CHARACTERIZATION OF ORGANIC NANOPARTICLES FOR THE PRODUCTION OF THE TOPICAL MEDICATIONS

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ABSTRACT

In this study, we present the experimental results of structural characterization of organic nanoparticles derived from Dendrobium moschatum, using combined techniques such as transmission electron microscopy (TEM), energy dispersive X ray fluorescence spectroscopy (EDS), and zeta potential in order to obtain the form, size and stability of these nanoparticles as well as know the chemical nature of the samples to support the current studies on the development of topical medications using these nanoparticles.

INTRODUCTION

This study discusses the results of structural characterization obtained for three qualities of organic nanoparticles: pure, diluted in alcoholic extract and nanoparticles with gel. The nanoparticles studied are organic, derived from plants such as *Allivium sativus*, *Dendrobium moschatum*, *Ginkgo biloba* and *Zingiber officinalis* in thermal water-based solution (A-complex) [1], whose production methods are protected by patents in the United States (US7335638B2, US7732410 and US7914781).

These nanoparticles, together with recombinant proteins of *Trypanosoma cruzi trans-sialidase* (B-complex) form the basis of a product being studied in the form of PTCTS gel (trans-sialidase particulate) which have not yet commercialized and protected by US patents (US7335638B2, US7674832B2 and US7906114B2). The effects observed are anti-oxidants and high anti-apoptotic activity, presenting anti-aging properties and skin healing by release of free radicals and proinflammatory cytokines, May nanoparticles neutralize free radicals and, consequently, reduce topical inflammations [2].

MATERIALS AND METHODS

Preparation of materials

In this study, three types of nanoparticles were analyzed: (A) pure organic, (B) diluted organic and (C) organic in gel. The organic nanoparticles of the alcoholic extract comes from the following preparation: plant extract – produced through a mixture of 30% of orchid flowers (*Dendrobium moschatum*), 60% ethanol and 10% water, remaining more than 1 month in a sealed bottle. After that, the flowers were removed and the extract was filtered in glass microfiber filters of 0.45 μm pore. When diluted in thermal water, such extract was mixture 1: 1.

The gel which makes the sample of organic nanoparticle is prepared according to the following proportions: 25% anion gel (currently is used commercial *Dermavita* gel, composition: carbomer, methylparaben, propylparaben, imidazolidinyl urea, propylene glycol, glycerin, triethanolamine, deionized water); 24.995% thermal water; 0.005% extract previously diluted (1: 1 in thermal water with 0.13% EDTA); 25% non-ionic gel (currently is used commercial *Dermavita* gel, composition: hidroxyetilcelulose, glycerin, propylenoglycol, imidazolidinyl urea, methylparaben, disodium EDTA, water); 24.995% ultrapure water; 0.005% active enzyme of trypanosome cruzi transialidase produced by recombinant *E.coli* bacteria, presenting around 3.0 mg/mL, diluted 1: 100,000 in sterile water.

Zeta Potential analysis

For the realization of measures, 1000 μL of pure organic nanoparticles extract was diluted with 4000 μL of ultrapure water and shaken in vortex tubes for 2 minutes. The resulting dispersion was added to zeta potential cuvette and inserted in the device. Zeta potential (ZP) is applied to quantify the charge of nanoparticles and forecast the stability in dispersions. The ZP is calculated with the Helmholtz-Smoluchowski equation determined by the measurement of the electrophoretic mobility [3,4]. Laser Doppler anemometry (*Zetasizer Nano ZS*, Malvern Instruments, UK) was used for ZP analysis. The measurements were performed in original dispersion medium (aqueous phase of the formulation) and in conductivity adjusted Milli-Q water (50 μS/cm) using 0.9% NaCl solution. Measurements were carried out as triplicate.

Transmission electron microscopy and Energy dispersive X ray fluorescence spectroscopy

The composition of the nanoparticles (and many other types of materials) may be identified by transmission electronic microscope (TEM) through a technique known as energy dispersive X ray fluorescence spectroscopy (EDS), a method of qualitative and quantitative analysis of elemental composition by the excitation of atoms and detect its characteristic X ray. When the electron beam focuses on the material, electrons belonging to the nearest layers of the atom's nucleus are excited, occurring change in energy levels. When they return to original position, they release the energy emitted with a certain wavelength, in the X ray spectrum, which is measured by a detector installed in the vacuum chamber of TEM. The components of the sample are distinguished by the fact that the electrons that make up an atom have different energies. EDS spectra have a spectrum due to copper or gold, as the holders for microscopy analysis is made of these materials. EDS analysis is not destructive to the sample.

These three types of nanoparticles were analyzed: (A) pure organic (B) diluted organic and (C) organic in gel. In the micrographs shown in this study, samples were prepared by depositing the solution containing the nanoparticles on screens support copper or gold, coated with a film *Collodion* and carbon and, after drying for approximately 72 hours, the bright field images were obtained from a microscope *MET JEOL JEM 2100*, operated at a DC voltage of 200 kV, at the Analytical Center of the Chemistry Institute of University of São Paulo. For analysis using EDS is the same samples used to MET and are performed in the same transmission electron microscope.

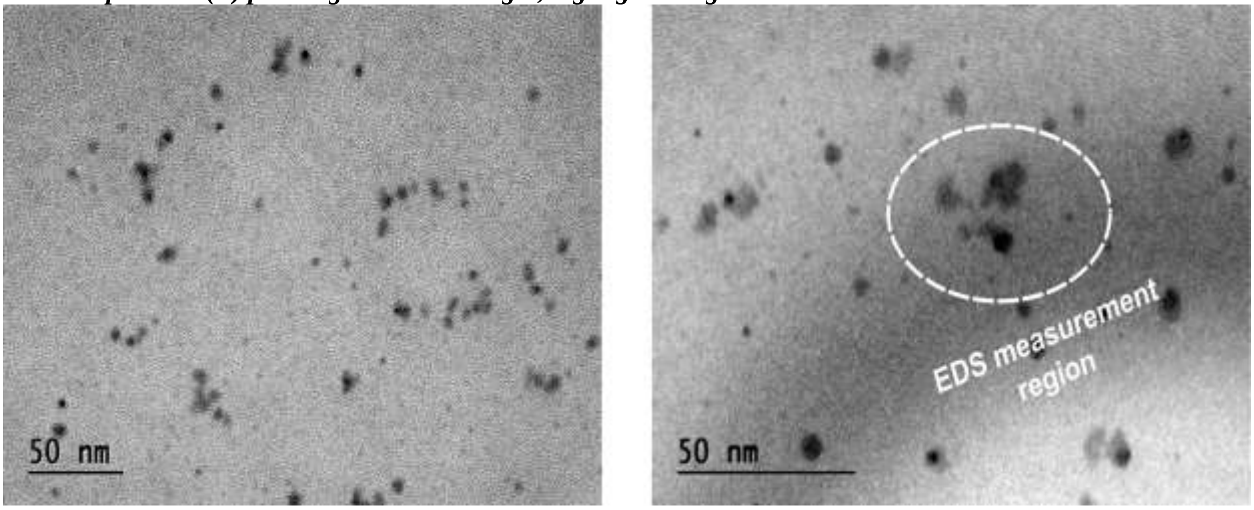
RESULTS AND DISCUSSION

Particle size and surface charge of nanoparticles govern the biodistribution and pharmacokinetic attribute of the nanoparticles in the body. Changing parameters such as size, morphology and charge can greatly influence on how a nanoparticle interact with and perform in biological environment. For the zeta potential, values above 30 mV, in module, in this case (-55 ± 3) mV, provide good stability by preventing aggregation of the nanoparticles due to repulsive forces between the particles with similar electric charge.

The Figure 01 shows the micrographs obtained for the nanoparticles (A) pure organic. We can see that the nanoparticles have a very small size. In this sample, five micrographs were taken of different areas used to estimate the particle size distribution. Note that the focus of the particles in the image is not perfectly suited for this sample. This is generally for organic samples, because the nature of these nanoparticles does not allow adequate light-dark contrast to afford perfect images, which does not happen, for example, metallic nanoparticles. To obtain images with excellent graphic quality using this technique requires the use of a substance that allows the acquisition with high contrast images. However, to do this, we may be changing the nature and composition of the sample to be analyzed.

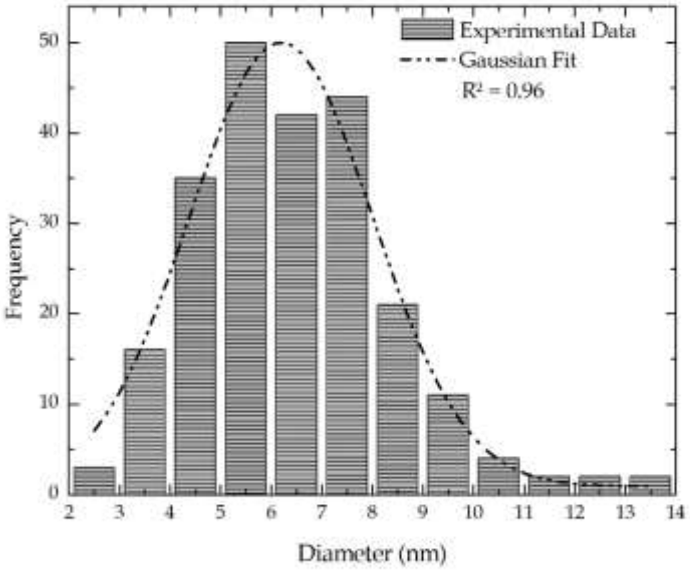
Histograms were constructed for the average size of the nanoparticles. The histogram for nanoparticles (A) pure organic shown in Figure 02. The images were processed with *Image J* software. This software has several plug-ins, with which many different studies can be executed. It has a plugin for particle size analysis, but images must be binarized. How nanoparticles are not very different from the image background, being a little dark, the edge boundary information would be easily lost. However, it is possible to use another method in the same software. Initially, makes an image calibration, converting pixel values in nanometers values, using as reference the scale bar provided by MET equipment. After this, draws up a straight manually on each diameter, automatically marking particles. The software gives as a result that marking various parameters, such as perimeter and area, however, here only used as a variable diameter to define particle size.

Figure 01: Micrographs obtained by transmission electron microscopy, showing morphology and size to nanoparticles (A) pure organic. On the right, highlighted region where was made the EDS measures.



For each type of nanoparticle approximately 200 counts were made (200 entries), using various micrographs, which allows to generate histograms with acceptable values. The average size of the nanoparticles was obtained by Gaussian parameter adjustments performed with the correlation coefficient, R^2, greater than 0.96 to nanoparticles (A) pure organic, $R^2 = 0.67$ for the nanoparticles (B) diluted organic and $R^2 = 0.96$ for the nanoparticles (C) organic in gel. The values were: nanoparticles (A) pure organic $\langle D \rangle = (7 \pm 2)$ nm and polydispersity index of 29%; nanoparticles (B) diluted organic $\langle D \rangle = (61 \pm 36)$ nm and polydispersity index of 59%. For the nanoparticles of sample (C) organic in gel, $\langle D \rangle = (30 \pm 8)$ nm and polydispersity index of 23%. In practice, the higher the polydispersity index, wider the size distribution of nanoparticles. This means that the organic nanoparticle in gel had the lowest polydispersity index, which is therefore more stable in this medium.

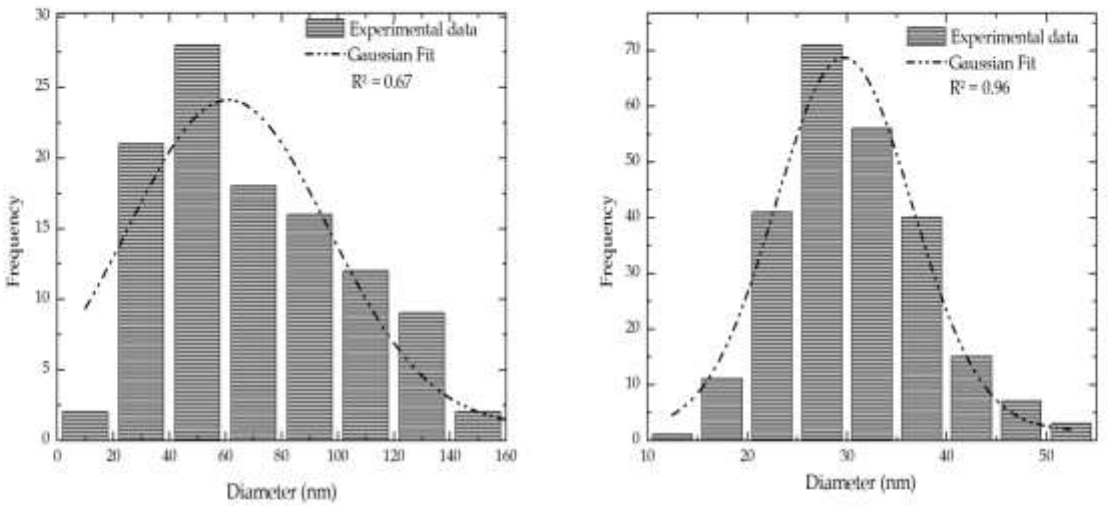
Figure 02: Size distribution histogram for the nanoparticles for sample (A) pure organic. The dashed line represents a Gaussian fit.



The histogram for nanoparticles (B) diluted organic and (C) organic in gel shown in Figure 03. We observed that sample (B) has the distribution more wide than the samples (A) or (C). This is due to the nanoparticles (sample B) are not homogeneous and are widely dispersed as the high polydispersity index suggests. Another interesting factor is that when diluted, nanoparticles behave form different species of forming "aggregates" of small particles (see Figure 04), and these inevitably have to be treated as a larger particle.

Furthermore, when they are in a semi-solid dispersion (gel) nanoparticles tend to become slightly more stable (see micrographs with these nanoparticles in the Figure 05). Therefore, the standard deviation value changes for these samples. The polydispersity index is obtained by ratio of the standard deviation, σ, and the average diameter, x_c, times 100%.

Figure 03: Size distribution histogram for the nanoparticles for: on the left, sample (B) diluted organic and on the right, sample (C) organic in gel. The dashed line represents a Gaussian fit.



The adjustment equation of Gaussian curves has been used to adjust the histograms is given by:

$$f(x) = f_0 + \frac{A}{\omega\sqrt{\frac{\pi}{2}}} exp\left\{-2\left[\frac{(x - x_c)}{\omega}\right]^2\right\}$$

where:
f_0 is the deviation from the baseline;
x_c is the center of the peak of the Gaussian curve;
ω is equal to 2 times the standard deviation of the distribution Gaussian (2σ) or about 0.849 of the peak width at half height;
A is the area under the peak.

EDS spectra (see Figure 06) showed the presence of non-metals such as carbon and oxygen, and minerals such as sodium and potassium. It is known that the thermal waters are rich in minerals [5], which leads us to believe that the presence of these two elements is strongly correlated with the solvent used to preserve the nanoparticles. The carbon, copper and gold, both samples is due to experimental support screen to conduct the sample, but for treating organic material, carbon is the chemical element, of course, should appear. Was also detected the presence of a weak band, referring to the silicon in the sample (B) dilute organic suspected that also is related to the type of thermal water used. According to [6], the presence of silicon has been investigated in thermal waters.

The Figure 06 (C) also shows the EDS spectrum for nanoparticles organic in gel. The excellent contrast of nanoparticles in the micrographs is evidenced by the increased concentration of copper found in these samples. The measurements were performed on screen support made of gold, to avoid losing the copper spectrum due to overlap with the copper screen spectrum.

Figure 04: Micrographs obtained by transmission electron microscopy, showing morphology and size to nanoparticles (B) diluted organic. On the right, highlighted region where was made the EDS measures.

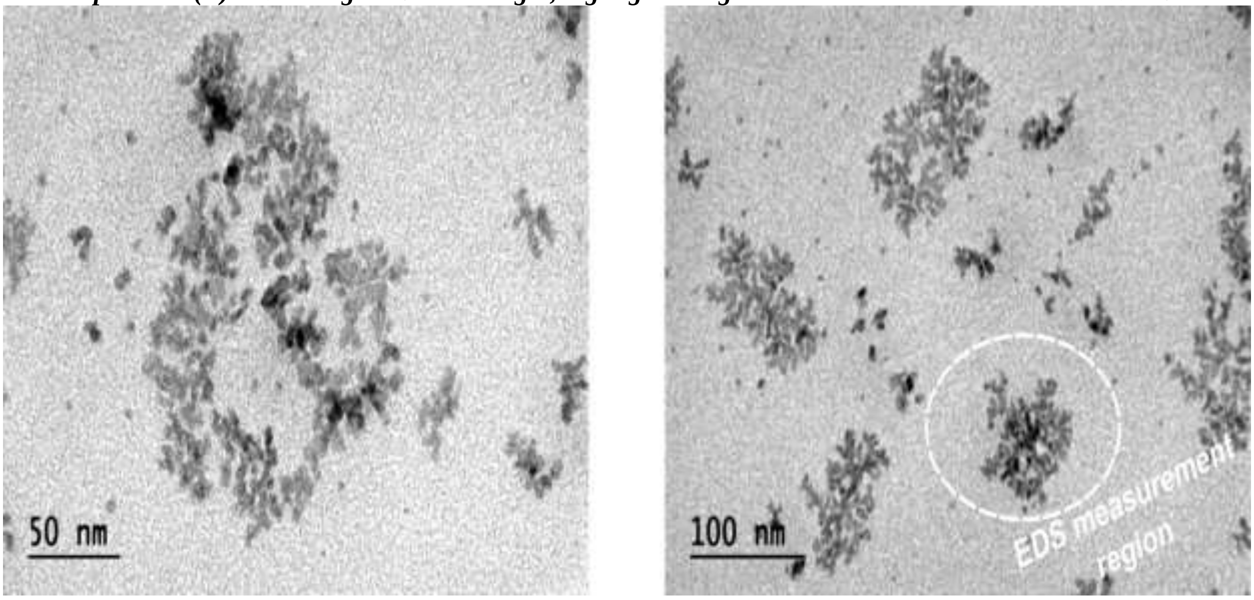


Figure 05: Micrographs obtained by transmission electron microscopy, showing morphology and size to nanoparticles (C) organic in gel. On the right, highlighted region where was made the EDS measures. The excellent contrast of nanoparticles in the micrographs is evidenced by the increased concentration of copper found in these samples.

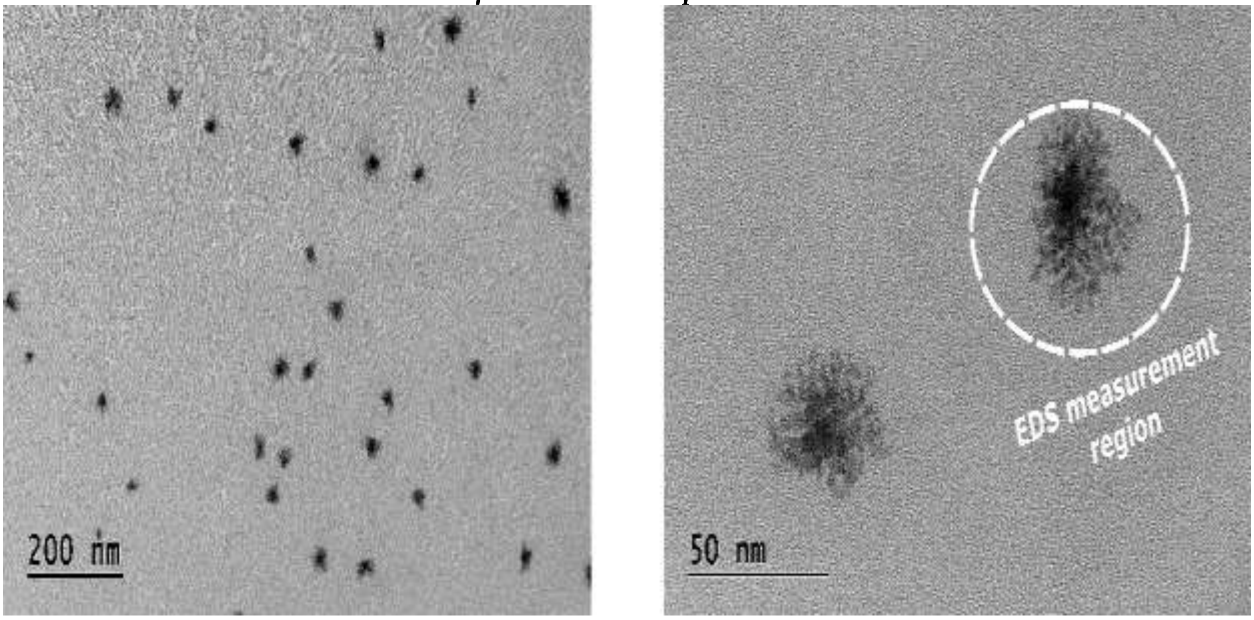
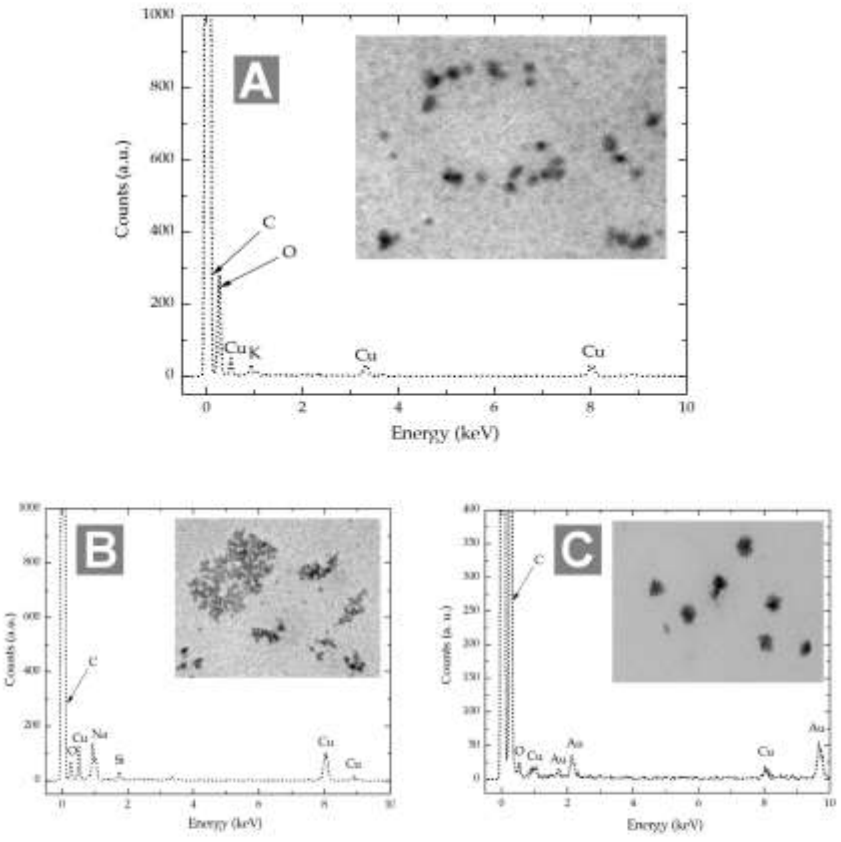


Figure 06: (A) EDS spectra for pure organic nanoparticles, for the region shown in Figure 01 (B) EDS spectra for diluted organic nanoparticles, for the region shown in Figure 04 and (C) EDS spectra for organic in gel nanoparticles to the region shown in the Figure 05.



CONCLUSION

This study was conducted as part of the proposed actions to characterize organic nanoparticles produced by plants. The chemical and structural characterization is basic and priority factor before working with a material whose composition, effects and damage is unknown. In this sense, the powerful technique of transmission electron microscopy coupled to energy dispersive X ray fluorescence spectroscopy and zeta potential makes it possible to obtain the form and structure of nanoparticles, evaluate the stability of the particles, in addition to the chemical elements, which makes it possible to study them separately, if required. Our analysis showed three different types of organic nanoparticles: pure nanoparticles showed a very small size, while the nanoparticles diluted in an alcohol solution had a greater aggregation state with a higher polydispersity index showing an enlargement in the size distribution. In addition, the gel nanoparticles showed a controlled size between 20 and 30 nm with a polydispersity index of 23%, the lowest presented for the studied samples, ensuring stability. EDS spectra for this sample showed a considerable amount of copper. EDS spectra for the other nanoparticles showed elements as mineral salts, silicon, carbon and oxygen in accordance with the expected source for organic samples. Zeta potential measurements in the solutions studied showed a great stability to the particles, at least in the short time.

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