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LIGHT AND COLOUR EFFECTS ON GOLD NANOPARTICLES

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- **UNDERGRADUATE DISSERTATION: Light and colour effects on gold nanoparticles.**
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ÍNDICE

1. ABSTRACT

A literature review has been made for this final degree project about gold nanoparticles (AuNPs). They are first introduced by describing the metallic nanoparticles, and then the different properties and characteristics that make AuNPs unique thanks to their nanometer-size, studying deeply the "Surface Plasmon Resonance". On the other hand, it has also been considered important to study their synthesis and their different applications, especially in the biomedical field. Special emphasis has been placed on the use of these nanoparticles as colorimetric reagents and the effects of light on AuNPs in radiotherapy, explained in the "results and discussion" section. To conclude are cited new advances that have been studied nowadays, among which the use of AuNPs in tests for Covid-19 stands out.

- **Keywords:** Gold nanoparticles, Surface Plasmon Resonance, Nanomedicine, Sensors, Radiation.

2. INTRODUCTION

The new branch of science that we are going to talk about is "nanotechnology." The term "nano" refers to dimensions 100 nanometers or less. The history of nanomaterials starts in 1959, thanks to a physicist named Richard P. Feynman who suggested that the key to future technology would be scaling down materials to nano level.

2.1 Nanoparticles

A nanoparticle (NP) is a particle with small dimensions that goes from one nanometer (nm) to 100 nm maximum. Thanks to their size, they have specifics characteristics and properties. Materials' properties change as their dimensions become smaller (even though these changes are not always desirable) and as the quantity of the atoms at their surface approaches to be significant.

Nanoparticles have a very high surface area volume relation (Fig.1); this is a very important fact for the diffusion phenomenon. Diffusion is even better with high temperatures. Referring to the melting temperature of nanoparticles, it is lower due to this fact. To sinter them can be at lower temperatures and shorter time than how it is for larger particles. So far, they have been made of metal, non-metal, polymeric and bio ceramic materials.

NPs have many potential applications in a wide range of areas as biotechnology, electronics, aerospace engineering, medicine, and many more. Their scientific research today is increasing due to all the things that characterize them as physicochemical and electrical properties. Most NPs that are used in medicine are: liposomes, dendrimers, and polyethylene glycol. They have been employed as delivery systems for many molecules such as DNA, monoclonal antibodies, proteins, or drugs.

Due to their nanometer-size, NPs have found different ways to go into human bodies and cross biological barriers. That way, NPs have been able to reach sensitive organs such as the liver, heart, brain, etc. Thanks to studies that scientists have done, we can compare a NP with a size of less than 10nm to a gas, in the sense of how they act entering human tissues, and how they modify or change the cell's normal biochemical environment.

So far, what we have seen is how NPs' sizes have good characteristics or applications, but now we are going to talk about toxicity. Comparing small NPs of the same chemical components to large-size nanoparticles, we can say that they have higher toxicity just because of their size, suggesting that toxicity is inversely proportional to the size of particles. This relationship can be explained by their high surface area to volume relation that we named at the beginning, which characterizes NPs. It also takes part in their increased surface reactivity, because they have an excess of atoms on their surface, making them, although thermodynamically more stable than bigger nanoparticles in relation to aggregation processes, more reactive. NPs' surface reactivity shows that compared to large-size particles, they have a better biological activity per given mass when they are absorbed by living organisms, if they are solid particles.

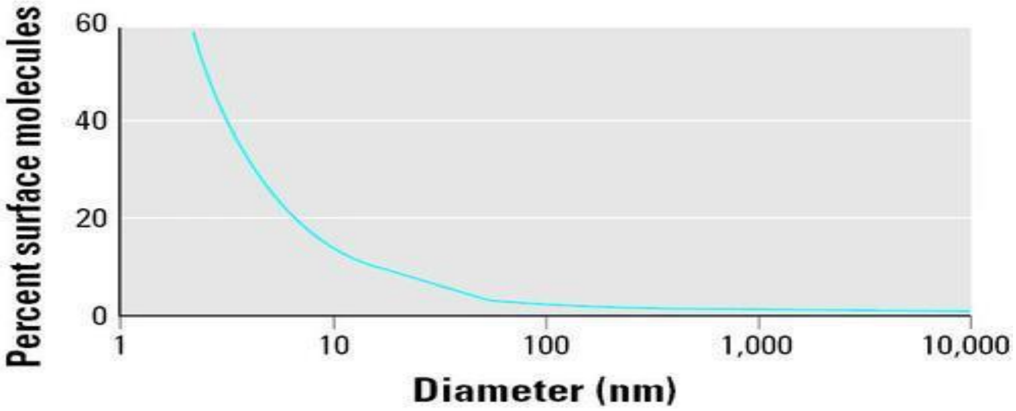

Figure 1. Surface molecules as a function of particle size. Surface molecules increases exponentially when particle size decreases < 100 nm.

The increased biologic activity can be positive and desirable (e.g., antioxidant activity, carrier capacity for therapeutics, penetration of cellular barriers), negative and undesirable (e.g., toxicity, induction of oxidative stress or of cellular dysfunction), or a mix of both. Figure courtesy of H. Fissan. (Oberdörster et al., 2005).

(Gwinn and Vallyathan, 2006) developed a scheme (Fig 2.) of interesting potential interactions of nanoparticles' transportations and their consequent events that happen in our body, focusing on cardiovascular, pulmonary, and other organ involvement.

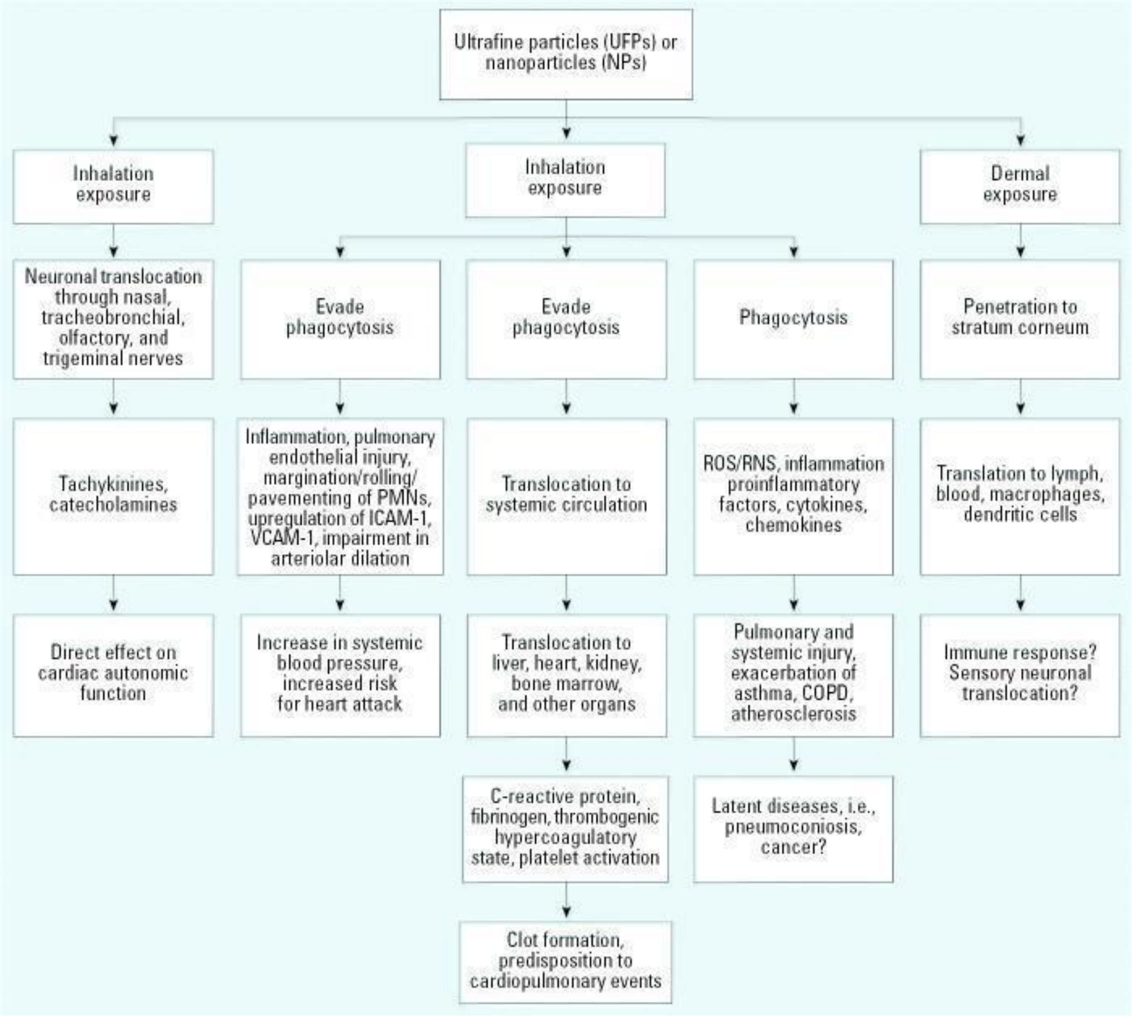

Figure 2. Hypothetical scheme of potential interactions that may occur via inhalation of UFPs and translocation to other organs.

(Abbreviations: ICAM-1, intracellular adhesion molecule-1; PMNs, polymorpho-nuclear leukocytes; RNS, reactive nitrogen species; VCAM-1, vascular adhesion molecule-1). “Scheme also shows suspected interactions (indicated by a question mark) leading to sequences of events that may cause cardiovascular and pulmonary morbidity and mortality” (Gwinn & Vallyathan, 2006).

2.2 Metallic nanoparticles

A long time ago, until the thirteenth century, metallic nanoparticles were mainly used to decorate facades, cups, and windows. Since scientists have discovered remarkable, unique properties and characteristics about nanoparticles such as mechanical strengths, high surface area, low melting point, optical properties, and magnetic properties, NPs have an important spot in nanotechnology, innovation, and science.

In 1727, John Herman Schulze first demonstrated that silver salts turned into black with exposure to light. In 1845, Michel Peyrone synthesized cisplatin being platinum containing anti-cancer drugs (Apostolou et al., 2013). In 1893, Alfred Werner elucidated the cisplatin structure, whereas Roserberg studied the antitumor activity of cisplatin. In the 17th and 19th century, gold nanoparticles were used to treat fever and syphilis, respectively.

It is said from different studies that experimental conditions, kinetics of interaction of metal ions with reducing agents and adsorption process of stabilizing agent, get to influence metallic nanoparticles characteristics and properties. Those characteristics are different from their corresponding bulk metal, and they make nanoparticles useful in many industrial applications. But not all NPs have good properties; they also have drawbacks like: instability, impurity, noxiousness, explosion, or difficulty in synthesis, because is extremely challenging to retain the nanoparticles' sizes in solution form.

- Instability: NPs are thermodynamically unstable, and keeping their structure becomes hard, which prompts a poor quality.
- Impurity: Because of their high reactivity, when they are being synthesized, there could be chemical compounds forming at the same time, which aggravate NPs' purity.
- Biologically harmful: nanomaterials have been reported toxic, carcinogenic, and to cause irritation as they become transparent to the cell dermic (Venkatesh, 2018).
- Explosion: exothermic combustion can lead to explosion, as fine metal particles act as strong explosives (Venkatesh, 2018).

Characterization of Metallic Nanoparticle

There are different techniques that give us information about NPs' characteristics. This way, we can identify them. Some of them are (Rafferty, 2019; Venkatesh, 2018):

a) Absorbance Spectroscopy: As metal NPs have bright colour, this technique permits to characterize them; it shows qualitative information about them by applying Lambert-Beer law. "Beer's law is a relation concerning the absorption of radiant energy by an absorbing medium. It states that the absorptive capacity of a dissolved substance is directly proportional to its concentration in a solution. The relationship can be expressed as $\boldsymbol{A = \varepsilon l c}$. Where A is absorbance, ε is the molar extinction coefficient (which depends on the nature of the chemical and the wavelength of the light used), l is the length of the path light must travel in the solution in centimeters, and c is the concentration of a given solution" (Rafferty, 2019).

b) Infrared Spectroscopy: This method can be useful to figure out information on organic layers surrounding metallic nanoparticles, and it also helps to identify their surface structure.

c) TEM: (Transmission electron microscope) It generally provides information about particle size, shape, crystallinity, and interparticle interaction. TEM is a high spatial resolution structural and chemical characterization tool.

d) SEM: (Scanning Electron Microscopy) It is used to get images of the material's surface, and it tells the purity of nanoparticle sample information.

e) AFM: (Atomic Force Microscopy) It was created to beat a fundamental downside with STM – it can only image conducting or semiconducting surfaces. The AFM has the benefit of imaging practically any kind of surface, including polymers, glass, and organic examples. It is better for nonconductive nanomaterials.

f) EXAFS: (Extended X-ray Absorption Fine Structure) This is one of the most solid and potent characterization methods to assess the structure of metallic nanoparticles. To increase proper data about the structure, the sample of metallic nanoparticles ought to be homogeneous.

g) XPS: (X-ray Photoelectron Spectroscopy) It is used to provide information on metal state. Suppose the oxidation state of metal on the surface. It is often oxidized by air. So, by using this method, zero-valency of surface metal must be confirmed.

2.3 Gold nanoparticles properties: SPR

Gold nanoparticles (AuNPs) have many different properties. Some properties are magnetic, optical like LSPR, high surface area to volume ratio, the good transmission of conduction, low melting point, excellent biocompatibility, and low toxicity. These properties make AuNPs an important tool in bionanotechnology (Yeh et al., 2012).

LSPR refers to the term "Localized surface Plasmon resonance," that is nanoparticles' resonant oscillation of their free electrons in the presence of light. That happens because their electrons form a "cloud" around the atomic cores, not being static; instead, it is mobile, allowing gold nanoparticles to transport charge. As we can figure from quantum mechanics, these electrons could act as particles or as waves with an energy value and finally produce resonance. When a metal in its surface absorbs light of a resonant wavelength, the electron cloud will vibrate, and it will dissipate energy. Those oscillations of the electron cloud of the metal are called "plasmons" (Winter, 2007). Nanoparticles have more potential for surface plasmon resonance (SPR) because of their surface area, that is composed of high proportions of their substance. This surface plasmon resonance occurs in the visible portion of the spectrum and some visible wavelengths can be absorbed and other ones can be reflected giving this metal a certain colour. In this sense: "AuNPs exhibit a range of colours (e.g., brown, orange, red and purple) in aqueous solutions as the core size increases from 1 to 100 nm, and generally show a size-relative absorption peak from 500 to 550 nm (Yeh et al., 2012) . The optical plasmon properties of metal NPs depend on the interparticle distance between pairs of NPs, large or small aggregates of metal NPs as compared to individual and well-spaced nanoparticles (Vilela et al., 2012). This absorption band arises from the collective oscillation of the conduction electrons due to the resonant excitation by the incident photons (Fig. 3), which is called a "surface plasmon band" (Mody et al., 2010). However, this band is absent in both small nanoparticles ($d < 2$ nm) and the bulk material. This phenomenon is influenced not only by size, but also by shape, solvent, surface ligand, core charge, temperature, and is even sensitive to the proximity of other nanoparticles," (Chithrani et al., 2010; Yeh et al., 2012).

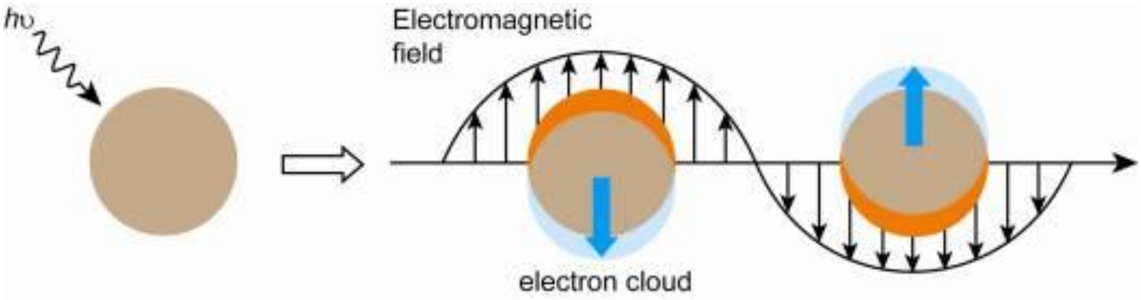

Figure 3. Schematic representation of the oscillation of conduction electrons across the nanoparticle in the presence of the electromagnetic field of the incident light.

With small wavelengths, 400-500 nm, in the spectrum we can find smaller nanoparticles absorbing light in the blue- green portion, instead with bigger wavelengths like 700nm light is reflected producing a red colour. If the size of the nanoparticle increases, their wavelengths of surface plasmon resonance of absorption will be longer absorbing red light and instead reflecting blue light (Fig. 4). If they keep increasing, it reaches a limit where their plasmon resonance wavelengths move into IR, giving these nanoparticles translucent colour.

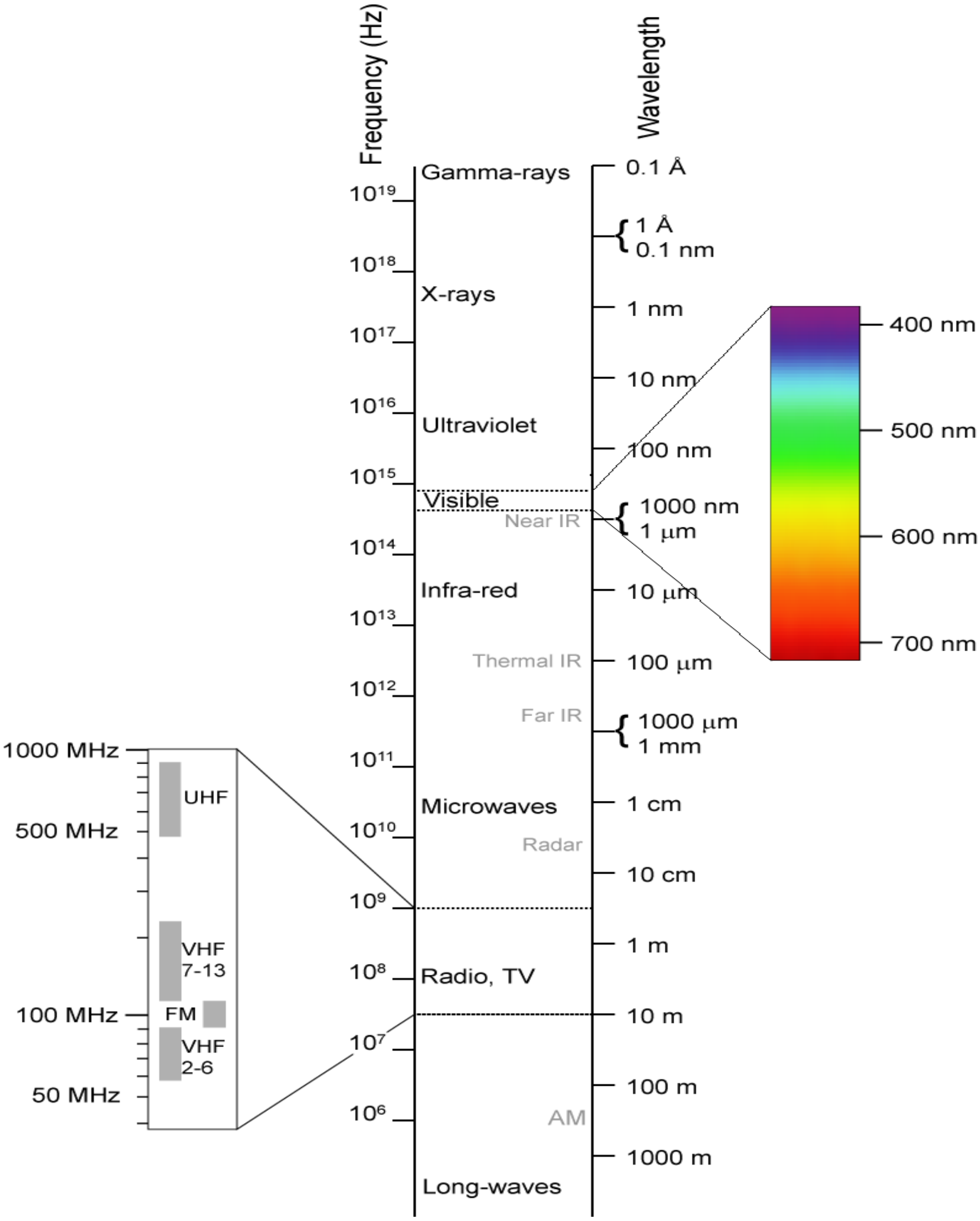

Figure 4. Electromagnetic Spectrum.

2.3.1 Gold nanoparticles' synthesis.

For the control of nanoparticles' size and morphology, synthesis conditions allow a way to tailor materials properties in explicit applications. Considerable efforts have been made towards developing new and promising methods for the synthesis of noble metal nanoparticles. In general, the strategies for nanoparticle synthesis can be grouped into "top-down" and "bottom-up" approaches (fig. 5) (Pareek et al., 2017). The first one is basically based on physical methods such as the thermal evaporation, the synthesis of gaseous clusters, the ion implantation, the chemical vapor deposition and the mechanical activation or mecanosynthesis; while in the second one, chemical methods are the main ones, like the chemical reduction method, microemulsion, electrochemical, laser ablation or microwave methods.

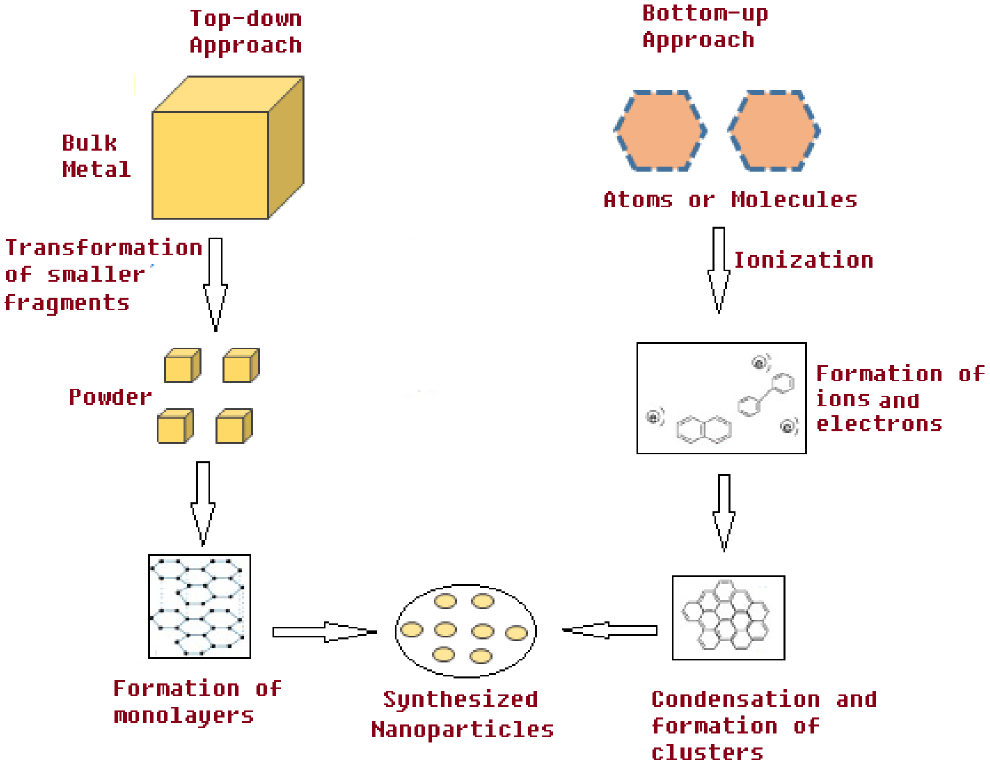

Figure 5. Top down and Bottom up approaches in Nano synthesis. (*Nano Technology - Elements, Types of Nano Fabrication, Applications, Advantage & Disadvantage*, n.d.)

The "top down" approach consists of dividing mass solids into smaller portions. This methodology may include grinding or wear, chemical methods, and volatilization of a solid followed by condensation of the volatilized components. Many methods that use the "top-down" approach require complex and complicated instrumentation, which makes them expensive (Zanella, 2014). Another drawback is the difficulty of obtaining homogeneous particle sizes with perfect crystallographic and surface structures, while this is the major advantage of bottom-up approach. There is also an advantage: it can synthesize nanomaterial in bulk quantities within a short span of time (Pareek et al., 2017)

The second approach, "from the bottom up," involves the reduction of metal ions from its oxidation state in solution to its elemental state, followed by a nucleation or aggregation process (Sifontes et al., 2010). This latter approach is much more popular in nanoparticle synthesis (Zanella, 2014). Furthermore, the synthesized particles have calculable stability which is exceptionally attractive for satisfying their applications. Here is a brief discussion, as an example, of the chemical reduction method for the preparation of NPs (Pareek et al., 2017):

The chemical reduction is a technique that remains as a first choice for the synthesis of noble metal NPs (Au, Ag and Pt). It includes reduction of an ionic salt utilizing different reducing agents in a suitable medium in presence of a stabilizing agent. A variety of reducing agents such as hydrogen, hydrazine, alcohols, carbon monoxide, $LiAlH_4$, $NaBH_4$, or $R_4N^+(Et_3BH^-)$ have been used to prepare metal colloids in the nanometer size range. Gold nanoparticles can be ordinarily prepared by the reduction of Au^+ particles in aqueous solutions. The citrate interceded reduction of aqueous solution of $HAuCl_4$ to synthesize gold nanoparticles of 20 nm size showed (Ma et al., 2015; Turkevich et al., 1951) (Fig.6). A progressively controlled and refined synthesis was created by differing the ratio of reducing/stabilizing agents (trisodium citrate to gold ratio) to control nanoparticle size. Other than the simplicity, this strategy requires extreme conditions, for example, high temperature, pressure, and a long time for completion of reaction. The most outstanding disadvantage is the nature of reactants used in the reaction system which are generally toxic chemical substances and results in severe potential risks for environment and health. The presence of stabilizing molecules on the surface of nanoparticles precludes their biological biomedical applications.

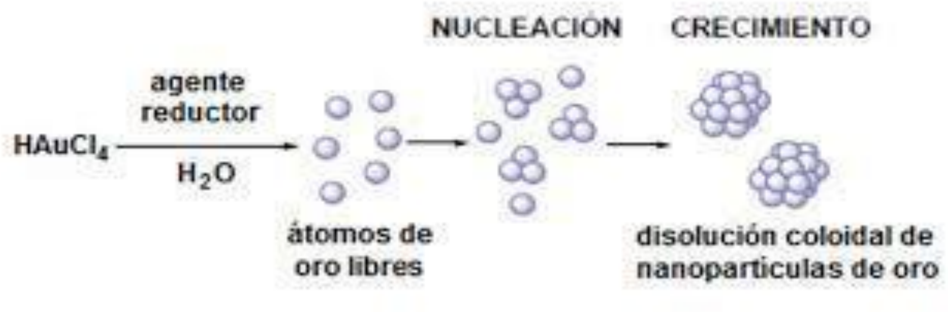

Figure 6. Scheme of gold reduction and NPs’ formation.

3.OBJECTIVES

The objective of this work is to evaluate the effects of light and colour on gold nanoparticles through different scientific publications and current news that deal with the subject. A first objective lies in describing the use of gold nanoparticles as colorimetric reagents. A second objective is to analyze the use of radiation through metallic nanoparticles for therapeutic purposes. Finally, the latest advances and future expectations are analyzed, with special emphasis on the current use of gold nanoparticles as coronavirus test kit Covid-19.

4. METHODOLOGY

The methodology followed in this bibliographic review, carried out between February and June 2020, has been done through several complementary lines:

- Attendance at the bibliography course.
- Literature review from scientific databases.
- Book consulting.
- Newspaper and magazine consulting.

I assisted with an event about Informational Competences for the Final Degree Project (TFG) for the Pharmacy degree which was taught in "CRAI Antonio de Ulloa" building by Marta Suárez Samaniego. There these things were explained: Bibliographic searches through CRAI's catalog, how to get to advanced research, and how to use a web application called Mendeley. Mendeley is a bibliographic reference manager with advanced features of social network that is integrated in commercial databases. Thanks to this application, an exhaustive work has been done, since it allows you to store all the articles, book chapters, and information of interest and thus, create your own personal library. It also facilitates this bibliographic review preparation when citing the sources of information, and so that in the bibliography, because they appear ordered and written following *Vancouver* rules.

Other highly reliable databases and scientific guides have also been used, where articles are found, most of them written in English. These search engines are:

- Google Scholar (https://scholar.google.es/).
- PubMed (http://www.ncbi.nlm.nih.gov/pubmed).
- Science direct (https://www.sciencedirect.com/).

In addition, *Google* has been used for information research, which redirected to specialized pages in order to be able to compare information and resolve doubts regarding articles. Also, *Google Images* has allowed the search of images for a better understanding of the explained content to be able to visually observe it.

5. DISCUSSION AND RESULTS

5.1 Gold nanoparticles as colorimetric reagents

One of the major applications of AuNPs is in chemical and biological sensing. Utilizing the intrinsic features, Au NPs have been used as efficient sensors for the detection of different analytes such as metal ions, anions, and molecules like saccharides, nucleotides, proteins, and toxins. The AuNPs sensors can be colorimetric, fluorescence-based, electrical and electrochemical, surface plasmon resonance, and many more. The fundamentals of colorimetric sensing is based on visible colour change due to the aggregation of AuNPs (Alex and Tiwari, 2015; Elahi et al., 2018; Moores and Goettmann, 2006).

Michael Faraday's work in the 1850s was based on a scientific evaluation of colloidal Au, which recognized that the colour observed was due to the size of the Au particles, and it made him interested in the interaction between light and matter. Colloidal Au has been used for the study of cerebrospinal fluid by using the Lange reaction, thanks to colloidal gold reaction that was used to detect infection of the central nervous system. Depending on the pathological conditions that must be investigated, a specific pattern of colour change of the colloidal auric solution happens. This application is the first recorded practical application of extremely small gold particles for biosensing (Aldewachi et al., 2018).

Biosensors are generally defined as sensors that consist of biological recognition elements, often called bioreceptors, or transducers. They have two basic principles different from conventional chemical sensors: the sensing elements are biological structures, such as cells, enzymes, or nucleic acids, and the sensors are used to measure biological processes or physical changes (Li et al., 2010). Biological processes and biomolecular interactions can be used to control the dispersion and aggregation of the NPs. Contingent upon the size of the AuNPs, controlled aggregation of the particles can result in colour changes, from pink to violet to pale blue. This wonder has been utilized in home pregnancy tests and in investigations for explicit gene sequences, and for colorimetric detection of an assortment of analytes (Aldewachi et al., 2018; Online, 2008).

The urine pregnancy test is based on an immunochromatographic technique. It is an easily available diagnostic test and one which, due to its low cost, ease per test, and the simple immunologic principle, has been produced numerously and is frequently used these days (Rojanathanes et al., 2008). By and large, the urine pregnancy test measures human chorionic gonadotropin (hCG), which is the hormone produced by pregnant women. AuNPs are bound to antibodies complementary to hCG. At the point when the stick is lowered in urine flow, if the hormone is present, it will bind to nanoparticles causing aggregates to form. The solution then passes through a paper filter. On the off chance that the pregnancy hormone is recognized (Fig.7), the aggregates will be caught by the filter creating a colored product. If the pregnancy hormone is not detected, the nanoparticles will pass through the filter because of their small size (Winter, 2007).

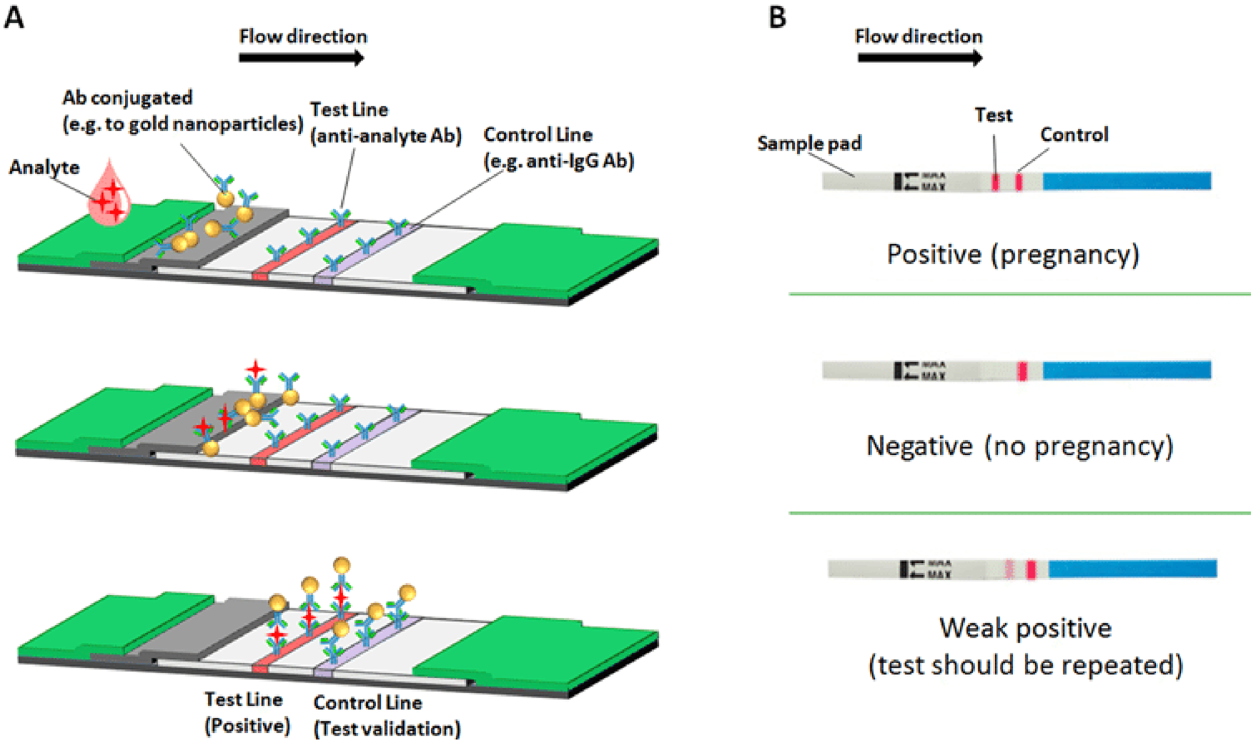

Figure 7. Schematic design of an urine pregnancy test.

For DNA sequence recognition and nucleic acid hybridization, nanoparticle-based detection systems have been commonly applied, specifically colloidal AuNPs with 15nm diameter because they can be easily synthesized and functionalized with thiolated oligonucleotides, generating DNA-functionalized gold nanoprobes (Au nanoprobes) (Eaton et al., 2007). The utilization of Au nanoprobes for this specific application was first suggested by Mirkin and his collaborators, building up a cross-linking assay using two distinctive functionalized DNA–Au–NPs that hybridize in adjoining places of the complementary DNA sequence, bringing about the development of a polymeric system of DNA and Au nanoprobes with a corresponding red-to-blue colour change (Elghanian et al., 1997).

Peter Eaton’s assay was based on a non-cross-linking hybridization method, where aggregation of the oligonucleotide-derivatized gold nanoparticles (Au nanoprobes) is induced by an increasing ionic strength of the assay mixture: the presence of a target complementary to the Au nanoprobe born sequence prevents aggregation and the solution remains red, whereas the presence of noncomplementary/mismatched targets does not prevent Au nanoprobe aggregation, resulting in a visible colour change from red to blue. The atomic force microscope (AFM) was used to study the biological colorimetric detection (Fig 8). Complementary and noncomplementary target sequences were utilized to contemplate the degree of specific interaction between the target and the Au nanoprobes. This permitted direct proof of the method of activity of the Au nanoprobes and in extra lowed evaluation of the frequency of nonspecific interactions between the Au nanoprobes and the target. After a salt addition, the "blank" and "negative" samples showed broad Au nanoprobe aggregation total perceptible by the blue shade of the corresponding solutions; while the "positive" sample containing the complementary DNA does not show that impact and the solution stays red (Eaton et al., 2007).

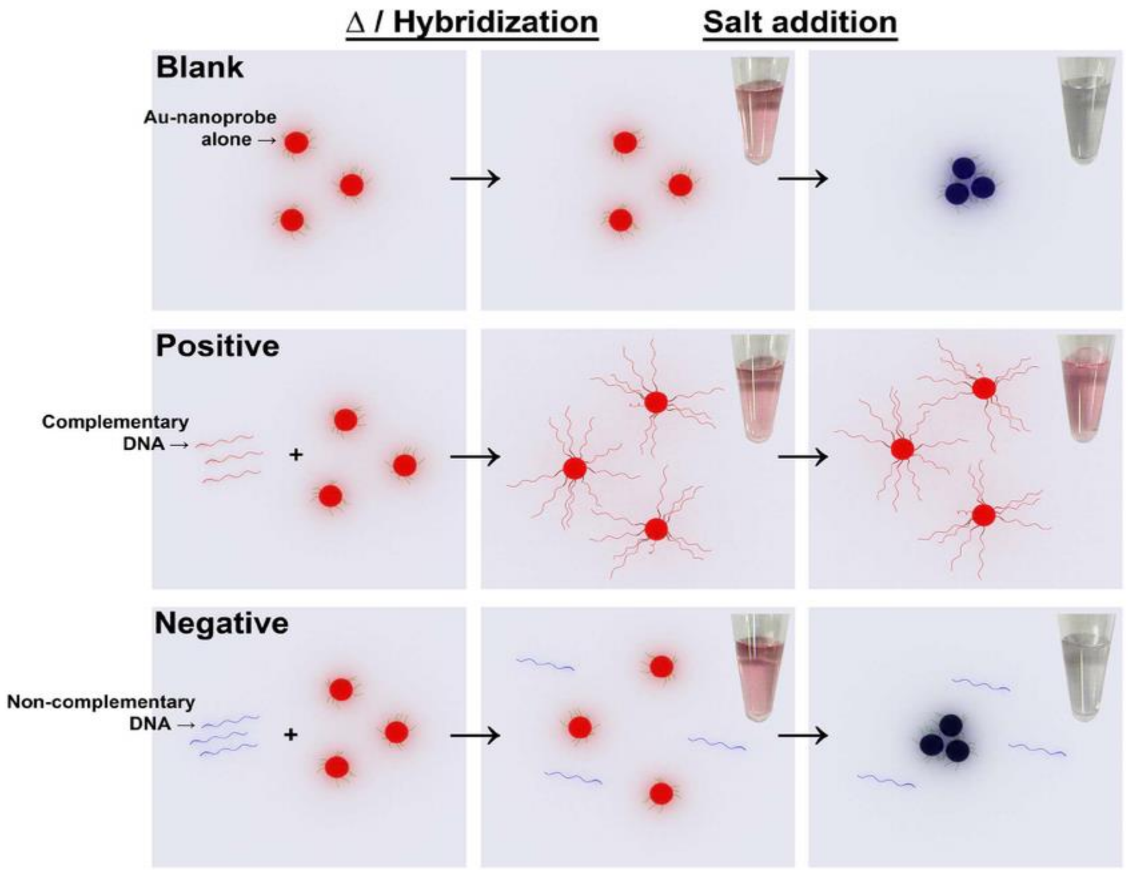

Figure 8. Specific DNA sequences detected by Au nanoprobes imaged by an AFM.

Visual cancerous cells and DNA sensing based on AuNPs aggregation is another example of another colorimetric assay, for an immediate identification of cancer by utilizing aptamer-conjugated Au NPs has been reported by (Vilela et al., 2012). Various samples were used, some of them with the target cancer cells, that showed a shading change from red to blue, and the non-target, did not show any colour change. The chemical principle depended on the utilization of an aptamer sequence with high selectivity and affinity to the cancer cells, and this aptamer was conjugated to AuNPs through a thiol functional group on its sequence. In this way, when there were cancer target cells, the aptamer-conjugated AuNPs aggregated on them indicating a blue shading. This assay indicated magnificent selectivity, being able to recognize cancerous from non-cancerous samples with simple instrumentation and in a rapid way.

5.2 Radiation therapy with AuNPs

Radiation is a therapy that is based on the cellular damage caused by ionizing radiation projected to biological tissues. Since its beginning, the objective of radiation therapy (RT) has been to cure cancer locally without excessive side effects and it is currently used in around 50% of cancer treatments. The most significant variables influencing the consequences of RT are the tumor type, its location and regional extent, the anatomic region of involvement and the geometric exactness with which a determined radiation dose is delivered. Although higher doses of radiation can produce better tumor control, the dosage which can be given is limited by the possibility of normal tissue damage (Haume et al., 2016; Mehta et al., 2010).

Ionizing radiations are delivered to tumors via an external beam directed toward the cancerous tissue. The idea of radiosensitization was presented around 30 years ago, when high-Z (atomic number) materials were proposed as dose enhancers for radiotherapy. The radiosensitization impact depends on the strong attenuation of photons by high-Z molecules, which prompts an expansion in the local dose deposition. There have been researches done in the development of new radiosensitization agents utilizing various components (e.g., gold, Z = 79) and as result, AuNPs have become an emerging class of radiosensitizers for oncological applications, and as such, they are widely investigated (Laprise-Pelletier et al., 2018). To optimize the technique of AuNP based radiation treatment for clinical application, a few investigations ought to be done to address the impact of photon energy and GNP size independently. Additionally, more biological tests on cell lines and creature models are required to explain the observed contrasts in dose enhancement effect concerning the magnitude of improvement effect of cell type in AuNP-based radiation treatment (Mesbahi, 2010).

When colliding with materials, photons can be attenuated by four major types of interactions: the photoelectric effect (Fig. 9), the Compton effect, pair production, and Rayleigh scattering. Based on the cross section (i.e., the probability of an interaction to occur) specific to each attenuation process, attenuation coefficients allow a quantification of each one of these mechanisms according to the energy of the incoming photons; this can be evaluated for every single chemical element being hit by the photons. For high-Z elements, the probability of interaction is higher because of their larger cross section. The total mass attenuation coefficient (μ / ρ) belongs to the sum of the individual attenuation coefficients (Fig. 10). It represents the probability of interaction per mass unit of a given material (in $cm^2 g^{-1}$). Figure 10 shows the mass attenuation coefficients (μ / ρ) for each of the processes, and for gold and water to compare them (Kwatra et al., 2013; Laprise-Pelletier et al., 2018).

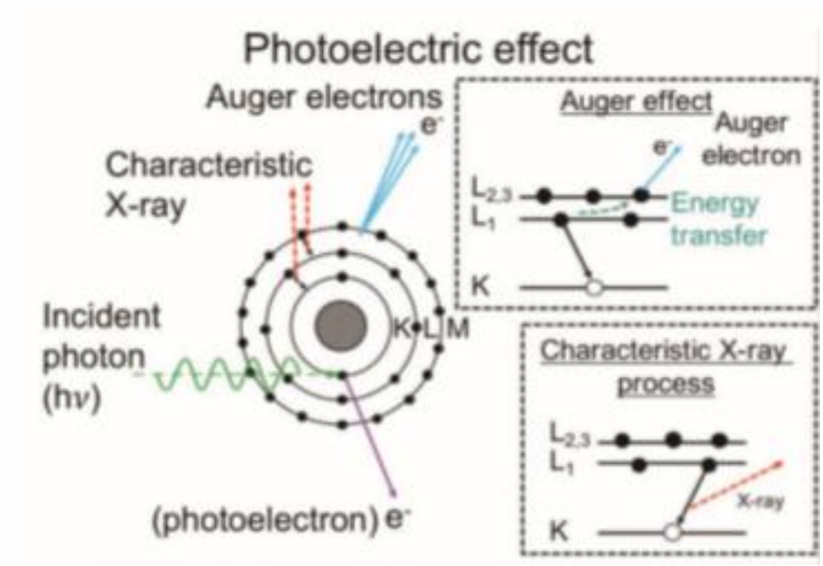

Figure 9. The photoelectric effect and the consequents auger effect and X-ray process.

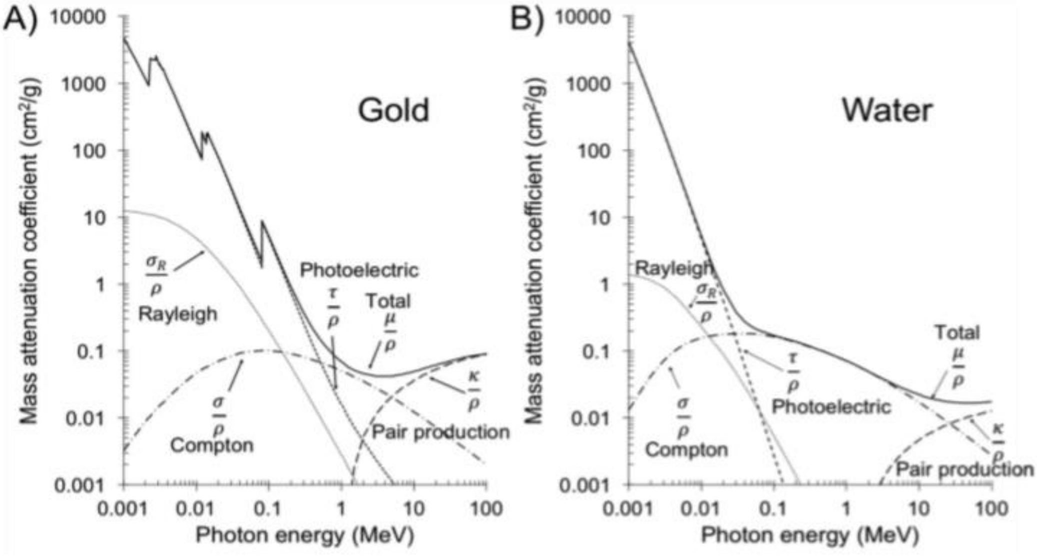

Figure 10. Mass attenuation coefficients (μ/ρ) for each of the processes, and for both gold and water.

For gold, the photoelectric effect is very strong at energies of 200 keV and below, whereas similar photoelectric interaction rates are reached in water at photon energies of 30 keV and below (corresponding to low-dose-rate energy ranges). It occurs when an incident photon collides with an electron belonging to one of the inner orbitals of the impacted atom. The photon is absorbed and the electron is ejected, also called “photoelectron”. When the photoelectron is ejected from the inner-shell orbital, an electron of the upper shell fills the vacant place, which leads to the emission of characteristic X-rays or Auger electrons (Laprise-Pelletier et al., 2018).

When the nanoparticles arrive at the tumor, their shape and size additionally assume a role in intratumor diffusion and intracellular uptake. In a systematic examination of the impact of nanoparticle size (10−100 nm) on passive targeting of tumors in vivo, Perrault and collaborators discovered that the penetration of nanoparticles inside the tumor is strongly dependent on the overall size of the nanoparticle, where larger nanoparticles seem to remain close the vasculature, while smaller, quickly diffuse all through the tumor matrix. (Perrault et al., 2009).

Focusing on AuNPs, Chithrani and colleagues investigated the size and shape reliance of AuNP uptake in mammalian cells. They showed that kinetics and saturation concentrations are highly dependent upon nanoparticles' dimensions. Specifically, results showed that within the size range of 2–100 nm, GNPs of diameter 50 nm demonstrate the highest uptake. Concerning shape, they also reported that spherical gold nanoparticles have a higher propensity to be internalized in vitro by HeLa cells compared with rod-shaped particles of similar dimensions (Chithrani et al., 2006; Chithrani, 2010).

Original work led by Hainfeld and collaborators gave the first trial proof of the radiation dose enhancement effects of AuNPs in vivo (in mice), which lighted exponential development in the field of AuNP radiosensitization (Hainfeld et al., 2004). Also, it is found in experimental studies that radiosensitization was dependent on the number of AuNPs internalized within cells. The 50-nm AuNPs with the highest internalization also showed the highest radiosensitization enhancement factor (1.43 at 220 kVp) compared with GNPs of 14 and 74 nm (enhancement factors of 1.20 and 1.26, respectively) (Ngwa et al., 2014).

Radiosensitization by AuNPs was at first believed to stem only from physical dose enhancement, exploiting the elevated photoelectric absorption of Au. However, additional chemical and biological contributions to radiosensitization became evident with more experimental data, suggesting roles for AuNPs in all three phases of interactions with IR. While the precise mechanisms underlying AuNP induced dose enhancement remain to be fully elucidated, several modes of radiosensitization have been proposed. These mechanisms are discussed in the context of the three different phases (Fig. 11) of radiation effects on biological systems (Her et al., 2017):

1. Physical enhancement: The primary reason for creating AuNP radiosensitizers depends on differences in the energy absorbing properties of Au compared to soft tissues, enabling physical dose enhancement when Au is present. In the kV energies, photons interact with matter mainly through the Compton effect or the photoelectric effect, that have been already explained before.
2. Chemical enhancement: In comparison to the physical and biological pathways of radiation enhancement by AuNPs, the chemical one, has not been widely explored. Despite the limited number of studies, discoveries to date propose an influence on the radiation impacts even at low concentrations of Au. AuNPs' role in the chemical phase of radiation exposure is through involvement in radical reactions that fix the harm, or by debilitating of DNA bonds, rendering DNA more susceptible to radiation-induced damage.
3. Biological enhancement: While kV radiation is used to treat patients with superficial tumors, it suffers from short penetration depth. Hence, most patients with deep tumors (e.g. cervical, pancreatic, prostate, brain) are commonly treated with MV radiation generated by a clinical linear accelerator (LINAC) to minimize excessive dose to skin (Mesbahi, 2010). For this reason, radiosensitization at MV energies would be necessary to expand the clinical utility of AuNP radiosensitizers. However, based solely on physical dose enhancement, radiosensitization with AuNPs is expected to be insignificant at MV energies due to the minimal contribution of photoelectric absorption of photons.

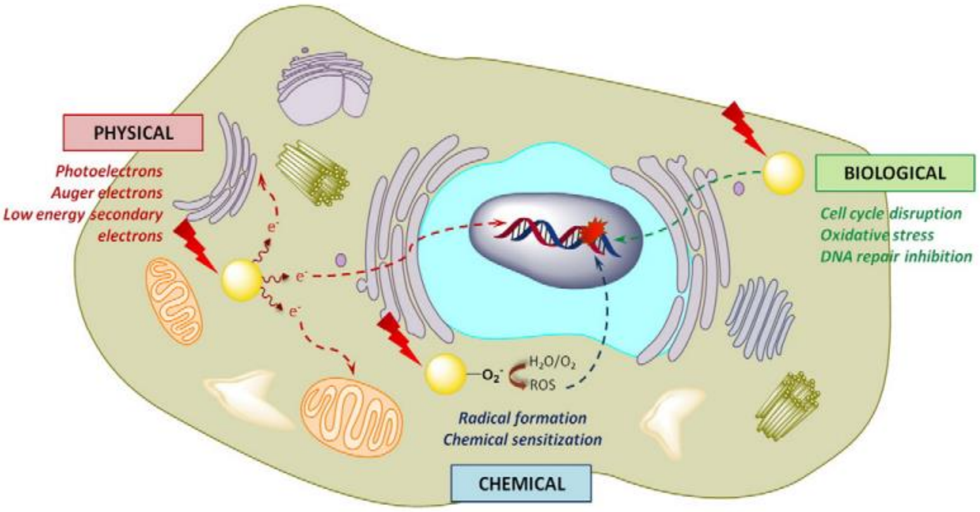

Figure 11. AuNPs enhance radiation effects via physical, chemical, and biological interactions with IR.

5.3 Gold nanoparticles applications

Gold nanoparticles may be utilized in different areas, one of most significant being the biomedical field. They have appropriate properties for controlled drug delivery, cancer treatment, biomedical imaging, diagnosis, and some others, because of their magnificent compatibility with the human organism, low toxicity and tunable stability, small dimensions, and capacity to interact with many substances. They additionally have optical properties, having the option to retain infrared light. Also, because of their large surface and the capacity of being coated with different therapeutic agents, gold nanoparticles have been shown to have an incredible potential to be utilized as drug delivery systems. AuNPs are intensively studied in biomedicine, and ongoing investigations revealed the fact that they can cross the blood-brain barrier, may interact with the DNA and produce genotoxic effects. Due to their ability of producing heat, they can target and kill the tumors, being utilized in photodynamic treatment (Cabuzu et al., 2015).

Described here are the most commons uses (Fig. 12) of AuNPs in the biomedical field (De et al., 2008; Elahi et al., 2018)**:**

- Photodynamic therapy: Photodynamic treatment (PDT) is viewed as a significant treatment for oncological illnesses and certain skin or infectious diseases utilize photosensitizers as light-sensitizing agents and a laser. Apoptosis or necrosis is induced in tumor cells by singlet oxygen and highly active free radicals produced via the energy of photosensitizers. Powerful fluorescence quenching and SPR absorption are the significant highlights of AuNPs which have been used in this treatment.

- X-ray imaging: AuNPs have attracted the main attention as an x-ray contrast agent because it represents a high X-ray absorption coefficient, ease of synthetic manipulation, nontoxicity, surface functionalization for colloidal stability and targeted delivery. Common vascular contrast agents such as iodinated molecules have low-molecular weight. The water solubility of these iodinated aromatics is high, and it is indicative of low toxicity. Even so, the blood circulation time is short and is rapidly eliminated. Therefore, a short imaging window may require multiple injections with the risk of developing thyroid dysfunction. The development of an imaging window due to longer vascular retention time compared with common agents is due to AuNPs' properties.

- Drug delivery: AuNPs are suitable for the delivery of the drugs to cellular destinations due to their ease of synthesis, functionalization, and biocompatibility. AuNPs have been used for the co-administration of protein drugs due to their ability to cross cellular membranes, possibly due to the interaction of GNPs with cell surface lipids (Tiwari et al., 2011). These effective nanocarriers can transfer different drugs such as peptides, proteins, plasmid DNAs, small interfering RNAs, and chemotherapeutic agents. New researchers have proposed stable colloidal gold nanorods as an appropriate agent for drug delivery. The other candidates are gold nanocages. By binding the cancer cell receptors to the nanocages surface conjugated with bioactive molecules such as antibodies, targeted drug delivery takes place.

- Sensing: One of the major applications of AuNPs is in chemical and biological sensing. Utilizing the intrinsic features, AuNPs have been used as efficient sensors for the detection of different analytes such as metal ions, anions, and molecules like saccharides, nucleotides, proteins, and toxins. According to the sensing strategy, the AuNPs sensors can be colorimetric, fluorescence-based, electrical and electrochemical, surface plasmon resonance, surface enhanced Raman scattering (SERS)-based and many more. Different types of nano-biosensors have employed special features of AuNPs, as it is said when biosensors are described earlier.

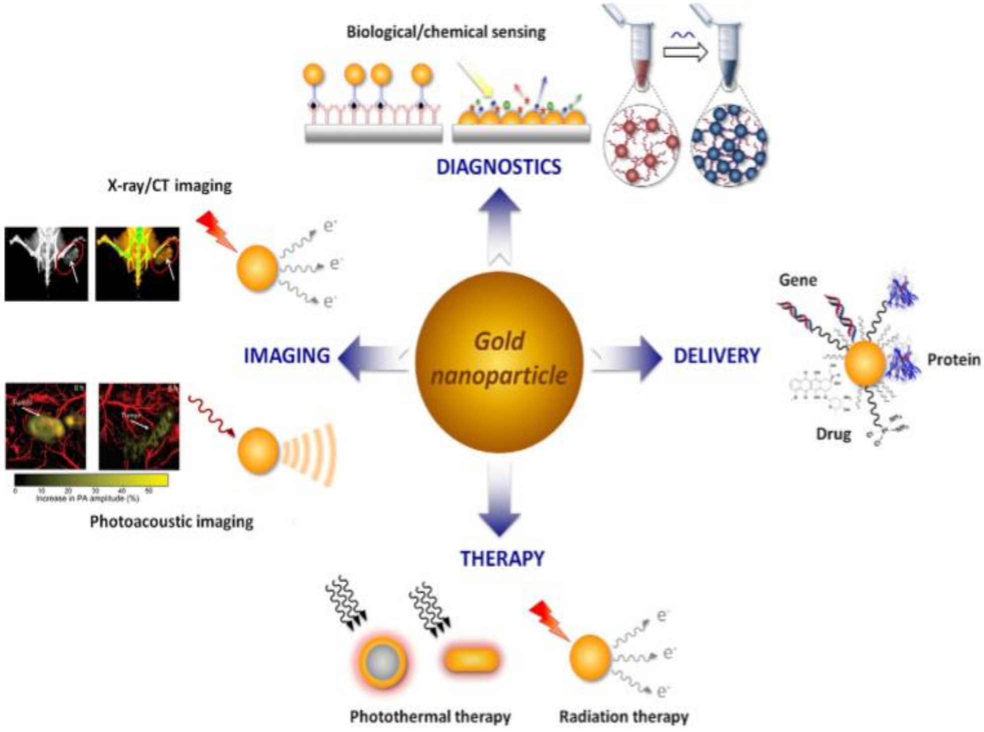

Figure 12. Wide range of biomedical applications of AuNPs owing to their unique physico-chemical, optical, and electronic properties.

5.4 Advances and future perspectives

In the latest research about AuNPs, different advances have been observed. This study has been focused on publications of the trending topic these days, the Covid-19 pandemic. Cancer advances and other clinical topics are also discussed.

According to a publication in the multimedia platform for scientific communication, SINC (Scientific News and Information Service) (Test colorimétrico con nanopartículas de oro para detectar el coronavirus, 2020) “the “Instituto de Salud Carlos III” is financing a project called "Colorimetric test with gold nanoparticles to detect coronavirus". Researchers from the IMDEA Nanoscience Institute are developing a sensor to identify the presence of the SARS-CoV-2 coronavirus using gold nanoparticles with DNA. This test includes a vial with a reddish aqueous solution due to the presence of the AuNPs. When coronavirus’ RNA is incorporated, these DNA-functionalized nanoparticles get aggregate and precipitate at the bottom, producing a distinct decrease in the colour of the solution. This can be seen with the naked eye” (Fig. 13).

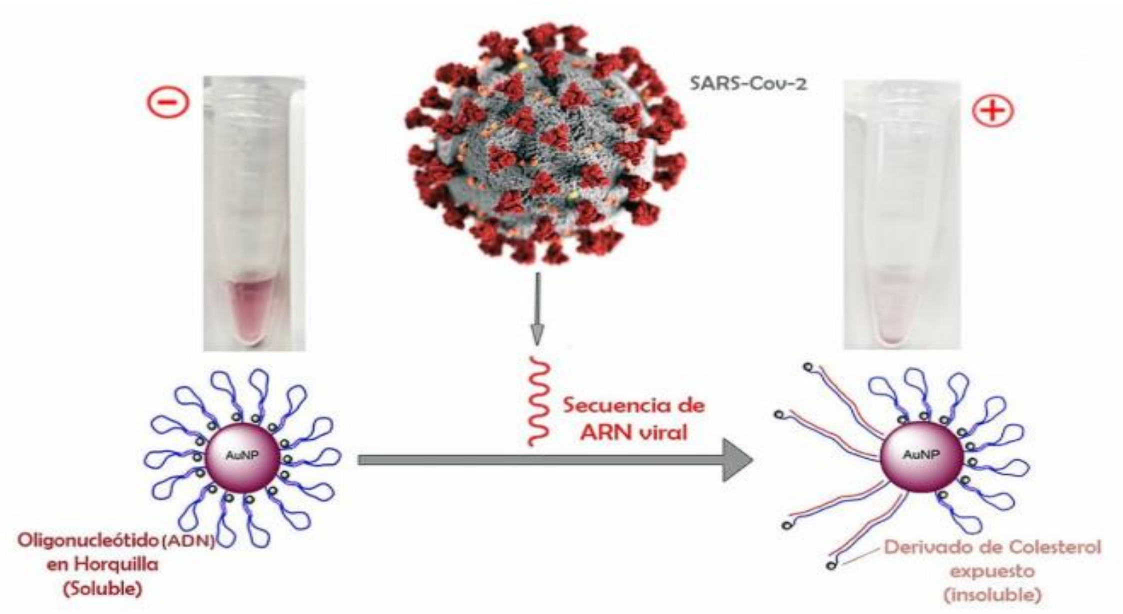

Figure 13. Scheme of the colorimetric test based on the use of gold nanoparticles with DNA.

Álvaro Somoza, the main investigator in this study, explains that “the AuNPs’ DNA chains are hairpin-folded and have a hydrophobic group (cholesterol) at the end, therefore, it is soluble in water. But when viral RNA is present, the DNA of the nanoparticle binds to the virus sequence, the hairpin opens, and cholesterol is exposed to the medium (water), resulting in a water-insoluble structure and causing the nanoparticles to precipitate. For this reason, the solution becomes colorless. At the moment, this has not been proved in real samples of coronavirus, but only in models and analogs sequences to the pathogen RNA, including a synthetic copy of the gene: RdRP. When prototypes will be ready, ISCIII scientists will test them with sequences from real viruses, first attenuated and then directly using samples from infected patients”.

Other publications have been done in May, both in Pharmatech, which is the institutional magazine of the Spanish Association of Pharmacists Industry (AEFI), that covers all the information and services in the Pharmaceutical sector, as well as in the ABC newspaper, in which it is indicated that the Technological Institute of Plastics of the Valencian Community (Aimplas) is working on the development of a “fast, economical, and efficient” diagnostic technique, based on the modification of gold nanoparticles to detect antibodies to SARS-CoV-2 or its antigen in urine, blood and respiratory fluids (INFOEDITA, 2020; Nanopartículas de oro para hacer millones de test rápidos de Covid-19 a bajo coste, 2020).

This technique is based on previous developments of the technological center used in the diagnosis of prostate cancer. It is planned to start the validation tests on real samples in collaboration with two research units of hospitals in the Valencian Community, specifically the “ISS La Fe” and the Research Foundation of the “Hospital General Universitario de València”. Currently, several detection methods are being used: PCR (Polymerase chain reaction) and immunoassay (INFOEDITA, 2020; Nanopartículas de oro para hacer millones de test rápidos de Covid-19 a bajo coste, 2020).

“Thanks to the NCYT Amazings source, another virology study has been found, published in March in the magazine: "The journal of physical chemistry C". This study is about a probe based on AuNPs that controls viral RNA in living cells of Influenza A. It is the first time in virology that experts use gold nanoparticles’ imaging tools that monitor mutations in real time of influenza, which is a highly contagious virus that mutates rapidly, becoming resistant to drugs and vaccines as which is replicated” (Dardir et al., 2020; Una nueva herramienta monitoriza las mutaciones en tiempo real de la gripe, 2020).

New advances related to light effects in gold nanoparticles have also been found in a newspaper called “Galicia press”, showing the use of AuNPs in radiation-protected lenses. These studies have been carried out by researchers from several universities and laboratories in Spain and Portugal. The results have been published in the journal “Applied Materials Today” and it is shown that the way light is absorbed by this material does not impair visibility. They have noted that the nanoparticles incorporated in contact lenses absorb intense light throughout the visible and IR spectrum and prevent damage to the eye. Researchers have considered feasible that these lenses can be marketed (Iglesia, 2020).

To finish with the last advance, there is an interesting study for the early detection of a very common disease these days that takes away many lives, cancer. This is a novel blood test that uses AuNPs to detect cancer, which identifies the signals released (EV) by cancer cells that could result in better treatment and earlier diagnosis. AuNPs that are discharged from cancer cells and health cells are called “VE”. These allow communication between cells. Cancer cells use nanoparticles to explode cells that surround them. Gold nanoparticles emit a unique signal when they are struck by laser light. This can be used to detect a patient-specific fingerprint. With ONJCRI (Olivia Newton John Cancer Research Institute) collaborators’ help, technology was tested on blood samples from 23 melanoma patients. The new device accurately detected cancer’s VEs in blood samples and successfully tracked how the cancer EV fingerprint changed in response to therapy for each patient (Ibarra, 2020) .

6.CONCLUSIONS

- ✓ Gold nanoparticles have shown to have specific characteristics because of their nanometer-size and that has enabled them to go into the human body. It is reflected in this literary review that the main property of the AuNPs is the LSPR, directly related with the high amount of atoms on the metal surface.
- ✓ They also have some drawbacks due to this nanometer-size such as toxicity, compared to larger particles, or as impurity, instability, or the difficulty of their synthesis.
- ✓ Many techniques based on AuNPs’ characteristics, such as absorbance spectroscopy or infrared spectroscopy, have been used to employ gold nanoparticles as biosensors.
- ✓ Different methods for their synthesis have been verified, being the most effective method, and therefore the most used, the chemical method: the bottom up.
- ✓ In the last section, the key aspects of this work have been discussed: AuNPs as colorimetric reagents are very useful in different tests, such as the pregnancy test or the Covid-19 test; in radiation therapy, the main objective of which is to cure cancer, has also been exemplified in the advances section.
- ✓ Because of their big potential, it has been found that their use is being increasingly demanded in many areas, and as we have seen previously, specially in biomedicine.

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