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GOLD AND SILVER NANOPARTICLES AS COMPUTED TOMOGRAPHY (CT) CONTRAST AGENTS PRODUCED BY A PULSED LASER ABLATION TECHNIQUE: STUDY *In-vitro* AND *In-vivo*

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ABSTRACT

High-purity metal nanoparticles such as gold and silver nanoparticles become an interesting subject for application in the medical field. Using the laser ablation approach, these nanoparticles were successfully synthesized in this present work. Experimentally, a pulse neodymium yttrium aluminum garnet laser (1064 nm, 30 mJ, 10 Hz) was used to irradiate high-purity metals immersed in an iodine liquid medium. Purple, yellow, and dark purple colloidal nanoparticles of Au, Ag, and Au-Ag were successfully synthesized. The results certified that all nanoparticles have a spherical shape; Au nanoparticles had an average diameter of 26 nm and a standard deviation of 4 nm, while Ag nanoparticles had an average diameter of 24 nm and a standard deviation of 5 nm. The diameter distribution of Au-Ag nanoparticles is extraordinarily vast, ranging from 18 to 80 nm. Colloids of mixed Au-Ag nanoparticles had the highest Hounsfield Units (HU) value in an in vivo test, with a value of 302. This was supported by the results of an in vitro contrast enhancement evaluation, which determined that a mixture of Au-Ag nanoparticles provided the best contrast enhancement compared to other colloids. These results confirmed that the Au-Ag nanoparticles are very suitable as contrast agents in CT.

Keywords: Pulsed Laser Ablation, Gold Nanoparticles, Silver Nanoparticles, Contrast Agents, in vitro, in vivo.
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INTRODUCTION

A computed tomography (CT) scanner is a medical imaging technology that quickly and accurately depicts tissue abnormalities and organ details in the human body. A CT scan is an x-ray device that combines tomography and digital technology to take multiple 2D images to create a 3D image.[1–3] In assessing the quality of CT-Scan images, spatial resolution and contrast are always important parameters that must be known. One of the requirements for a good CT image is an image that can clearly distinguish each organ with the image as close and clear as possible to the original organ.[4] However, some body parts such as soft tissues are still difficult to differentiate because of the similarity in density. Therefore, a contrast agent is needed for better results of regions of interest (ROI).[5] Iodine-based contrast agents (IBCA) were the standard x-ray computed tomography contrast agents until recently. Iodine has a high atomic number (Z=53) and a good ability to block x-rays. IBCA has numerous negative effects on the human body, including nausea, high temperature, low blood pressure, etc. IBCA also has a limited circulation time, which limits its applicability.[5,6] Based on this case, new variations of X-ray CT contrast agents are currently receiving increased attention. Specifically, metallic nanoparticles with high atomic numbers and excellent x-ray attenuation qualities, such as gold and silver nanoparticles.[7,8] Interestingly, a nano-sized contrast agent

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(NSCA) exhibits excellent biodistribution, low toxicity, and excellent pharmacokinetics. Additionally, NSCA has longer circulation times than iodine-based contrast agent (IBCA). It can be administered to a broader range of target tissues.[5] Both gold and silver nanoparticles are noble metals. They have excellent stability in the form of nanoparticles, which are expected to be good for delivery. They also fulfill the properties of X-ray agent contrasts like having high atomic numbers with Z=79 for gold and Z=47 for silver and having exceptional X-ray attenuation properties.[8,9] Pure nanoparticles are necessary to generate ideal contrast agents that are less toxic to the body.[8] Therefore, in this study, we used a physical method that can produce pure nanoparticles. This is called the pulsed laser ablation (PLA) method. PLA is used because of its ability to produce nanoparticles with high purity, small size, and high concentration in a short time compared to chemical methods that are difficult to manufacture and leave contaminants in the product.[10] Gold and silver nanoparticles are known for their similarity in their physical properties. Therefore, in this study, we would like to compare the mixture of colloidal nanoparticles between them. We compared their physical and contrast agent properties by variating their composition. We also analyzed their ability as contrast agents by using in vivo and in vitro methods.

EXPERIMENTAL

Synthesis

The following materials were derived from 99.95% pure gold (Au) and silver (Ag) plates for the manufacture of gold (Au) and silver (Ag) nanoparticles (Au-Ag NPs): Five distinct sample compositions were synthesized in this work utilizing the PLA technique. Its composition consists of 100 % gold, combinations of Au-Ag NPs with a ratio of Au-Ag NPs (Au: Ag) of 50 % to 50 %, and 100 % silver. Pulse Nd: YAG laser (New Polaris II) with 1064 nm fundamental wavelength, 50 mJ laser energy, 10 Hz pulse repetition rate, and 7 ns pulse width was used as an energy source. By focusing and irradiating the laser beam on the plate's surface with a quartz convex lens, a luminous plasma was generated in the surface of the metal plate. The surface of the material had a laser flux of 4 J/cm^2. The laser beam was bombarded at the surface of the sample for 10 minutes each sample until it ablated and generated nanoparticles in the iodine liquid medium. The liquid holding the plate was regularly shifted in the XY direction to obtain homogenous colloidal Au, Ag, and Au-Ag nanoparticles.

Characterization

The produced nanoparticles were analyzed using procedures that examine nanoparticle properties. SEM-EDX technology (SEM-EDX, JEOL JED-2300) was used to examine morphological traits. The optical properties were found using UV-Vis spectroscopy. The particle size and size distribution were investigated utilizing SEM-EDX data by employing an imageJ software.

In-vitro Measurement

For the in-vitro contrast agent assay, colloidal gold and silver nanoparticles with different compositions were deposited into phantom tube vials aligned as shown in Fig.-1. They were then scanned with a CT scanner employing a high voltage dose of 135 kV and a tube current of 200 mAs utilizing the head CT protocol option. After getting the exposed image, the Hounsfield Unit (HU) value of the ROI is obtained from the software called MicroDicom. Then, the contrast enhancement is calculated. A comparative analysis of the HU value will be carried out due to the composition as an object of exposure to the image results obtained. The contrast enhancement equation in the phantom image is:

$$C_{enh} = \left|\frac{I_{post} - I_{pre}}{I_{post}}\right| \times 100 \quad (1)$$

I_{post} represents the mean signal intensities of the ROI with nanoparticles, while I_{pre} represents the mean signal intensities of the ROI without nanoparticles.[11]

In-vivo CT Imaging

Colloidal gold, silver, and mixture of gold-silver nanoparticles that have been synthesized in iodine liquid are then used as contrast agents on CT scan. A contrast agent test was carried out in-vivo on white rats. A comparative study was performed using various contrast agents synthesized in this study. They were iodine contrast agent, colloidal silver nanoparticle contrast agent in an iodine medium, colloidal gold nanoparticle contrast agent in an iodine medium, and colloidal gold-silver contrast agent nanoparticles in an iodine medium. All the samples were at a concentration of 26 ppm. All of them were scanned using a CT scanner employing 120 kV of high voltage and 300 mAs of tube current.

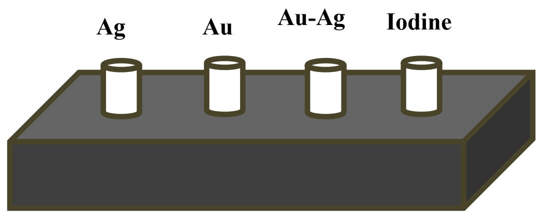


Fig.-1: Colloidal Gold and Silver Nanoparticles in Phantom Tube Vials

RESULTS AND DISCUSSION

Synthesis of Au-Ag Nanoparticles

Figure-2 shows the photographs of colloidal nanoparticles (a) gold (Au), (b) silver (Ag), and (c) a mixture of gold-silver (Au-Ag) obtained by using the pulse laser ablation method. The visible coloration of the colloids appears in all nanoparticles. The colors of colloidal Au, Ag, and Au-Ag were light purple, yellow, and dark yellow, respectively. The dark yellow color in Au-Ag is the mixture of the purple and yellow colors contributed by the Au and Ag nanoparticles, respectively.

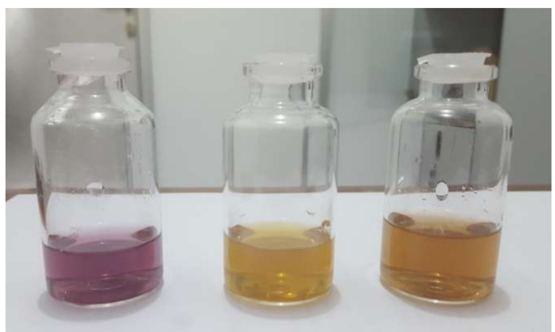
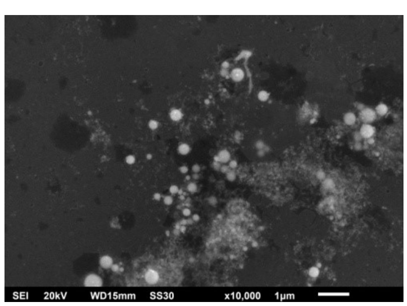
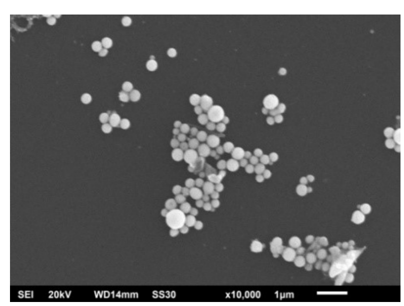


Fig.-2: Photographs of Colloidal Nanoparticles of (a) Gold (Au), (b) Silver (Ag), and (c) Gold and Silver Mixed (Au-Ag) Obtained by the Present PLA Method

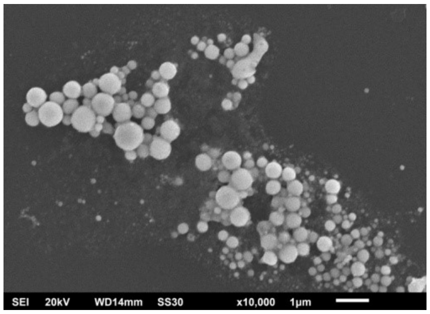
The morphological characteristics of the synthesized nanoparticles are observed by using the SEM-EDX technique. Figures-3(a), (b), and (c) display the photographs of nanoparticles Au, Ag, and Au-Ag, consecutively. To produce the nanoparticles of Au and Ag, the fundamental Nd: YAG laser was irradiated on the surface of pure metals for 20 minutes, while for Au-Ag production, the laser was focused on Au metal first for 10 minutes and consecutively irradiated on Ag metal in the same liquid medium. The ratio of the combined colloids Au-Ag is 1:1. All nanoparticles have a spherical shape with different size distributions. However, it should be pointed out that the distribution of nanoparticle size is very large in the case of Au-Ag (Fig.-3c).



(a)



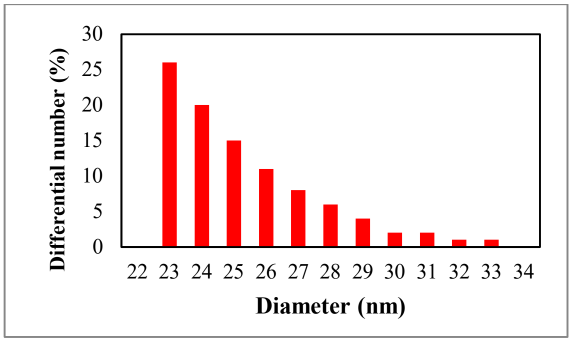
(b)



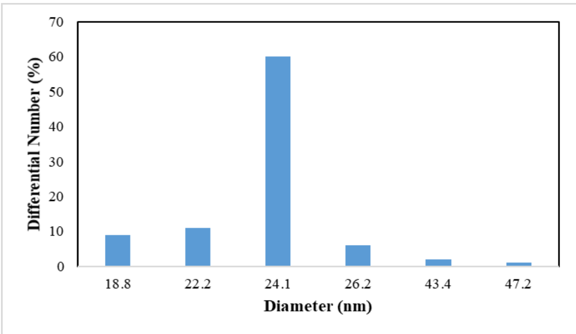
(c)

Fig.-3: Morphological Characteristics of (a) Gold (Au), (b) Silver(Ag), and (c) Mixture of Gold-Silver (Au-Ag) Nanoparticles Obtained by SEM-EDX Technique

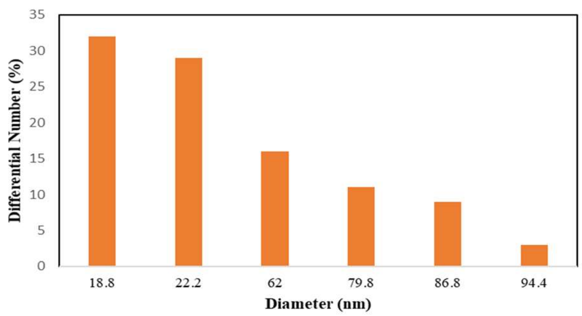
Figure-4 depicts the particle size distribution of manufactured nanoparticles. As shown in Fig.-4, the average size of Au nanoparticles is 26 nm with a standard deviation of 4 nm. Figure-4(b) depicts the size distribution of Ag nanoparticles with a mean size of 24 nm and a standard deviation of 5 nm. Figure-4(c) depicts the average size of Au-Ag nanoparticles. The nanoparticle size distribution peaked at 18 nm and 80 nm. The lower size is attributed to Ag nanoparticles, whereas the larger size is attributable to Au nanoparticles. It should be noted that the nanoparticle size distribution of Au-Ag is much broader than that of monometallic Au and Ag nanoparticles. This is the same result reported by Peng *et al*.[12] The nanoparticle size distribution of gold-silver (Au-Ag) is notably larger than that of Au and Ag nanoparticles (Figs.-4(a) and 4(b)). The size of Ag nanoparticles is smaller than that of Au nanoparticles. Ag nanoparticles are significantly smaller than Au nanoparticles because their melting and boiling temperatures are significantly lower. Consequently, Ag nanoparticles reach their melting and boiling temperatures before Au nanoparticles, resulting in smaller Ag nanoparticles.



(a)



(b)



(c)

Fig.-4 Size Distribution of (a) Au, (b) Ag, and (c) Au-Ag Nanoparticles Analyzed from SEM-EDX Technique

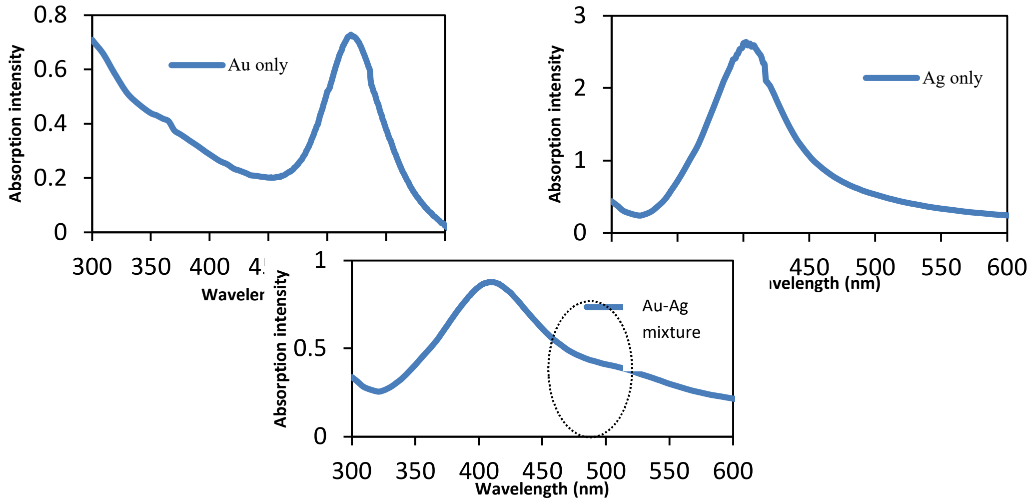


Fig.-5: UV-Vis Spectroscopy Absorption Spectra of (a) Au, (b) Ag, and (c) Au-Ag Nanoparticles, clockwise

Utilizing UV-Vis spectroscopy, the optical characteristics of Au, Ag, and Au-Ag nanoparticles were determined. The absorption spectra of colloidal Au, Ag, and Au-Ag nanoparticles are depicted in Fig.-5. Figure-5(a) illustrates the average Au absorption spectrum. The peak of Au nanoparticles' absorption spectra occurs at 520 nm, which is a property of surface Plasmon resonance (SPR). Ag nanoparticles are responsible for the 420 nm peak in Fig.-5(b) SPR spectrum. Figure-5 depicts the absorption spectra of a colloidal Au-Ag combination. The ablation time ratio for Au and Ag is 1:1, with each metal requiring 10 minutes. Compared to the spectrum produced from monometallic colloids, the peak corresponding to Au can be seen to be quite weak and broad. According to Vinod *et al.*[13], this is the collective nature of surface plasma resonance caused by Au and Ag nanoparticles. It may be determined from the absorption intensity, which is greater for the Ag intensity, that a large number of tiny Ag nanoparticles were formed. The smaller Ag nanoparticles can be found in the fluid on their own or with other nanoparticles. The absorbance at 406 nm is influenced by both isolated and connected Ag nanoparticles.

In-vitro Imaging Analysis of Synthesized Au-Ag Nanoparticles

Based on Figure-6, the greatest contrast enhancement is due to the Au-Ag nanoparticles mixture which is 197% enhanced over standard iodine (iopromide). On the other hand, the contrast enhancements of Au and Ag nanoparticles are almost the same, 145% and 144% respectively compared to the standard. This contrast enhancement is proportional to the increase in HU value where the highest HU value is owned by a mixture of Au-Ag nanoparticles of 23 HU, followed by Ag and Au 17 HU and iodine 11 HU. All solutions were prepared at the same concentration of 15 ppm. All the DICOM images were processed with windowing soft tissue.

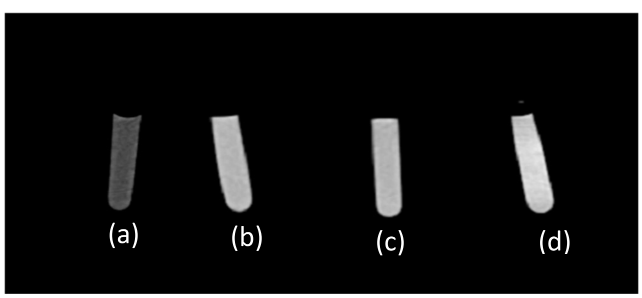


Fig.-6: Images of contrast vial tube phantoms containing (a) iopromide (b) AgNPs (c) AuNPs, and Au-Ag NPs

***In-vivo* Imaging Analysis**

The colloidal gold nanoparticles from this study were then tested preclinically as a contrast agent in CT scan modalities with rats. Comparative studies of CT scan image results were also carried out by comparing the results of the image using a colloidal gold nanoparticle contrast agent as a result of the study with a standard contrast agent using liquid iodine. 7(a), is an image taken of a rat using only iodine as a contrast agent on a CT scan. Figure-7(b), is an image taken from a rat animal using the results of research on colloidal silver nanoparticles wrapped in iodine as a contrast agent on a CT scan. Figure-7(c), is an image taken from a rat animal using the results of research on colloidal gold nanoparticles wrapped in iodine as a contrast agent on a CT scan. The three contrast agents, colloidal silver, gold, and iodine nanoparticles have the same concentration of 26 parts per million (ppm). By using colloids, the CT scan image looks brighter than the image using an iodine contrast agent. Colloidal nanoparticles can produce images with a Hounsfield Unit (HU) value of 236 in gold and 196 in silver, greater than the HU value in an image using an iodine contrast agent with the same concentration (HU value 91). Therefore, the colloids from this study are very good to be used as contrast agents. In addition to colloidal gold nanoparticles, a colloidal mixture of gold nanoparticles and silver nanoparticles was also tested as a contrast agent in CT scan modalities with rats. Figure-7(d) shows a CT scan image of rats treated with a colloidal mixture of gold and silver nanoparticles. From the image, it is clear that the image given a colloidal contrast agent mixed with gold nanoparticles and silver nanoparticles has a higher contrast level than the image using an iodine contrast agent. This is confirmed by the HU value of the image with a contrast agent mixed with gold and silver nanoparticles produces a HU value of 302. This *in vivo* study strengthens the results of *in vitro* studies which show that a mixed colloid of gold and silver nanoparticles produces a better contrast than the colloid of each material alone.


(a)

(b)


(c)


(d)

Fig.-7: Images of White Mice Taken Using a CT Scan Using Contrast Agent (a) 26 ppm Liquid Iodine, (b) Colloidal Silver Nanoparticles, (c) Colloidal Gold Nanoparticles, and (d) Colloidal Gold-Silver Nanoparticles

CONCLUSION

Gold, silver, and mixture of gold and silver nanoparticles were successfully synthesized by using the laser ablation method employing pulse Nd: YAG laser at 1064 nm. The results confirmed that the diameters of Au and Ag nanoparticles are 26 nm and 24 nm, respectively. The diameter distribution of Au-Ag nanoparticles is extraordinarily vast, ranging from 18 to 80 nm. In vitro assessment of mixed Au-Ag results in most excellent in contrast enhancement compared to other colloids with a percentage enhancement of 196%, where the highest HU value was owned by a colloidal mixture of Au-Ag nanoparticles of 302.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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REFERENCES

1. J. T. Bushberg, *The Essential of Medical Imaging: Second Edition*. Lippincott Williams & Wilkins, Philadelphia, (2002).
2. J. Baruchel, J.-Y. Buffiere, and E. Maire, *X-ray Tomography in Material Science*, Hermes science publications, Paris, (2000).
3. L. W. Goldman, *Journal of Nuclear Medicine Technology,* **36(2)**, 57(2008), https://doi.org/10.2967/jnmt.107.044826.
4. E. Samei and N. J.Pelc, Computed Tomography: Approaches, Applications, and Operations. Springer, Switzerland, (2020).
5. N. Lee, S. H. Choi, and T. Hyeon, *Advanced Materials,* **25(19)**, 2641(2013), https://doi.org/10.1002/adma.201300081
6. N. Aslan, B. Ceylan, M. Mehmet, and F. Findik, *Journal of Molecular Structure,* **1219**, 128599(2020), https://doi.org/10.1016/j.molstruc.2020.128599
7. H. Wang, X. Mu, H. He, and X. D. Zhang, *Trends in Pharmacological Sciences,* **39(1)**, 24(2018), https://doi.org/10.1016/j.tips.2017.11.003
8. D. Xi, S. Dong, X. Meng, Q. Lu, L. Meng, and J. Ye, *RSC Advances,* **2(33)**, 12515(2012), https://doi.org/10.1039/C2RA21263C
9. H. Liu, H. Wang., R. Guo, X. Cao, J. Zhao, Y. Luo, M. Shen, G. Zhang, and X. Shi, *Polymer Chemistry,* **1(10)**, 1677(2010), https://doi.org/10.1039/C0PY00218F
10. M. Dell'Aglio, R. Gaudiuso, O. De Pascale, and A. De Giacomo, *Applied Surface Science,* **348**, 4(2015), https://doi.org/10.1016/j.apsusc.2015.01.082
11. A. Alipour, Z. Soran-Erdem, M. Utkur, V. K. Sharma, O. Algin, E. U. Saritas, and H. V. Demir, *Magnetic Resonance Imaging,* **49**, 16(2018), https://doi.org/10.1016/j.mri.2017.09.013
12. Z. Peng, B. Spliethoff, B. Tesche, T. Walther, and K. Kleinermanns, *Journal of Physical Chemistry B* **110(6)**, 2549(2006), https://doi.org/10.1021/jp056677w
13. M. Vinod and K. G. Gopchandran, *Progress in Natural Science: Materials International,* **24(6)**, 569(2014), https://doi.org/10.1016/j.pnsc.2014.10.003

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