

## PROPERTIES AND OBTAINING COPPER, GOLD AND SILVER QUANTUM DOTS SUPPORTED IN CARBON NANOTUBES

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### ABSTRACT

The synthesis of Au, Ag and Cu metal nanoparticles in 2-ethoxyethanol and 2-propanol supported in carbon nanotubes is reported. The synthesis was carried out by chemical liquid deposition (CLD) at 77K to obtain metal colloids. The metal supported in carbon nanotubes was obtained by solvated metal atom dispersed (SMAD) method. The composites were characterized by electron microscopy with electron diffraction, scanning electron microscopy, elemental analysis, thermogravimetry, infrared spectroscopy (FTIR). The quantum dots size in 2-propanol and carbon nanotubes supported are: silver 4.37 nm and copper 2.56 nm. In 2-ethoxyethanol solvent: silver 4.14 nm, copper 2.47 nm and gold 5.30 nm. The transmission electron microscopy (TEM) images confirm the presence of quantum dots inside and outside the carbon nanotubes. The samples demonstrate a thermal stability until 295°C. The FTIR exhibit the presence of solvent residues in the samples. The electron diffraction is observed in the silver and copper in 2-propanol supported in carbon nanotubes and in the silver sample for 2-ethoxyethanol. The histological analyses of rainbow trout (*Oncorhynchus mykiss*) after being injected with gold quantum dots in 2-ethoxyethanol, in concentrations 0.5 and 0.3x10<sup>-3</sup> M do not exhibit cell disruption showing hepatocytes with morphology according to the normal for that specie.

**Keywords:** Chitosan, Carbon nanotubes, quantum dots, Fish toxicity.

### 1. INTRODUCTION

There is a great interest in nanotechnology, which can be defined as the discipline that encompasses those fields where materials and / or substances of very small dimensions are studied, manipulated and obtained, we are talking about nanoscale sizes. The importance of nanometric substances and materials lies in the large number of applications that can be obtained in different areas, because by decreasing the geometry of the materials, they acquire new and important properties [1]. These materials have a radius of less than 100 nm and are called nanoparticles [2]. Nowadays, particles smaller than 10 nm are called quantum dots.

The scale of the processes that nanotechnology deals with is of a barely conceivable smallness. The interesting thing is that by reducing the geometry and changing the properties of the materials, processes can be carried out with extraordinary precisions, such as increasing resistance, improving catalysis and others. Nanotechnology promises potential applications in almost all aspects existing in nature from the area of medicine to the improvement of industrial processes [1]. Examples include administering medications that are able to target specific organs and creating clothing that does not absorb dirt or odors and paint for cars that have the ability to clean themselves.

Medical applications of nanoscale technologies have the potential to revolutionize healthcare by providing powerful tools to diagnose and treat diseases at a molecular level. In the US in mid-2006 there were already 103 drugs and delivery systems in different stages of development, all of them based on nanotechnology [3]. Within the world of nanomaterials, the so-called carbon nanotubes can be identified, which have among their qualities: high hardness, toughness, elasticity and flexibility, in addition to the ability to easily penetrate cells, which facilitates the transfer process of an exogenous body, without interacting with the drug they are carrying. All this makes nanotubes offer a new possibility for their use as nanovectors in the area of biomedicine [4]. Therefore, they can be applied in conjunction with nanoparticles offering new therapies to diseases that do not have definitive treatments such as arthritis and / or osteoarthritis, among others.

According to Red Health Catholic University, arthritis is a disease that affects the joints, causing pain, swelling and stiffness. The cause is not known, but it is inferred that there are environmental, hormonal and genetic factors that may contribute to the development of it. Therapies include medications, exercises, and lifestyle changes. On the other hand, osteoarthritis has been defined as a disease caused by an injury to the cartilage causing pain and loss of normal movement. Causes include aging, obesity, and overuse. Currently there is no therapy for osteoarthritis, only medications for the relief of symptoms and delayed evolution. Around 15.000 people are treated annually for conditions associated with inflammatory diseases such as arthritis and osteopenia in the country. This disease can present varied manifestations with varying degrees of

discomfort and limitations. By not controlling it, it causes massive destruction of the affected joints with associated deformations, causing significant progressive muscle skeletal disability. Current pharmacological therapies mainly include the use of anti-inflammatories, analgesics and methotrexate all of them with side effects [5].

Gold is the most resistant metal to all the oxidizing agents that exist and the most chemically inert. Therefore, it is considered a noble metal [6]. Arthritis is a disease known to cause inflammation in the joints causing pain in many cases disabling. Joints constitute a structure that allows two objects to move independently to a certain point. Cartilage has the ability to compress and expand. When pressure is applied to the joints, they are compressed. When the pressure ceases, the body relaxes and the cartilage expands again allowing the circulation of nutrient-carrying fluid through the joint. Arthritis causes at any level the loss of nutrient-carrying fluid and a certain thinning of the cartilage, which eventually ends up softening due to the loss of fluid in the fundamental substance. However, to date it is not known what causes the arthritic process [7].

In the early twentieth century, gold began to be used as a treatment for tuberculosis due to the discovery of bacteriostatic properties. At that time, it was believed that rheumatic arthritis was also caused by the bacillus of tuberculosis and therefore began gold therapies against that disease. Later it was found that gold was not effective for the treatment of tuberculosis but did show favorable results against rheumatic arthritis [8].

At present, the mechanism of action of gold compounds is not well known at present and this is mainly because the causes of arthritis formation are not fully identified. However, it can be inferred how gold compounds will be transported into the bloodstream. The explanation lies in the fact that gold compounds have a great affinity for S-giver ligands, causing the displacement of the thiolate ligand (AtGS) of the gold derivative, thus forming a new complex with proteins containing SH groups or sulfur atoms such as albumin (AlbumSH) [8].

One of the hypotheses raised about the mechanisms of action of gold compounds in inflamed areas proposes that gold has the ability to inhibit the action of enzymes that cause the degradation of inflamed areas by coordinating gold to thiol groups. On the other hand, there is evidence that gold has the ability to inhibit activated oxygenated species, such as hydroxyl radicals or super oxides that are produced in inflammatory processes [8]. There are many studies that today are carried out with nanoparticles in the area of medicine. Furthermore, the Plasmonic Nano-optics group, within the ICFO (Institute attached to the Polytechnic University of Catalonia) investigates the optical and thermal properties of gold nanoparticles in order to use them as nano sources of heat and light on diseased tumor cells, selectively destroying the tumor. The revolution of this method is that, unlike radiation therapy or chemotherapy, it does not affect the healthy tissues surrounding the patient's tumor.

## 2. EXPERIMENTAL

### 2.1 Synthesis of Nanoparticles Supported In Carbon Nanotubes

It is known that micro-organisms are developing resistance to the antibiotics that are being used today, causing a partial or total loss of effectiveness against bacteria. For this reason, new proposals are emerging to combat existing diseases and among them is colloidal silver as an important alternative to the use of conventional antibiotics. There are studies that support the use of colloidal silver since its effectiveness has been proven in a large number of germs making it impossible to create resistance mechanisms. Colloidal silver has great efficacy against a wide variety of bacteria including Gram positive and Gram negative, plus it is not only limited to bacteria but can also be used against fungi and yeasts.

The way colloidal silver acts consists of the inhibition of the enzymes involved in the respiratory process of oxide-cell reduction of bacteria, which causes their death in a few minutes. One of the great advantages of silver is that it does not inhibit the rest of the enzymes involved unlike what happens with antibiotics, it also has no toxicity in any case and is eliminated entirely by feces. It can be established that silver is safe for man and all multicellular living beings [9].

There are studies in bacteria to analyze the bactericidal effect of silver on them. Campos et al. carried out a study in facultative and strict anaerobic bacteria from inside the root canal of temporary teeth with a diagnosis of pulp necrosis. Sensitivity tests were assessed by forming the inhibition halo and measuring its halo. After analyzing 20 samples, they were able to conclude that the bactericidal effect in both groups of bacteria was not the same since in strict anaerobes the halo was much greater than in facultative anaerobes [10].

In Peru, silver nanoparticles have been made to be used in clay filters, which can integrate the nanoparticles into their internal structure after a thermal process and then be used to disinfect contaminated water. While smaller is the silver nanoparticle, its bactericidal action is many times greater and if an adequate distribution is also achieved within the nanoporous matrix of the clay, very high performance filters can be obtained. It was concluded that nanotechnology is a great contribution to the development of new materials [11]. Colloidal silver is becoming increasingly known as it is cheap, effective and its preparation is not complicated. It has the ability to inactivate all the enzymes of oxygen metabolism of bacterial cells, fungi and viruses [12].

Other study showed cellulose and silver nanoparticles were prepared by the metal-vapour synthesis method. TEM has shown that the size of silver particles in a nanocomposite varies from 8 to 12 nm. All experimental nanocomposites had pronounced antibacterial activity against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* FDA 209P and acid resistant *Bacillus coagulans* ATCC 6633, but no fungicidal effect against *Aspergillus niger* ATCC, ATCC 2091 and *Candida albicans* ATCC 2091 was found [13].

On the other hand, copper it is a paramagnetic element and has high thermal and electrical conductivity. It's moderately hard. Thermal and electrical properties depend largely on the physical conditions, temperature and grain size of the metal. The possible uses of copper nanoparticles are multiple and varied. They are used to inhibit the proliferation of microorganisms on its surface. It is estimated that many infections could be avoided using materials that possess biocidal surface activity [14].

Researchers and scientists from various disciplines are beginning to have better expectations in nanotechnology as possible treatments in diseases such as cancer. Copper nanoparticles can be used as a transport of poison that can eliminate cancer cells, or carry some metal that can be combined with radioactive waves to burn malignant cells [15].

The Institute of Materials Sciences in Madrid developed a system to make a crystalline powder with copper nanoparticles. They point out that the cell surface of the microorganisms adheres to the copper nanoparticles present in the dust, later they observed by microscopy that the copper is internalized by the microorganism causing cell death. They also add that the range of application is very large since they measured the effectiveness of both Gram positive and Gram negative bacteria [16]. In Chile, the company Full House, Science for Money highlights among its achievements, the development of copper powder, formed by copper microparticles of size 0.2  $\mu\text{m}$ . The uses of this powder are varied, among them are: friction materials, charcoal brushes, electrical and electronic items [17].

On the other hand, at the Universidad Nacional Mayor San Marcos in Peru, the preparation of a bactericide of copper nanoparticles, coated with silver, is being developed for use in the treatment of water for human consumption. This material was chosen because it currently registers high production rates and is also considered durable and recyclable, among other properties [18].

The colloids of Cu, Ag and Au were prepared by the method called Chemical Liquid Deposition (CLD), which consists of the physical co-deposition at 77 K of metal vapors (Cu, Au and Ag) with organic vapors (2-propanol, 2-ethoxyethanol) in a reactor of metal atoms [19].

The solvent is placed in a degassing flask, to later attach it to the outlet of the vacuum network; wait until the vacuum (0.05 atm) is reached and the solvent is frozen with  $\text{N}_2(l)$ . Once frozen it is allowed to thaw slowly. The freeze-thaw cycle is repeated again until no gas emission is observed. The corresponding amounts of copper, gold and silver are weighed. They are then deposited in the crucible and connected to the lower ends of the electrodes. It weights the corresponding amounts of nanotubes and are activated for 30 minutes in a muffle at 400°C. They are then deposited at the bottom of the reactor. The degassing flask is connected to one of the outlets with the solvent (2-propanol, 2-ethoxyethanol), then the glass reactor is attached to the head by vacuum and is expected to reach a vacuum equal to or less than 0.05 atm. Once the necessary vacuum is reached, the Dewar is placed with  $\text{N}_2(l)$ , maintaining a temperature of 77 K.

About 5 ml of solvent is allowed to evaporate to form a layer of it on the walls of the reactor preventing the metal from adhering to the walls of the reactor. The amperage of both crucibles is increased slowly until the metal begins to evaporate simultaneously in conjunction with the co-condensation of the organic solvent.

It is important to reach the end of the reaction with an excess of solvent, that is, the metals must evaporate completely a little before passing all the solvent. When the solvent and metals have evaporated, the  $\text{N}_2(l)$  is removed keeping the reactor inside the Dewar for 1 hour. The Dewar is then removed and purged with  $\text{N}_2(g)$  for 12 hours under constant stirring. Once the aforementioned times have been completed, the colloid obtained is subtracted by means of a siphon from the bottom of the reactor, in an environment of  $\text{N}_2(g)$ . The sample is under vacuum to evaporate all liquid waste and obtain the active solid.

### 2.2 Scanning Electron Microscopy

A JSM 638LV-JEOL microscope with voltage acceleration of 20 kV is used.

### 2.3 Transmission Electron Microscopy

A JEOL JEM 1200EXII Microscope with 4 Å resolution is used.

### 2.4 Electron Diffraction

From the resulting interference pattern it is possible to deduce the structure of crystal that produces this pattern [20]. Operate the JEOL JEM 1200 EXII microscope in ED mode. Obtention of ring patterns corresponding to the samples of interest. Finally, the values of interplanar spaces were measured.

### 2.5 Infrared Spectrophotometry with Fourier Transform (FT-IR)

A Nicolet Magna 550 FTIR is used. Mix the solid sample with KBr to obtain a final concentration of 2%. The region of the mid-infrared spectrum is used. Analyze the accumulation spectrum obtained at room temperature using the Omnic 5.2 program.

### 2.6 Thermogravimetry and Differential Scanning Calorimetry

In addition, it provides the same information on by-products that may be formed with these variations and the composition of the residue that remains at the end of the analysis.

TGA TA instruments Q50 was used. This study uses dynamic thermogravimetric analysis, which consists of heating a sample under controlled atmosphere conditions with a previously set temperature ramp [21]. Add to the sample holder of the TGA equipment between 0.5 to 1.0 mg of the sample. Set a temperature range of 0°C to 500°C, with a heating rate of 10°C/min and a flow of  $\text{N}_2(g)$  of 50 mL/min. The critical temperature of the sample is then determined by the Fourier derivative. Finally, the mass losses in the temperature range are determined.

## 2.7 Acute Toxicity Bioassay

Acute toxicity tests consist of exposing an organism for a certain time to chemical substances and evaluating the effect it produces on the species under study [22]. The analyses in this study are performed with the rainbow trout species. Gold nanoparticles supported in carbon nanotubes in 2-ethoxyethanol solvent are used. Uses a NaCl 0.9% solution. 20 fish genus salmon. The fish are placed in containers with distilled water. The aeration system is installed and the fish are leave in the adaptation stage minimum 3 days. 0.2 mL of 0.9% NaCl solution + nanoparticles supported in different concentrations are injected in the intraperitoneal area of the fish. Fish are counted at a minimum exposure of 72 hours and mortality is accounted for. Table 1 summarizes the parameters of the toxicity bioassays.

**Table 1.** Experimental conditions test in fish.

Experimental conditions of the bioassay	
Type of test	Static with spare parts
Duration of the test	2 weeks
Temperature	20°C
Light quality	Ambient lighting laboratory
Water replacement	Every 48 hours
Number of organisms per container	5
N° of aftershocks by concentration	1
Feeding regime	No feeding
Vol./Experimental unit	30 L
N° of treatments	4
Dilution water	Distilled water
pH	7.2

Administration concentrations are:  $1.0 \times 10^{-3}$  M,  $0.5 \times 10^{-3}$  M and  $0.3 \times 10^{-3}$  M in the first experience. In the second, the concentrations of the nanoparticles administered are modified based on the results obtained in the first experimental situation. The new concentrations administered are:  $0.7 \times 10^{-3}$  M,  $0.5 \times 10^{-3}$  M and  $0.3 \times 10^{-3}$  M. The control group is injected with 0.9% NaCl solution.

## 2.8 Histological Analysis of Rainbow Trout Liver

For histological analysis, very fine cuts of the samples are made in such a way that they allow their microscopic analysis without the components overlapping visually. This technique is based on consecutive stages: sample collection, fixation, dehydration, clarification and infiltration, cutting, assembly and staining [23].

### Sample collection:

The autopsy of the dead fish is performed and the organ of interest is removed with a scalpel. The liver is kept in 10% formalin buffered with calcium carbonate for 48 hours.

**Histological cut:** a 3 mm cut is made to each organ. It is left in the sample holder and in water for 10 minutes.

**Inclusion:** Place the sample holders in alcohol 70% for 1.5 hours. Locate the sample holders in alcohol 96% (I, II and III) for 1.5 hours.

Place the sample holders in 100% alcohol (I, II and III) for 1.5 hours.

Locate the sample holders in xylol (I, II, III) for 1.5 hours.

Locate the sample holders in paraffin (I, II) for 1.5 hours.

A 5-micron cut of the tissue is made into a microtome. The cuts are left at H<sub>2</sub>O at 37 °C until the stretch of the sample is visualized. Then a slide was taken and albumin was added. To fix the sample was placed in the stove for 15 minutes.

### Staining:

Xylol, 100% ethanol, 96% ethanol, hematoxylin, eosin and distilled water.

## 3. RESULTS

### 3.1 Reaction of Formation Of Nanoparticles Supported In Carbon Nanotubes.

**Table 2.** Reaction mixture for formation of supported nanoparticles.

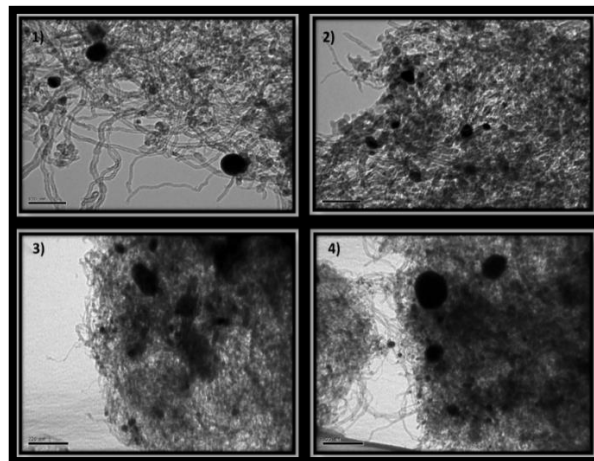
Metal	Amount (g)	Reagent	Concentration (M)	Nanotubes (g)	Reagent volume (mL)
Gold	0.0098	2-propanol	$1 \times 10^{-3}$	0.1400	50
Silver	0.0054	2-propanol	$1 \times 10^{-3}$	0.0770	50
Copper	0.0032	2-propanol	$1 \times 10^{-3}$	0.0457	50
Gold	0.0109	2-etoxyethanol	$1 \times 10^{-3}$	0.0780	50
Silver	0.0056	2-etoxyethanol	$1 \times 10^{-3}$	0.0770	50
Copper	0.0035	2-etoxyethanol	$1 \times 10^{-3}$	0.0455	50

### 3.2 Scanning Electron Microscopy

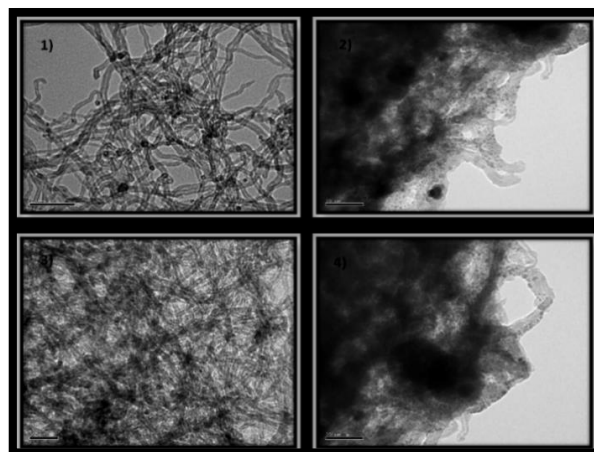
The SEM studies they do not contribute to knowing the morphology of carbon nanotubes doped with metallic nanoparticles. For this reason, no micrographs are presented.

### 3.3 Transmission Electron Microscopy

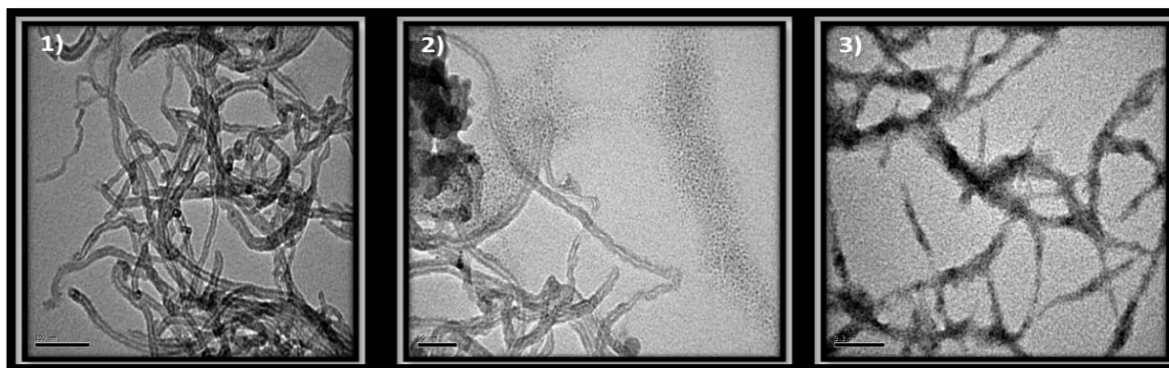
The images below show nanoparticles supported in carbon nanotubes in solvent 2-propanol by the technique of transmission electron microscopy with varying degrees of magnification.



**Figure 1.** Transmission electron microscopy silver nanoparticles supported in carbon nanotubes in 2-propanol solvent.

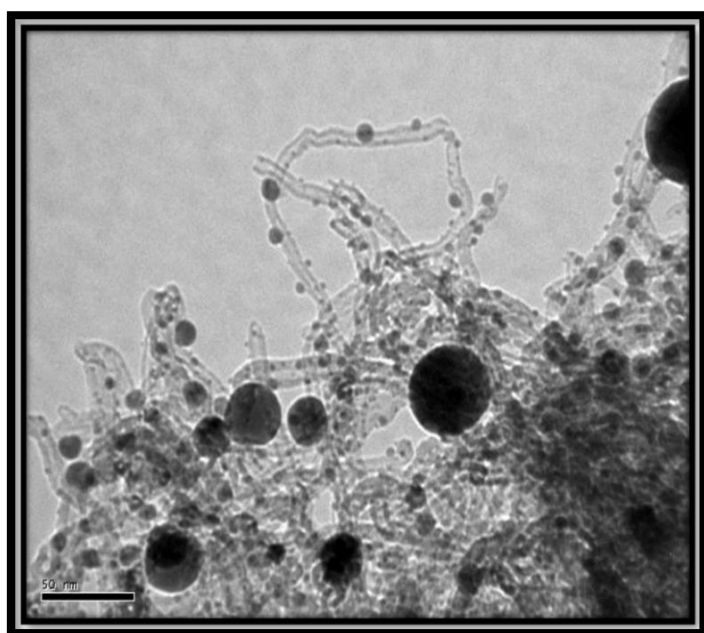


**Figure 2.** Transmission electron microscopy copper nanoparticles supported in carbon nanotubes in 2-propanol solvent.



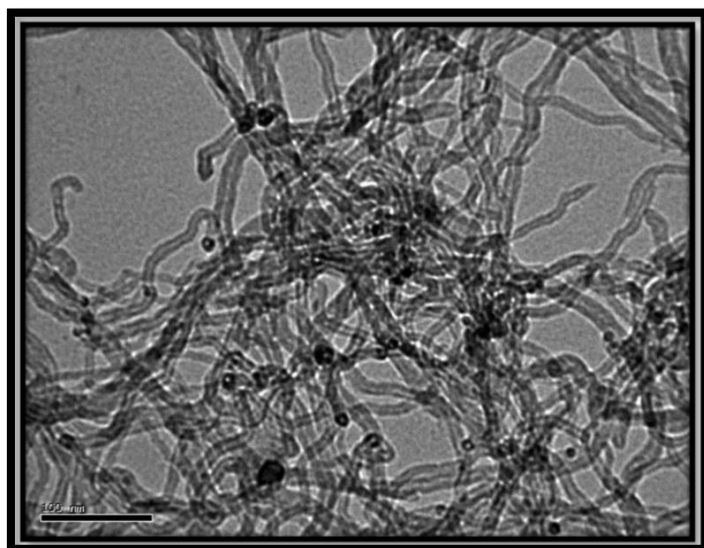
**Figure 3.** Electron microscopy of transmission nanoparticles of gold supported in carbon nanotubes in solvent 2-propanol.

The images presented below have been analyzed using a computer program called MacBiophotonics Image J to make measurements of the nanoparticles and establish their size.



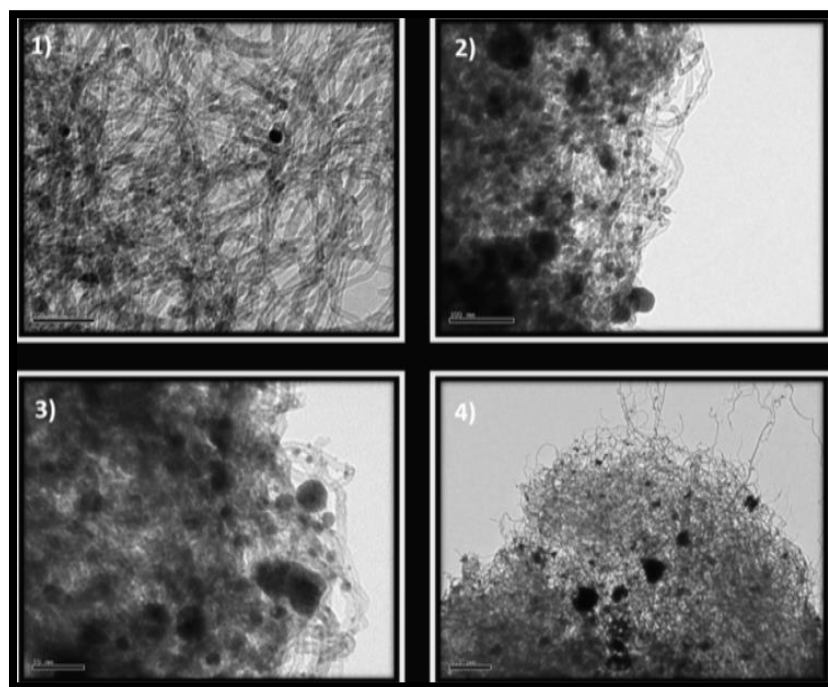
<b>Min</b>	1.47 nm
<b>Max</b>	11.24 nm
<b>S.D.</b>	2.10
<b>Average</b>	4.37 nm

**Figure 4.** TEM silver nanoparticles supported in carbon nanotubes in 2-propanol solvent (left). Summary table size silver nanoparticles (right).

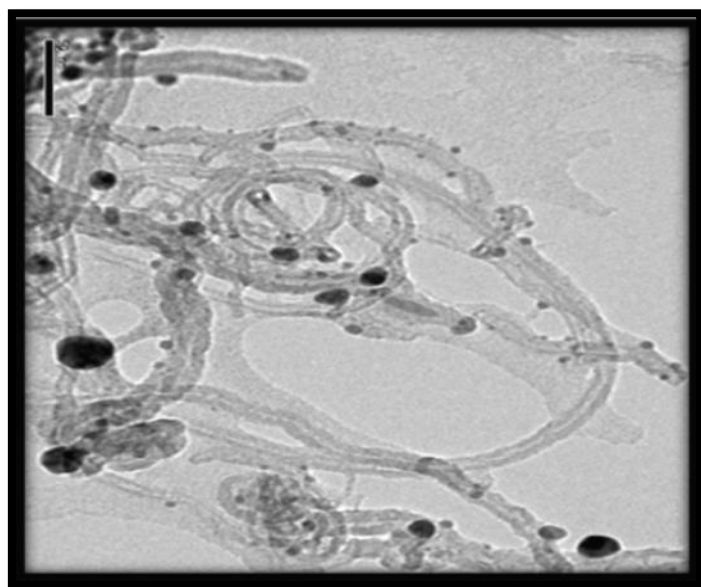


<b>Min</b>	1.03 nm
<b>Max</b>	5.13 nm
<b>S.D.</b>	0.96
<b>Average</b>	2.56 nm
<b>Increase</b>	400000

**Figure 5.** TEM copper nanoparticles supported in carbon nanotubes in 2-propanol solvent (Left). A table shows the average size of copper nanoparticles (right).

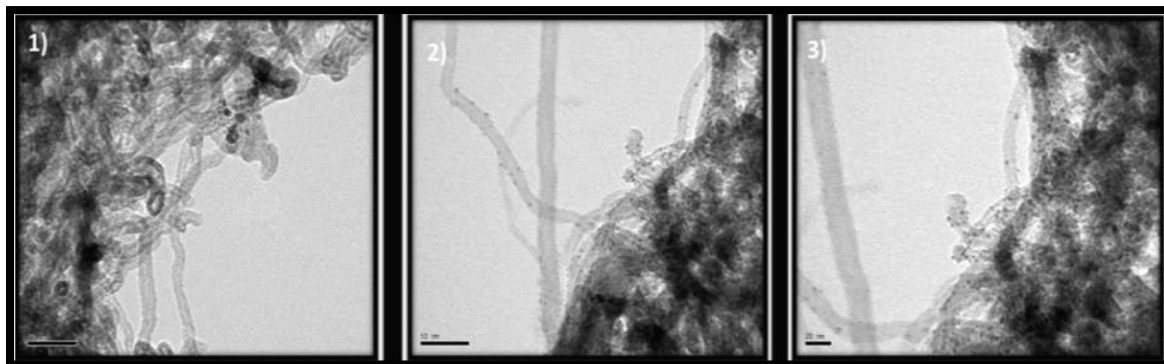


**Figure 6.** Transmission electron microscopy of silver nanoparticles supported in carbon nanotubes in 2-ethoxyethanol solvent.

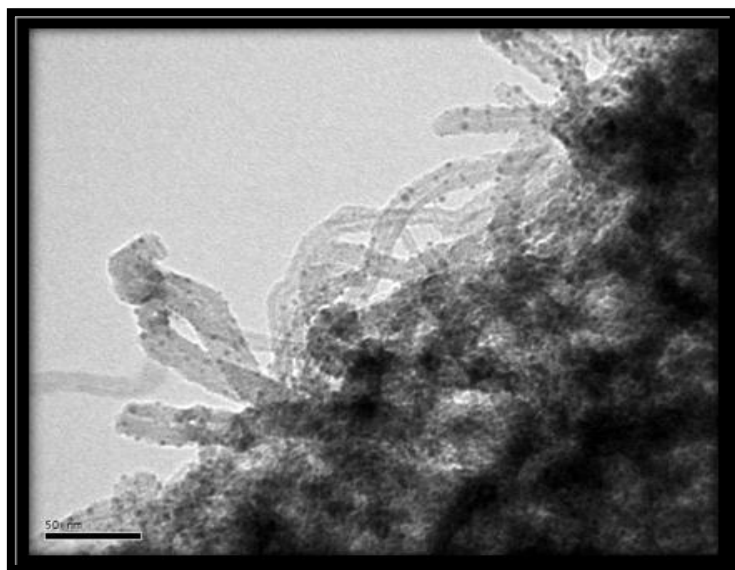


<b>Min</b>	1.54 nm
<b>Max</b>	8.73 nm
<b>S.D.</b>	1.80
<b>Average</b>	4.14 nm
<b>Increase</b>	400000

**Figure 7.** TEM silver nanoparticles supported in carbon nanotubes in 2-ethoxyethanol solvent (left). A table with size of nanoparticles shows the average (right).

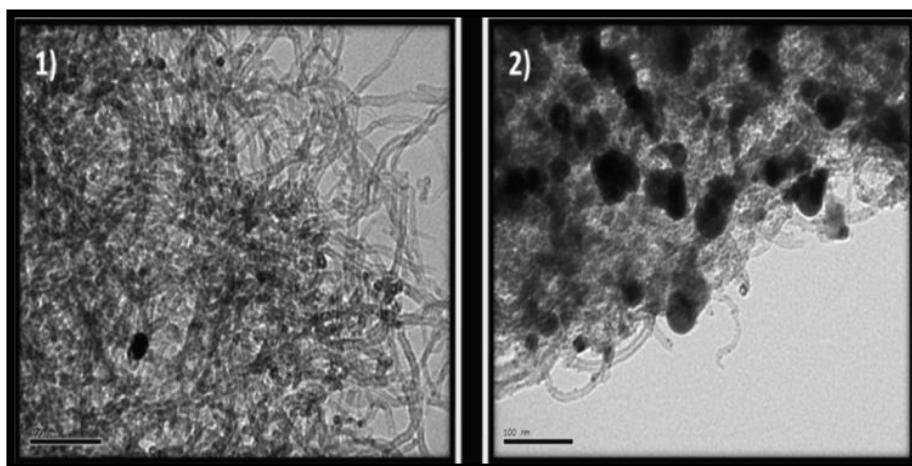


**Figure 8.** Transmission electron microscopy copper nanoparticles supported in carbon nanotubes in 2-ethoxyethanol solvent.

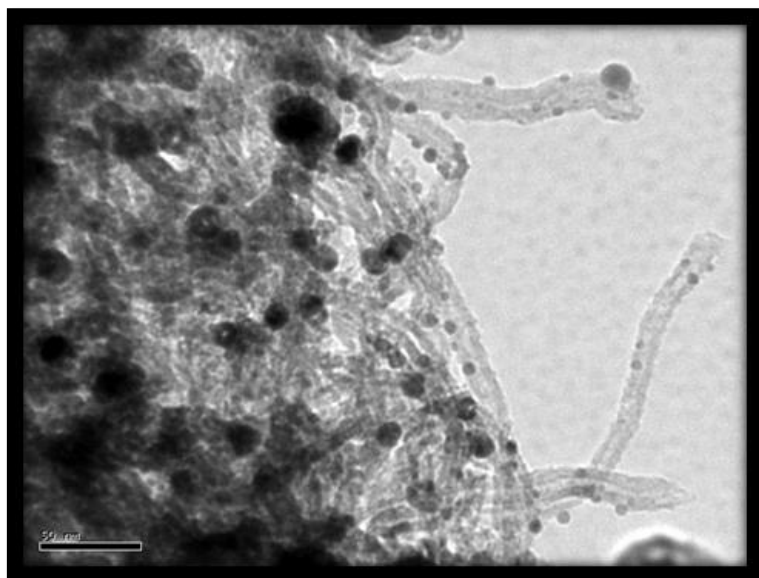


<b>Min</b>	1.03 nm
<b>Max</b>	4.65 nm
<b>S.D.</b>	0.78
<b>Average</b>	2.47 nm
<b>Increase</b>	400000

**Figure 9.** TEM copper nanoparticles supported in carbon nanotubes in 2-ethoxyethanol solvent (left). Summary table of copper nanoparticles size shows the average size.



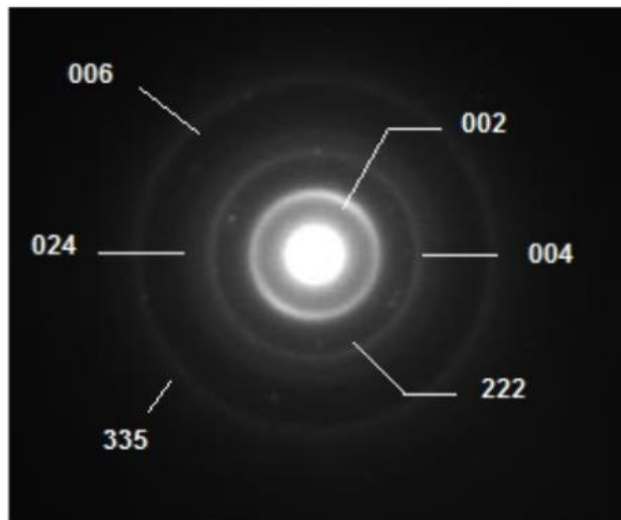
**Figure 10.** Transmission electron microscopy gold nanoparticles supported in carbon nanotubes in 2-ethoxyethanol solvent.



<b>Min</b>	2.12 nm
<b>Max</b>	9.80 nm
<b>S.D.</b>	1.62
<b>Average</b>	5.30 nm
<b>Increase</b>	400000

**Figure 11.** TEM gold nanoparticles supported in carbon nanotubes in 2-ethoxyethanol solvent (left) Summary table size of gold nanoparticles (right).

### 3.3 Electron Diffraction

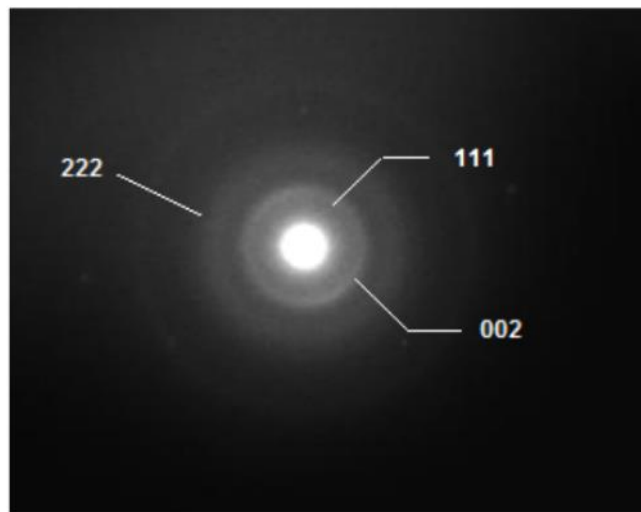


**Figure 12.** Diffraction of silver nanoparticles supported in carbon nanotubes in 2-propanol solvent.

The following table summarizes the most important data from electron diffraction on figure 12.

**Table 3.** Measurements of silver nanoparticle rings supported in carbon nanotubes in 2-propanol solvent from electron diffraction.

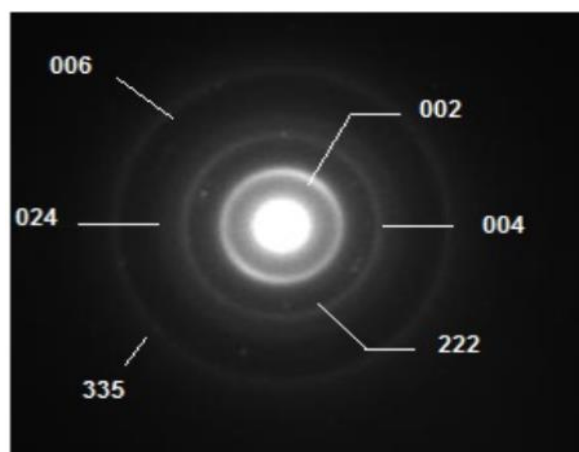
Miller index	Value obtained (Å)	Actual value (Å)	Error %
002	2.027	2.044	0.83
222	1.199	1.180	1.61
024	0.889	0.914	2.7
004	1.060	1.022	2.78
006	0.682	0.681	0.23
335	0.626	0.623	0.48



**Figure 13.** Diffraction of electrons copper nanoparticles supported in carbon nanotubes in 2-propanol solvent.

**Table 4.** Measurements of copper nanoparticle rings supported in carbon nanotubes in 2-propanol solvent from electron diffraction.

Miller index	Value obtained (Å)	Actual value (Å)	Error %
111	2.089	2.087	0.09
002	1.723	1.808	4.66
222	1.044	1.044	0



**Figure 14.** Electron diffraction to sample silver nanoparticles supported in carbon nanotubes in 2-ethoxyethanol solvent.

**Table 5.** Measurements of silver nanoparticle rings supported in carbon nanotubes in 2-ethoxyethanol solvent from electron diffraction.

Miller index	Value obtained (Å)	Actual value (Å)	Error %
002	1.997	2.044	2.26
222	1.158	1.180	1.79
133	1.060	1.022	3.79
006	0.682	0.681	0.23
026	0.638	0.646	1.17

### 3.4 Infrared Spectrophotometry with Fourier Transform (FT-IR)

The following table summarizes the most relevant bands for the composites.

**Table 6.** Classification of present bonds nanoparticles of silver, copper and gold supported in carbon nanotubes in solvent 2-ethoxyethanol.

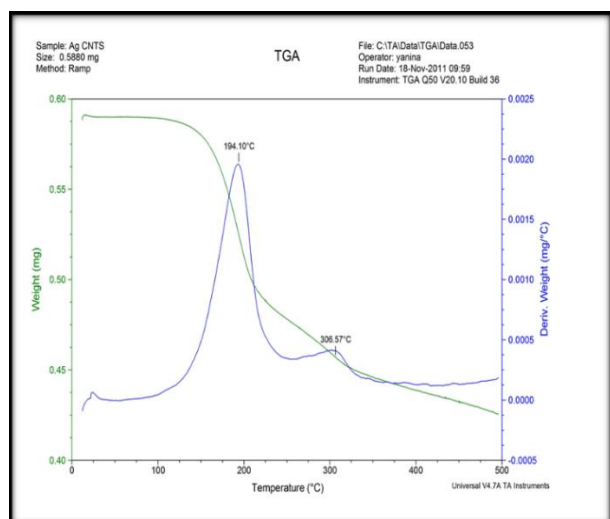
Metal	Silver	Metal	Copper	Metal	Gold
Frequency (cm <sup>-1</sup> )	Type of bond	Frequency (cm <sup>-1</sup> )	Type of bond	Frequency (cm <sup>-1</sup> )	Type of bond
3433	O-H	3434	O-H	3434	O-H
2909	C-H	2917	C-H	2926	C-H
2848	O-H	2852	C-H	2860	C-H
1720	C=O	1722	C=O	1729	C=O
1643	C=C	1642	C=C	1595	-
1521	-	1558	-	1457	-
1450	-	1448	-	1379	-
1239	C-O	1368	-	1276	C-O
1150	C-O	1252	C-O	1125	C-O
1102	C-O	1108	C-O	1075	C-O
1021	C-O	796	-	801	-
950	-	-	-	745	-

### 3.5 Thermogravimetry

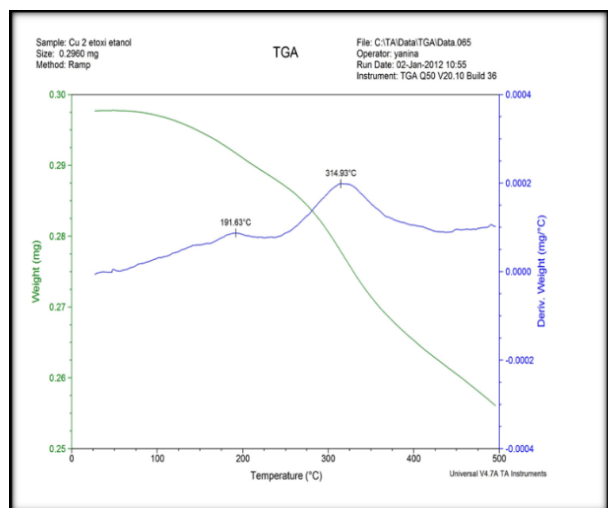
Below are the results of TGA and DTGA analyses performed on gold, silver and copper samples supported in carbon nanotubes in the 2-propanol solvent.

**Table 7.** Percentages of mass loss and critical temperature of gold, silver and copper samples supported in carbon nanotubes in 2-propanol solvent.

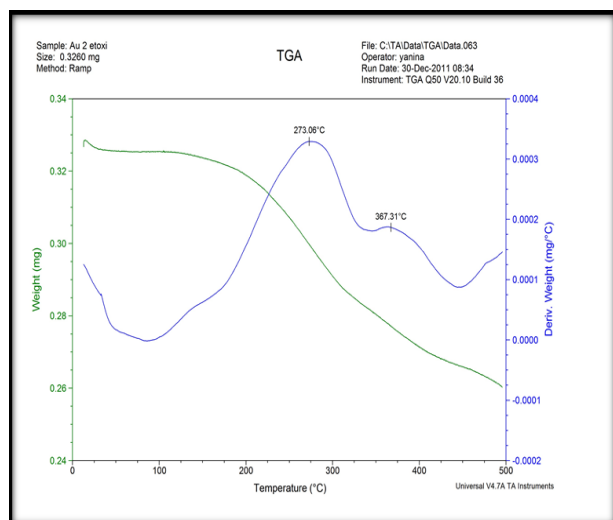
Sample	Silver	Copper	Gold
Weight (mg)	0.289	0.253	0.534
Loss %	47	19	59
Residue %	0.153	0.205	0.219
Critical temperature (°C)	397.44	298.97	410.47



**Figure 15.** TGA (green line) analysis and DTGA (blue line) shows a decomposition temperature at 194 and 306.57°C for silver sample supported in carbon nanotubes in 2-ethoxyethanol solvent.



**Figure 16.** TGA (green line) and DTGA (blue line) showing a DTGA at 191 and 314.93°C for copper sample supported in carbon nanotubes in 2-ethoxyethanol solvent.



**Figure 17.** TGA (green line) and DSC (blue line) for gold sample supported in carbon nanotubes in 2-ethoxyethanol solvent.

**Table 8.** Exhibit the TGA data.

Sample	Silver	Copper	Gold
Weight (mg)	0.588	0.296	0.326
Loss %	28	13.5	19
Residue %	0.425	0.2562	0.264
Critical temperature 1 (°C)	194.10	191.63	273.06
Critical temperature 2 (°C)	306.57	314.93	367.31

### 3.7 Acute Toxicity Bioassay

Below is a table with mortality rates of juvenile rainbow trout performed in the first toxicity test administering different concentrations of gold nanoparticles supported in carbon nanotubes in the solvent 2-ethoxyethanol.

**Table 9.** Table mortality first analysis in fish exposed to gold quantum dots supported in carbon nanotubes in 2-ethoxyethanol.

Concentration	Total fish	Live fish	% live fish	Dead fish	% dead fish
$1 \times 10^{-3}$ M	5	1	20	4	80
$0.6 \times 10^{-3}$ M	5	4	80	1	20
$0.3 \times 10^{-3}$ M	5	5	100	0	0
Control	5	5	100	0	0

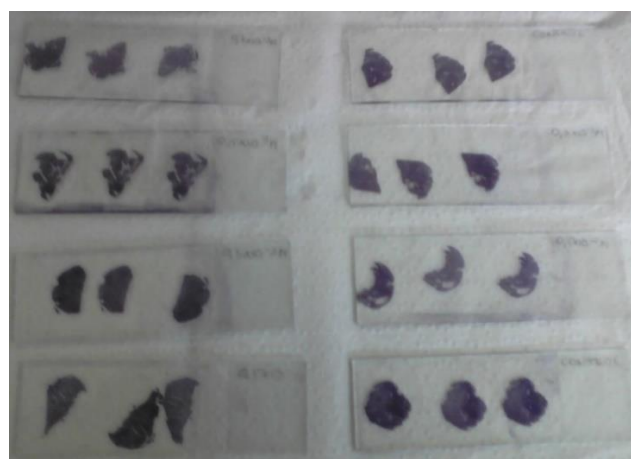
In the second study the results obtained were the following:

**Table 10.** Table mortality second analysis in fish exposed to gold quantum dots supported in carbon nanotubes in 2-ethoxyethanol.

Concentration	Total fish	Live fish	% live fish	Dead fish	% dead fish
$10.7 \times 10^{-3}$ M	5	1	20	4	80
$0.5 \times 10^{-3}$ M	5	5	100	0	0
$0.3 \times 10^{-3}$ M	5	2	40	3	60
Control	5	2	40	3	60

### 3.8 Histological Liver Analysis in Rainbow Trout

The images below show the cuts of fish liver that were injected with gold quantum dots supported in carbon nanotubes in 2-ethoxyethanol solvent at different concentrations. Subsequently, the cuts were deposited on slides and subjected to staining, generating the product shown below, for visualization and analysis under a microscope. Samples are exhibited in figure 18.



**Figure 18.** Histological cuts liver rainbow trout for analysis under microscope

## 4. DISCUSSION

The images showed the term of the reaction involving the formation of quantum dots in the solvent 2-ethoxyethanol and the support in carbon nanotubes. The presence of nanotubes (black) is displayed at the bottom of the reactor. The second micrograph shows the sample once it has been subjected to heat to evaporate the entire solvent.



The six reactions were performed following the same protocol described in the materials and methods section. It is necessary to check the formation of nanoparticles from metals in the state in which they are found in nature and the formation of bonds with carbon nanotubes.

It is necessary to carry out a series of analyses in order to determine the size, location, presence or absence, contamination and other aspects, of the materials with which work began at the beginning of the reaction.

Transmission electron microscopy was performed on reactions involving gold, silver and copper. The images show the presence of nanotubes, which are those structures that are observed in an elongated way that can be compared with a string, nanoparticles on the contrary are those circular structures of different sizes that sometimes represent large black dots. The large black heads are due to agglomerations of nanoparticles. In addition, the location of the nanoparticles is observed both outside and inside the nanotubes.

All the samples made in the solvent 2-propanol were taken with different degrees of magnification in order to find an image that would allow in the first instance to identify the presence of the nanoparticles and then clearly differentiate the nanoparticles from the nanotubes. Not all the images managed to clearly show the difference between nanotubes and nanoparticles, discarding those that presented little sharpness for the determination of particle size.

Presence of the nanoparticles was verified by electron microscopy, the size of the silver, copper and gold quantum dots was determined with the MacBiophotonics ImageJ computer program. The measured nanoparticles were those visualized in their entirety, the X axis was taken as a reference for the measurement of the diameter, which has been designated as the length of the nanoparticle.

For silver quantum dots and nanoparticles in 2-propanol solvent, the particle size ranges from 1.47 nm to 11.24 nm. The mean size was 4.37 nm with a standard deviation of 2.10 nm. The image magnification was  $4.2 \times 10^5$ . Distribution graphs show that quantum dots sizes with a higher percentage of distribution were: 2.61; 3.73 and 3.36 each with a percentage of 14.3%.

In the image of silver in solvent 2-ethoxyethanol, the particle size ranges from 1.54 to 8.73 nm. The mean size was 4.14 nm with a standard deviation of 1.80 nm. The image magnification was  $4.0 \times 10^5$ . The histogram shows that the quantum dots sizes with the highest percentage were 3.08 and 3.59 nm with 12.5%.

The copper nanoparticles in 2-propanol solvent are ranging from 1.03 to 5.13 nm, with an average size of 2.56 nm and a standard deviation of 0.96 nm. The image magnification was  $4.0 \times 10^5$ . The distribution graph shows that the nanoparticles with a higher percentage of distribution were 1.5 nm with a 14% percentage followed by a size of 2.05 nm with a 10% percentage.

Copper quantum dots in 2-propanol solvent, the particle size ranges from 1.03 nm to 4.65 nm, with an average size of  $2.47 \pm 0.78$  nm. The image magnification was  $4.0 \times 10^5$ . The distribution graph shows a higher percentage of distribution for the nanoparticle size of 2.15 nm with a 24% percentage followed by the size 1.54 nm with 16%.

Gold quantum dots were measured in 2-ethoxyethanol solvent, of which the size ranges from 2.12 nm to 9.80 nm. The mean size was  $5.30 \pm 1.62$  nm. The image magnification used was  $4.0 \times 10^5$ . The histogram shows that the highest percentage was 13% for the nanoparticle size of 5.6 nm.

The sizes obtained for the silver, copper and gold samples in both solvents do not exceed 12 nm. Then, we can conclude that the products obtained are a mixture of quantum dots and nanoparticles [24].

Thermogravimetry analyses were performed for gold, silver and copper quantum dots in order to determine the thermal stability of the new products generated. The temperature ranges chosen were from 25°C to 500°C. In this range, only mass loss should be detected by the solvent residues used, 2-propanol and 2-ethoxyethanol, since their boiling temperatures are 81°C and 135°C, respectively) [25].

All the samples presented interference which was reflected in the calculation of the weight derivative. The interference was electrostatic which was evident to the naked eye when seeing how the nanomaterials reacted in the presence of any

metallic object that approached them. By calculating the derivative, the critical temperature at which the product begins to lose mass was obtained. Below are the temperatures of the samples made in the solvent 2-propanol, silver: 397.44°C; copper: 298.97°C and gold: 410.47°C. In solvent 2-ethoxyethanol, two critical temperatures were recorded for each sample, silver: 194.10°C and 306.57°C, copper: 191.63°C and 314.93°C, gold: 273.06°C and 367.31°C.

It is not possible to associate the melting points of metals with the critical temperatures obtained by TGA since these indicate that the most heat-resistant metal is copper with a melting temperature of 1083°C, followed by gold: 1064°C and finally silver: 962°C [26]. If we look at the critical temperatures in the solvent 2-propanol, they indicate that gold is the most resistant followed by silver and copper. The same situation can be observed with the solvent 2-propanol since its melting temperature is recorded at 81°C and in no sample is there a loss of mass at that temperature.

The samples obtained in the solvent 2-ethoxyethanol also do not allow to associate the solvent losses to the critical temperatures indicated because the boiling temperature of this solvent is 135°C and the lowest recorded is 191.63°C. Recording these critical temperatures suggests the presence of a new compound produced by the interaction of the solvent 2-ethoxyethanol with air. This assumption was not proven in this study.

The sample that recorded the greatest weight loss in 2-propanol solvent was gold with 59%, followed by silver with 47% and finally copper with 19%. In the solvent 2-ethoxyethanol the greatest mass loss was recorded in the silver sample with 28%, followed by gold with 19% and finally copper with 13.5%. The temperatures obtained in both solvents did not allow to obtain a comparison pattern between them because in the 2-propanol solvent the greatest loss was recorded in the gold sample and in the 2-ethoxyethanol solvent it was recorded in the silver sample. If we analyze the samples based on the compounds present we can see that the stability of the samples is not related to the presence of metals since they have melting points much higher than 500°C. The observed temperatures reflect the presence of solvent residues that have interacted with the environment.

The FTIR measurements were made in the mid-infrared spectrum for gold, copper and silver samples in both 2-propanol solvent and 2-ethoxyethanol. The results for 2-propanol present 3 characteristic bands [27]. Based on this, the presence of bonds in silver and copper samples in the presence of the solvent 2-propanol has been observed. Vibrations appear at  $3400 \text{ cm}^{-1}$  ( $\nu$ -O-H),  $3000 \text{ cm}^{-1}$  ( $\nu$ -C-H), and  $1,000\text{-}1200 \text{ cm}^{-1}$  ( $\nu$ -C-O). In silver samples have been observed bonds with vibrational energies in the frequencies  $2962 \text{ cm}^{-1}$ ,  $2902 \text{ cm}^{-1}$  and in copper  $2922 \text{ cm}^{-1}$ ,  $2855 \text{ cm}^{-1}$  by the presence of the C-H bond, which they are very close to the frequency of  $3000 \text{ cm}^{-1}$  so they can be identified as bands of the solvent. The same goes for bands in the range  $1000 - 1200 \text{ cm}^{-1}$ .

It is interesting to remark the presence of C=O stretching in the 2-ethoxyethanol system which means reduction of alcohol function by metal quantum dots. No information can be obtained in the middle range about metal-oxygen interactions. The rest of the absorptions generated are motivated by the movements of the bonds other than tension, such as their flexion, but these signals have not been classified in this study [28].

These analyses demonstrate the presence of residues of the solvents 2-propanol and 2-ethoxyethanol in the carbon nanotubes.

Electron diffraction was performed on all samples, however, not all of them managed to generate diffraction. The rings were measured with the MacBiophotonics ImageJ program. In the sample of silver nanoparticles supported in carbon nanotubes in 2-propanol solvent, the following interplanar distances were obtained:  $2.044 \text{ \AA}$ ;  $1.180 \text{ \AA}$ ;  $0.914 \text{ \AA}$ ;  $1.022 \text{ \AA}$ ;  $0.681 \text{ \AA}$  and  $0.623 \text{ \AA}$ , with these data it was possible to identify the Miller indices to which they corresponded: 002, 222, 004, 024, 006 and 335 respectively. From the Miller indices, using tabulated data, the interplanar spaces to which they corresponded were searched and a similarity was seen in these values with those obtained with errors less than 5%. In the sample of silver nanoparticles supported in carbon nanotubes in 2-ethoxyethanol solvent it was possible to identify 5 interplanar distances, where the measurements obtained were:  $1.997 \text{ \AA}$ ;  $1.158 \text{ \AA}$ ;  $1.060 \text{ \AA}$ ;  $0.682 \text{ \AA}$  and  $0.638 \text{ \AA}$ , which correspond to the following Miller indices: 002, 222, 133, 006 and 026 respectively. When comparing the silver nanoparticles in both solvents we see that the interplanar spaces  $2.044 \text{ \AA}$ ;  $1.180 \text{ \AA}$  and  $0.681 \text{ \AA}$  were found in both samples. These distances that are characteristic for each element allow the identification of the material of interest, in this study the presence of silver atoms is corroborated.

The sample of copper nanoparticles supported in carbon nanotubes in 2-propanol solvent has the following interplanar spaces: 2.089 Å: 1.723 Å and 1.044 Å. These measurements correspond to the Miller indices of copper: 111, 002 and 222, respectively. These interplanar distances made it possible to identify copper atoms present in the sample. Both diffraction studies allow to characterize the interplanar distances between the copper and silver atoms present in the samples supported in carbon nanotubes, however, in this study the analysis of electron diffraction was not deepened.

The different concentrations of gold nanoparticles supported in carbon nanotubes in 2-ethoxyethanol solvent administered have provided partial data on the ranges that are acceptable and non-toxic for the species under study (rainbow trout). The experiments showed that the concentration  $1 \times 10^{-3}$  M, is highly toxic for fish with a mortality percentage of 80% of these species, so it will not be considered in subsequent tests. The concentration  $0.6 \times 10^{-3}$  M administered instead showed a mortality of 20% that corresponds to a single dead fish so it is acceptable to administer and consider in future trials. The concentration of  $0.3 \times 10^{-3}$  M administered to the fish showed zero mortality indicating that this dose is not fatal to the fish. The control group to which NaCl was administered 0.9% registered zero mortality rate therefore no death should be associated with stress conditions in the new habitat, disease or poor handling in the injection of solutions.

In the second experimental situation, different concentrations were considered based on what was granted in the first experience. The concentration  $1 \times 10^{-3}$  M was discarded and the highest administered was  $0.7 \times 10^{-3}$  M, followed by  $0.5 \times 10^{-3}$  M and finally  $0.3 \times 10^{-3}$  M. In this experience, deaths of 80% were generated in the control group, therefore, the deaths that occurred in other fish with different concentrations of nanoparticles administered could not be attributed to this fact. It was ruled out in this experience that the injection was one of the causes of death through observation in the bioassay laboratory.

The mortality percentages in this experience were: for the concentration of  $0.7 \times 10^{-3}$  M of 80%, for the concentration  $0.5 \times 10^{-3}$  M zero, and the concentration  $0.3 \times 10^{-3}$  M together with the control group of 60%. In this experience the only concentration that met the expected was  $0.5 \times 10^{-3}$  M, from here all the lower doses administered should have presented zero mortality rate. Possible causes of death are discussed below.

The presence of fungal contamination was evident. The fish presented superficial wounds and whitish skin coloration in part of the organism. This fungal infection is common in freshwater fish and not so much saltwater fish because salt is an excellent fungicide. The stress conditions to which the fish were subjected, when extracted from their natural habitat, transported and subsequently subjected to a new medium, is a possibility of death along with fungal infection, because stress, which usually increases the production and release of the hormone cortisol, is one of the largest causes of deaths in fish (Aquanovel life aquarium).

The histological sections obtained were analyzed by microscope. Two types of magnification were used for each display: 4X and 10X. The histological architecture of the organ under study (liver) with 4X magnification, for the control fish demonstrated distribution of liver structures according to what was expected for species and age status. The 10x increase warned of mild central vein congestion. This situation was repeated in the two control species and for one of the fish injected with a concentration of  $0.3 \times 10^{-3}$  M. The second liver analyzed of concentration  $0.3 \times 10^{-3}$  M with increase 4x presented a congestion with a degree greater than the control, noticing a considerable number of erythrocytes at the sinusoidal level.

Each of the five livers injected with nanoparticle concentration  $0.5 \times 10^{-3}$  M, four had the same characteristics as the control group and one had little congestion. By congestion, it refers to the accumulation of blood elements not associated with the administration of nanoparticles. On the other hand, the liver with a nanoparticle concentration of  $0.7 \times 10^{-3}$  M presented zones of cytoplasmic vacuolizations with nuclear displacement with a certain predominance of location bordering the central veins. This damage covers 30% to 35% and is the only one that can be associated with the administration of an excessive dose of nanoparticles to the fish organ. Therefore, a deeper acute toxicity test should not be ruled out.

In general, the livers did not present cellular alteration, except for the concentration  $0.7 \times 10^{-3}$  M and the hepatocytes exposed a morphology according

to what was expected for the species. Based on the first basic toxicity studies, gold nanoparticles supported in carbon nanotubes in 2-ethoxyethanol solvent can be proposed as a future drug. This certainly needs to be supplemented by other, more rigorous studies.

## CONCLUSIONS

Nanoparticles of different sizes were obtained for the gold, silver and copper compounds supported in carbon nanotubes in solvent 2-propanol and 2-ethoxyethanol. The average size in 2-propanol solvent for silver was 4.37 nm, for copper 2.56 nm. The size obtained in the solvent 2-ethoxyethanol for silver was 4.14 nm, for copper 2.47 nm and for gold 5.30 nm.

Thermogravimetry and DSC analyses provided the critical temperatures of the compounds, these being for silver nanoparticles supported in carbon nanotubes in 2-propanol solvent: 397.44°C, copper: 298.97°C and gold: 410.47°C. In the solvent 2-ethoxyethanol two critical temperatures were recorded for each sample, being for the silver metal: 194.10°C and 306.57°C, for copper: 191.63°C and 314.93°C, for gold: 273.06°C and 367.31°C. The mass losses in 2-propanol solvent were; silver: 47%, copper: 19% and gold: 59% and solvent 2-ethoxyethanol, silver: 28%, copper: 13.5% and gold 19%. No relationship was found between critical temperatures and boiling temperatures of solvents. The presence of new compounds generated from solvents and interaction with the environment is inferred.

The FTIR analysis indicate the presence of residues of the solvents used. The characteristic frequencies of the C-H bonds close to frequency of 3000  $\text{cm}^{-1}$ , OH at the frequencies of 3400  $\text{cm}^{-1}$  (only in solvent 2-ethoxyethanol) and C-O at the frequencies of 1200 to 1000  $\text{cm}^{-1}$ , also C=O presence is observed in 2-ethoxyethanol. Most characteristic bands of organic solvents, were observed.

X-ray diffraction allowed to characterize the interplanar distances present in the study samples in both copper and silver. The distances obtained for silver supported in carbon nanotubes in 2-propanol solvent were: 2.044 Å: 1.180 Å: 0.914 Å: 1.022 Å: 0.681 Å and 0.623 Å. For the silver samples supported in carbon nanotubes in 2-ethoxyethanol solvent the distances obtained were: 1.997 Å: 1.158 Å: 1.060 Å: 0.682 Å and 0.638 Å. Copper samples gave the following distances: 2.089 Å: 1.723 Å and 1.044 Å.

All these distances were obtained experimentally, then they were compared with the tabulated data and gave errors of less than 5%. This analysis characterizes and confirms the presence of silver and copper atoms in the samples obtained.

The acute toxicity analysis gave the fish mortality rates for the different concentrations of gold nanoparticles supported in carbon nanotubes in 2-ethoxyethanol solvent. Mortality rates were:  $1 \times 10^{-3}$  M: 80%,  $0.6 \times 10^{-3}$  M: 20%,  $0.3 \times 10^{-3}$  M: zero. With these results, the first parameters of concentrations that must be used and will not be fatal for the species under study are established. In the second study there was death in the control group, it is inferred that by fungal infection, corroborated with the naked eye. The stress conditions to which the fish were subjected in transport and adaptation to a new medium were added. The mortality percentages in this experience corresponded to 60% in the control group and to the fish with concentration  $0.3 \times 10^{-3}$  M, zero for the concentration  $0.5 \times 10^{-3}$  M and 80% for the concentration  $0.7 \times 10^{-3}$  M.

The analysis of histological sections showed a mild central vein congestion for the control group, one of the livers of the concentration  $0.3 \times 10^{-3}$  M and 4 livers of the concentration  $0.5 \times 10^{-3}$  M. For the other liver of the concentration  $0.3 \times 10^{-3}$  M a more severe congestion was evidenced and the remaining liver of concentration  $0.5 \times 10^{-3}$  M showed a less than mild congestion. However, the liver analyzed for  $0.7 \times 10^{-3}$  M showed areas of cytoplasmic vacuolization with nuclear displacement with a certain predominance of location bordering the central veins, this being the only damage that could be associated with the nanoparticles. In general, the livers did not show cellular alteration exposing hepatocytes with morphology according to what was expected to the species.

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