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Bactericidal Properties of Carbohydrate-Stabilized Platinum Oxide Nanoparticles

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***Abstract*—**Platinum oxide nanoparticles were prepared by a simple hydrothermal route and chemical reduction using carbohydrates (Fructose and sucrose) as the reducing and stabilizing agents. The crystallite size of these nanoparticles was evaluated from X-ray diffraction (XRD), atomic force microscopy (AFM) and transmission electron microscopy (TEM) and was found to be 10 nm as shown in figure 1, which is the demonstration of EM bright field and transmission electron microscopy. The effect of carbohydrates on the morphology of the nanoparticles was studied using TEM (Figure 1). The nanoparticles (100 µg/ml) were administered to the *Pseudomonas Stutzeri* and *Lactobacillus* cultures and the incubation was done at 35 ^{o}C for 24 hours. The nanocomposites exhibited interesting inhibitory as well as bactericidal activity against *P. Stutzeri* and and *Lactobacillus* species. Incorporation of nanoparticles also increased the thermal stability of the carbohydrates.

***Keywords*—**Platinum oxide, *P. Stutzeri*, *Lactobacillus*, bactericidal effect.

I. Introduction

METAL nanoparticles have different properties from those of bulk metal because of their small sizes and thus these materials can be employed in various photoelectronic, catalytic, magnetic, sensor and biomedical applications [1-3]. In particular, Pt is known to inactivate microbes by interacting with their enzymes, proteins or DNA to restrain cell proliferation or cell division. It also binds to the negatively-charged bacterial cells to change the functionality of the cell membrane, thereby preventing bacterial regeneration [4]. Nanoparticles have gained prominence as ideal synthetic building blocks in bottom-up approaches for constructing a plethora of nanomaterials.1–5 "Nuts and bolts" for constructing new and useful nanomaterials include nanoparticles of uniform size, shape, and capping ligands. Among all, capping ligands play a vital role in transforming the spherical or triangular shaped nanoparticles to nanodevices or nanosensors of any desired shape [5]. Much of the recent research efforts have focused on developing new strategies to fabricate nanoconstructs with carbohydrates [6, 7] as capping ligands because of their potential applications in the design and development of nanoscale devices and nanosensors for biomedical applications [8]. Carbohydrates contain many hydroxyl and carbonyl groups; these groups offer sugar coated nanoparticle a unique H-bonding capabilities in constructing supramolecular architecture.

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Upon surface coating with nanoparticles they provide attractive nano construction abilities for building smart nanomaterials. For example, nanowires of platinum or tellurium have been constructed from glucose stabilized platinum nanoparticles or starch stabilized tellurium nanoparticles [9-11]. Despite large number of applications, there are only very few reports on direct generation of Pt nanoparticles (PtNPs) in a sucrose matrix. Current strategies for carbohydrate functionalization of Pt nanoparticles utilize thiol tailored sugars as synthons [10, 12]. However, a unified non-thiol synthetic approach applicable for all carbohydrates in functionalizing gold nanoparticles would be highly useful for constructing nanomaterials. Wallen and coworkers have developed a "green" method to synthesize and stabilize platinum nanoparticles in starch matrix using glucose as the reducing agent [13].

In this work, we have developed an easy method to produce platinum oxide composites with homogeneous size distribution and then these materials were stabilized with sucrose. This structure provides a robust, highly-porous and self-sustaining structure with large surface area, which is essential to facilitate incorporation of the Pt ions in the metallization process to give a high Pt loading content. Characterization studies were done via XRD, AFM and EM. These stabilized nanoparticles were then used as the antimicrobial agents against *Pseudomonas Stutzeri* and *Lactobacillus* cultures in further experiments.

II. Experimental

A. Chemicals

The sucrose was supplied by Fibrocel - Produtos Biotecnológicos Ltda. (Ibiporã, Brazil). Platinum oxide and other reagents were commercially purchased from Aldrich company and used as received. Aqueous solutions and bacterial suspensions were prepared using distilled deionized sterile water. Agar, tryptone and yeast extract of Bacto™, used for the microbiological culture media, were purchased from Himedia. The *Pseudomonas Stutzeri* [14] and *Lactobacillus* [15] strains were taken from the Central

B. Synthesis of Platinum Oxide Nanoparticles (PtONPs)

All reagents were of analytical grade and used without further purification. In a typical experiment, 1.7 g (1.0×10^{-2} mol) of platinum oxide (Aldrich, 99.9+%) was dissolved in 100 ml of deionized water. Then the solution platinum oxide was precipitated with 0.62 g (1.55×10^{-2} mol) of sodium hydroxide (Aldrich, 99+%). An obtained precipitate of platinum oxide was filtered and dissolved in 100 ml of

aqueous ammonia (0.4% w/w, 2.3 $\times 10^{-2}$ mol) until a transparent solution of platinum - ammonium complex was formed. Next, 2.5 g (8.9×10^{-3} mol) of oleic acid (Sigma–Aldrich, 99+%) was added dropwise into the obtained complex and the resulting solution was gently stirred for 2 h at room temperature until the complete homogeneity of the reaction mixture was achieved [16]. Finally, 2 g (1.11×10^{-2} mol) of glucose was added to the mixture at room temperature with gentle stirring. The reduction process of platinum complex solution (in quartz glass) was initiated with UV-irradiation. UV treatment was carried out for 8 h under vigorous stirring without additional heating. An UV lamp (k = 365 nm, 35 W) was used as light source to stimulate the reduction process [17]. After 8 h of irradiation the transparent dispersion of oleic acid stabilized platinum oxide nanoparticles (platinum oxide concentration = 10 mg ml^{-1}) was obtained. The synthesis of platinum oxide NPs was also successfully conducted with the final Pt concentrations in the range of 0.1–2%.

C. Transmission electron microscopic evaluation of Pt oxide nanocomposites

The TEM images of the Pt oxide nanoparticles were taken by the following method: at first, a solution of Pt oxide was prepared and diluted 100 times with 50% ethanol. A drop of this diluted solution was added to fomvar/carbon-coated grids (400 meshes) and after drying, viewed under the TEM (Brazilian Synchroton Light Laboratory), operating at 80 kV.

D. Characterization of Pt oxide *nanocomposites*

X ray diffraction patterns (XRD) were obtained in a Siemens Kristalloflex diffractometer using nickel filtered CuKα radiation from 4 to 70° (2θ angle) as shown in Figure 1. Thermogravimetry (TG) was conducted using dried samples in SDT 2960 device from TA Instruments. Samples were heated in open alumina pans from 40 to 600 °C, under an oxidant atmosphere (O_2), using flux of 50 mL/min, at a heating rate of 10 °C/min. The Pt oxide content of the BC/Pt composites was estimated from the residue at 600 °C.

E. Bactericidal Evaluation

The antimicrobial activity of sucrose modified Pt oxide nanoparticles was investigated against *Pseudomonas Stutzeri* and *Lactobacillus*. The following solution and media were prepared to study the antimicrobial activity: a) NaCl aqueous solution (2.7 g NaCl dissolved in 300 mL of distilled water), sterilized by autoclaving at 120 °C for 20 minutes; b) culture medium (LB) containing 1.0 g NaCl, 1.0 g tryptone, 0.5 g yeast extract of Bacto™ and 100 mL distilled water, sterilized by autoclaving at 120 °C for 20 minutes; and c) solid Luria-Bertani (LB) medium, used to prepare Petri dishes containing 3 g NaCl, 3 g tryptone, 1.5 g yeast extract of Bacto™, 4.5 g Agar and 300 mL distilled water. The solid Luria-Bertani (LB) medium was sterilized by autoclaving at 120 °C for 20 minutes. An aliquot (100 µL) of the bacterial suspension prepared previously was transferred to a test tube with 9.9 mL of lysogenic broth medium (LB) and homogenized. This tube contained a concentration of 10^7 cells.ml^{-1}. This bacterial suspension was diluted in a saline solution to obtain a suspension containing about 10^5 cells.mL^{-1}. Aliquots of 1000 µL of the suspension containing about 10^5 cells.mL^{-1} were transferred to sterile test tubes containing the samples. The test tubes were incubated in a stirring incubator at 37 °C and stirred at 120 rpm for 24 hours. After incubation, the content of the tubes was transferred to four Erlenmeyer flasks filled with 50 mL of sterile NaCl aqueous solution at 0.9% [18]. An aliquot (100 µL) of the content of each Erlenmeyer flask was transferred to a microtube filled with 900 µL of sterile NaCl aqueous solution. The microtubes were then vortexed. This dilution sequence was repeated four times to obtain four dilutions. Aliquots (100 µL) of these dilutions were then spread on a nutrient agar plate and incubated at 37 °C for 24 hours, after which the colony-forming units (CFU) were counted and the average was taken of the three plates corresponding to a particular sample [19]. The antibacterial activity of the platinum nanoparticles impregnated in bacterial cellulose was determined at different concentrations (10^3, 10^4, 10^5, 10^6 and 10^7 cells.ml^{-1}). The experiments were carried out in triplicate. Statistical analysis was performed using Student's *t*-test and the percentage reduction in bacterial count was calculated by the Equation 1:

$$[(\text{viable CFU at 0 hour} - \text{viable CFU at 24 hours}) / \text{viable CFU at 0 hour}] \times 100\% \quad (1)$$

III. RESULTS AND DISCUSSION

Pt oxide nanoparticles could be successfully synthesized in the sucrose matrix. The reduction was fastest in the case of sucrose followed by waxy corn and soluble starch while stability of nanoparticles followed the order soluble starch > waxy corn starch > sucrose (data not shown). Thus the size and molecular weight were important for stabilization of the nanoparticles. Figure 1 shows the TEM image of Pt oxide nanoparticles alone and the Figure 2 shows the TEM image of Pt oxide modified with sucrose.

The sucrose reduction occurred only after heating at 80 ^oC for 4 h [20, 21]. This is required because the more hydrolyzed the carbohydrate the better its ability to act as a reducing agent. UV Visible illumination was used to enhance the reduction capacity ofsucrose where it is hydrolyzed and the hydrolyzed products then reduce the metal. The time taken for reduction is also very short (30 s).

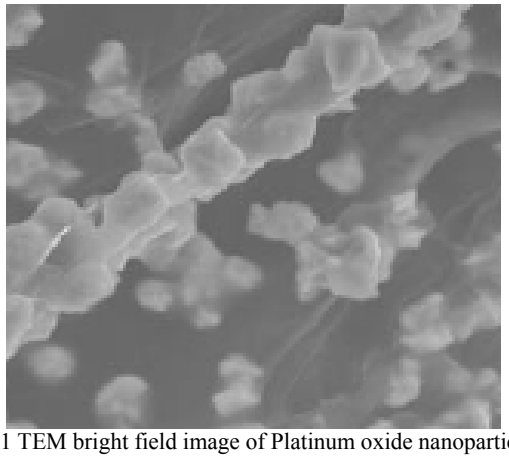

Fig. 1 TEM bright field image of Platinum oxide nanoparticles.

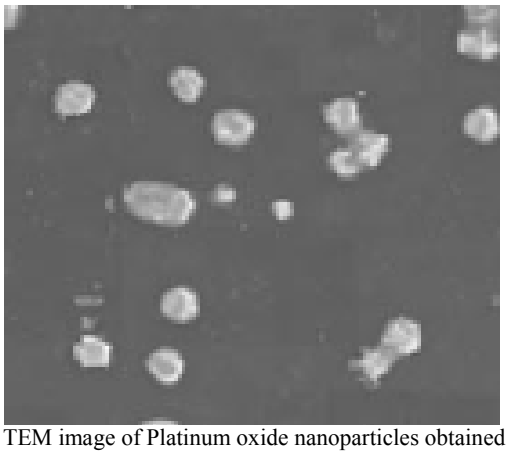

Fig. 2 TEM image of Platinum oxide nanoparticles obtained using sucrose as a reducing agent.

UV–vis absorption spectrum of carbohydrate-stabilized Pt oxide nanoparticles in aqueous solution was recorded after sufficient dilution as demonstrated in Figure 3. The characteristic absorption peak due to the surface-plasmon resonance (SPR) of Pt colloids was observed within the range of 415–425 nm.

Although the exact mechanism of the formation of the nanostructures is difficult to know [22], we think that the simple structure of sucrose could serve as a directing template for the growth of Pt nanoparticles.

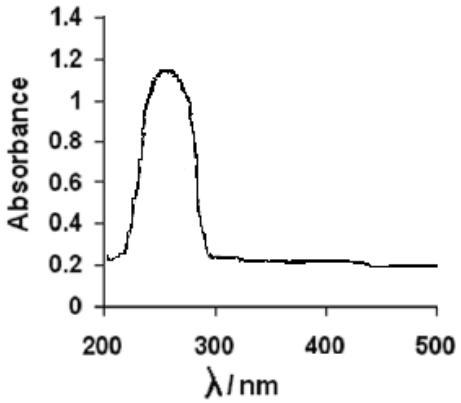

Fig. 3 UV-visible spectrum of Pt oxide nanoparticles modified with sucrose.

Figure 4 demonstrates the simulated XRD patterns of sucrose-stabilized Pt oxide nanoparticles. All Bragg's reflections representing b111N, b200N, b220N and b311N planes of fcc crystal structures of due to metallic Pt are observed [23]. About 7% of Pt is present in nanocomposites and oxygen comes from the surrounding organic matter [24-26].

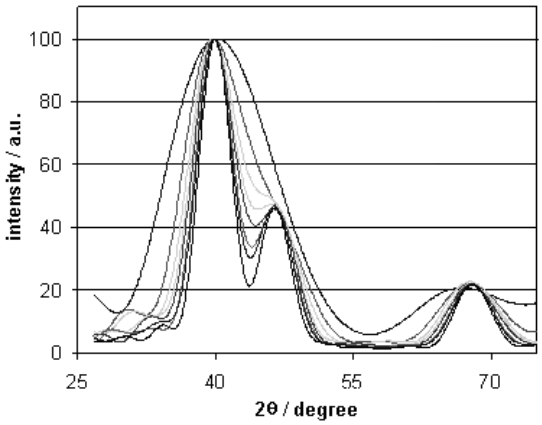

Fig. 4 Simulated XRD 2θ / Degree Intensity / a.u. patterns of sucrose modified Pt nano-particles.

Figure 5 shows the Atomic Force Microscopic image of the sucrose stabilized Pt oxide nanoparticles. The carbohydrate-stabilized aqueous solution of Pt oxide nanoparticles exhibited antibacterial activity against *Pseudomonas Stutzeri* and *Lactobacillus* bacteria even at concentrations as low as 0.049 mg/l. The photographs in Figures 6 and 7 clearly show the zone of inhibition for carbohydrate-Pt nanocomposites against *Pseudomonas Stutzeri* and *Lactobacillus*. Similar results were obtained against *S. aureus* (data not shown).

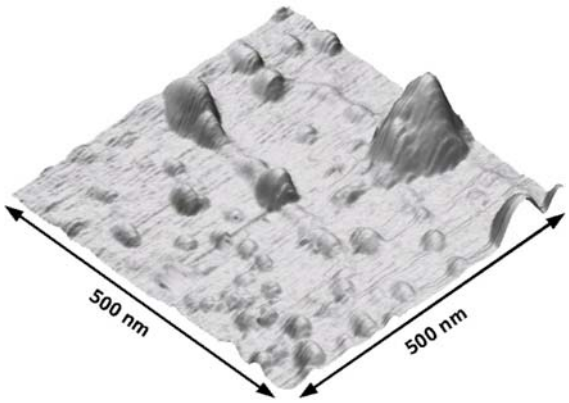

Fig. 5 AFM image of the platinum oxide nanoparticles

The digital photograph in Figure 6a represents MIC and MBC tests in Pt oxide-sucrose nanocomposite against *Pseudomonas Stutzeri*.

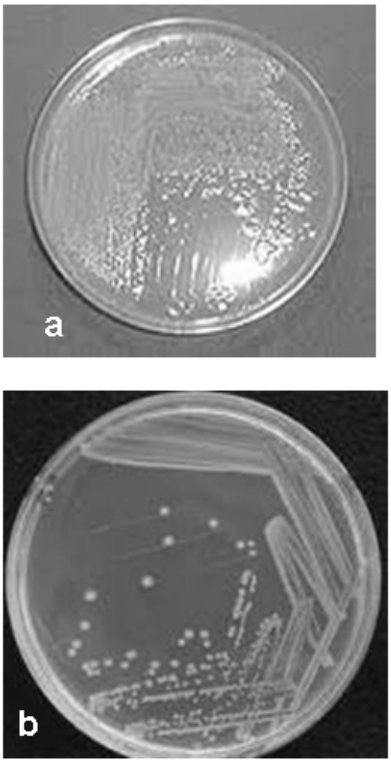

Fig. 6 Digital photographs representing (a) MIC and (b) MBC tests against *Pseudomonas Stutzeri* in Platinum-sucrose nanocomposite incubated at 37 ^{o}C for 24 hours.

While, Figure 7a and b show the same parameters against *Lactobacillus* strain. The effectiveness of sucrose nanocomposites was proven to be highly effective. Therefore, it could be concluded that Pt oxide nanoparticles could be released through aqueous carbohydrate solutions owing to the stable dispersion at molecular level and the slow diffusion from the stabilizing medium [27, 28].

IV. CONCLUSIONS

Platinum oxide nanoparticles were obtained by a green approach using sucrose as a reducing as well as stabilizing agent. The nanoparticles exhibited interesting morphology when synthesized under hydrothermal conditions in the matrix of sucrose-rich medium. These nanocomposites exhibited high thermal stability as well as bactericidal effect against *Pseudomonas Stutzeri* and *Lactobacillus*. Thus the carbohydrates can serve as a carrier for platinum oxide nanoparticles and the nanocomposites can have potential biological applications.

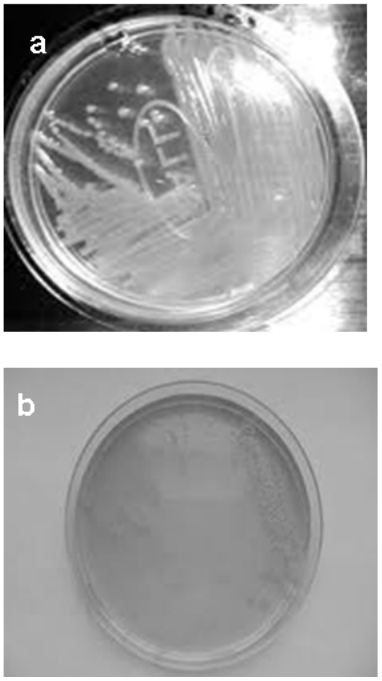

Fig. 7 Digital photograph representing (a) MIC and (b) MBC tests against *Lactobacillus* in Platinum-sucrose nanocomposite incubated at 37 ^{o}C for 24 hours.

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