

# Silver Nanoparticles As Fluorescent Probes: New Approach For Bioimaging

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**Abstract:** Nanotechnology is the study of the controlling of matter on an atomic and molecular scale. Generally nanotechnology deals with structures of the size 100 nanometers or smaller in at least one dimension, structural features are intermediate in between isolated atoms and bulk materials, in the range of  $10^{-9}$  to  $10^{-10}$  meters, the objects often display physical attributes substantially different from those displayed by earlier atoms or bulk materials. There are many methods for Nanoparticles synthesis but chemical reduction method is frequently used and characterized using NMR, XRD and FT-IR. Fluorescent molecules enable scientists to detect particular subunit of biomolecular complex, such as live cells with exquisite sensitivity and selectivity fluorescent. Photobleaching is a photochemical reduction of a dye or a fluorophore signal. Photobleaching creates complications in the observation of fluorescent molecules, since they will be eventually destroyed by light exposure necessary to stimulate them into fluorescing. This phenomenon is problematic in Time-lapse microscopy or fluorescent microscopy. So, Nanoparticles are new subject for bioimaging rather than fluorescent molecules because of its long fluorescent signal stability. These concerns have led to a debate among advocacy groups and governments on whether special regulation of nanotechnology is warranted.

**Index Terms:** Fluorescent, Fluorescent microscopy, FT-IR, Photobleaching, Time-lapse microscopy, XRD.

## 1 INTRODUCTION

Nanoparticles research is currently an area of intense scientific research, due to its wide variety of biomedical, electrical and optical applications. The principal parameters of nanoparticle are their shape, size and the bulk chemical compositions. These can be present in form of aerosol, suspension or an emulsion. The antibacterial property of silver has been known for thousands of years with the ancient Greeks cook from silver pots. Recent research has found that impregnating other materials with silver nanoparticles is a practical way to exploit the germ fighting properties and photo properties of silver.

The extremely small size of nanoparticles results in the particles having a large surface area relative to their volume. In the case of silver nanoparticles, this allows them to easily interact with other particles and increases their antibacterial properties. It has been observed that when synthesized at nanoscale level, silver particles are not only able to promote enhanced optical activity, but they can also display visible fluorescence of very high intensity. Silver nanoparticles absorb and scatter light very efficiently. This strong interaction with light is due to a phenomenon called Surface Plasmon Resonance in which the conduction band electrons collectively oscillate when they are excited by light of a specific wavelength. Detection of single biological molecules requires illuminating the sample with an intense light to avoid the background noise. This gives rise to photobleaching. The strong light scattering property of silver nanoparticles decreases the required excitation intensity and fluorescence time, which reduces the rate of photobleaching significantly. These optically enhanced silver nanoparticles can be developed for a range of bio analytical applications such as biolabelling, fluorescent tagging and as biosensors

## 2 MATERIALS AND METHODS

### 2.1 Materials and Instruments

Commercially available analytical grade chemicals from Sigma and Merck were used for chemical synthesis of nanoparticles without purification. All the chemical reactions were monitored were Shimadzu spectrophotometer and characterized by FT-IR, SEM and NMR. FT-IR measurements were accomplished on a Jasco FT/IR-4200 spectrophotometer. Fifty Scans were signal averaged with a resolution of  $4\text{ cm}^{-1}$  at room temperature. All chemical reactions were performed in open tubes or vessels without any mechanical support.

### 2.2 Synthesis of Silver Nanoparticles

Silver nanoparticle was prepared by chemical reduction method of  $\text{AgNO}_3$ . An aqueous solution of  $\text{AgNO}_3$  and  $\text{NaBH}_4$  was taken in different conical flask. Take 30ml of 0.002M  $\text{NaBH}_4$  (freshly prepared) in a 100ml conical flask and placed the flask in ice bath on a magnetic stirrer for 20

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minutes. After cooling, add 2 ml of 0.001M  $\text{AgNO}_3$  into the stirring solution at approximately 1 drop/sec either by burette or a pipette. The stirring was stopped as soon as  $\text{AgNO}_3$  was added. The formation of colloidal silver particles can be observed by the formation of a brilliant yellow color solution. The yellow color is due to the light reflection by the (Plasmon resonance) free electrons on the surface of silver nanoparticles. This colloidal solution was test with pinch of NaCl.

### 3 RESULTS AND DISCUSSION

#### 3.1 Synthesis of Silver Nanoparticles

A solution was synthesized by the above procedure, light yellow in color. Light yellow color is because of light reflection by free electrons on the surface of metal nanoparticle.  $\text{AgNO}_3$  reaction final concentration was  $2.5\mu\text{moles}$  and  $\text{NaBH}_4$  final concentration  $65\mu\text{moles}$  were used for chemical reduction. For silver nanoparticle colloidal testing, pinch of NaCl was added to light yellow suspension and it converted to black color due to aggregation of silver nanoparticles.



Fig.1 Silver nanoparticles after synthesis

#### 3.2 Absorption Spectra of Silver Nanoparticles

The absorption spectrum of the pure colloidal solution was taken from 200nm to 800nm immediately after synthesis by the UV-spectrophotometer. Optical density (O.D) at  $\lambda_{\text{max}}$  (380nm) was 0.870. Single peak indicated homogeneity of nanoparticles in the solution. The solution was kept at room temperature for 6 days and then the spectrum was taken. After 6 days,  $\lambda_{\text{max}}$  shifted to 395nm and optical density was measured 0.532 which indicates aggregation of silver nanoparticle in different ratios.

#### 3.3 Structural and Functional analysis of silver Nanoparticles

Silver nanoparticles were characterized using the spectroscopic techniques like NMR (Nuclear Magnetic

Resonance Spectroscopy), FT-IR (Fourier transform Infrared spectroscopy).

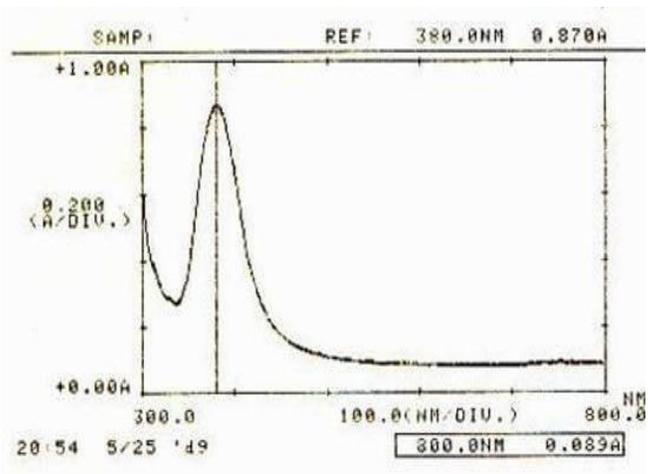


Fig.2 Absorption spectrum of the colloidal solution after synthesis

Nanoparticles pellet was lyophilized and mixed with KBr for Fourier Transform Infrared Spectroscopy for functional analysis. FT-IR spectrum of silver nanoparticles shows characteristic bands at  $3400\text{cm}^{-1}$  corresponding to particular stretching.  $^1\text{H}$  NMR spectrum of dilute colloidal dispersion of nanoparticles shows broad signals in the aromatic region.

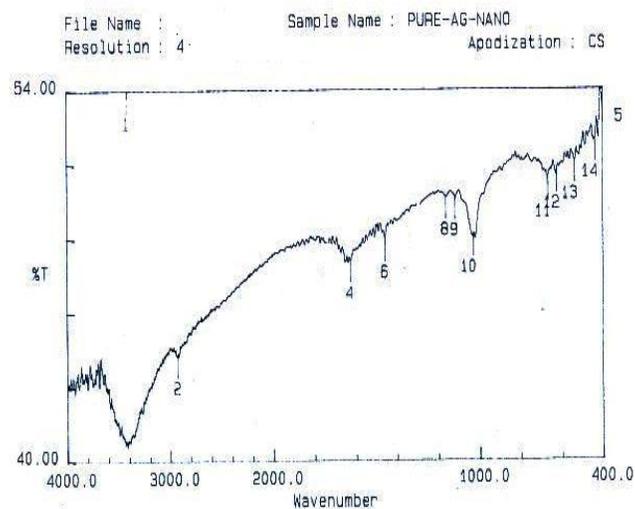


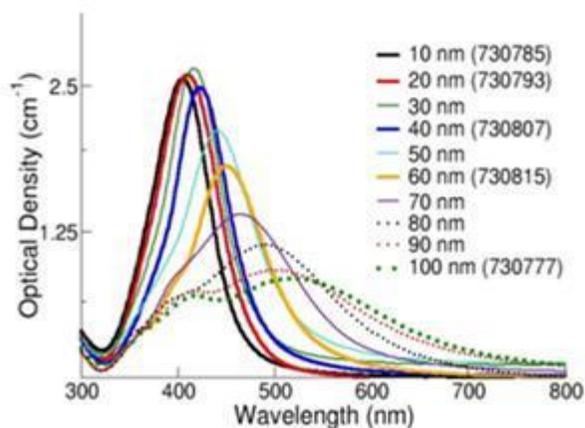
Fig.3 FT-IR for silver nanoparticles

**Table 1**  $^1\text{H}$  Nuclear Magnetic Resonance signal list

S.No.	PROTON ( $\text{H}^+$ ) PEAK TYPES	PROTON PEAK IN VARIOUS SAMPLE IN PPM SCALE				
		PureAg-NP	Glutathione(G SH)	Ag-GSH	Ag-GSH-RhoB	Rhodamine B
1	QUADRET	-----	3.55	3.55	3.690	3.686
			3.53	3.53	3.671	3.663
			3.51	3.51	3.653	3.645
			3.49	3.49	3.634	3.627
2	TRIPLET	-----	1.06	1.06	1.205	1.198
			1.04	1.05	1.188	1.180
			1.03	1.03	1.170	1.162
3	AROMATIC PROTON PEAKS	-----	-----	-----	-----	7.171, 7.147, 6.852, 6.847, 6.636, 6.630
4	SOME OTHER PEAKS ASSOCIATED WITH WATER PEAKS	4.705 Water peaks only	4.70, 4.90, 4.85, 4.80, 4.75, 4.70, 4.60	Water peak suppression	Water peak suppression And 4.896, 4.857, 4.853, 4.848, 4.844, 4.820, 4.808, 4.797	
5	CONDO EFFECT PEAK	0.14	-----	0.14	0.0000	0.006
6	OTHER PEAKS	-----	-----	-----	2.654, 2.601	-----

Comparison of  $^1\text{H}$  NMR spectrum with that of constituent monomers clearly indicates similar resonances in the nanoparticle dispersion in addition to other resonances. From NMR results, we can easily understand by comparison and concluded as silver nanoparticles were successfully coupled with Glutathione and Rhodamine-B. The below results are not exactly same to each other because of chemical shifting.

### 3.4 Fluorescence Studies of Silver Nanoparticles



**Fig.4** Absorption spectra of different size silver nanoparticles (<http://www.sigmaaldrich.com/materials-science/nanomaterials/silver-nanoparticles.html>)

Optical absorption of silver nanoparticles is size dependent; it shifts from shorter to longer wavelengths and broadens as size increases. For nanoparticles larger than 80nm, a second peak becomes visible at a shorter wavelength than the primary weak. This secondary peak is due to quadrupole resonances which yields a unique spectral fingerprint. When silver nanoparticles aggregate, metal peptides become electronically coupled to it and this coupled system has a different SPR than the individual particles. In case of a multi nanoparticle aggregate, the Plasmon resonance in the red region having longer wavelength than that of individual nanoparticles and aggregation is observable as an intensity increase in the red/infrared region of the spectrum (from Sigma Aldrich). Glutathione is a tri-peptide with a gamma linkage between the amine group of cysteine and the carboxyl group of the glutamate side chain. We coated silver nanoparticles with glutathione and coupled it to Rhodamine-B dye for fluorescent studies. Rhodamine-B is a chemical dye. Rhodamine dyes fluoresce and can thus be detected easily and inexpensively with instruments called fluorometers. Rhodamine dyes are used in many biotechnology applications such as fluorescence microscopy, flow cytometry, fluorescence correlation spectroscopy and ELISA.



**Fig.5** Rhodamine-B dye coupled nanoparticles under Fluorescent Microscope

## 4. CONCLUSION

By this present work, we successfully synthesized silver nanoparticles using chemical reduction of silver nitrate and characterized using NMR and FT-IR. We coupled glutathione coated silver nanoparticles with Rhodamine-B dye for fluorescent study of silver nanoparticles. By using this modal, Antibodies, drugs or some other proteins can be coupled with glutathione coupled silver nanoparticles for intracellular or extracellular imaging or detection in Fluorescent microscopy. We have stabilized the nanoparticles that can now be modified in a number of diverse fashions accordingly.

## 5. ACKNOWLEDGEMENTS

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## 6. REFERENCES

- [1]. A. Henglein (1993) "Physicochemical properties of small metal particles in solution: "Microelectrode" reactions, chemisorption, composite metal particles, and the atom-to-metal transition", *J. Phys. Chem.*, 97, 5457-5471.
- [2]. A. Hilger, N. Guppers, H. Tenfelde and U. Kreibig (2000) "Surface and interface effects in the optical properties of silver nanoparticles", *Eur. Phys. J. D*, 10, 115-118.
- [3]. Alqudami Abdullah and S. Annapoorni (2005) "Fluorescent silver nanoparticles via exploding wire technique", *Pramana - J. Phys.*, 65 (5), 815-819.
- [4]. Alqudami Abdullah, S. Annapoorni, S. Lamba, P. C. Kothari and R. K. Kotnala (2006) "Magnetic properties of iron nanoparticles prepared by exploding wire technique", *J. Nanosci. Nanotech.*, revised.
- [5]. A Mooradian (1969) "Photoluminescence of metals", *Phys. Rev. Lett.*, 22 (5), 185-187
- [6]. Anant Kumar Singh et al, "Synthesis of highly fluorescent water-soluble silver nanoparticles for selective detection of Pb(II) at the parts per quadrillion (PPQ) level", *Chem. Commun.*, 2012, 48, 9047-9049 DOI: 10.1039/C2CC34027E
- [7]. A. S. Edelstein and R. C. Cammarata (1996) "Nanomaterials: Synthesis, Properties and Applications", IOP Pub., London. [3] U. Kreibig and M. Vollmer (1995) *Optical Properties of Metal Clusters*, Springer, Berlin.
- [8]. C. Burda, X. Chen, R. Narayana and M. A. El-Sayed (2005) "Chemistry and properties of nanocrystals of different shapes", *Chem. Rev.*, 105, 1025-1102.
- [9]. D. Ievlev, I. Rabin, W. Schulze and G. Ertl (2000) "Light emission in the agglomeration of silver clusters", *Chem. Phys. Lett.*, 328, 142-146.
- [10]. G. T. Boyd, Z. H. Yu and Y. R. Shen (1986) "Photoinduced luminescence from noble metals and its enhancement on roughened surfaces", *Phys. Rev. B*, 33 (12), 7923-7936.
- [11]. J. A. Creighton and D. G. Eadon (1991) "Ultraviolet-visible absorption spectra of the colloidal metallic elements", *J. Chem. Soc. Faraday Trans. 87*, 3881-3891.
- [12]. Jun Lin Li, Xue Qing An and Yin Yan Zhu, "Controllable synthesis and characterization of highly fluorescent silver nanoparticles", *J Nanopart Res* (2012) 14:1325 DOI 10.1007/s11051-012-1325-3
- [13]. J. Zheng and R. M. Dickson (2002) "Individual water-soluble Dendrimer-encapsulated silver nanodots fluorescence", *J. Am. Chem. Soc.*, 124, 13982-13983.
- [14]. M. Treguer, F. Rocco, G. Lelong, A. L. Nestour, T. Cardinal, A. Maali, and B. Lounis (2005) "Fluorescence silver oligomeric clusters and colloidal particles", *Solid State Sci.*, 7, 812-818.
- [15]. N. Toshima (2003) in L. M. Liz-Marzan and P. V. Kamat (Ed.), "Nanoscale Materials", Kluwer Academi Pub., London, pp. 79-96.
- [16]. P. Gangopadhyay, R. Kesavamoorthy, S. Bera, P. Magudapathy, K. G. M. Nair, B. K. Panigrahi and S. V. Narasimhan (2005) "Optical absorption and photoluminescence spectroscopy of the growth of silver nanoparticles", *Phys. Rev. Lett.*, 94, 047403-1-4.
- [17]. R. W. Chantrell, J. Popplewell, and S. W. Charles (1978) "Measurements of particle size distribution parameters in ferrofluids", *IEEE Trans. Mag. MAG-14*, 975-977.
- [18]. S. Link and M.A.El-Sayed (2003) "Optical Properties and ultrafast dynamics of metallic nanocrystals", *Annu. Rev. Phys. Chem.*, 54, 331-366.
- [19]. U Kreibig and M Vollmer, "Optical properties of metal clusters in: *Springer series in materials science*" (Springer, Berlin, 1995) Vol. 25.

**Table 2** Fourier transform Infrared spectroscopy signal list

S No.	BOND	SPECIFIC TYPE OF BOND	STANDARD ABSORPTION PEAK	ABSORPTION PEAK IN OUR SAMPLES			
1	C-H	Methyne	1350-1330(Bend)	—	1334.86	1341.30	1355
2	C-H	Methylene	2935-2915(Stretch)	2926	2920	2926	2920.49
				—	—	2851.05	2860.59
3	C-H	Methyl	2970-2950(Stretch)	—	—	—	2960
				—	—	—	2860.59
4	C-H	Aromatic C-H, out of plane	900-670(Several types)				
		Disubstitutive(ortho)	770-735	—	—	—	758.09
		Meta	810-750+	—	—	—	760.09
		Para	900-860	—	—	—	890
			860-800	—	—	—	—
5	C-O-C	Cyclic ethers, large rings, C-O stretch	1140-1070	—	—	—	1120
							1080
6	N-H	Aliphatic primary amino	3400-3380+	—	3348.73	3350.65	3393.09
			3345-	—	—	—	—
7	C-N	Aliphatic primary amino	1090-1020(Stretch)	—	1074.45	1083.71	1055,1080
8		Ammonium ion	3300-3030/1430-1390	—	3250.35/1	3294.71/1	3270
					394	396	
		-CH <sub>2</sub> S-thiol or thioesters (Stretch)	710-685	—	713.60/64	713.73	695
					2.35		
9		-NH-CO- Amide	1680-1630	—	1657.00	1647.36	1640
10		-COOH/CO- Carboxylic acid/ketone	1725-1700/1725-1705	—	1714.87	—	—