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Synthesis and characterization of fluorophore attached silver nanoparticles

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Abstract. **Silver nanoparticles stabilized by soluble starch were synthesized and characterized. *In vivo* studies in rats showed no toxicity and revealed their distribution in various tissues and permeability across BBB. This starch stabilized silver nanoparticles have good biological characteristics to act as a potential promising vector for gene/drug delivery.**

Keywords. **Silver nanoparticles; fluorophore; rhodamine; *in vivo* studies; toxicity studies.**

1. Introduction

Metal nanoparticles are applied in biology as biosensors in protein detection (Nam *et al* 2003), labeling agents (Tkachenko *et al* 2003) and cancer therapeutics (Hirsh *et al* 2003). Among inorganic antibacterial agents, silver has been employed most extensively since ancient times to fight infections and control spoilage. Approximately 22–300 mg of silver per day from natural sources in food and water are ingested by humans. Silver based drugs are the most documented universal, broad spectrum antimicrobial agents in modern history. The antibacterial and antiviral actions of silver, silver ion and silver compounds have been thoroughly investigated by researchers (Tokamaru *et al* 1984; Oka *et al* 1994; Oloffs *et al* 1994).

Noble metal nanomaterials have been synthesized using a variety of methods, including hard – template, bio-reduction and solution phase – synthesis. Silver nanoparticles used in such studies were synthesized using organic solvents and toxic reducing agents like hydrazine, sodium borohydride and *N*,*N*-dimethyl formamide. All these chemicals are highly reactive and pose potential environmental and biological risks. In earlier reports, natural polymers like starch (Raveendran *et al* 2003) and chitosan (Huaung *et al* 2004) were shown to stabilize silver nanoparticles and separate reducing agents were used. Interest is now growing for synthesis of metal nanoparticles using green chemistry principles for application in biology. Recently the concept of green nanoparticle preparation using B-D-glucose as the reducing agent was reported by Raveendran *et al* (2003) and later by Vigneshwaran *et al* (2006).

In the present study, silver nanoparticles were synthesized using starch as both reducing and stabilizing agent. Such starch stabilized silver nanoparticles were attached with rhodamine 6G for *in vivo* studies. So far no *in vivo* study was done for silver nanoparticles in animals except for some *in vitro* studies by Arora *et al* (2008, 2009). The present study was aimed to test the toxicity and tissue distribution of Rhodamine 6G attached silver nanoparticles in rat.

2. Experimental

2.1 *Synthesis of silver nanoparticles*

Starch stabilized silver nanoparticles were synthesized as reported by Vigneshwaran *et al* (2006) with slight modification. Soluble starch was dissolved in distilled water. After complete dissolution, 100 mM aqueous solution of silver nitrate was added and autoclaved for 5 min. Solutions with different volumes of silver nanoparticles were prepared and added to the same volume of rhodamine 6G (50 μg/ml) which was dispersed in water/ ethanol mixture.

2.2 *Characterization of synthesized silver nanoparticle*

Silver nanoparticles synthesized were characterized by UV-Visible absorption spectroscopy, transmission electron microscopy and fluorescence spectroscopy.

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2.3 *Animal studies*

500 μl of Rhodamine 6G attached silver nanoparticles was administered to three Wistar rats while keeping two Wistar rats as control. All the rats were maintained in standard cage and fed with standard diet. After two weeks animals were sacrificed, pooled (control and experimental separately) and blood and tissue samples were used for further studies.

2.4 *Toxicity study*

The levels of sugar, protein, calcium, cholesterol, bilirubin from blood and phosphorus, urea, sugar, protein and creatinine from urine were determined following the regular clinical kit method. Blood biochemistry analysis included alkaline phosphatase activity, Na^+K^+ ATPase activity (Ronner *et al* 1977) GSH level (Beutler *et al* 1963) catalase activity (Beers and Sizer 1952) and LPO activity (Ohkawa *et al* 1979).

3. Results and discussion

Silver nanoparticles synthesized were stable in solution at room temperature (approx 25°C) and showed no signs of aggregation.

It necessitates high temperature/high pressure treatment to expand the starch molecule making it more accessible for silver nanoparticles to get embedded and stabilized (Doi *et al* 2002; Han *et al* 2003). Also, elevated temperature accelerates the reduction process by aldehydes (Nath *et al* 2004). The extensive number of hydroxyl groups present in soluble starch facilitates the complexation of silver ions to the molecular matrix (Raveendran *et al* 2003) while the aldehyde terminals helped in reduction of the same.

UV-Visible absorption spectrum was taken for the yellow coloured solution of starch stabilized silver nanoparticles (figure 1). The typical peak at 419 nm corresponds to the characteristic surface plasmon resonance of silver nanoparticles. The plasmon band is symmetric, which indicates that the solution does not contain many aggregated particles.

It is well known that colloidal silver nanoparticles exhibit absorption at wavelengths from 390–420 nm due to Mie scattering (Kleemann 1993). Hence, the band at 419 nm can be attributed to Mie scattering which responds only to silver metal (Aoki *et al* 2003) and will not include the protecting agent starch.

Transmission electron micrographs of starch stabilized silver nanoparticles given in figures 2 and 3 show the presence of particles at an average size range of 5–10 nm. The silver nanoparticles were also monodispersed in a uniform manner.

Among organic dyes, rhodamine 6G dye is one of the most important dyes which has remarkably high photo stability and high quantum yield (0·95). The interaction of rhodamine 6G with silver nanoparticles resulted in quenching and enhancement of luminescence intensity of dye molecules with varied concentrations of silver nanoparticles. Hence our work was aimed at attaching rhodamine 6G with appropriate concentration of starch stabilized silver nanoparticles for imaging.

UV-Visible absorption spectra for dye alone and dye with silver nanoparticles are shown in figure 4. Starch stabilized silver nanoparticles showed surface plasmon resonance at 419 nm and dye molecules showed an intense peak at 526 nm.

In order to evaluate the toxic effect of silver nanoparticles, rhodamine 6G attached silver nanoparticles were injected intra-peritoneally into rats and after two weeks various biochemical parameters were analysed in blood of control and experimental rats.

During the study period (15 days), treatment with rhodamine attached silver nanoparticles did not cause any

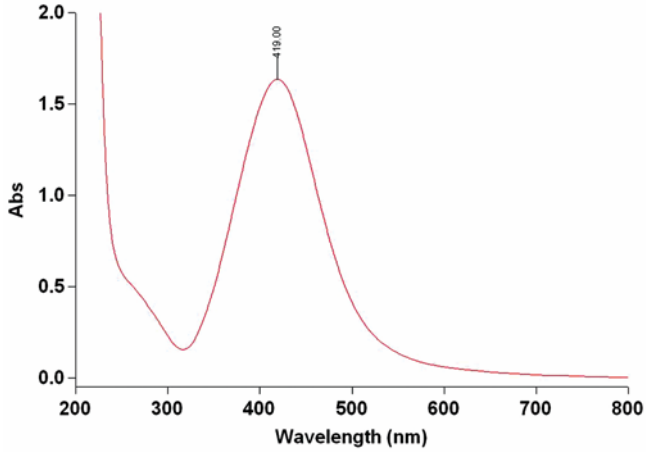

Figure 1. UV-Visible absorption spectroscopy of starch capped silver nanoparticles.

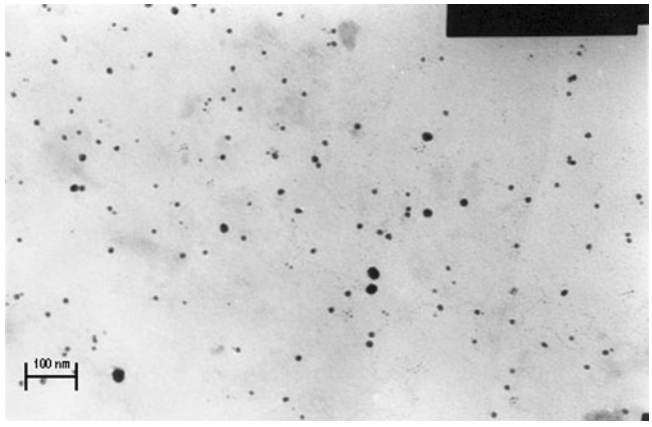

Figure 2. Transmission electron image of silver nanoparticles at 1,00,000 ×.

adverse effects on growth, because no significant differences in the body mass gain were observed between the silver nanoparticle treated mice and control mice. No abnormal clinical signs and behaviors were detected in both the control and treated groups.

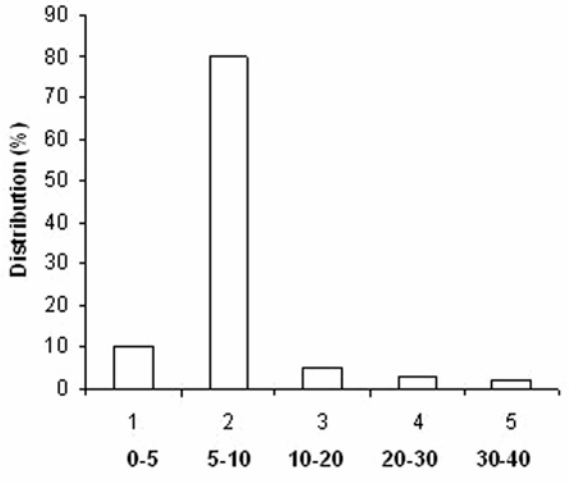

Figure 3. Distribution (%) of silver nanoparticles (nm).

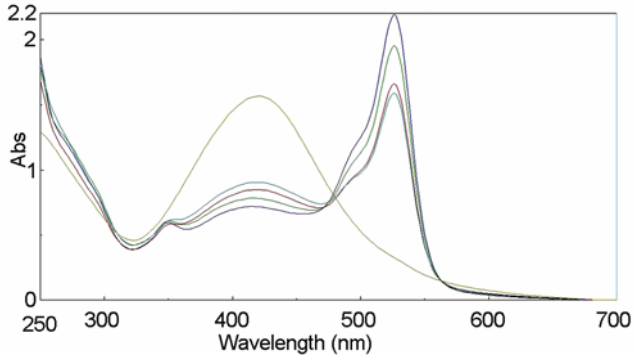

Figure 4. Overlay of Ag-rhodamine 6G at different concentration.

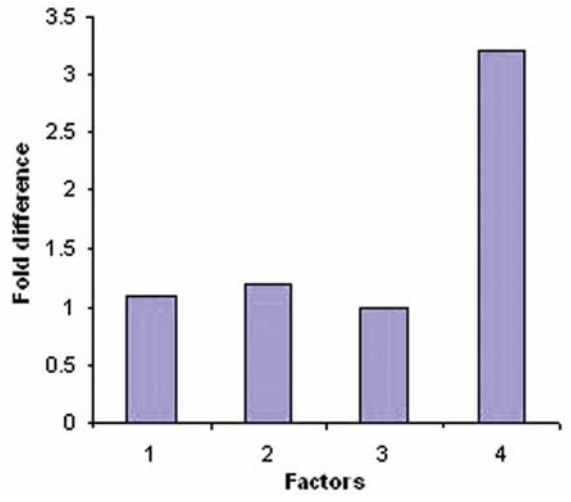

Figure 5. Fold difference of various enzyme activities in rat blood. 1. Alkaline phosphosphatase 2. Catalase 3. GSH level 4. Na^+K^+ATPase activity.

Various biochemical parameters analysed in blood of control and experimental rat showed no significant differences (data not shown). The enzyme activities such as alkaline phosphatase and catalase showed approximately a one fold increase and Na^+K^+ ATPase activity increased 3·5 folds in experimental animal than in control animals (figure 5).

Oxygen free radicals (OFRs) are generated by stimulating H_2O_2 *in vitro* and *in vivo*. OFRs scavengering enzymes normally respond to conditions of oxidative stress with a compensatory mechanism that increases the antioxidative enzyme activity (Wills 1966). The present study shows increased level of catalase activity in nanosilver injected experimental animals than controls.

Na^+K^+ ATP channels are normally closed by a high ATP/ADP ratio generated by the metabolism of glucose and the resulting synthesis of ATP. Closing of the Na^+K^+ ATP channel results in depolarization of the plasma membrane and the activation of Ca^{2+} ions (Azuma *et al* 1991). Increased Na^+K^+ ATPase activity and reduced level of calcium were observed in the present study revealing polarization of plasma membrane by the activation of Na^+K^+ ATP channel.

Silver nanoparticles were detected in various organs such as the brain, liver, lungs, kidneys, and spleen (figure 6). The distribution pattern as observed by the UV-Visible spectral scan is shown in figure 7. Maximum concentration was observed in spleen and brain followed by lungs and liver. Minimum absorption was observed in the kidney. Similar pattern was observed in the fluorescence spectroscopic study. The comparative percentage distribution of silver nanoparticles as observed by UV-Visible scan and fluorescence scan is shown in figure 8.

In general the spacing of cell membranes is in the range of 6–10 nm and macromolecular contrast agents with a molecular size of less than 8 nm in diameter are cleared from blood by glomerular filtration and by tubular excretion of the kidney although the electrostatic charge properties of those particles also have a significant role in their ability to penetrate the glomerular basement membrane (Kobayashi *et al* 2004). Hence, in the present study as the size of the synthesized silver nanoparticle is larger (10 nm), minimum absorption was observed in kidney.

Silver nanoparticles were seen in blood even after two weeks of injection in rat. Increased half life of ultra small particles were shown by Quan-Yu Cai *et al* (2007) with reference to gold nanoparticles.

The nanoparticles were detected in the brain indicating that silver nanoparticles have the ability to penetrate blood brain barrier and also in lungs without any apparent toxicity. The distribution pattern of silver nanoparticles in suspended cells from different tissues was confirmed by the fluorescence microscopic observation (figure 9).

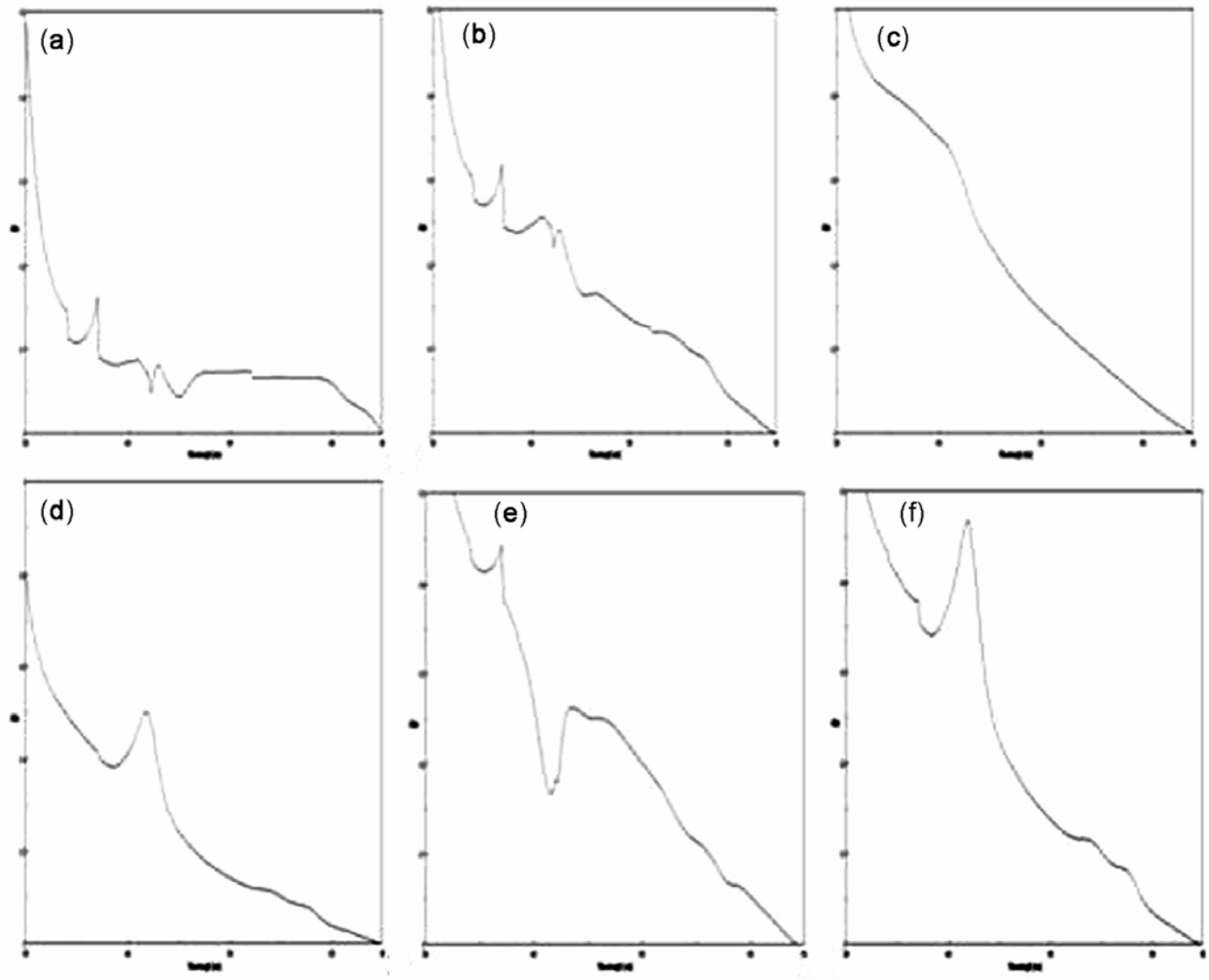

Figure 6. UV-Visible scan pattern of tissue homogenate. (**a**) Kidney; (**b**) Liver; (**c**) Lung; (**d**) Spleen; (**e**) Brain; (**f**) Blood.

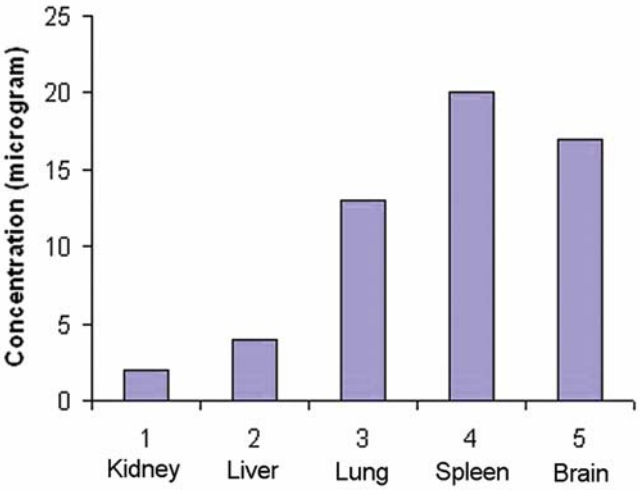

Figure 7. Distibution pattern of silver nanoparticles in various tissues.

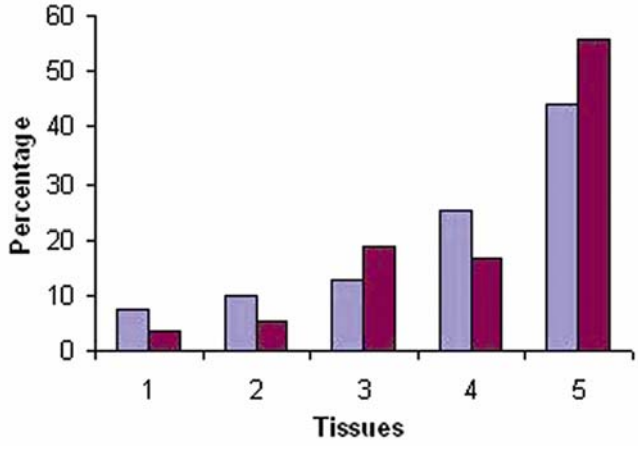

Figure 8. Percentage distribution of silver nanoparticles in various tissues as observed by UV-Visible spectroscan (clean bar) and fluorescence spectrophotometric study (dotted bar).

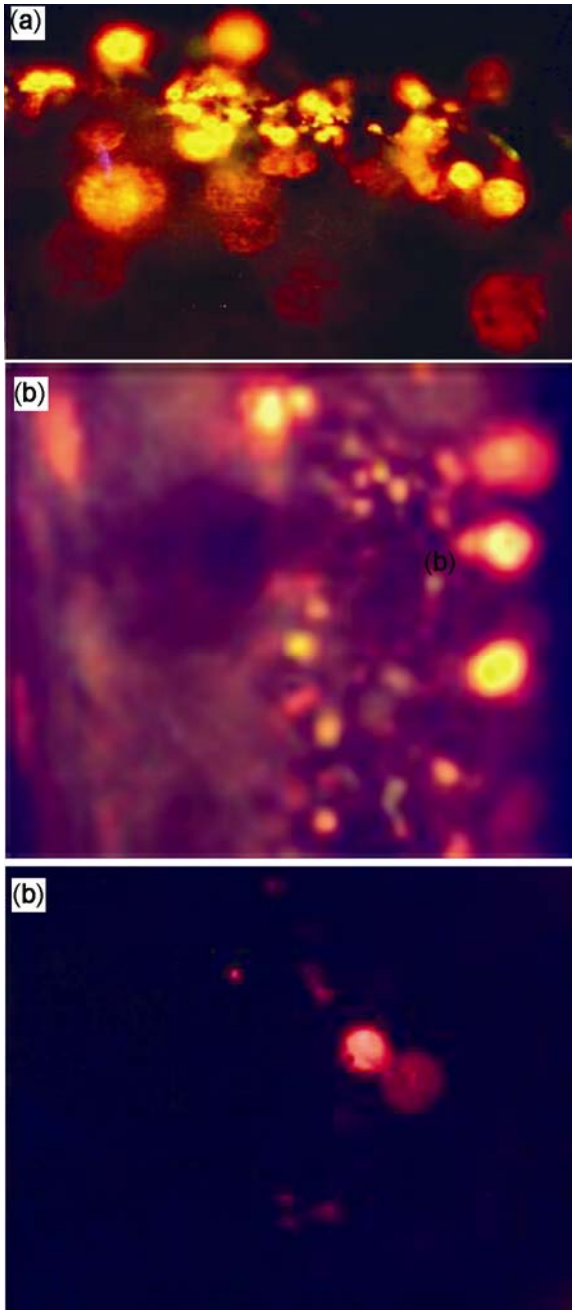

Figure 9. Fluorescent microscopic images of rat tissue cells in suspension. (**a**) Brain cells (**b**) Splenocytes (**c**) Hepatocytes.

4. Conclusions

The above results clearly indicate that no toxicity developed against starch stabilized silver nanoparticles and it could penetrate all tissues including the brain through BBB excluding the kidney. Green chemistry aims at the total elimination of toxic reducing agents with no potential environmental and biological risks. The starch stabilized silver nanoparticles could act as a potential promising vector for gene/drug delivery.

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