FABRICATION OF ANTIBACTERIAL MATERIAL USING DIFFERENT ACETATE SALTS

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Abstract: Ammonium, Cadmium, Cobalt, Sodium and Magnesium acetates were diluted inside both distilled water and Isopropanol alcohol separately and then tested as antibacterial material against (Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella sp. and Candida albicans). Among diluted salts in water only Cadmium and Cobalt acetate had inhibition zones. The particles sizes inside distilled water were 5.6 and 25.5 nm for diluted Cadmium and Cobalt acetate respectively as measured by particle size distribution test. The addition of all used acetate salts on Isopropanol alcohol increased its ability for killing tested bacteria. All diluted acetates in Isopropanol alcohol had inhibition zones, both Cadmium and Cobalt acetates had equal or larger inhibition zones than those diluted in water. Minimum bactericidal concentration (MBC), minimal inhibitory concentration (MIC) and biofilm inhibition% were measured for Cadmium acetate and cobalt acetate.

Key words: Acetate salts, inhibition zone, Maximum bactericidal concentration, minimal inhibitory concentration and biofilm inhibition.

1. Introduction

Today nano-structured materials are good candidate for different application including biotechnology. This is due their quantum confinement effects and high surface area to volume ratio [1],[2]. The numbers of diseases-causing organisms (which are killed by nano materials) are more than that are killing by antibiotics [3]. In biological and pharmaceutical applications; nano particles have excessive attention due to their ability for doing definite processes and selectivity [4]. Due to the revolution, some pathogens are becoming untreatable and their resistance against drugs increase such as methicillin-resistant Staphylococcus aureus (MRSA) [5]. So humans are continuously in a state of struggle and fight against these harmful microorganisms. Only way forward to survive and to win this war is discovery and search of unusual means against these microorganisms. Metal and metal compounds nanoparticles are considered as a good pathogens killers due to their high bactericidal activity at very little particle concentrations [6]. The aim of this contribution is investigation of antibacterial activities of five dissolved acetate salts in distilled water and isopropanol alcohol.

2. Materials and Methods

2.1 preparation of nanoparticles

Five acetate salts (Ammonium acetate, Sodium acetate, Cobalt acetate, Cadmium acetate and Magnesium acetate) were dissolved separately in distilled water to get five solutions with concentration 2.5mg/ml. The same salts were also dissolved in isopropanol alcohol to get the concentration 2.5mg/ml.

2.2 The measurement of antibacterial activities

The procedure of evaluation the samples' antibacterial activities against pathogenic bacteria (Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli and Klebsiella sp.) and Candida albicans was stated by [7]. Inhibition zones were recorded in a millimeter.
2.3 Measurement of MBC and MIC

The details of these measurements were fully explained by [8].

2.4 Particle size measurement

Using scattering theory, particle size of diluted acetate salts was measured. This was done by utilizing a device from Brookhaven instrument corp.

3. Results

3.1 Materials characterization

Figure 1 shows the size distributions of diluted cobalt and cadmium acetates in water. Median diameters were 5.6 and 25.5 nm for Cadmium acetate and Cobalt acetate respectively.

![Lognormal distribution for diluted: A- Cadmium acetate  B- Cobalt acetate](image)

3.2 The antibacterial of Cadmium acetate and Cobalt acetate

The inhibition zones for five diluted acetates in distilled water with control against the pathogenic bacteria are measured and listed in Table 1, see Figure 1. There are no effects belong to diluted (Ammonium acetate, Sodium acetate and Magnesium acetate) against tested bacteria. But there are obvious activities for Cadmium acetate and Cobalt acetate. Heavy metals are documented to be great inhibitors of biodegradation activities [9]. Cadmium is a heavy and a non-biodegradable metal [10]. The entering of heavy metal ions like Cd$^{2+}$ to the metabolic system of an organism is one of the mechanisms of microorganism killing [11]. Active source of oxygen species creation is the accumulation of these ions and then it destructs the RNA, DNA and proteins of bacteria, Cd is one of elements that cause the release of free oxygen radicals that destruct and stimulate of sensitive macromolecules [12], [13].

On the other hand; Seniya et al. confirmed that the resistance of bacterial growth increased by the stress of heavy metal [14].

Cadmium ions have the required properties for effective contact killing like: the oxidation of the Cd under ambient circumstances, high solubility of cadmium oxide, high thiophilicity of the dissolved metal ions. The first two properties cause a high level of ion release from the solid metal surface, whereas third condition is related with the toxicity of metal ions to bacteria [15].

| Sample       | Inhibition zones for diluted acetates in distilled water against Bacteria. |
|--------------|--------------------------------------------------------------------------|
|              |                                                                          |

Table 1: Inhibition zones for diluted acetates in distilled water against Bacteria.
Another active element in killing the tested bacteria in table 1 is Cobalt. Co particles can affect the integrity of DNA both by inhibiting the base excision repair system and by producing activated oxygen species. Cobalt compounds were testified to: (1) sister chromatid exchanges, (2) DNA protein cross links, (3) gene mutations, (4) induce DNA damage, and (5) aneuploidy in vitro studies on animal and human cells [16].
In biological systems, the main biochemical role of Co is its contribution in vitamin B12, a co-enzyme in some biochemical processes. This metal and some of its complexes have been found to display antimicrobial activities [17].

Table 2 shows the values of inhibition zones for the same used acetate salts in table 1 but with dilution in Isopropanol alcohol instead of water, see Fig. 3.

**Table 2. Inhibition zones for diluted acetates in Isopropanol alcohol against Bacteria**

| Sample                         | Ammonium acetate | Cadmium acetate | Cobalt acetate | Sodium acetate | Magnesium acetate | Control |
|-------------------------------|------------------|-----------------|----------------|----------------|-------------------|---------|
| The code                      | 1                | 2               | 3              | 4              | 5                 | 6       |
| Staphylococcus aureus         | 40               | 45              | 40             | 46             | 42                | 35      |
| Staphylococcus epidermidis    | 31               | 32              | 30             | 33             | 32                | 26      |
| Escherichia coli              | 31               | 35              | 28             | 36             | 39                | 25      |
| Klebsiella sp.                | 32               | 33              | 28             | 25             | 25                | 21      |
| Candida albicans              | 25               | 28              | 30             | 36             | 35                | 21      |
By comparison between the values of zones in table 1 and 2, it can conclude that, against most tested bacteria there is increment of the activities for both Cadmium and Cobalt acetates after dilution in Isopropanol alcohol but at different rates. Also table 2 shows that the activity of Isopropanol alcohol in killing the tested bacteria was increased after addition of Co and Cd acetates inside it. Table 3 shows the values of MBC and MIC for tested bacteria when diluted Cadmium acetate and Cobalt acetate in water and Isopropanol alcohol were used separately.

Table 3. MBC and MIC of diluted acetates in water and Isopropanol alcohol

| Microbes              | MBC and MIC (μg/ml) | Chemical compounds/ Dilution factor |
|-----------------------|---------------------|-------------------------------------|
|                       |                     | Cadmium Acetate / water             |
|                       |                     | Cadmium Acetate / Isopropanol alcohol |  |
| Cobalt Acetate / water| Cobalt Acetate / Isopropanol alcohol |
|-----------------------|---------------------|-------------------------------------|
| Staphylococcus aureus | MBC 2500            | 1250                                |
|                       | MIC 1250            | 625                                 |
| Staphylococcus epidermidis | MBC 2500 | 2500                                     |
|                       | MIC 1250            | 1250                                |
| Escherichia coli      | MBC 2500            | 1250                                |
|                       | MIC 1250            | 625                                 |
| Klebsiella sp.        | MBC 156             | 156                                 |
|                       | MIC 78              | 625                                 |

Results in present study showed after 24h incubation that the lowest MBC and MIC of diluted Cadmium and Cobalt acetates nanoparticles were recorded against Klebsiella sp. Results in table 4 revealed that the maximum inhibition rate was 50% against Escherichia coli and the minimum inhibition rate was 30% against Escherichia coli. Ali et al. examined the biofilm production in clinical isolates, and demonstrated that highly number of Klebsiella spp. biofilm producers was isolated from sputum specimens [18]. Kudhier et al. found that the percentage inhibition adhesion of Pseudomonas aeruginosa bacteria increased from 68.8% for undoped TiO2 to 90% for 9% Ag-doped TiO2 nanofibres [19]. Salman et al. tested the antibiofilm activity of levan, and demonstrated that was able to decrease Candida albicans biofilm formation to 70.58% at 27 hrs incubation time [20].

Table 4. Biofilm inhibition%

| Microbes             | Inhibition rate% |
|----------------------|------------------|
|                      | Cadmium Acetate / water | Cadmium Acetate / Isopropanol alcohol | Cobalt Acetate / water | Cobalt Acetate / Isopropanol alcohol |
| Staphylococcus aureus| 35%               | 40%                             | 45%               | 45%                             |
4. Conclusions

Among five acetate salts only Cadmium and Cobalt acetates had antimicrobial activities when the dilution was done in water. The killing ability of bacteria by Isopropanol alcohol was increased by addition of Cadmium and Cobalt acetates in it.

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5. References

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