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Use of Solid Nanoparticles as Contrast Agent for Ultrasound Imaging

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Abstract: The feasibility of using solid nanoparticles as ultrasound contrast agent have been called into question on the ground that the size of a nano-particle is much smaller than the wavelength of ultrasonic wave, therefore, backscattering from these particles will obviously be poor and hence, poor echogenicity would give poor image quality. However, appropriate size metal nanoparticles exhibit some important properties, such as, bio-conjugation and Enhanced Permeability Retention (EPR) effect.

Due to different acoustic impedance of solid nanoparticles than that of the biological tissues, the solid nanoparticles become a better ultrasound contrast agent than that of microbubbles. When solid nanoparticles are exposed to ultrasound waves during imaging they remain stable. In present work, we have synthesized solid nano-particles of gold to study its applications as Ultrasound Contrast Agent (USCA). With existing ultrasound technology, it appears that gold nano-particles of size 10 nm may not be suitable for Ultrasound Contrast Agent (USCA) as it failed to improve image brightness or even image quality. The size of nanoparticles are very small, therefore, it circulate inside the region of interest (human body) for sufficient duration to complete the imaging process.

Keywords: Bioconjugation, Echogenicity, Drug delivery, Nanoparticles, Solid nanoparticles, Ultrasound Contrast Agent (USCA).

I. Introduction

The ultrasound (US) wave reflected or backscattered form images of soft tissues. In this imaging system, the ultrasound wave is partially reflected from the layers between different tissues or scattered from smaller structures i.e. ultrasound is reflected from the surface wherever there are changes in acoustic impedance. In general, we can classify contrast agent into two main categories such as (a) microbubbles and (b) non microbubbles. There are wealth of literature available on microbubbles based contrast agent. A microbubble consists of gas-liquid emulsions. When ultrasound signals are incident on these bubbles. They are backscattered and the gaseous core of microbubbles produce strong echogenic effects, which results is a good contrast to the tissue background.

In the field of ultrasound imaging, the nanoparticles may lead to early diagnosis and prompt treatment. Ultrasound Contrast Agent (USCA) has expanded tremendously in recent years, and it is a rapidly growing field of bio-medical research.

Solid nanoparticles produces significant backscattering, for example gold, silver, silica, iron oxide etc. The stronger is the backscattered signal better will be the quality of the image. At present there are a number of nanomaterials such as liposomes, micelles, polymers, dendrimers, emulsions, quantum dots and solid nanoparticles etc. which are under investigation for their respective applications as USCA.

Nowadays, nanoparticles are emerging as contrast agents because of its unique properties at smaller dimensions. The physical, optical and chemical properties of the material changes drastically at nano-dimensions [1]. Gold nanoparticles, on account of their surface activities, bio-compatibility, less toxicity, may appear suitable for injection inside the human body. Its high atomic number and X- ray absorption coefficient make it useful for therapy and imaging. In present work, we have synthesized solid nano-particles of gold to study its applications as Ultrasound Contrast Agent (USCA).

II. Contrast Enhancement

An appropriate size metal nanoparticles exhibits some important properties, such as, Enhanced Permeability Retention (EPR) effect and bioconjugation. Nanoparticles can target tumor cells by an accumulation and entrapment process, known as permeability and retention effect. Nanoparticles exhibits EPR, which makes them accumulate in tumor tissues to higher extents than in normal tissue due to the leaky tumor blood vasculature [2, 3]. Nanoparticles targets the tumours passively [4, 5]. Moreover, they retain nanoparticles in tumor tissues for longer duration i.e. more than twenty four hours [6]. Several investigators also, observed that EPR based tumor targeting

strategy using nanoparticles, micelles, liposome etc., exhibited more than 10-200 times higher concentrations in tumor than that in normal tissues [6-11].

The gold nanoparticles had been used in combination with magnetic nanoparticles to target specific cell types for efficient imaging of cancer cells. In case of gold nanoparticles of size smaller than 4 nm the chemical reactivity becomes predominant, and this may cause oxidative damage to cells [12].

III. Bioconjugation

Bioconjugation is the process of coupling two biomolecules together in a covalent linkage. Nanoparticles are known to communicate with biomolecules. Therefore, synthetically modified biomolecules can have diverse functionalities, such as tracking cellular events, reveal enzyme function, determining protein bio-distribution, imaging specific biomarkers, and delivering drugs to targeted cells [14-17]. Recently, bioconjugation has also gained importance in nanotechnology applications such as bioconjugate quantum dots [18].

Nanoparticles also follow some sort of bioconjugation protocols. Metal nanoparticles can be made pathology specific by bioconjugating them with monoclonal antibodies or antibody fragments and peptides [13-17]. On account of these properties small size nanoparticles might be used as contrast agent for ultrasound imaging system.

IV. Solid Nanoparticle

It appears according to theory that the nanoparticles cannot be used as contrast agent because the size of a nanoparticle is much smaller than the wavelength of ultrasound, therefore, it would become a poor scatterer as echogenicity would decrease with the decrease in size of the particle.

However, solid nano-sized particles, on account of, its different acoustical properties such as density, speed of ultrasound, attenuation and acoustic impedance could become, a better contrast agent [19]. When solid nanoparticles are exposed to ultrasound they remain stable. Since the sizes of nanoparticles are very small, therefore, it circulate inside the region of interest (human body) for sufficient duration to complete the imaging process. The larger size particles do not circulate within the human body for longer duration, they are cleared off rapidly, and therefore, sufficient time window is not available for imaging the organs. In addition to this, nanoparticles have many advantages for specific targeting [20, 21]. Nanoparticles are suitable for medical imaging, multi imaging technology and even therapy. While passing through the capillary nanoparticles can accumulate inside the tumour, target the cancer cells and pass through the blood- brain barrier [21, 13-17]. Therefore, nano-contrast agents are drawing attention of several investigators working in the field of medical imaging.

V. Material and Methods

A. Preparation of Gold Nanoparticles

Gold nanoparticles were synthesized by the process known as the citrate reduction method i.e. reduction of tetrachloroauric acid ($HAuCl_4$) by trisodium citrate dihydrate.

For preparing gold nanoparticles, 1.0 g $HAucl_4$ was dissolved in 250 mL distilled water to make our stock solution. We then took 25 mL of the stock and diluted it with 225 mL of distilled water, yielding 250 mL of 1.0 Mm concentration. Further, 0.5 g Trisodium Citrate Dihydrate (TCD) was dissolved in 50 mL distilled water.

Now we started heating the $HAucl_4$ to a boil, in present case, it took 4.5 minute to boil the solution. When the $HAucl_4$ boiled, we add the Trisodium Citrate Dihydrate (TCD). We observed that the solution turned clear within a few seconds, the solution will turn black. Further, heating of solution was continued. The solution will begin to lighten and finally turned deep wine red. Then we remove from heat when the solution turns deep wine red and we got gold nanoparticles.

B. Preparation of Phantom Material

For ultrasound imaging, we developed ultrasound phantom material from Agar Agar gel dissolved in pure distilled water. In order to control speed and attenuation of ultrasound in the medium certain amount of ethanol and propanol was added to it. We, then dispersed 1-2.5 % mass concentration of gold nanoparticles on phantom in liquid state. The phantom material was allowed to be cooled and stored in refrigeration. Another set of phantom material were prepared with silica nanoparticles of size 400 nm, 600 nm and 800 nm at the same concentrations.

C. Gold Nanoparticle and Estimation of Its Size

The absorption spectrum of gold nanoparticles are as indicated in Fig. 1. It is clear that the absorption spectrum peaks up at the wavelength 525 nm, which is consistent with the surface plasmon resonance of GNPs.

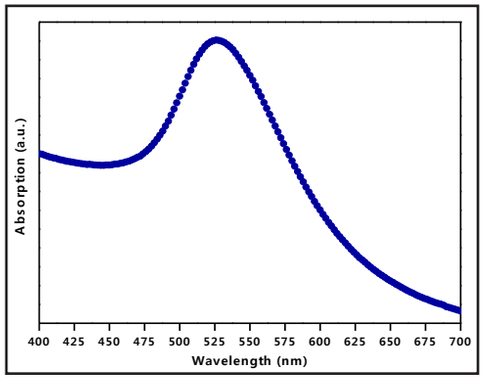

Fig. 1: Absorption Spectra (Ultraviolet-Visible Region) of Gold Nanoparticle

Further, the size of the gold nanoparticles were estimated using X-Ray Diffraction (XRD) analysis. Fig. 2 presents the XRD spectrum of GNPs. It is clear that there are diffraction peaks are observed at 38.078°, 44.256°, 64.376°, 77.312°, and 81.449° respectively corresponding to the different planes (111), (002), (022), (113), and (222), respectively.

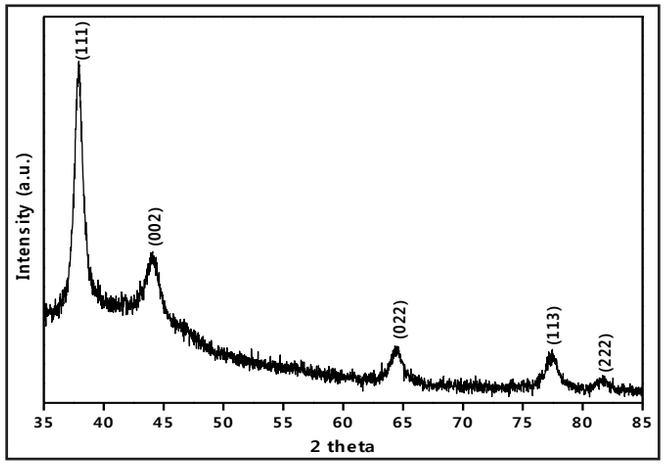

Fig. 2: XRD Pattern of Gold Nanoparticles

With the help of Scherrer equation we calculated size of the gold nanoparticles. The crystallite size of the gold nano particles turns out to become around 10 nm (9.76 nm).

VI. Results and Discussion

It appears that nano size particles (1-100 nm) does not contribute much to the image. In present study of ultrasound imaging using gold nanoparticles of size 10 nm, we failed to observe any significant change in image brightness within the range of concentration. This is because nano-size particle produces low backscattering amplitudes and thus they may not be useful with respect to current ultrasound technology.

However, the silica nanoparticles of size 400 nm, 600 nm and 800 nm in phantom material at different concentrations, contributes significantly to image brightness. Image brightness depends on size as well as concentration of particles. It was also confirmed by other investigators [19, 22]. Jun *et al.* [20] studied the fissibility of nano-contrast agent. He studied on in vitro system and concluded that the image quality increased owing to application of nano-contrast agent [20].

Liu *et al.* [19] studied the effect of silica nanoparticles (100-200 nm) and concluded that image brightness increases with size and concentration of the nano-particles. The same observations were also confirmed for polystyrene nanoparticles having different sizes and concentrations [19, 20]. These results indicate that the nanoparticles might turn out to become suitable for ultrasound imaging [23].

However, on the basis of phantom study, we observed that the gold nanoparticles of sizes 10 nm may not be suitable for contrast agent because size of these scatterers (the gold nanoparticles) were so small in comparison to the wavelength of ultrasonic wave that the backscattering was very poor hence poor image quality.

VII. Advantages

Most of the imaging techniques use ionizing radiation and hence they may produce harmful effects on biological system but radiation free nature of ultrasound makes it safer than the other imaging techniques [24]. Nano-particle contrast agents allow real-time blood flow assessment [24]. Among the various imaging techniques available today (MRI, PET, SPECT) ultrasonic imaging technique is quick, safe, instant and cost effective [25]. Ultrasonography requires low amount of contrast agent than that of MRI [25]. In biological system blood and surrounding tissues mainly consists of water, therefore, backscattering from both would be similar and hence similar echogenicity. This would produce poor contrast, which makes it difficult to differentiate between the tissues. In order to differentiate between them some contrast agent is added to the system to visualize the interface between the blood and the tissue. The contrast agents thus improve image quality and visualization [26].

VIII. Conclusion

With existing ultrasound technology, it appears that gold nanoparticles of size 10 nm may not be suitable as contrast agent for ultrasound imaging as it failed to improve image brightness or even image quality.

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