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GOLD NANOPARTICLES STABILIZED WITH GUM ARABIC FOR CANCER TREATMENT

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ABSTRACT

Cancer is a group of diseases characterized by uncontrolled growth and abnormal spread of cells. The number of deaths due to cancer is higher than the ones caused by AIDS, tuberculosis and malaria combined. Among the different options of cancer treatment radiotherapy (teletherapy and brachytherapy) stands out. The presence of gold nanoparticles (AuNPs) may enhance energy deposition for teletherapy treatment. The use of nanoparticles for brachytherapy have been studied, and AuNPs is a good option once they can easily permeate tumor vasculature and remain in tumors. However, the tumor uptake of AuNPs may be significantly reduced due the attenuation with the formation of the protein coronas. The objective of this work is present the functionalization of AuNPs with arabic gum (GA). GA is a biocompatible, nontoxic, water soluble, natural gum obtained from *Acacia senegal* tree. In this study, a synthesis of gold nanoparticles (AuNPs), based on Turkevich method, using citrate (NaCit) solution as reducing agent and a HAuCl4 solution, under vigorously stirring and boiling temperature, going from a light yellow to a wine red in three minutes. The functionalization of the nanoparticles was performed with Arabic gum solutions, in three different concentrations, which were added under stirring to the AuNPs already obtained. Samples were characterized to measure the size of the samples. Lower concentrations o GA in the solution presented smaller coated particles (up to 45 nm).

1. INTRODUCTION

Cancer is one of the leading causes of death in the world. This term describes a large set of diseases characterized by the anomalous reproduction of cells [1]. For 2018, World Health Organization (WHO) estimated 18.1 new cases of cancer and 9.6 deaths as a result of cancer [2]. Nowadays, there are different cancer treatments available, and choose of treatment will depend of a set of factors such as the type of cancer, the stage of the illness, the health and preferences of the patient among others. Surgery, chemotherapy, hormonotherapy, and radiotherapy are examples of cancer treatment [3].

Although radiotherapy is not a treatment developed recently, every day more and more new research has been emerged in this field. The treatment uses a radiation source to kill cancerous cells and shrink the tumors. Radiotherapy may be divided in two: teletherapy and brachytherapy [3].

Differently from teletherapy, in brachytherapy the radioactive source take place into to the tumor or near of it, thus allowing the physicians deliver the dose of radiation in a smaller area, affecting less healthy tissues. Brachytherapy may be used as a treatment for cancer in different parts of the body such as prostate, head, uterus, eye, among others [4].

In recently years, nanotechnology has driving new research, including for the medical field. For cancer treatment particles with a nanometric size, also known as nanoparticles (NPs), has been studied as a possibility to improve existent therapies or even been using in new treatments types [5], [6], [7], [8].

The NPs have been causing revolutions in different researches fields, once they may have different properties when compared with their macroscopic versions. For cancer treatment they may present advantages such as multimodal imaging, superior targeting for specific sites, theranostic properties, the possibility of modulate the surface properties and last but not least size adjustment [7], [8] [9]. Recently, some researches about the use of NPs as the treatment source in brachytherapy has arisen.

1.1. Gold Nanoparticles

Gold nanoparticles (GNPs) has been already used for some therapeutic uses, and some studies has shown the benefits of unite teletherapy and GNPs [10]. These NPs has also an excellent biocompatibility with antibodies, antigens, and other biomolecules. GNPs with functionalized surface may also accelerate the reaction antibody-antigen [11].

The GNPs may be obtained by many differents synthesis, by chemical route Turkevich (citrate reduction) method stands out as a classic, and for this work this was the method chosen. In this synthesis, ordinarilly citrate is used as the reductor agent but literature report some variants of this method using changing the reductor agents, pH, temperature, reagents proportions, among others [12].

Due the different properties of gold in nanoscale, when compared with metallic gold, new applications have arisen, in medical field some can be cited, such as in photodynamic therapy as heat source, or as a sensor for protein detector, or even as carrier of therapeutic agents [12].

New studies, targeting use radioactive nanoparticles for brachytherapy instead of the milimetrics seeds has been emerged. An isotope of interest for this application is the 198-Gold, once it presents a half-life of 2.69 days, and emits a desirable β particles (maximum energy = 0.96 MeV) and a γ (411 keV in ca. 96 %) [12] [13].

The scientific community focused on nanobrachytherapy may be considered still small once the resources for this kind of development can be restrictive, so some researches still in intial stages, such as synthesis development, studies about coatings functionalization [12].

1.2. Arabic Gum

Arabic gum (GA) is a natural gum obtained from *Acacia senegal* tree. This gum biocompatible, nontoxic, water soluble. It is a complex polysaccharide that is being extensively used as stabilizing, emulsifying and thickening agent in the food industry. It is also an important hydro soluble ligand, known as biologically safe, and also has a low cost. For GNPs Arabic gum has a stabilizer function, which will prevent that NPs undergo changes.[14]

The aiming of this work is to functionalize GNPs with Arabic gum and evaluate the obtained results, keep in mind that in future it will be a previous stage for radioactive GNPs, trying to optimizing the functionalization process.

2. MATERIALS AND METHODS

As a first step, all glassware was rinsed with aqua-regia ($HCl:HNO_3$ 3:1), and then washed with ultrapure water - type 1, obtained in a Milipore Direct- Q3 UV System (18.2 mΩ·cm at 25 °C). All solutions used in this work were prepared using ultrapure water – type 1.

Gold nanoparticles wer obtained following the method described by Daruich et al, 2018 [15]. In a flask with reflux, under a strong agitation, 50 ml of a solution 0,001 M of $HAuCl_4$ (puchased from Sigma-Aldrich) were added and heated up to 130 °C, then 5 ml of 0,038 M sodium citrate (purchased from Sigma-Aldrich) solution was added and few seconds after the reaction started, this take about 2 minutes until lost the initial yellow color, and remain colorless for a few seconds and finally change to an intense wine color, and that is an indicative of the end of the reaction[15].

When the GNPs reached room temperature, the coating process with gum Arabic (purchased from Synth) was proceeded. In an attempt to reach nanoparticles with the smaller hydrodynamic size, three solutions of GA with different concentration were prepared: 1) 10 ml [$1x10^{-5}$ M], 2) 10 ml [$1x10^{-6}$ M], 3) 10 ml [$1x10^{-7}$ M].GNPs functionalization with GA (GA-GNPs) were performed under a slowly magnetic agitation, for different time periods, and in different proportions of GA and GNPs as can be seen in Table 1. The solution of GA was always added in the GNPs colloid, not the contrary.

Table 1. GA-GNPs functionalization parameters

	Sample	GA:GNPs	Agitation (min)		Sample	GA:GNPs	Agitation (min)		Sample	GA:GNPs	Agitation (min)
$1x10^{-5}$ M GA concentration	A1	1:1	1	1x10-6 M GA concentration	B1	1:1	1	1x10-7 M GA concentration	C1	1:1	1
	A2	1:1	5		B2	1:1	5		C2	1:1	5
	A3	1:1	10		B3	1:1	10		C3	1:1	10
	A4	1:1	20		B4	1:1	20		C4	1:1	20
	A5	2:1	1		B5	2:1	1		C5	2:1	1

2.1. Characterization

Dynamic light scattering

The hydrodynamic diameter and the Polydisperse Index (PdI) of aqueous suspensions of all samples were measured by dynamic light scattering (DLS) with a Litesizer™ 500 from Anton Paar Instruments.The mesurements were performed in a fixed temperature of 25 ºC. The hydrodynamic diameter was calculated from the average of three measurements.

UV-Vis

UV-visible spectra of nanoparticles suspensions were performed with a Shimadzu UV-1600 spectrophotometer, with a spectral range of 400 to 700 nm, using a 10 mm quartz cell.

3. RESULTS AND DISCUSSION

Gold colloids presented a reddish color, even after coating as can be see in Figure 1, this color change can also be observed with an UV-Vis spectrometry. GNPs should display a single absorption peak in the visible range between 510-550 nm, because of surface plasmon resonance of GNPs it presented an absorption peak at 525 nm (figure 2) [11] .

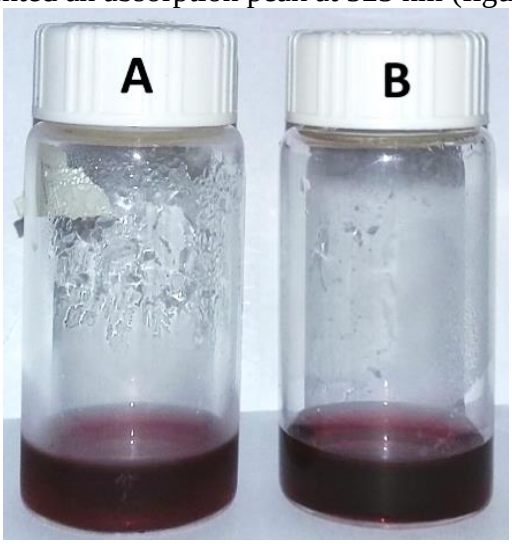

Figure 1. A) GNPs with GA, B) GNPs without GA

In Figure 1, presents GNPs and a GA-GNPs colloids; the size obtained for GNPs without GA was 29 nm, which considered an optimal size for biological applications. Several studies have shown that nanoparticles design can cause differential cell signaling when compared with free ligand in solution. According literature the optimal size for coated nanoparticles should be between 30 and 50 nm of diameter, in this range the ligand density is optimal, and the called “sweet spot” occur. The Sweet spot is the process which induces the membrane to wrap around the nanoparticle to form a closed-vesicle structure [16].

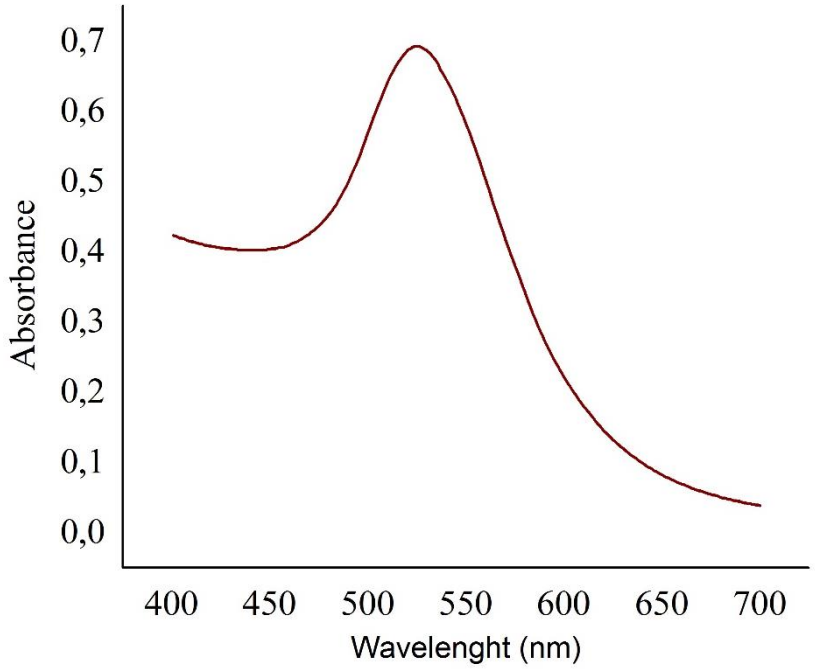

Figure 2. UV/visible surface plasmon absorbance spectrum for GNPs sample.

GA-GNPs prepared with the solutions with GA solutions with higher concentration presented also the biggest sizes, even considering that the initial size of the particle is ca. 29 nm, as shown in Table 2. This result may be due an agglomeration of the Arabic gum polysacarides in the NPs surface. Organic materials may exhibit strong attraction to each other in aqueous media. When nanoparticles coated with organic molecules approach other organic molecules, the water molecules in contact with them tend to be displaced into the solution and thus reduce the total free energy of the system, leading to a favorable interaction between organic molecules.

Table 2. Size measurements with DLS for GA-GNPs

	Sample	DLS size (nm)		Sample	DLS size (nm)		Sample	DLS size (nm)
$1x10^{-5}$ M GA concentration	A1	86,84 ±1,23	1x10-6 M GA concentration	B1	82,52 ±1,62	1x10-7 M GA concentration	C1	45,41 ±1,12
	A2	85,65 ±0,93		B2	79,06 ±0,40		C2	81,81 ±1,64
	A3	88,00 ±2,00		B3	71,10 ±1,95		C3	57,86 ±1,07
	A4	77,58 ±2,77		B4	83,16 ±8,63		C4	64,30 ±1,32
	A5	90,92 ±1,88		B5	82,97 ±1,79		C5	67,86 ±1,89

Thus in the less concentrated solutions of GA, a smaller size of the nanoparticles was observed, therefore, the agglomeration by free molecules was smaller [17]. Other important contribution to the agglomeration is the presence of hydrogen bonded with amylopectin rings

which are capable of folding around the nanoparticles and, thus, are expected to prevent the aggregation of GNPs [18]. A comparison of the smallest NPs obtained for each GA solution, which for the aiming of this studied will be considered the best of each, is shown in Figure 3.

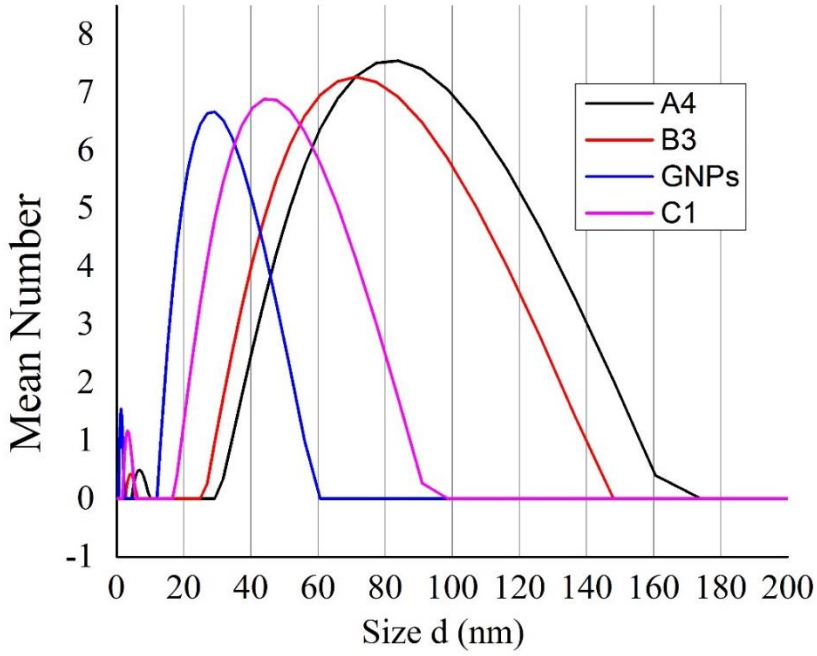

Figure 3. Comparison of GNPs and GA-GNPs size by DLS.

4. CONCLUSIONS

For this work a methodology of nanoparticles surface functionalization with Arabic gum was evaluated. Gold nanoparticles were synthesized using a citrate reduction to provide the necessary material for the work development, and the nanoparticles presented a hydrodynamic size of 29 nm.

In this work the optimal coating concentration and time agitation were found to be $1x10^{-7}$ M GA and 1 minute, in this condition GA-GNPs of ca. 45 nm were obtained. Our investigations indicated that gum Arabic has a potential to be an excellent backbone for the stabilization of GNPs.

For future works a wider evaluation of GA potential as a coating should be evaluated, such as physical-chemical analysis, stability tests among others to make feasible in-vitro and in-vivo.

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