Biological Synthesis of TiNi-TiO_2 Nanocomposite and Their Characterization

L. Reyes, J.L. Cavazos, T. Garza and I. Gómez

***Abstract*— The results of the extracellular synthesis of TiNi-TiO_2 nanocomposite by a native strain of fungus resistant to heavy metals at room temperature are presented. The nanocomposite were characterized by X-Ray Diffraction, Differential Scanning Calorimetric (DSC), FE-SEM and AFM. X-Ray diffraction analysis shows that phases obtained were TiNi in martensite (M) and austenite (A) polymorphs. The phases for TiO_2 in two polymorphs were found: Anatase and Rutile. Differential Scanning Calorimetric analysis revealed a value of the transformation hysteresis Af–Ms equal to 12ºC. FE-SEM showed that particles obtained have size already of 15-200 nm. AFM analysis show nanoparticles with spherical morphology formed at the edge of hyphae. The nanocomposite was sintered at 500ºC. After, it was characterized as biomaterial under physiological serum and presents the formation of hydroxyapatite.**

***Index Terms*— TiNi, TiO_2, nanocomposite, biosynthesis**

I. Introduction

Some metal-tolerant microorganisms present in their normal metabolism mechanism of removal of metals by precipitation. When the organism is grown in the presence of metal salts can generate reducing enzymes, intra or extracellular, which convert the metal to its insoluble form, creating crystals of different shapes and sizes [1-3]. Therefore, scientists have seen the microorganisms as a potential source in the synthesis of nanoparticles of different metals, oxides and sulfides.

Some fungi have been studied in the field of materials for their ability to produce nanoparticles of different metals. These have made good nano-dispersibility and well-defined dimensions.

It has been shown to be carried out bio-reduction $AuCl^{4-}$ aqueous ion using the fungus Verticillium sp obtaining gold nanoparticles with excellent monodispersed and well-defined size [4]. These results show that $AuCl^{4-}$ ions are trapped at the cell surface and occur by electrostatic interaction with positively charged groups (such as lysine residues) due to enzymes present in the cell wall.

The study was compared with research in bacteria. The results indicated that fungi could be a potential source for the production of large quantities of nanoparticles. This conclusion was due the ability to have the fungus to secrete high proportion of protein, and therefore, have considerable productivity nanoparticles in the biosynthetic approach.

The elucidation of the mechanism of formation was followed in vitro. The NADH-specific reductase, released by the Fusarium oxysporum, successfully performed the reduction of ions $AuCl^{4-}$ obtaining gold nanoparticles.

Based on the properties of Fusarium oxysporum, this was used in the formation of an extremely stable silver hydrosol [5]. The fungus Verticillium sp acidophilus has the ability to produce both gold nanoparticles and silver ions, incubating $AuCl^{4-}$ and Ag^{+} [4]. However, it is a new biological method of synthesis in obtaining intra and extracellular silver nanoparticles with fungi, Fusarium oxysporum and Verticillium sp. This has opened the possibility of production of nanoparticles can get trapped in the biomass in film form or produced in solution. Both have a significant commercial potential [6-7]. The fungus, Aspergillus flavus has been studied for its ability to accumulate silver nanoparticles on the surface of the cell wall when incubated with a solution of silver nitrate [8].

Some parameters as the minimum time, miniaturization and non-hazardous process are critical to any kind of technology. In this effort, Bhainsa and D'Souza (2006) [9] obtained nanoparticles with high mono-dispersed silver in a span of 10 minutes using Aspergillus fumigatus. This was the first report of the rapid synthesis of nanoparticles with mushrooms, being the fastest production compared to the physical processes and chemical synthesis. This may be useful in developing a biological method for mass production of nanoparticles. The nanoparticles of tetragonal barium titanate ($BaTiO_3$) with dimensions of 10 nm were produced by Fusarium oxysporum in observing environmental conditions is an economic method of synthesis and friendly nanomaterials in obtaining technological interest [10]. The presence of ferroelectric properties in these nanoparticles will revolutionize the electronics industry. Also, the synthesis of CdSe quantum dots as the room temperature was recently conducted by the fungus, Fusarium oxysporum when incubated with a mixture of $CdCl_2$ and $SeCl_4$ [11]. Recently, the fungus Fusarium oxysporum has also been used in the synthesis of SiO_2 and TiO_2 nanoparticles by exposing to a mixture of TiF_6^- and SiF_6^- respectively. The extracellular hydrolysis of anionic complexes with protein synthesis causes crystalline particles [12]. Currently, studies by Schabes-Retchkiman

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(2006) [13], indicated that the Alfalfa (Medicago sativa) when exposed to metal salts of Ni and Ti produce nanoparticles of TiNi, obtaining a reduction of particle sizes of 1-4 nm, with structure type face-centered cubic (fcc).

The TiNi alloy has unique properties that can be very useful in surgical applications. The superelasticity, biocompatibility and good mechanical properties make these alloys may behave differently to ordinary metallic implants [14, 15]. It has also been reporting that good cell-material response is due to TiNi in the presence of biological fluids alloy is formed spontaneously passive a thin layer of titanium oxide (TiO_2) stabilizing and providing high resistance to corrosion and high biocompatibility of TiNi implants [16, 17]. When the alloy of TiNi is implanted, TiO_2 is active biofilm facilitates implant osseo integration through a series of surface reactions with the environment. Phenomena that occur in these reactions include the formation of an amorphous layer of hydroxyapatite, which ends reinforcing and encouraging tissue reaction to the material [18].

Based on the described above, this paper proposes the biosynthesis of a nanocomposite of TiNi-TiO_2 using a strain of Penicillium sp.

II. Experimental Procedure

A. Microbial strain

Penicillium sp was obtained from culture collections. Microbial culture was isolated from ecosystem containing Cd (II), Zn (II) and was maintained on potato dextrose agar (PDA). Individual colonies was picked and further purified on agar plates.

B. Synthesis of TiNi using Penicillium sp

The culture broth was prepared from peptone from casein, yeast extract, sucrose, and malt extract; all company AD BIXION. The reaction medium was prepared from K_2TiF_6 (97% purity) and $NiCl_2$ (98% purity) both purchased from ALFA-AESAR.

To prepare 1L of culture broth, 3g of yeast extract, 3g of malt extract, 10g of sucrose and 5g of casein peptone were solved in water. The solution was sterilized and it was inoculated with a strain of fungus Penicillium *sp*. The fungus was keep for 2 to 3 days at a 25ºC in a shaker for the growing process. The obtained biomass was vacuum-filtered and it was stored for further use.

For the synthesis, a freshly prepared reaction medium was used. The reaction medium was prepared with 25mL of each of the metal solution. The metal dissolution concentration was $1.54x10^{-4}$ for both dissolutions. Then 5 g of biomass were placed in the reaction medium.

The reaction time was 2 hours at 25ºC. After that time, the mixture was vacuum-filtered in order to separate the biomass. The solid phase was discarded and the liquid phase was separated. Then the solvent was evaporated and the TiNi and TiO_2 particles were obtained.

C. Characterization

The Characterization of the particles was carried out by different techniques. X-Ray Diffraction (XRD) analysis of the samples was recorded on a Siemens D5000, using Cu Kα radiation and a graphite monochromator. The patterns were recorded from 10 to 85º 2θ with a 0.05º 2θ step and 2 s per step. Differential Scanning Calorimetry was carried out for thermal analysis of the samples in a Shimadzu DSC-50. The analysis was carried out from 30 to 300 °C. Field Emission Scanning Electron Microscopy (FE-SEM) was carried out with a FEI Nova NanoSEM 230.The images were recorded in the secondary electron mode at high vacuum. For the analysis by FE-SEM the samples were treated by heating at 250 and 500°C in an electric oven. Atomic Force Microscopy (AFM) in a NP-20 Veeco AFM in no contact mode was used.

III. Results and Discussion

Diffraction pattern shows the X-ray reflections characteristic for TiNi, in martensite and austenite phases. There also a martensite precursor present known as the R-phase transition. Also shows reflections of TiO_2. Is important note that by solid state processing are reported martensite phase at 20°C and austenite phase are obtained in temperatures of 120°C [19]. However, in our experiments by biosynthesis at room temperature are obtained both phases. In relation to TiO_2 obtained; in our experiments both rutile and anatase polymorphous were obtained too. This spectrum shows the reflections characteristic for martensite-TiNi found in larger proportion compared to austenite. By Scherrer equation was calculated an average particle size of 14 nm for martensite-TiNi and 21nm for R-TiO_2.

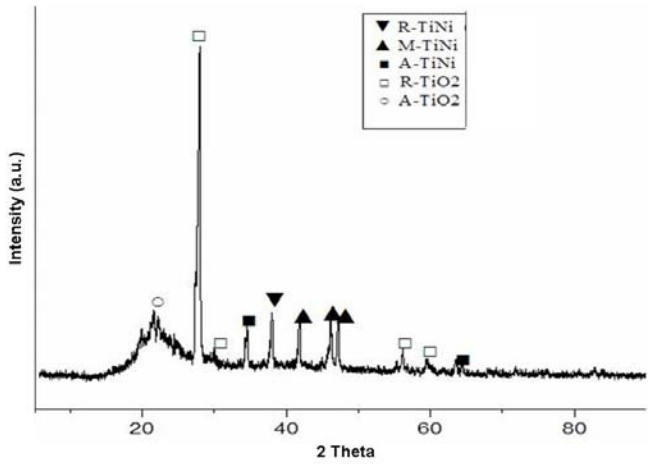

Figure 1 Powder XRD patterns of the prepared samples that corresponds to TiNi martensite (JCPDS 35-1281), TiNi austenite (JCPDS No.19850), TiO2 rutilo (JCPDS No. 211276), TiO2 anatase (JCPDS No. 211272) and the peak labeled with "R-TiNi" correspond to (JCPDS 41-1379).

Figure 2 presents the calorimetric curve obtained for one sample. In the first cycle both the direct transformation on cooling and the reverse transition on heating are realized in one stage. This figure shows two endothermic peaks in the heating cycle, one peak at 75°C another peak at 200°C. This analysis was doing from 23°C to 300°C in order to observe thermal effects of all phases presented in the sample, according to results by XRD. However the temperature for transition from martensite to austenite is reported already of 60-90°C [19,20].

The transformation temperatures measured by differential scanning calorimetry (DSC) are M_s = 117.55ºC, M_f = 45.16ºC, A_s = 46.53ºC, A_f = 109.3ºC, where Ms, Mf, As and

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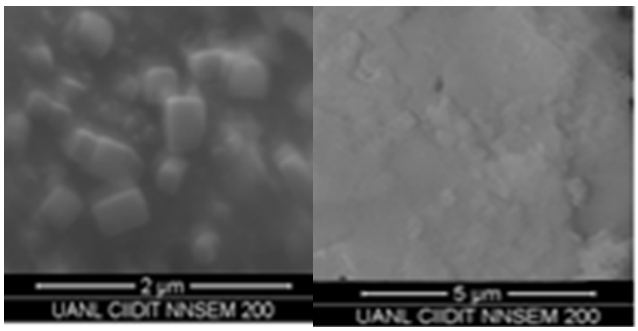
Af are the starting and finishing temperatures of martensitic transformation, and those of reverse martensitic transformation, respectively. The value of the transformation hysteresis *A*f–*M*s is equal to 12◦C. And normally this transformation hysteresis is already of 20°C [21].

Figure 3a show a scanning electron micrography after to carry out a thermal process at 250ºC in the sample. In it was possible to observe nanoparticles with morphologies clearly defined, that possibly are from TiNi alloy and TiO_2. These nanoparticles present sizes from 20 to 200nm. Then was conducted a further thermal process at 500ºC, in Figure 3b show a scanning electron micrography. In it was possible to observe sintering process in the sample.



a) b)

Figure 3 FESEM images from samples sintered at 250ºC (a) and 500ºC(b)

Figure 4 show image by AFM of a sample biosynthesized, without any thermal treatment. In it is possible to observe agglomerates of nanoparticles with spherical morphology. This characteristic allow us to elucidate extracellular mechanism of biosynthesis due this nanostructures are over hyphae.

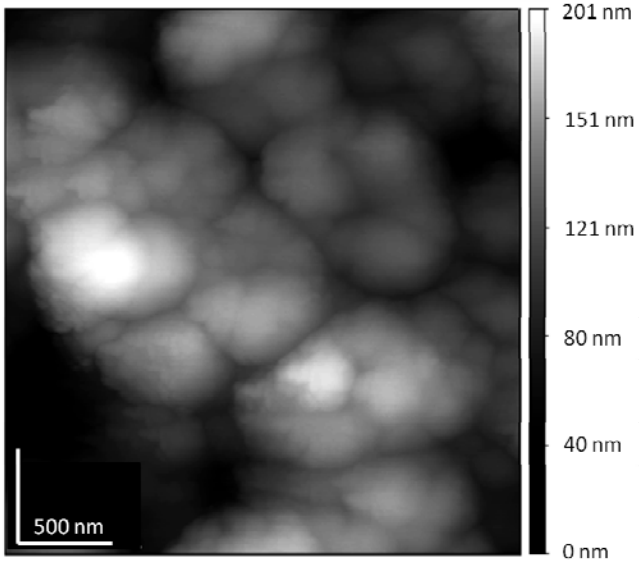


Figure 4 AFM image for sample biosynthesized.

In order to probe the potential application of the nanocomposite as biomaterial a thermal treatment at 500C was doing. After of it, the sintered sample was studied under saline artificial for 7 days, as reported in the literature by De Aza et al [14-15]. The results by FTIR show typical signals of hydroxyapatite, which is evident due to the signal that occurs near 1000 cm-1. In addition, signals can be observed between 1300 -1800 cm^{-1} which are consistent with signs of TiO_2, which have low percentage of transmittance. This indicates that, as reported [15], the sample to be in contact with the saline solution has an oxidation reaction that allows the appearance of hydroxyapatite on the exposed surface. To corroborate the results obtained by FTIR analysis was performed by X-ray diffraction and scanning electron microscopy for samples calcined after being in contact with the artificial saline. Figure 5 shows the diffraction pattern of sample calcined after being in contact with the saline solution, where were observed the presence of the reflections of TiO_2 and TiNi in different crystallographic arrangements, and also appreciate the reflections characteristic of hydroxyapatite.

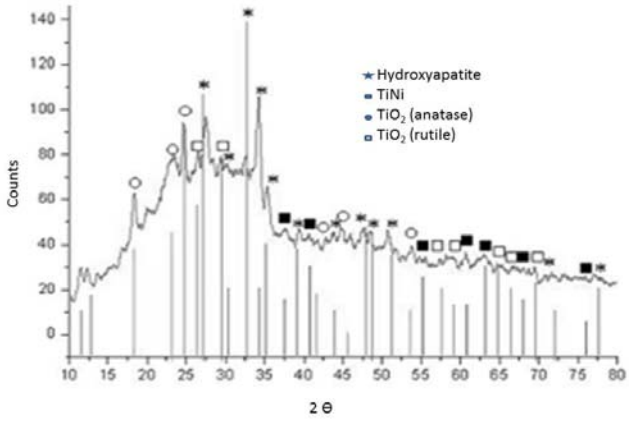


Figure 5 XRD for the nanocomposite used in saline solution, TiO2 (JCPDS

CONCLUSION

The biosyntheses of TiNi-TiO_2 nanocomposite were possible using Penicillium sp. The XRD exhibits the patterns of diffraction corresponding of TiNi and TiO_2 with different polymorphs. DSC analysis shows a transformation hysteresis Af–Ms equal to 12ºC. FE-SEM analysis shown nanoparticles with sizes already from 20 to 200nm. The AFM analysis shown nanoparticles synthesized with spherical morphology. AFM and SEM analysis corroborated that the synthesis was carried out by extracellular mechanism.

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