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ACKNOWLEDGEMENTS. I thank ISRO for providing a grant under the CAWSES India project for the above study; the anonymous reviewers for valuable suggestions and Prof. J. N. Goswami, Director, Physical Research Laboratory, Ahmedabad, for providing library facility. The ozone profiles and total ozone were retrieved from WOUDC. The 5-day back trajectories at different pressure levels were retrieved from ECMWF. Tropospheric column ozone, carbon monoxide and methane data were obtained from TES on NASA's EOS spacecraft. Tropospheric NO_2 column data from the SIAMACHY instrument were obtained from the website www.temis.nl. The vertical pressure velocity and geopotential maps were retrieved from NCEP reanalysis.

Received 11 April 2010; revised accepted 16 September 2010

Reducing biofouling on titanium surface by electroless deposition of antibacterial copper nano films

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The main objective of this work is to study the antibacterial properties of copper thin nano films on titanium surface deposited by electroless plating technique for biofouling free condenser tube applications. The electroless deposition of copper nano films on titanium substrates was done and Cu films were also post-annealed for 1 h at 600°C under vacuum condition to increase the particle size of the films. Surface characteristics of the films were studied using GIXRD, SEM and AFM. Antibacterial properties of the surface were evaluated by exposure studies in seawater using total viable count and epifluorescence microscopic techniques. Excellent antibacterial activity was exhibited by the electroless plated copper nano film on the titanium surface showing more than two orders decrease in the bacterial density compared to titanium surface with no copper film.

Keywords: Annealing, antibacterial properties, biofouling, copper nano film, electroless plating.

COPPER and its alloys were used as condenser materials to resist biofouling due to copper toxicity. However, in the new 500 MW Prototype Fast Breeder Reactor coming up at Kalpakkam, India, titanium is the condenser material in order to achieve zero corrosion in the steam generator material where a single wall separates sodium and water. Any through wall pitting and leaks can cause catastrophic accidents. Copper ions leaching out from the steam side of condenser materials can cause under deposit corrosion on the steam generator walls.

One of the main challenges in the use of titanium, particularly for heat exchanger tube applications, is its biofouling[1]. Biofouling is the undesirable growth of living organisms on a surface, which will reduce its efficiency and lifetime. The principal methods used to control biofouling are mechanical cleaning techniques to detach the biofoulers and chemical treatment techniques to kill the biofoulers using various oxidizing and non-oxidizing biocides[2]. Since surface properties of the substratum influence initial adhesion and growth of bacterial cells on materials, modification of the surface of the condenser materials like stainless steel, titanium, etc. can further supplement the present treatment programmes. The present study is an attempt to use nanotechnology methods for surface modification aimed at improving the antibacterial properties of these condenser tube materials.

Many of these coatings on the surface provide a barrier between the surface and the environment, and also provide fouling resistance to the surface[3]. This fouling resistance is mostly due to toxic metallic ions on the surface, making it inhospitable to most marine organisms by blocking the respiratory enzyme system of these microorganisms in addition to damaging microbial DNA and the cell well[4].

Copper is known to have excellent toxicity to marine organisms and thereby provide good resistance to biofouling, and hence is used extensively as condenser material in power plants[5]. However, titanium has been chosen as condenser material for the new fast breeder reactor at Kalpakkam to avoid steam-side corrosion problems. Thus this study is focused on exploiting the excellent potential of copper nano coatings on the surface of titanium to con-

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trol biofouling[6,7]. Copper-containing coatings or paints are not feasible for condenser applications, as they will affect the heat transfer property.

Various techniques have been used for the copper nano coatings, such as chemical vapour deposition (CVD), physical vapour deposition (PVD), and electrochemical and electroless plating. CVD and PVD techniques are relatively expensive and sophisticated[8]. Electroless deposition has emerged as the method of choice for metallic coatings in recent years due to its low cost, fast deposition, good filling capability, good uniformity and low processing temperature. It is the easiest method to deposit metallic films on arbitrary shapes with uniform thickness[9]. The main advantage of electroless plating is the uniformity over the surface.

Therefore copper nano thin films have been grown on titanium substrate using electroless plating technique to enhance its antibacterial properties by enhancing surface activity. This layer was characterized and tested for antibacterial activity by exposure studies. The copper nano films were annealed in vacuum condition in order to increase particle size and thereby confirm the effect of nano particles on antibacterial activity[6].

The commercially pure titanium (grade 2) specimens used were obtained by cutting titanium sheets into small coupons of size 30 mm × 20 mm × 3 mm. The surfaces were mechanically polished up to 1200 grit by silicon carbide papers, cleaned, degreased and air-dried. Polished titanium substrates were anodized in orthophosphoric acid (H_3PO_4) solution at 30 V DC for 1 h at room temperature. After the anodization process titanium coupons were thoroughly cleaned with acetone and then washed using distilled water. After this activation process, electroless copper was deposited on the titanium substrate.

The copper electroless bath solution contained copper sulphate ($CuSO_4 \cdot 5H_2O$) which serves as a source of copper ions, sodium potassium tartarate ($C_4H_4KNaO_6 \cdot 4H_2O$) that acts as a complexing agent, sodium hydroxide (NaOH) as a pH controller and formaldehyde (37 wt%) as a reducing agent[10]. Electroless copper deposition on titanium substrate was done under thermostatic condition and pH of the solution was controlled at 12.5–13 using NaOH.

The deposition temperature was around 50–60°C with a deposition time of 5 min. After deposition the samples were thoroughly cleaned with deionized water and dried.

The copper-deposited substrates were post-annealed under vacuum condition at 600°C for 1 h in order to increase the grain size of the particle.

The thickness of electroless deposited films was measured by weight gain method. The crystal structure of the copper films deposited by electroless plating before and after annealing was analysed using a STOE (Germany) diffractometer and a $CuK_\alpha = 1.5406$ Å radiation source. Surface morphology of the deposited films was observed using a scanning electron microscope (SEM; XL30ESEM, Philips). The specimen topography was characterized by atomic force microscope (AFM) using a SLOVER PRO, multimode scanning probe microscope (M/s NT-MDT, The Netherlands).

The seawater used for laboratory exposure studies was obtained from the outfall of Madras Atomic Power Station (MAPS) having about 0.1–0.3 ppm residual chlorine. The source of seawater for the cooling water system of MAPS was the coastal water of Kalpakkam belonging to the Bay of Bengal. This exposure medium was selected for the present study, as the new reactor will also use this water in its cooling water system. MAPS outfall water (500 ml) was collected in a cylindrical glass vessel and biofilm-forming contents were concentrated by Millipore filtration (0.45 μm Millipore paper) of 1.5 l of this water and adding the filtrate into it. Then 1% glucose was added to the medium for maintaining the viability of microbes for 72 h of static exposure time. A glass rod positioned centrally in the glass vessel supported the glass pegs, which bore the specimens. Five sets of control titanium and surface-modified titanium specimens were exposed for 72 h in this medium.

After 72 h, the specimens in triplicate (control, electroless copper and annealed copper) were removed from the medium and gently washed to get rid of loosely adhering cells. The bacterial cells on the specimens were dispersed into 15 ml sterile phosphate buffer (0.0425 g KH_2PO_4, 0.19 g $MgCl_2$/l) by ultrasonication for 10 min. Serial dilutions of the bacterial cell suspension were prepared and 0.1 ml of each dilution was plated onto zobell marine agar (ZMA; from Hi Media M 384). The plates were incubated for 24–48 h at 32°C and the total viable count (TVC) was estimated[11]. Statistical analysis of the data was carried out using MYSTAT software. Three replicates were analysed for each experimental condition. Student's t test was performed to assess significance in the difference between bacterial counts on control and film-deposited surfaces.

Specimens in duplicate exposed in the same laboratory media were used for direct microscopic observation using epifluorescence microscopy[12]. The specimens were gently washed with sterile water and air-dried in a sterile chamber and the surface was flooded using 0.1% acridine orange (AO) in distilled water. After 2 min, the excess stain was drained-off and the specimens were washed in sterile water, dried and observed under a Nikon Eclipse E600 epifluorescence microscope (excitation filter BP 490; barrier filter O 515). When AO intercalates with DNA it emits green fluorescence upon excitation at 480–490 nm, and when AO complexes with RNA orange-red fluorescence is obtained.

The thickness of electroless deposited films was measured by weight gain method and was around 120 nm.

Figure 1 shows the GIXRD pattern of electroless copper and annealed copper on titanium. The diffraction peaks were matched with the characteristic peaks of metallic copper with the JCPDS File no 040836. In as-deposited film the peaks corresponding to 38.3° and 40° are those of the titanium substrate. The calculated particle size of the as-deposited copper film and annealed film was around 22 and 25 nm respectively. After annealing treatment of thin films, the diffraction peak intensity was found to increase compared to as-deposited film. This shows that the annealed electroless copper-plated films are highly crystalline in nature compared to as-deposited copper on titanium surface. The absence of substrate peaks in the annealed surface shows the complete coverage of the copper film over it due to annealing treatment.

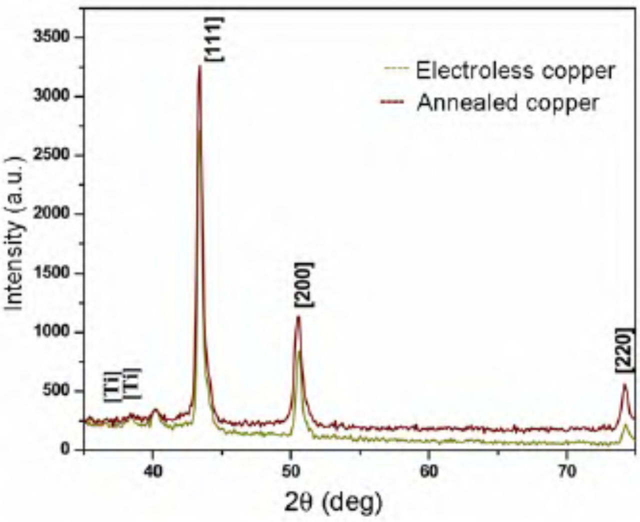

Figure 1. GIXRD of electroless copper film and annealed copper film on titanium substrate.

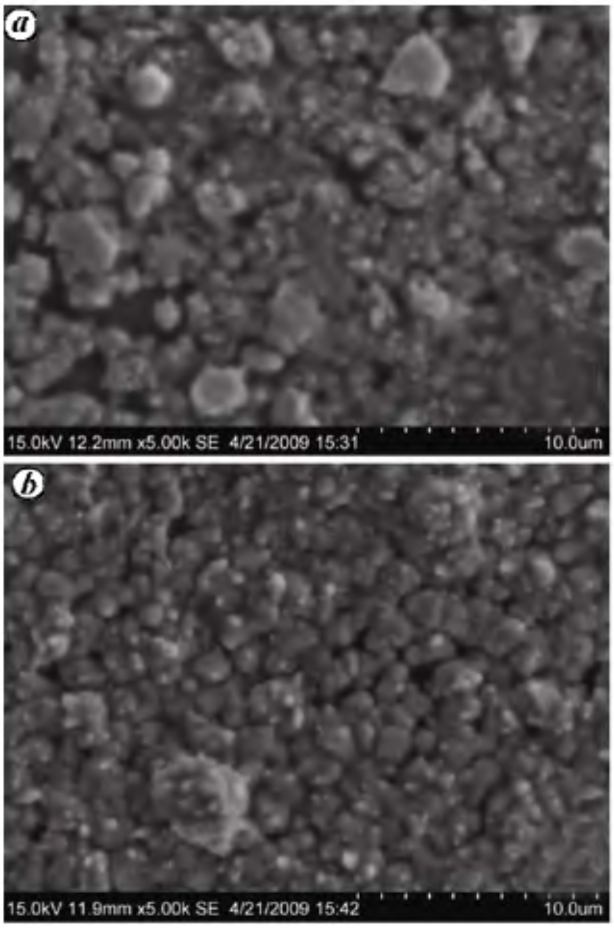

Figure 2. SEM photomicrograph showing (***a***) electroless copper and (***b***) annealed copper thin film on titanium substrate.

The surface morphology of the deposited electroless copper film and annealed copper film is shown in Figure 2. SEM of as-deposited surface shows Cu thin film formation on the surface. It consists of several copper grains of nanometre size. Subsequently, the sample annealed at 600°C for 1 h shows grain growth of the copper particles. Aggregation of copper particles gradually increases with the annealing effect. So the size of the grains increases with annealing treatment leading to complete coverage over the surface. The as-deposited films were porous and become dense with the annealing treatment[13].

The surface topography of the deposited electroless copper film and annealed copper film is shown in Figure 3. The average grain size of the particles in electroless copper and annealed copper was around 300 and 700 nm respectively. The annealed copper film surface became smoother compared to as-deposited films due to the distribution of the particles over the surface.

Figure 4 shows the comparative results of bacterial count on titanium control, electroless copper and electroless annealed copper on titanium substrate. The error bar is the standard deviation of the mean and this clearly shows that the variation between the different experimental conditions is significant. Student's *t* test performed using MYSTAT software gave a highly significant P value of <0.0001.

Epiflourescence microscopy showed apparent decrease in the bacterial attachment compared to growth of biofilm on control titanium, electroless copper film and annealed copper film on titanium substrate (Figure 5). A clear increase in attachment on the annealed surface was also observed compared to as-deposited electroless copper film, confirming enhanced antibacterial activity at the nanolevel of copper particles.

Copper-based alloys are suitable candidates for seawater condenser applications due to their excellent biofouling resistance owing to copper toxicity to micro- and macroorganisms[14]. Many recent studies have also demonstrated copper toxicity on microorganisms[15]. This has motivated the present study into the possibility of using nano layers of copper deposited on titanium surfaces to make them antibacterial and thereby reduce biofouling.

Both GIXRD and AFM studies have confirmed that the copper thin films obtained in the present study are smooth and within the dimensions of a nanofilm consisting of small clusters of copper nanoparticles. More than two orders decrease in the bacterial density on copper-coated surface and epifluorescence micrographs depicting

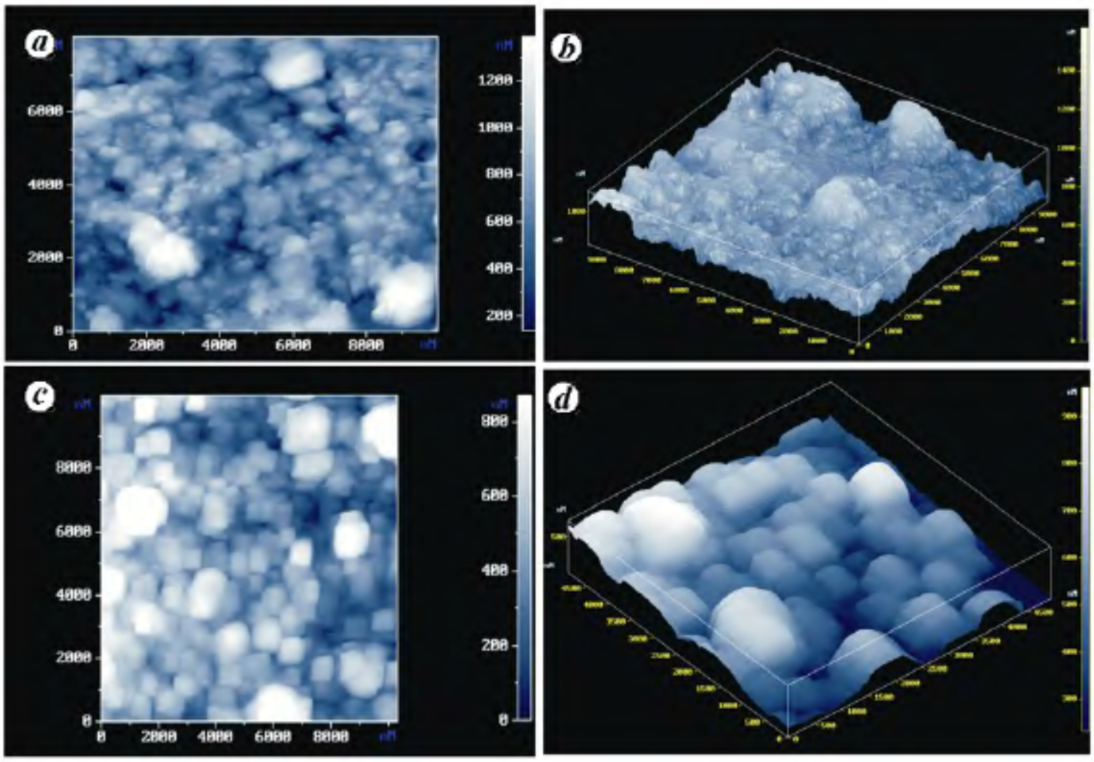

Figure 3. AFM photomicrograph of (***a*** and ***b***) electroless copper plating and (***c*** and ***d***) annealed copper film on titanium.

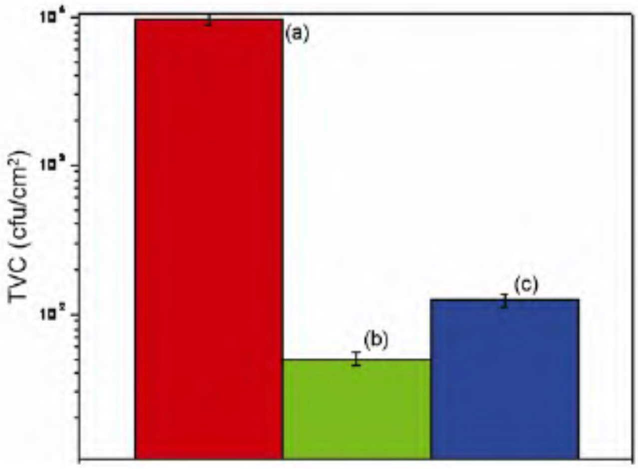

Figure 4. Bacterial count on titanium control (a), electroless copper (b) and annealed electroless copper (c) on titanium.

few fluorescing cells clearly demonstrate the superior anti-bactericidal capability of nanocrystalline copper thin films.

The major mechanism through which copper ions manifest antibacterial properties is by attaching to and penetrating the bacterial cell wall and affecting cellular activity due to their toxicity. They get attached to the surface of the cell membrane and drastically disturb its functions, like permeability and respiration. After damaging the cell wall they are able to penetrate inside the cell and cause further damage by possibly interacting with biomolecules, mainly enzymes. The negative charge of the surface membrane of bacteria and positive charge of copper ions can easily bind, thereby enhancing the antibacterial properties[4].

In our earlier studies we have used different techniques for making copper nano films on titanium surfaces like DC magnetron sputtering[16] and PLD technique[7]. In all these studies we obtained nanostructured thin films and good antibacterial resistance. However, to enable the engineering scale utilization of this copper coating technique, it was decided to use a more industrially feasible process such as electroless deposition. Surface characterization studies have revealed that in electroless plating, the crystalline nature and particle size of the copper films decreased compared to the sputtered and PLD-deposited films, as shown by in GIXRD results. The size of the grains was in the nanometre range and evidently the antibacterial properties increased more than two orders than only one order in other techniques compared to the control surface. This can be related to the release of copper ions which are known to be reactive towards bacteria and to the peculiar morphology at the nanoscale resulting in a large effective surface area[17].

The application of nanotechnology to develop antibacterial copper nano coatings has led to a highly effective antibacterial film to control biofouling of the titanium surface by industrially feasible electroless plating technique. Copper nano thin films were deposited using elec-

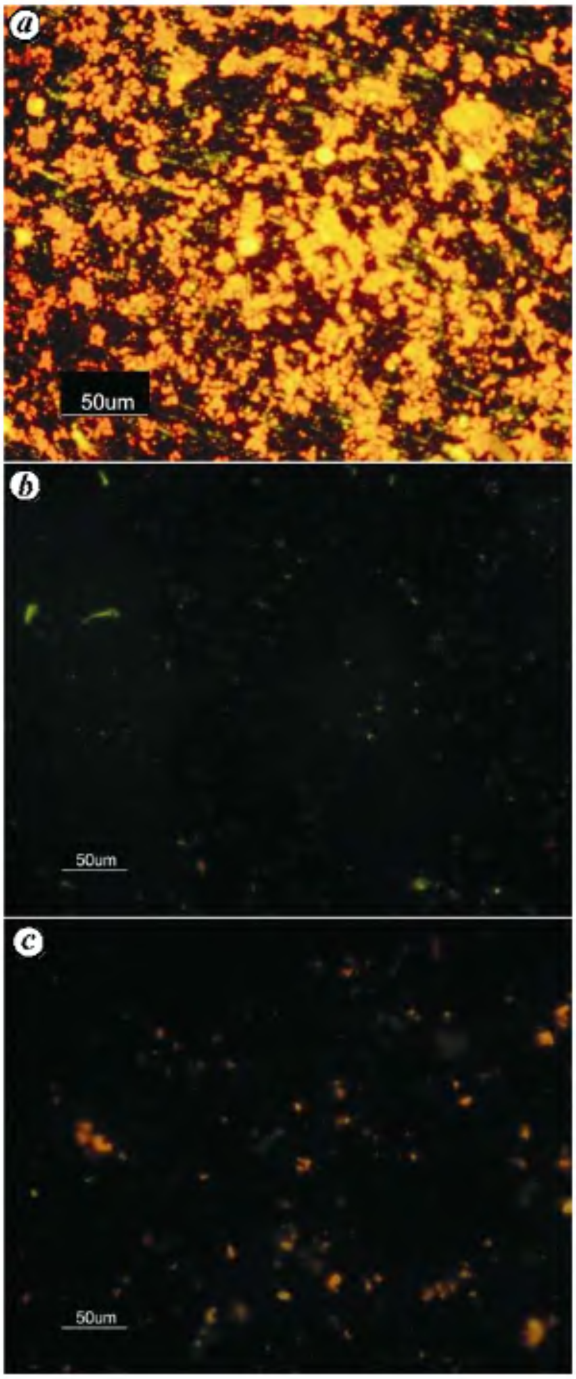

Figure 5. Epifluorescence photomicrograph of (***a***) titanium control, (***b***) electroless copper on titanium and (***c***) annealed electroless copper on titanium.

troless plating technique. GIXRD, SEM and AFM studies confirmed the nanocrysatlline nature of the film. Crystalline nature and particle size of the annealed copper films increased compared to the as-deposited films. Antibacterial activity of the films slightly decreased by thermal annealing in vacuum due to the slight increase in grain size of the copper particles.

This study clearly shows that inhibition capability against biofilm formation increases with decrease in copper nanoparticle size.

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ACKNOWLEDGEMENTS. We thank Dr Jeppiaar, Chancellor, Sathyabama University and Dr Baldev Raj, Director, Indira Gandhi Centre for Atomic Research, Kalpakkam, for financial support and guidance.

Received 7 April 2010; revised accepted 14 September 2010