Chapter 3

Multi-Technique Approach for Characterization of Gold Nanoparticles

3.1. Introduction

Nanoparticle characterization is necessary to establish understanding and control of nanoparticle synthesis and applications [1,19,158-160]. The need to fine-tune different nanoparticle properties to make them suitable for specific applications has evolved a large number of worldwide research efforts aimed at their tailoring. However, full use of these structures in these applications requires more detailed information and a feedback of data coming from reliable characterization techniques. In general as discussed in chapter 1, there are number of techniques for the characterization of nanomaterials, for the understanding of their structure, properties and interactions. For example, spectroscopic techniques (e.g. UV-visible, photoluminescence, IR and Raman spectroscopy) are employed for the confirmation of the presence of molecular species and electronic transitions, monitoring phase transitions and band gap calculations, studying luminescence, fluorescence and chemical species etc [1,19,158-160]. Microscopic techniques (e.g. SEM, TEM, STM and AFM) give the direct visualization of the morphology, particle size, phases, defects etc [1,19,158-160]. Scattering techniques (e.g. XRD, DLS, SAXS and SANS) are extremely reliable for finding the particle size, shape, number density, interactions and crystal structure [1,19,158,159]. Each technique has its own advantages and disadvantages to get the required information under the required conditions. Therefore, many times combination of different techniques is used for obtaining complementary results.
A multi-technique approach combining spectroscopy, microscopy and scattering techniques has been used in this thesis for the characterization of gold nanoparticles. The use of different techniques is required for understanding the role of different components and tuning of the synthesis. The different techniques used are UV-visible spectroscopy, TEM, DLS, SAXS and SANS. This chapter gives the details of these techniques and the kind of information that can be obtained from them is discussed.

3.2. UV-Visible Spectroscopy

UV-visible spectroscopy is based on the absorption of photons by the samples to produce electronic transitions from the ground state to an excited state. As a result the absorption spectra are generated reflecting the identity and concentration of molecules in solution [161]. Some of the nanoparticles display UV-visible spectrum because of the existence of the surface plasmon resonance (SPR) that is sensitive to size, shape, concentration, agglomeration state and refractive index near the nanoparticle surface, which makes UV-visible spectroscopy a valuable tool for characterization of nanoparticles [162].

3.2.1. Surface Plasmon Resonance

At the nanometer scale, particles display properties that are not inherent in individual atoms or to those in the bulk macroscopic material [1,19,158-160]. The optical properties of gold nanoparticles depend on their size and shape, interparticle distance, type of attachment (e.g. protein), dielectric constant of the dispersion medium etc [159-162]. In a nanoparticle, many of the atoms are located on the surface due to large surface-to-volume ratio, hence unusual properties are observed which are not present in the bulk or in individual atoms [1,19,158-160]. One of these optical phenomena is surface plasmon resonance. The deep coloured nature from orange through to deep purple, of colloidal gold is a result of the surface
plasmon. At this nanometer scale the ‘quantum size effect’ becomes significant as the de Broglie wavelength of the valence electrons is in the same size range as the particle itself. The particles are then considered to be zero-dimensional quantum dots or quantum boxes and are subject to quantum mechanical rules. Freely mobile electrons are trapped effectively in quantum boxes and hence exhibit a collective oscillation frequency associated with the plasmon resonance in the presence of light [160-162].

Surface plasmon resonance manifests itself when the electric field of the incoming radiation induces a dipole on the nanoparticle. When a gold nanoparticle, with a diameter of between about 10 and 100 nm, is irradiated by light with a wavelength much greater than the particle diameter, the conduction band free electrons (e⁻ cloud) are displaced relative to the fixed background of immovable positive ions by the oscillating electric field of the incident light. As a result, a net charge difference appears on the surface at one side of the particle. Its attraction with the lattice ions on the opposite side leads to a restoring force. It tries to compensate for this dipole, so that a unique resonance frequency is formed which matches the electron oscillation frequencies within the particle and this results in an oscillation of the e⁻ cloud known as a dipole plasmon resonance. This frequency of oscillation is equal to the frequency of resonant light and as the particle is much smaller than the wavelength of incoming light it is homogeneously polarised [160-162]. The plasmon resonance band is observed at approximately 530 nm for gold nanoparticles of diameter 5–20 nm [159]. The resonance frequency is mainly determined by the strength of the restoring force. This force depends on the separation of the surface charges i.e. the particle dimensions and the polarizability of the medium between and around the charges. In other words the frequency, intensity and bandwidth of the SPR absorption and scattering depend on the incident wave, metal composition, nanoparticles size and shape, dielectric properties of surrounding medium/substrate, spaced particles inter-particle interaction and particle-to-particle
Chapter 3: Multi-Technique Approach for Characterization of Gold Nanoparticles

interactions [163,164]. These different information thus regarding nanoparticles can be obtained by the UV-visible spectroscopy [159-162].

The nanoparticle systems because of surface plasmon effects can appear different coloured as given in Table 3.1. When particles are small (~ 10 nm) they appear red due to the absorption of blue-green light at around 520 nm. If these particles are allowed to coalesce in a controlled fashion by the addition of electrolytes, a colour change is observed. The red goes through violet and blue to green as the nanoparticles grow. This is due to a change in the absorption spectrum upon aggregation, causing the increased absorption of red light.

Table 3.1. The complementary observed colours of nanoparticles to that absorbed in SPR.

<table>
<thead>
<tr>
<th>Wavelength of the Maximum Absorption (nm)</th>
<th>Colour Absorbed</th>
<th>Colour Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>380 – 420</td>
<td>Violet</td>
<td>Green-Yellow</td>
</tr>
<tr>
<td>420 – 440</td>
<td>Violet-Blue</td>
<td>Yellow</td>
</tr>
<tr>
<td>440 – 470</td>
<td>Blue</td>
<td>Orange</td>
</tr>
<tr>
<td>470 – 500</td>
<td>Blue-Green</td>
<td>Red</td>
</tr>
<tr>
<td>500 – 520</td>
<td>Green</td>
<td>Purple</td>
</tr>
<tr>
<td>520 – 550</td>
<td>Green-Yellow</td>
<td>Violet</td>
</tr>
<tr>
<td>550 – 580</td>
<td>Yellow</td>
<td>Violet-Blue</td>
</tr>
<tr>
<td>580 – 620</td>
<td>Orange</td>
<td>Blue</td>
</tr>
<tr>
<td>620 – 680</td>
<td>Red</td>
<td>Blue-Green</td>
</tr>
<tr>
<td>680 – 780</td>
<td>Purple</td>
<td>Green</td>
</tr>
</tbody>
</table>

3.2.2. Experimental Details

The schematic diagram for a UV-visible spectrophotometer is shown in Figure 3.1. Radiation from the source passes through monochromator which select and transmits the light of
particular wavelength. The transmitted light is splitted into two beams by beam splitter and each splitted beam passes through sample and reference to photo detector. The signals are amplified and spectrum is displayed in a computer. Intensity of light ($I_0$) at a given wavelength passes through a sample solution that absorbs light in certain wavelength. Intensity of transmitted light ($I$) from the sample is detected by the detector [160]. The amount of radiation absorbed can be measured either by transmittance or absorbance, where transmittance $T = P / P_0$ and absorbance $A = \log_{10} (P_0/P) = - \log_{10} T$. $P_0$ and $P$ are the radiant power of the incident and transmitted radiation, respectively. Further, the absorbance ($A$), concentration of the absorbing species $c$ (in mol/lt or M) and path length of the sample cuvette $b$ (in cm) are related through Beer-Lambert law as $A = \varepsilon cb$, where $\varepsilon$ is the molar absorptivity or molar extinction coefficient (M$^{-1}$cm$^{-1}$) [165,166].

Figure 3.1. Schematic diagram for a UV-visible spectrophotometer.

In this thesis, the measurements were carried out using 6505 Jenway UV-visible spectrophotometer. This instrument is suitable for measurements in the scanning wavelength range between 190 nm and 1100 nm (with an accuracy of ±1 nm). It makes use of tungsten halogen lamp as a visible light source and deuterium discharge lamp for the UV light source.
The instrument can operate in spectrum mode with a wavelength interval 1 nm and the samples held in quartz cuvettes of path length 10 mm.

3.2.3. Characterization of Gold Nanoparticles by UV-Visible Spectroscopy

Gold nanoparticles show a characteristic SPR peak around 540 nm observed in the UV-visible spectrum as shown in Figure 3.2. The SPR profile can be used to obtain information on the nanoparticle composition, its size and shape and dielectric properties of the surrounding medium [163,164]. Figure 3.2 also shows the UV-visible spectra of gold nanoparticles for different sizes of nanoparticles. The SPR peak position shifts to higher wavelength with the increase in the particle size and can be explained by the particle in a box problem in quantum mechanics. The absorption energy of quantum dots (QDs) shifts to higher wavelength \( E_g(R) > E_g \) with increasing radius of QDs \((R)\) with a dependence of \(1/R^2\) [167].

![Figure 3.2](image)

**Figure 3.2.** UV-visible spectrum of gold nanoparticles for different sizes \((c > b > a)\).

UV-visible absorption spectra are known to be strongly depending on the shape of the nanoparticle. In particular, shape anisotropy results in an anisotropic response towards
incoming light, which allows the further modulation of optical effects through alignment [163,164]. Figure 3.3 shows variation of UV-visible spectra with the change in the particle morphology. One-peak SPR peak for spherical nanoparticles transforms to two-peaks SPR for nanorods which typically display longitudinal and transverse plasmon resonances (electron oscillations along or across their long axis, respectively). Rod length affects longitudinal plasmon wavelength (a strong extinction peak in the upper visible or near-infrared parts of the spectrum). As the aspect ratio increases, the position of the longitudinal plasmon band red-shifts and the transverse plasmon band position stay relatively constant. Thus, the particle shape dictates what wavelengths of light can be absorbed, and elastically scattered; gold nanorods of moderate aspect ratio (2 – 5) display plasmon bands with tunable maxima from ~700 to 900 nm and high-aspect ratio nanorods exhibit a longitudinal plasmon band past 1500 nm [163,164,167].

![UV-visible absorption spectra](image)

**Figure 3.3.** UV-visible absorption spectra of different gold nanostructures, e.g. nanoparticles/spheres, decahedra and nanorods.

UV-visible absorbance spectra can also be used to investigate different ligands (proteins, antibodies etc.) on the nanoparticles (Figure 3.4). The adsorption of ligand onto gold nanoparticle surface results in red shift in absorbance peak for the nanoparticle. It is
believed to be due to the changes in the dielectric nature (modification of the refractive index) surrounding the nanoparticles after the formation of ligand layer on the surface of the gold nanoparticles [163,164,168].

![UV-visible spectra of gold nanoparticles](image)

**Figure 3.4.** The change in UV-visible spectra of gold nanoparticles on their conjugation with ligand.

### 3.3. Transmission Electron Microscopy

For the study of structure and morphology of nanomaterials with magnifications down to the atomic scale transmission electron microscopy is an important direct method. TEM uses electrons instead of light waves to generate magnified images. It has higher resolution than optical microscopes (due to $\Delta \lambda$) and allows one to visualize objects that are as small as 1 nm, hence atomic resolution is possible. In this way, it bridges the 1 nm - 1 μm gap between X-ray diffraction and optical microscopy [54,160,169,170].

#### 3.3.1. Experimental Details

In transmission electron microscopy, a beam of electrons is passed through the sample with high accelerating voltage. It uses magnetic lenses to deflect the electron beam where the
electrons are transmitted through the sample and detected by a camera before being focused by an objective lens. The resulting pattern of electron reflection and absorption is magnified on a fluorescent screen and an image appears on the screen corresponding to the size and shape of the particles present in the sample [54,160,169].

![Schematic of a transmission electron microscope.](image)

**Figure 3.5.** Schematic of a transmission electron microscope.

Schematic of a typical transmission electron microscope is shown in **Figure 3.5.** The electron gun, a hairpin-shaped tungsten filament (cathode) is usually used as the source of electrons through thermo-ionic emission. An accelerating voltage (fixed amount of negative high voltage) is applied to the surrounding cathode cap and a small emission current is then applied to the filament to achieve the release of electrons. The anode located below the gun
assembly, is electrically at ground, thus creating a positive attraction for the negatively charged electrons, which overcome the negative repulsion of the cathode cap and accelerate through the small hole in the anode. As glass lenses impede electrons, electromagnetic converging lenses are used to direct and focus the electron beam inside the column. A tightly wound wrapping of copper wire produces the magnetic field that is the essence of the lens. The electron moves through the centre hole in this solenoid. The electrons follow a tight spiral path as they are accelerated through the lenses. The path and trajectory taken by the electrons are influenced by the lens current as they pass through a small opening in the lens. Then there is an opening to insert specimen holder into the high-vacuum chamber for observation. A series of pumps are used to accomplish an adequate vacuum as electrons are easily deflected by hydrocarbons or gas molecules. The final image is viewed by the projection onto a phosphorescent screen which gives off photons when irradiated by the electron beam. A film camera beneath the phosphorescent screen or a CCD camera is used to capture the image. An operation panel for sample alignment, beam focusing and image magnification gives the control over the instrument [54,160,169].

3.3.2. Gold Nanoparticles as Characterized by Transmission Electron Microscopy

The uniformly suspended nanoparticles in the liquid solvent are first obtained by stirring the solution in an ultrasonic stirrer. For the TEM sample preparation, few drops of the sample are put on a carbon amorphous film coated microscopic copper grid. Carbon film coated copper grid and the specimen holder are shown in Figure 3.6. Then the sample is dried in air allowing the solvent to evaporate under ambient conditions. Alternatively, a drop of sample solution can be attached on one side of the copper grid, which is then gently placed on filter
paper with the opposite site facing down. We have used JEOL 2000 FX transmission electron microscope for the characterization of gold nanoparticles in this thesis. All the TEM microphotographs were taken at acceleration voltage 160 kV, recorded on a photographic film. A typical TEM micrograph of gold nanoparticles is shown in Figure 3.7. A large number of such micrographs are usually taken to determine the size distribution of the nanoparticles [170].

**Figure 3.6.** Carbon film coated copper grid as put on the specimen holder in TEM measurements.

**Figure 3.7.** (a) TEM micrograph of gold nanoparticles and (b) calculated nanoparticle size distribution.
3.4. Dynamic Light Scattering

Unlike static light scattering (SLS) which is sensitive to the length scale that of order of wavelength of light, dynamic light scattering (DLS) can measure sizes down to 1 nm. SLS measures the time averaged scattered intensity as a function of the scattering angle. On the other hand, DLS gathers information on the Brownian motion in a sample by analyzing the fluctuations of the scattered light intensity. This time dependence of the fluctuations in the net scattered intensity forms the basis of the DLS which yields structural information of the particle by measuring its diffusion coefficient [63,64,171-175].

3.4.1. Theory of Dynamic Light Scattering

The signal generated by the light scattering from diffusing particles can be analyzed by its intensity autocorrelation function $G^I(\tau)$ as given by

$$ G^I(\tau) = \langle I(t)I(t+\tau) \rangle \quad (3.1) $$

where $I(t)$ is the scattered light intensity at time $t$ and $I(t+\tau)$ is the scattered light intensity at some later time $(t + \tau)$. Figure 3.8 shows a typical pattern of intensity fluctuations as a function of time. The normalized intensity autocorrelation function $g^I(\tau)$ is

$$ g^I(\tau) = \frac{G^I(\tau)}{\langle I(t) \rangle^2} \quad (3.2) $$

The electric field autocorrelation function $g^E(\tau)$ is related to the normalized intensity autocorrelation function by

$$ g^I(\tau) = 1 + B[g^E(\tau)]^2 \quad (3.3) $$

where $B$ is experimental parameter which mainly depends on the detection optics and alignment. It is roughly equal to the inverse of the number of speckle from which light is collected (a speckle pattern is a random intensity pattern produced by the mutual interference
of a set of wave fronts). For a monodisperse system of particles, \( g^{E}(\tau) \) follows a simple exponential decay with decay constant \( \Gamma \)[173],

\[
g^{E}(\tau) = \exp[-\Gamma \tau]
\]  

(3.4)

The apparent diffusion coefficient \( (D_a) \) is obtained from relation \( \Gamma = D_a Q^2 \) \( (Q = 4\pi n \sin \theta \lambda, \) where \( 2\theta \) is the scattering angle, \( n \) is refractive index of medium and \( \lambda \) is wavelength of light) and the corresponding effective hydrodynamic radius \( R_H \) is given by the Stokes-Einstein relation [173],

\[
R_H = \frac{k_B T}{6\pi \eta D_a}
\]

(3.5)

where \( k_B \) is Boltzmann constant, \( T \) is the temperature and \( \eta \) is the solvent viscosity.

![Intensity fluctuations at the detector due the Brownian motion in the sample volume.](image)

**Figure 3.8.** Intensity fluctuations at the detector due the Brownian motion in the sample volume.

**3.4.2. Experimental Details**

DLS experiment involves scattering of a monochromatic beam of light from the sample through an angle \( 2\theta \) and then recording the scattered photon intensity at small intervals (~ 50
ns) of time. A photon correlator card is used to store these intensities in different channels and to generate the intensity correlation spectrum $g^I(\tau)$ [174]. The schematic of the dynamic light scattering instrument is shown in Figure 3.9.

![Figure 3.9. Schematic of a dynamic light scattering set up.](image)

A typical DLS setup consists of a laser source such as He-Ne (632.8 nm) or Ar-ion (514.5 nm) is used to obtain a monochromatic beam of light. The beam is further converged by using a lens. Light is scattered by the sample at all angles. However, a DLS instrument uses a single detector and the measurement is usually done at one particular angle. The scattered light is focused on the detector using a lens. The detector comprises a photomultiplier tube (PMT) and a photon counter. PMT is used to amplify the photon signal, whereas photon counter does the actual counting job. The intensity fluctuations of the scattered light are converted into electrical pulses, which are fed into a digital correlator. This generates the autocorrelation function.

We have carried out DLS studies using Autosizer 4800 (Malvern Instruments, UK) equipped with 7132 digital correlator and coherent (Innova 70C) Ar-ion laser source operated at wavelength 514.5 nm with a maximum output power of 2 W.
3.4.3. Characterization of Gold Nanoparticles by Dynamic Light Scattering

Figure 3.10. The variation of intensity correlation functions with nanoparticle size. The decay of the function slows down for larger sized particles.

Small particles diffuse rapidly and yield fast fluctuations, whereas large particles and aggregates generate relatively slow fluctuations. The rate of the fluctuations is determined through the autocorrelation analysis technique. The calculated autocorrelation function \([g_I(\tau)]\) enables the determination of the diffusion coefficient, which then can be converted to a size using the Stokes-Einstein relationship [63,171-175]. The calculated variation in the intensity autocorrelation function with increasing nanoparticle sizes is shown in Figure 3.10. The profile broadens as the size of the particle increases [175].

3.5. Small-Angle Scattering

Small angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS) have been referred to as small-angle scattering. The basics and the data analysis methods used in the two techniques are similar and the only difference is in the radiation used [64]. The contrast factor
for a system could be different because of differences in their interactions with matter and therefore provide complementary information.

3.5.1. Theory of Small-Angle Scattering

The scattering of radiation (X-ray/neutron) by a scatterer is characterized by a single parameter $b$ referred as the scattering length. If the wave vectors of incident and scattered waves are $k_i$ and $k_f$ respectively, a wave scattered by a scatterer at a point $r$ in the sample will thus be phase shifted with respect to that scattered at the origin by a phase factor $e^{iQr}$, where $Q = k_f - k_i$ is the wave vector transferred in the scattering process. The integral scattering cross-section for a nucleus is given by $\sigma = 4\pi b^2$ and it can be looked upon as an effective area presented by the scatterer to the incident radiation. The scattering cross-section describing the flux scattered into the solid angle $d\Omega$ and normalized to the irradiated sample ($V_T$) volume is called macroscopic differential scattering cross section and is expressed for an assembly of scatterers in a macroscopic sample as [176-179]

$$\frac{d}{d\Omega}(Q) = \frac{1}{V_T} \left\langle \sum_j b_j \exp(-iQr_j) \right\rangle^{2}$$

(3.6)

where $b_j$ is the bound scattering length and $r_j$ is the position vector of $j^{th}$ scatterer in a sample, and the bracket represents an average over all possible orientations.

Since SANS deals with the study of large scale heterogeneities rather than locating the individual scattering centers, the summation over $b_j$ can be replaced by a volume integral over scattering length density $\rho(r)$ as defined as

$$\sum_j b_j = \int_{V_T} \rho(r)dr$$

(3.7)

The summation in the above equation extends over all the nuclei in the volume $V_T$.

For the two-component system, particles dispersed in a medium having scattering length densities $\rho_p$ and $\rho_m$, respectively, equation simplified to
\[
d\frac{\sum d\Omega}{d\Omega}(Q) = n(p_p - p_m)^2 V^2 P(Q)S(Q) \quad (3.8)
\]

where \( n \) is the number density of particles in the sample and \( V \) is the volume of the single particle. \( P(Q) = \langle |F(Q)|^2 \rangle \) is intra-particle structure factor and \( S(Q) \) is the inter-particle structure factor. \( F(Q) \) is the form factor associated with the particle and \( F(Q) \) is defined as

\[
F(Q) = \frac{1}{V} \int V \exp(-iQr)dr \quad (3.9)
\]

and is normalized so that \( |F(0)|^2 = 1 \).

In the case particles are randomly oriented in the sample and the scattering is isotropic, \( S(Q) \) is given by

\[
S(Q) = 1 + \frac{1}{n} \left( \sum_k \sum_{k'} \exp[-iQ.(R_k - R_{k'})] \right) \quad (3.10)
\]

where \( R_k \) is the position vector of the \( k^{th} \) particle.

The typical functionality of \( P(Q), S(Q) \) and \( d\Sigma/d\Omega(Q) \) as a function of \( Q \) are plotted in Figure 3.11.

**Determination of Intra-Particle Structure Factor**

For some of the regular shapes, having an axis of symmetry, the analytical expressions for \( P(Q) \) are available in literature [180,181]. It is assumed that the particles are randomly oriented in the sample so that the theoretical form factors for anisotropic particles have to be averaged over orientation. \( P(Q) \) expressions for some standard shapes are given below:

(i) **Spherical Particle**

For spherical particle of radius \( R \) and having uniform scattering length density the form factor is given as [176-181]

\[
P(Q) = \left[ \frac{3(\sin QR - QR \cos QR)}{(QR)^3} \right]^2 \quad (3.11)
\]
Figure 3.11. Typical curves for (a) $P(Q)$, (b) $S(Q)$ and (c) $d\Sigma/d\Omega(Q)$.

(ii) Spherical Shell (Core-Shell Particle)

The form factor for spherical shell with inner radius $R_1$ and outer radius $R_2$ ($= R_1 + t$), where $t$ is thickness can be obtained by subtracting the empty core of radius $R_1$ from sphere of radius $R_2$ with proper weighting by the volumes. $P(Q)$ for spherical shell is written as [180,181]
\[
P(Q) = \left[ (\rho_c - \rho_{\text{shell}})V_1 \frac{3j_1(QR_1)}{QR_1} + (\rho_{\text{shell}} - \rho_m)V_2 \frac{3j_1(QR_2)}{QR_2} \right]^2 \quad (3.12)
\]

where \(\rho_c\), \(\rho_{\text{shell}}\) and \(\rho_m\) are the scattering length densities of core, shell and solvent, respectively, \(j_1\) is first order Bessel function.

(iii) Prolate Ellipsoidal Particle

The expression for form factor of prolate ellipsoidal particle with semi-major axis \(a\) and semi-minor axis \(b = c\) is given by [176-181]

\[
P(Q) = \int_0^1 F(Q, \mu)^2 d\mu \quad (3.13)
\]

where \(F(Q, x) = \left[ \frac{3(\sin x - x \cos x)}{x^3} \right]^2\), \(x = Q \left[ a^2 \mu^2 + b^2 (1 - \mu^2) \right]^{1/2}\) and \(\mu\) is the cosine of the angle between the direction of major axis and wave vector transfer \(Q\).

(iv) Rod-like/Cylindrical Particle

The form factor of randomly oriented cylindrical particles with the radius \(R\) and length \(L (= 2l)\) is given by [176-181]

\[
P(Q) = \int_0^{\pi/2} \frac{4j_1^2(Q R \sin \theta) \sin^2[(QL) \cos \theta]}{Q^2 R^2 \sin^2 \theta \cos^2 \theta} \sin \theta \, d\theta \quad (3.14)
\]

where \(j_1(x)\) is first order Bessel function and \(\theta\) is the angle subtended by the principal axis of the cylinder with \(Q\).

\(P(Q)\) variation on log-log scale shows a linear region in the intermediate \(Q\)-range \(1/l < Q < 1/R\). The slope of the linear region is \(-1\). On the other hand, \(P(Q)\) for a disc-like particle having radius \(R\) and thickness \(t\) has a slope of \(-2\) in the intermediate \(Q\)-range of \(1/R < Q < 1/l\).
(v) Gaussian Coil

The form factor of Gaussian chain coils with the radius of gyration $R_g$ is given by [176-181]

$$P(Q) = \frac{2(e^{-x} + x - 1)}{x^2}, \quad x = Q^2 R_g^2$$  \hspace{1cm} (3.15)

**Determination of Inter-Particle Structure Factor**

When the concentration of particles is high they start interacting with each other. The nature of $S(Q)$ depends on the structure and the organization of the particles and the type of interactions between the particles. In general, $S(Q)$ shows several maxima and minima of decreasing amplitude. The first peak in $S(Q)$ occurs at $Q_{max} \approx 2\pi/d$, where $d$ is the average distance between the particles.

The expression for $S(Q)$ depends on the relative positions of the particles. For an isotropic system, $S(Q)$ can be written as

$$S(Q) = 1 + 4\pi n \int (g(r) - 1) \frac{\sin qr}{qr} r^2 dr$$  \hspace{1cm} (3.16)

where $g(r)$ is the radial distribution function. $g(r)$ is the probability of finding another particle at a distance $r$ from a reference particle centered at the origin. The details of $g(r)$ depend on the interaction potential $U(r)$ between the particles [181-185]. Thus, one has to have the knowledge of $U(r)$ for calculating $S(Q)$. This in turn implies that measured $S(Q)$ can be used to obtain information about the interaction potential $U(r)$.

**3.5.2. Small-Angle X-Ray vs. Neutron Scattering**

The fundamental difference between X-rays and neutrons is the mechanism by which the incident radiation interacts with matter. X-rays are scattered by electrons surrounding atomic nuclei whereas neutrons by the nuclei. It is seen that as one goes across the periodic table, the X-ray scattering lengths increase with the atomic number of the atom, whereas the neutron
scattering lengths vary in a random way (Figure 3.12). For neutrons, neighbouring elements as well as isotopes of the same element can have different scattering lengths. For example, the scattering length of hydrogen is negative (−0.3741 × 10^{-12} \text{ cm}) and that of deuterium is positive (0.6674 × 10^{-12} \text{ cm}) [186]. To complement the SAXS data that has higher sensitivity for the gold nanoparticles, SANS has been used to highlight the structural features of the block copolymer phases in our multi-component colloidal systems.

![Scattering length variation for X-rays and neutrons.](image)

**Figure 3.12.** Scattering length variation for X-rays and neutrons.

**Concept of Contrast in SAS**

In SAS, the term \((\rho_p - \rho_s)^2\), the square of the difference of scattering length density of a particular component of the system and solvent is called as contrast factor and decides the visibility of that component in the measurement [186,187]. The values of \(\rho_p\) and \(\rho_s\) depend on the chemical composition of the particle and the solvent. Scattered intensity in a SAS experiment depends on the contrast factor. This factor, however, depends on the radiation used. Since scattering length is proportional to the atomic number in the case of X-rays, there
is very low contrast for hydrogenous systems such as block copolymer whereas higher Z elements such as gold show a strong contrast. In case of neutrons, due to the difference in the scattering length between H and D, it is possible to have a very good contrast between the hydrogenous particle and the solvent by deuterating either the particle or the solvent. The contrast between the particle and the solvent can be varied continuously by using mixed hydrogenated and deuterated solvents. Various possibilities of contrast variation by using D$_2$O as solvent are shown in Figure 3.13.

![Figure 3.13](image.png)

**Figure 3.13.** Various possible contrast variations in SANS experiment.

Figure 3.13(a) shows a hydrogenous spherical particle suspended in H$_2$O. Let the scattering length densities be $\rho_p$ of the particle and $\rho_H$ of the solvent. In this case $\rho_p = \rho_H$ and thus the particle is not visible for neutrons. However, the solvent can be replaced with D$_2$O, so the scattering length density of D$_2$O is $\rho_D$ which is different from $\rho_p$ and thus the SANS distribution is determined from the particle. Figure 3.13(b) shows a spherical core-shell particle suspended in H$_2$O. The constituents of the inner core are different from those of the outer shell of the particle. Let $\rho_{pc}$ and $\rho_{ps}$ be the scattering length densities of the core and shell. The solvent has a scattering length density $\rho_m$, which can be varied by varying the relative amounts of H$_2$O and D$_2$O in the solvent. Thus $\rho_m$ can be either matched with $\rho_{ps}$ so that the SANS distribution is determined by the core alone or $\rho_m$ can be matched with $\rho_{pc}$ so
that the SANS distribution is determined by shell alone. Deuterium labeling is another way of contrast matching as shown in Figure 3.13(c) where the same spherical shell particle is now placed in a D₂O solvent, by deuterating the core of the particle, the $\rho_{pc}$ gets equal to $\rho_D$, thus for neutron only the shell is visible.

3.5.3. Experimental Details

3.5.3.1. Small-Angle X-Ray Scattering

There exist both laboratory instruments based on more conventional sources and synchrotron based instrumentation for SAXS measurements. The schematic of a laboratory-based SAXS instrument is shown in the Figure 3.14. In a SAXS experiment, the incident neutron beam is collimated with a pin-hole arrangement. This X-ray beam is scattered by the sample and the angular distribution of scattered radiation is recorded using a 2-D detector [176]. We have carried out SAXS measurements using a Bruker Nanostar instrument equipped with 18 kW rotating anode generator. The X-rays are collimated through a 3 pin-hole system and data can be acquired using a 2-D gas filled detector over a $Q$-range of 0.01 to 0.2 Å⁻¹.

3.5.3.2. Small-Angle Neutron Scattering

SANS requires a neutron source i.e. a nuclear reactor or an accelerator-based spallation source and therefore the experiments are performed at large scale facilities. The small-angle neutron scattering experiments presented in this thesis have been performed at the SANS diffractometer at the Guide Tube Laboratory, Dhruva Reactor, Bhabha Atomic Research Centre, India [37] and at the SANS-I facility, Swiss Spallation Neutron Source SINQ, Paul Scherrer Institute, Switzerland [38]. Figure 3.15 shows the schematic of SANS diffractometer installed at the Guide Tube Laboratory, Dhruva Reactor, BARC. It makes use of polycrystalline block of beryllium oxide (BeO) filter as monochromator. The mean
wavelength of the monochromatized beam is 5.2 Å with a spread of $\Delta \lambda / \lambda \sim 15\%$. The angular distribution of neutrons scattered by the sample is recorded using a 1 m long one-dimensional He$_3$ position sensitive detector. The instrument covers a $Q$-range of 0.015 – 0.35 Å$^{-1}$ [188].

**Figure 3.14.** The schematic of a SAXS instrument.

**Figure 3.15.** Schematic of SANS facility at BARC.

The samples requiring high signal to background and wide $Q$-range were measured at the SANS-I facility at Swiss Spallation Neutron Source SINQ, Paul Scherrer Institute, Switzerland (Figure 3.16). This is a 40 m-long state-of-art instrument. It makes use of
velocity selector and the sample-to-detector distance can be varied from 1 to 20 m. The scattered neutrons are detected using two-dimensional 96 × 96 cm² detector. This instrument can collect the data in the $Q$-range of 0.001 to 1 Å⁻¹ [189].

Figure 3.16. Schematic of the SANS-I facility at PSI, Switzerland.

3.5.4. Gold Nanoparticles as Characterized by Small-Angle Scattering

Small-angle scattering has been widely used for the determination of the structure and interaction in variety of nanoparticle systems [64]. The shape information of the particles is obtained from the fact that the $Q$-dependence of scattering is different for different particles. 

Figure 3.17 shows the comparison of scattering for spherical, rod-like and disc-like particles. The rod-like particles show $1/Q$ dependence in the intermediate $Q$-range whereas $1/Q^2$ dependence is observed for disc-like particles. The cut-offs of these $Q$-dependent power-law scattering are decided by the dimension of the particles. The interaction of the particles from
Chapter 3: Multi-Technique Approach for Characterization of Gold Nanoparticles

Figure 3.17. $P(Q)$-dependence for different shapes of the particles.

Figure 3.18. SAS data from a charged nanoparticle system in presence of varying ionic strength. The increase in the value of cross-section in low-$Q$ region corresponds to the increasing ionic strength.

SAS data is obtained from the structure factor $S(Q)$. The SAS data usually show a correlation peak for interacting systems, whose position and width is decided by the number density and interaction of the particles, respectively. Figure 3.18 shows typical SAS data from a charged nanoparticle system in presence of varying salt (e.g. NaCl) concentration. It is seen that the
correlation peak broadens while the peak position remains unchanged. The broadening of peak is as a result of screening of charge between the particles and corresponds to increasing compressibility with increase in salt concentration.

3.6. Summary

This chapter has provided details on a number of techniques involving spectroscopy, microscopy and scattering methods, which can be used for obtaining complementary results for understanding and tuning of the gold nanoparticle synthesis. The different techniques of interest have been discussed are UV-visible spectroscopy, TEM, DLS, SAXS and SANS. UV-visible spectroscopy measures the absorbance of the sample solution to characterize nanoparticles through the characteristic SPR peak whereas the changes in peak height and peak width relates changes in the shape, size and number density of the gold nanoparticles. Time-dependent UV-visible spectra can be used to measure the evolution and the stability of gold nanoparticle during the synthesis. The high contrast of gold nanoparticles for electrons enables TEM to determine directly the shape and size of gold nanoparticles. A range of scattering techniques (DLS, SAXS and SANS) probes the systems under native conditions. In all these scattering techniques the radiation (light, X-ray or neutron) is scattered by the sample and the resulting scattering pattern is analyzed to provide information about the structure (shape and size), interaction and the order of the components of the samples. In DLS, analysis of intensity fluctuations enables the determination of diffusion coefficients associated with different structures in the system through the measurement of time-dependent fluctuations in the intensity of the scattered light. X-rays are scattered by the electron density fluctuation, which is proportional to the atomic number and enables SAXS to determine the size distribution of the nanoparticles. SANS can be used to study the role of different components on the synthesis of gold nanoparticles in these systems by varying the contrast of the individual components using suitable solvents. The results of a multi-technique approach by combining above different methods for the characterization of block copolymer-mediated synthesis of gold nanoparticles in this thesis are reported in Chapters 4–7.