that a low concentration of OHMG.HCl mainly damaged the outer membrane structure, while no significant damage to the intracellular structure was observed. In contrast, after exposure to a high concentration of OHMG.HCl, although the general morphological structure of the cells was still retained, the integrity of the cell wall layer structure was destroyed, most of which was collapsed, and obvious gaps could be seen even in some cells. It is concluded that the membrane damage was dose-dependent and local, and the disorganization of the membrane lipid structure was local and not global; the concave collapses observed in SEM and gaps observed in TEM indicated that the formation of local pores was possible. Bisbiguanides antiseptics, alexidine and chlorhexidine, could be adsorbed to E. coli cells and to isolated membrane components (Fig. 4a), which could lead to phospholipid phase separation and domain formation. So, it was possible that OHMG.HCl was adsorbed (Fig. 4b), bound to the phospholipid of cell membrane and led to the formation of the local pores (Fig. 4c); as a result, the cells lost their viability.

Oligoguanide were rapidly adsorbed to the negatively charged bacterial cell surface, then impaired the outer membrane and caused domain formation of the acidic phospholipids of the cytoplasmic membrane with an increase in the cytoplasmic membrane permeability; suggested that the uptake of chlorhexidine by *E. coli* was very rapid. concluded that death of cells and cytoplasmic membrane damage were directly associated and were a direct result of biocide action rather than mediated through the induction of autolytic enzymes.

### 3.2.4 Results of LC-MS

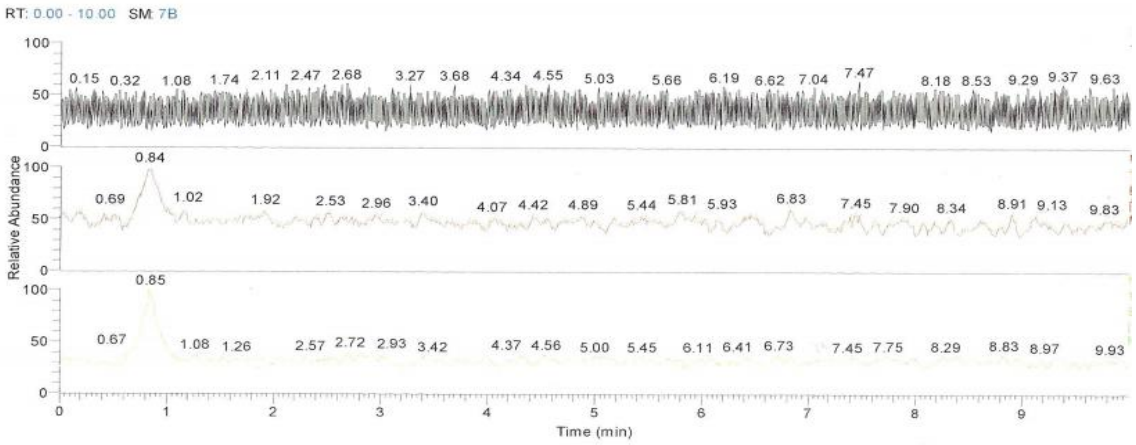
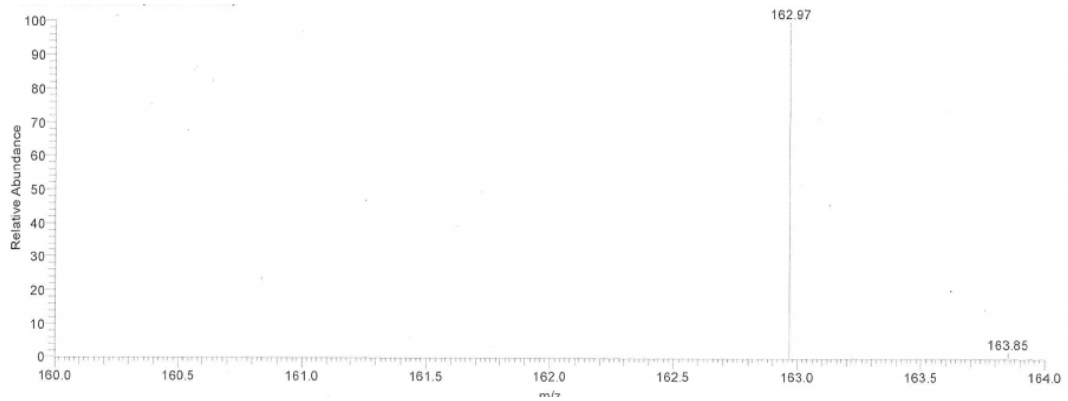



**FIGURE 5. LIQUID CHROMATOGRAPHY OF TEST SAMPLE USING OHMG.HCL AND CHLORAMINE B**

The results of liquid chromatography of the two samples showed that for the bactericidal agent (OHMG.HCl), no retention time was observed for the extra compounds such as in the prototype with chloramine B at 0.84 and 0.85 min). Corresponding to the retention time at 0.84 and 0.85 minutes the obtained MS spectrum is shown in Figure 6.



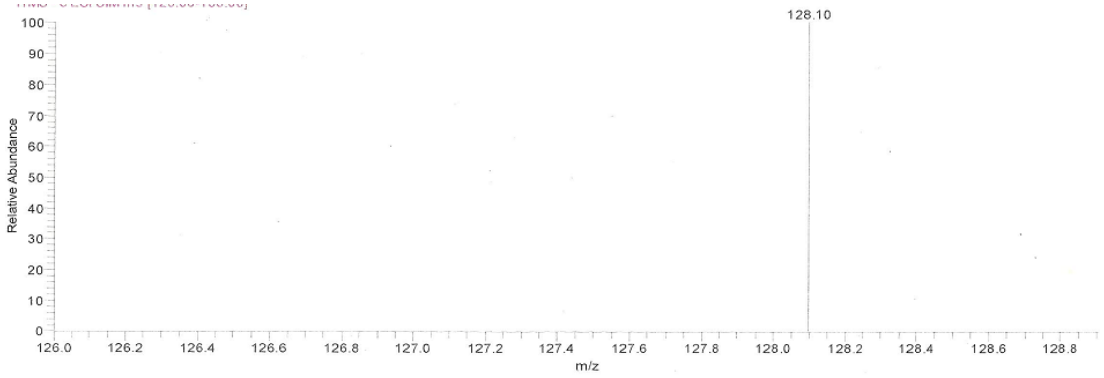



**FIGURE 6. MS SPECTRUM OF TEST SAMPLE USING CHLORAMINE B**

With the MS spectrum, the two retention times at 0.84 and 0.85 minutes exhibit the corresponding values of m / z of 162.97 and 128.10 respectively for corresponding two fragments as $CHCl_3COO^-$ and $CHCl_2COO$.

As compared to chloramine B, OHMG.HCl is a highly effective, non-toxic bactericidal agent, which does not create dangerous by-products in the process. Particularly when chloramine B used, there is the appearance of the by-products haloacetic acid in the water affecting human health.

## IV. CONCLUSION

Oligoguanidine OHMG.HCl was synthesized by a two-step polymerization consisting of condensation and cross-linking to obtain the antimicrobial oligomer with a higher molecular weightand cationic form with the higer positive charge density than pre-polymer. The Oligoguanidine synthesized has a chain structure, good water solubility and suitable for use as a disinfectant in wastewater treatment. Antimicrobial ability of OHMG.HCl was adjusted by varying alkyl monomer ratios at the same content of guanidio groups when they are absorbed into the cell walls of the bacteria

Results of the bactericidal efficiency comparison with Chloramine B showed that the synthetic OHMG.HCl has a fast killing time, low consumption environmental friendliness and not cause a toxic effect on health.

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