Shape controlled synthesis of gold nanoparticles and their efficacy in yield of fluorescence in Fluorescein dye molecule

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ORIGINAL RESEARCH ARTICLE

ABSTRACT

Conformational variation in the intrinsic chemical structure of the polymer poly(N-vinyl-2-pyrrolidone) (PVP) when dissolved in different solvents changes its reducing/capping ability to a large extent. This altered reducing/capping facilitates the synthesis of diverse anisotropic size/shaped gold nanoparticles with unique physico-chemical properties. Sincere efforts were undertaken in correlating the nanogold optical plasmonic signatures with the corresponding TEM measurements. XRD measurements quantify not only the phase purity of the as-synthesized Au nanoparticles but also explain the shape evolution of gold nanoparticles. On interaction with the conventional dye molecules in solution, our gold nanostructures effortlessly tailor the emission behavior of dye through the well-known fluorescence resonance energy transfer (FRET), thereby corroborating the fluorescence quenching/enhancement of the dye molecules with reference to the size/shape of the host gold nanoparticles, yielding ultra sensitive optical sensing/imaging. Herein, we systematically illustrate the FRET phenomena of the fluorescein dye molecules adsorbed on different gold nanoparticle morphologies, the results which could be extended similarly to various other relevant dye molecules as well with ease.

KEYWORDS

gold nanoparticles; fluorescence; optical absorption spectra; shape controlled synthesis; XRD

1 INTRODUCTION

Now days, researchers are trying to understand dye-metal nanoparticles hybrid systems due to their wide applications in bio-imaging (Tao et al., 2014), sensors (Achilefu et al., 2000; Ghosh and Pal, 2009) and single molecule detection (Bharadwaj et al., 2007). As dye-nanoparticle hybrid systems is necessity for many of industrial and technological applications and thus systematic study of these mixture are of prime importance (Tam et al., 2007). In general, these systems follow Fluorescence resonance energy transfer (FRET) in which enhancement or quenching of luminescence of adsorped molecules depends upon a number of factors including nanoparticle’s shape and size (Bardhan et al., 2009; Nakamura and Hayashi, 2005). Thus, a study on dependence of metal nanoparticles surface morphology on the fluorescence enhancement/quenching is of immense importance. Although a large number of metal nanoparticles are synthesized as reported in literature, gold nanoparticles are the most capable for application point of view owing to their surface plasmon resonance in wide range of visible range in electromagnetic spectrum (Kedia and Kumar, 2012), low cytotoxicity (Hong and Kang, 2006), biocompatibility (Davis et al., 2008), ease in synthesis and surface functionalization (Verma et al., 2016).

Basically, when a fluorophore is placed in the vicinity of metal nanoparticles possessing a strong plasmon’s field, its fluorescence emission may change extensively (John et al., 2015; Yun et al., 2005). The interaction between fluorophore and metal nanoparticles can change fluorescence emission level drastically (Kochuveedu and Kim, 2014; Raut...
et al., 2014). Deducing the relationship between the plasmonic field of a nanoparticles and resulting fluorescence emission of coupled dye molecule can be beneficial in developing highly efficacious contrast agents for bio-sensing/imaging (Hong and Kang, 2006). Enhancement of fluorescence can offer a excellent sensitivity and signal-to-noise ratio for molecular sensing/imaging, especially for the fluorophore having low quantum yield while conditional quenching of fluorescence may be effectively used for negative sensing (Tao et al., 2014). Interestingly if somehow both quenching and enhancement of a fluorophore can be achievable in a single system, then those types of system could be a highly specific and highly sensitive optical contrast agent (Anger et al., 2006).

From literature point of view quenching and enhancement of fluorescence intensity of fluorophore by using metal nanoparticles plasmonic’s appeared to be studied separately from each other. But in last couple of years, more researchers believed that both quenching and enhancement of fluorescence can be caused by the metal nanoparticle (Anger et al., 2006; Kang et al., 2011). Basically, gold nanoparticles when illuminated by visible light show intense surface plasmon’s resonance which generates high electric field intensities near their surface and thus interaction of these plasmonic particles with dye molecule could extensively change the optical properties of fluorophore (Dulkeith et al., 2002; Hutter and Fendler, 2004). Moreover, localized surface plasmon’s resonance (LSPR) is highly dependent on the nanoparticles shape and size (Kedia and Kumar, 2012; Verma et al., 2016), so tuning size and shape of gold nanoparticles not only changes the plasmonic’s properties of metal nanoparticles but also changes the fluorescence properties of fluorophores (John et al., 2015).

In this report we have synthesized gold nanoparticles of two different morphology namely spherical shape and star shape by wet chemical synthesis route. XRD pattern shows crystalline nature of synthesized nanoparticles and derived calculations closely agree with other spectroscopic techniques. Fluorescence spectra of fluorescein dye molecule in absence and in the presence of both spherical and star shaped gold nanoparticles were studied.

3. RESULTS AND DISCUSSION

3.1. Absorption spectroscopy and TEM observations

Optical absorption spectra (Figure 1 (b)) delineate the formation of pseudospherical/spherical gold nanoparticles at a PVP to metal ion ratio of ~ 3333 in the alcoholic methanol medium. The addition of metal ions in PVP-methanol complex results in the change in solution color from pale yellow to ruby red within 3 h indicates the formation of spherical
gold nanoparticles. Absorption spectra of spherical shape shows a single surface plasmon resonance (SPR) peak at 532 nm (Figure 1 (b)) due to isotropic nature of spherical shape as confirmed from the TEM images (Figure 1 (c)). Size histogram from the TEM micrograph shows a quite monodisperse distribution with average size around 42 nm (Figure 1(e)). On the other hand, for tuning the shape of gold nanoparticles we use polymer solvent interaction (Kedia and Kumar 2012) by using dimethylformamide (DMF) as solvent for the reaction and in this case we get anisotropic multibranched star shaped nanostructures as shown in TEM micrographs (Figure 1 (d)). Absorption spectra of star shaped nanoparticles shows two peaks (Figure 1 (b)), one around 540 nm while other around 900 nm. In this case, transverse plasmon band at lower wavelength (higher energy) arises due to the core of metal nanoparticles, whereas the longitudinal band at higher wavelength (lower energy) occurs due to surface tips/edges. In this case also, size histogram shows a quite monodisperse distribution with average size around 90 nm (Figure 1(f)).

Reduction mechanism of metal precursor by a reducing agent to form metal nanoparticles in wet chemical route can be well explained with La Mer's theory which relates the size and shape of metal nanoparticles with nucleation and growth rates of nanoparticles. In general, fast nucleation leads to smaller sized particles while slow nucleation leads to bigger size particles. Moreover growth rate of nanoparticle can easily depicts the mono-dispersity and shape of nanoparticles and establish the fact that anisotropic shapes grows slowly than isotropic ones because of presence of defect structures. In our case we have plotted concentration of Au0 zero-valent atom with reaction time to study the nucleation and growth of nanoparticles formed by reduction of HAuCl4 by PVP in different solvents. Early nucleation in case of spherical nanoparticles than star shaped nanoparticles can be well correlated with small size of spherical particles than star shaped nanoparticles obtained. Analytically, slope of these curves can be related to growth rate of nanoparticles. Larger value of slope means fast growth of nanoparticles which tend to from isotropic shapes and vice-versa. In Figure 2, slope of star shaped particles is less than that of spherical ones which shows that slow growth was necessary for
formation of anisotropic multi-branched star shaped nanoparticles.

**Figure 2.** Change in concentration of reduced gold with reaction time for spherical and star shaped nanoparticles.

### 3.2. Shape evolution of Nanoparticles through XRD Studies

XRD studies of as synthesized particles (Figure 3(A)) shows that both types of particles were highly crystalline in nature. Diffraction peaks at 38.40°, 44.65°, 64.79° and 77.99° arises due to <111>, <200>, <220> and <311> reflections respectively of fcc crystal structure of gold which matches with JCPDS card number-04-0784. The intensity ratio of diffraction peaks <200>/<111>, <220>/<111> and <311>/<111> is (0.25), (0.1) and (0.1) respectively which shows that spherical nanoparticles contains a large share of growth along <111> axis which also explains the fast synthesis time for spherical particles because being a low energy surface, growth on <111> surface is kinetically favored. On the other hand, in case of star shaped nanoparticles, peaks at 38.25°, 44.40°, 64.89° and 78.99° arises due to <111>, <200>, <220> and <311> reflections respectively of fcc crystal structure of gold nanoparticles. Interestingly in this case, intensity ratio of diffraction peaks <200>/<111>, <220>/<111> and <311>/<111> is increased drastically to values (0.45), (0.25) and (0.5) respectively which delineates the polycrystalline nature of star shaped nanoparticles formed by slow growth process in comparison to spherical shaped nanoparticles (Table 1).

Instrumental corrected broadening $\beta_{hkl}$ corresponding to each diffraction peak have component of both size broadening as well as strain induced broadening. The Debye-Scherrer equation shows the size induced broadening by relation

$$D = \frac{K\lambda}{\beta_{hkl} \cos \theta}$$

(1)

where $\beta_{hkl}$ is integral half width, $K$ is constant equal to 0.90, $\lambda$ is wavelength of incident X-ray ($\lambda=0.154$ nm), $D$ is average crystallite size, and $\theta$ is the Bragg angle. Whereas strain induced broadening in nanocrystals due to crystal imperfection and distortion can be calculated by

$$\varepsilon = \frac{\beta_{hkl}}{4 \tan \theta}$$

(2)

Now the total peak broadening is represented by the sum of contributions of crystallite size and strain present in the material and can be written as

$$\beta_{hkl} = \beta_D + \beta_\varepsilon$$

(3)

where $\beta_D$ is contribution due to crystallite size, $\beta_\varepsilon$ is contribution due to strain induced broadening and $\beta_{hkl}$ is the width of half-maximum intensity of instrumental corrected broadening. Now if we assume that particle size and strain contributions to observed line broadening are independent to each other and both have a Cauchy like profile, the observed line width can be written as

$$\beta_{hkl} = \frac{K\lambda}{D \cos \theta} + 4\varepsilon \tan \theta$$

(4)

The equation 4 is williamson-Hall equation which represent uniform deformation model. The values of $\beta_{hkl} \cos \theta_{hkl}$ on y-axis were plotted as a function of $4\sin \theta_{hkl}$ on x-axis, and from the linear fit the data, the crystallite size $D$ was estimated from the y-intercept and microstrain $\varepsilon$ from the slope of the linear fit. Williamson- Hall plot of spherical particles and star shaped nanoparticles were plotted separately (Figure 3(c and d)) and calculated crystallite size and microstrain were tabulated along with other calculated parameters of XRD measurement (Table 1). Crystallite
sizes of both spherical shaped and star shaped nanoparticles calculated from the W-H plots were found to be well correlated with TEM observations. Growth rate of nanoparticles retards in presence of strain on crystallographic facets. Interestingly, microstrain of star shaped nanoparticles calculated from W-H plot was found to be greater than that of spherical nanoparticles which again explains the higher synthesis time and thus overall anisotropic nature of shape.

For further fine details of growth mechanism, microstrain on each crystallographic axis was plotted against another parameter called Nelson-Riley function (Figure 3(b)) given by

$$F(\theta) = \frac{\cos^2 \theta + \cos^2 \theta}{2} \left( \frac{\sin \theta}{\theta} \right)$$

(5)

From Nelson-Riley function we can analyze that low value of NRF corresponds to high energy crystallographic facets. For spherical nanoparticles it was observed that microstrain increases with decreasing values of NRF which means that microstrain is least in case of low energy facets like <111> and <200> so most of the crystal growth preferably take place along these crystallographic axis. Being low energy facets, <111> facet facilitates the fast and uniform growth which explains less synthesis time and symmetrical shape of final nanoparticles. On the other hand, in case of star shaped nanoparticles microstrain decreases with decreasing values of NRF (Figure 3(b)). This shows that star shape nanoparticles preferably grow along high energy facets due to less value of microstrain which explains the slow growth as well as highly asymmetrical three dimensional morphology of star shaped nanoparticle.

3.3. Fluorescence Studies

Basically quantum yield of a fluorophore depends upon a large number of factors like concentration of fluorophore, solvent, pH, chemical bindings with other molecules and many more factors (Siejak and Frackowiak, 2005; Shang et al., 2007). But in this communication we focus only on the effect of nanoparticles-dye interaction on the fluorescence yield of “fluorescein” dye molecule. Increase or decrease in quantum yield of fluorescence of dye molecule in presence of other species can be well explained with Jablonski diagram (Figure 4).
A species (donor) when excited to higher electronic levels can be relaxed via Radiative or non Radiative processes after being vibrationally relaxed and ratio of Radiative emission to initial absorption gives the quantum yield of species. But if another species (acceptor), optically active in the emission range of donor species is in close proximity then energy transfer can take place from donor to acceptor, and in this process donor species can also be relaxed via this mechanism which decreases the quantum yield of donor species. On the other hand, when acceptor species got excited via this mechanism, its emission increases drastically which leads to increase in quantum yield of acceptor state.

The energy transfer efficiency equation is dependent on various factors (Swierczewska et al., 2011) and is given by

\[
\text{Efficiency} = \frac{I_{\text{acceptor}}}{I_{\text{donor}}} \times \frac{1}{\eta_{\text{donor}}}
\]

**Figure 4.** Jablonski diagram explaining resonance energy transfer between donor and acceptor species.

**Figure 5.** Absorption and emission spectra of fluorescein dye molecule in absence of any gold nanoparticles (a and b), emission spectra of fluorescein dye in presence of spherical and star shaped gold nanoparticles respectively (c and d), change in fluorescence intensity ratio with change in gold nanoparticles concentration with different shapes (e), picture of Nanoparticle – dye mixture under UV and visible light (f).
where \( \kappa \) is dipole orientation factor, \( \Phi_{dye} \) is quantum yield of dye, \( N_a \) is Avogadro number, \( n \) is medium refractive index, and \( J(\lambda) \) is the spectral overlap integral between donor and acceptor absorption. In our present study as we constraint ourselves to see the effect of shape of gold nanoparticles on fluorescence resonance energy transfer by mixing same concentration of dye molecule (\( \Phi_{dye} \)) in same solvent (\( n \)) with different shapes of nanoparticles. So, by changing shape of nanoparticles, both dipole orientation factor (\( \kappa \)) and spectral overlap integral between donor and acceptor absorption \( J(\lambda) \) changes which changes the energy transfer efficiency.

Absorption spectra of pure fluorescein dye molecule dispersed in ethanol shows peaks at 452 nm (2.74 eV) and 480 nm (2.58 eV) (Figure 5(a)). When excited at 450 nm (2.75 eV), fluorescein dye (0.2 mM) dispersed in ethanol gives emission peak at 515 nm (2.40 eV) (Fig. 5(b)). When same concentration of dye was mixed with 5 fold centrifuged spherical gold nanoparticles dispersed in ethanol having concentration (0.1 mM) and (0.2 mM), fluorescence emission of fluorescein enhances with increasing concentration of gold nanoparticles and green shifts to 520 nm (2.38 eV) (Figure 5(c)) which shows that in case of spherical gold nanoparticles, gold nanoparticle act as donor while dye molecule acts as acceptor and energy transfer takes place from metal surface to dye molecule (Figure 6). On the other hand, when (0.2 mM) of fluorescein was mixed with 5-fold centrifuged star shaped nanoparticles dispersed in ethanol having concentration of (0.1 mM) and (0.2 mM), yield of fluorescence quenches with increasing concentration of gold nanoparticles and emission peak blue shifts to 508 nm (2.44 eV) (Figure 5(d)) which essentially dictates that in this case star shaped gold nanoparticles acts as “acceptor” and dye molecule acts as “donor” and hence dye molecule can relax to ground state directly by loosing energy to gold nanoparticles instead of fluorescence and hence quenching was observed (Figure 6).

So, in these conditions as stated above, energy transfer efficiency is dependent on two factors, (i) dipole orientation which is highly dependent on asymmetry of nanoparticle morphology and (ii) spectral overlap between donor and acceptor absorption which in turn depends upon shape of gold nanoparticles. As seen from Figure 1(b), spherical gold nanoparticles shows LSPR at around 530 nm in visible region, absorption spectral overlap between gold nanoparticles and dye molecule in this case was very large so energy transfer takes place from gold nanoparticles to dye molecule and hence excellent fluorescent enhancement was observed in this case. While on the other hand, star shaped gold nanoparticles shows two LSPR peaks at 540 nm and 900 nm. Intensity of longitudinal peak (900 nm) of star was much larger than transversal peak due to presence of very sharp tips and overall highly asymmetric shape. Due to low absorption cross section of star shaped nanoparticles near the active optical region (ca. 500 nm) of dye molecule, absorption spectral overlap between star gold nanoparticles and dye molecule was negligible and hence energy transfer takes place from dye molecule to gold nanoparticles and hence fluorescent quenching was observed in this case. Fluorescence intensity ratio was plotted against gold nanoparticles concentration for spherical and star shape particles. FIR decreases to as low as (0.05) in case of 20 μM star shaped nanoparticle while FIR increases

<table>
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<tr>
<th>Shapes</th>
<th>Planes</th>
<th>2θ</th>
<th>β</th>
<th>“d” spacing</th>
<th>Intensity</th>
<th>Particle size (nm)</th>
<th>Microstrain from W-H analysis</th>
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<td>38.4025</td>
<td>0.1574</td>
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<td>100</td>
<td>51.5</td>
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<td></td>
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<td>0.3149</td>
<td>2.029</td>
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<tr>
<td></td>
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<td>64.7920</td>
<td>0.6298</td>
<td>1.438</td>
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<td></td>
<td>&lt;311&gt;</td>
<td>77.9935</td>
<td>0.7680</td>
<td>1.224</td>
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<tr>
<td>Star shaped Nanoparticles</td>
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<td>2.352</td>
<td>100</td>
<td>86.5</td>
<td>10.62 X 10^{-3}</td>
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<tr>
<td></td>
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<td>0.2558</td>
<td>2.044</td>
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<td></td>
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<td>64.8955</td>
<td>0.2755</td>
<td>1.430</td>
<td>25</td>
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<td></td>
<td>&lt;311&gt;</td>
<td>78.0936</td>
<td>0.2880</td>
<td>1.221</td>
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</table>
up to 2.8 in case of 20 μM spherical nanoparticles (Figure 5(e)). So, we can tune the fluorescence intensity in a wide range by changing concentration as well as shape of nanoparticles. Nanoparticle-dye mixture kept under visible and UV light (Figure 5(f)) contrasts the difference between fluorescence enhancement and fluorescence quenching.

**Figure 6.** Schematic of fluorescent enhancement and fluorescent quenching in presence of spherical and star shaped nanoparticles, respectively.

### 4. CONCLUSIONS

In brief, we have used polymer-solvent interaction to synthesize different shape nanoparticles by using versatile nature of polymer PVP in reducing and stabilizing of gold nanoparticles. With the help of XRD measurement and rigorous calculations we have deduce the shape formation mechanism for different shaped gold nanoparticles. We have synergistically identified the role of size/shape of gold nanostructures synthesized/stabilized by the polymer, PVP and its strategic effect on their properties to enhance or quench the photoluminescence yield of “fluorescein” dye. The central role of shape and size of as-prepared gold nanoparticle was not only giving distinct plasmonic signatures, but also in dictating their ability to interact with adsorbed analytes in a meticulous manner has been well-established analytically in a clear cut manner beyond doubt.

### ACKNOWLEDGEMENTS

Authors are thankful to University of Delhi for providing space and fund under Innovation project (SSNC – 302, 2015-16). Authors also thank the University Science Instrumentation Centre (USIC) for material characterization. We are grateful to Mr. P. Senthil Kumar for his special guidance in the project, Mr. Apoorva, Mr. Jeetendra for providing TEM and PL data and Ms. Kamalesh Nehra for helping us in the project. Special thanks to Mr. Jasjeer Singh and other project group members.

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