CHAPTER 2

Experimental methods
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This chapter deals with the key experimental techniques used for the structural studies of supramolecular aggregates and nanomaterials. The basic principles and the experimental details of these techniques are explained in brief.

2.1 Light Scattering

Application of light scattering technique as a probe of macromolecular properties like size, shape, molecular weight, etc. has attracted considerable interest in colloid and material chemistry. The angular distribution of the scattered light intensity from a colloidal solution depends on various factors like size and shape of the colloidal particle, optical properties of the scatterer such as its polarizability, refractive index, etc., and the wavelength of the incident light. In a basic light scattering experiment, one measures the average scattered intensity \( I_s \) (that is the average rate of flow of energy per unit area of cross section) or the energy spectrum of intensity \( S(\omega) \) where \( \omega \) is the angular frequency of the scattered light. Both \( I_s \) and \( S(\omega) \) are measured at various angles as they are angle dependent. The \( I_s \) bears information about the static properties of the scatterer whereas \( S(\omega) \) bears information regarding the dynamics of the scatterer. Hence, the light scattering experiments can be classified under two broad categories, namely the static light scattering in which one measures the average scattering intensity at various angles and the dynamic light scattering in which the energy spectrum of intensity or the time dependence of the intensity is measured. Dynamic light scattering has been used in the present study.

2.1.1 Dynamic Light Scattering (DLS)

In a classical light scattering experiment one assumes that the particles are at fixed positions in space, but in reality the suspended particles are not stationary; rather they diffuse
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in a random walk fashion known as the Brownian motion. Since the net intensity seen by the
detector is a result of the superposition of all the waves scattered from the scattering volume,
the intensity fluctuate randomly in time as the phases of the scattered waves fluctuate
randomly due to the random fluctuations in the relative positions of the particles which scatter
light. This time dependence or the fluctuations in the net scattered intensity forms the basis of
the Dynamic Light Scattering (DLS) experiment [26].

The key concept underlying in a DLS experiment is the fact that time scale of these
fluctuations depends on the size of the diffusing particles. Small particles diffuse in the
solution relatively rapidly resulting in a rapidly fluctuating intensity signal as compared to the
larger particles which diffuse more slowly. Figure 2.1 shows representative intensity-time
profiles for small and large size particles as observed on the same time scales for both the
particles, as a result of variations in the position of an essentially fixed number of particles in
the scattering volume. A mathematical procedure known as the autocorrelation is used to

![Diagram showing typical fluctuations in scattered intensity for 'large' and 'small' particles](image)

**Figure 2.1** Typical fluctuations in scattered intensity for 'large' and 'small' particles when
observed in the same time scale.
obtain quantitative information from these kinds of fluctuations in the scattered intensity. Hence this technique is also known under the name photon correlation spectroscopy.

The autocorrelation function, denoted by $C(\tau)$, represents the correlation between the values of the scattered intensity at a given time $t$ and at a later time $(t+\tau)$. By representing intensity at an arbitrary time as $I(o)$ and those at a later time $\tau$ as $I(\tau)$, the autocorrelation function can be written as

$$C(\tau) = \langle I(o).I(\tau) \rangle$$

(2.1)

When the sampling interval $\tau$ becomes very large, there should not be any correlation between the pairs of sampled intensities and hence the above equation reduces to

$$C(\infty) = \langle I(o) \rangle^2$$

(2.2)

An autocorrelator accepts the digital photo counts from the detector which represents the light scattering intensity $I(t)$ and computes the second order correlation function, normalized with the long time correlation data $\langle I \rangle^2$. The normalized time correlation function $g^{(2)}(t)$ of the scattered intensity is given by

$$g^{(2)}(\tau) = \frac{\langle I(o)I(\tau) \rangle}{\langle I \rangle^2}$$

(2.3)

For photo counts obeying Gaussian statistics, the relationship between $g^{(2)}(\tau)$ and the first order correlation function of the electric field $g^{(1)}(\tau)$ is given by the Siegert relationship

$$g^{(2)}(\tau) = \beta + A \left| g^{(1)}(\tau) \right|^2$$

(2.4)

where $\beta$ is the baseline and $A$ is an adjustable parameter which is dependent on the scattering geometry and independent of $\tau$.  

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For a suspension of monodisperse, rigid, spherical particles undergoing Brownian diffusion, the correlation function decays exponentially as

\[ g^{(1)}(\tau) = \exp(-Dq^2\tau) \]  
(2.5)

where \( D \) is the translational diffusion coefficient and \( q \) is the wave vector transfer, \( q \) ——, where \( 2 \) is the scattering angle and \( \lambda \) is the wavelength). For small, dilute, non-interacting spheres the hydrodynamic radius \( R_h \) can be obtained from the translational diffusion coefficient using the Stokes-Einstein relationship given by

\[ D = kT / (6\pi\eta R_h) \]  
(2.6)

where \( k \) is the Boltzmann’s constant, \( \eta \) is the solvent viscosity, and \( T \) is the absolute temperature. If the particle is nonspherical then \( R_h \) is taken as the apparent hydrodynamic radius. A lay out of the experimental setup of DLS is shown in Figure 2.2.

![Block diagram of a typical DLS instrument.](image)

**Figure 2.2** Block diagram of a typical DLS instrument.

2.1.1a Effect of polydispersity
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In most of the practical systems, it is almost impossible to have all the particles having the same size. It is thus important to consider the effect of polydispersity in such systems. When a broad distribution of diffusion coefficient is present in the system, each D contributes its own exponential and the mathematical equation 2.5 will be modified as

\[ g^{(1)}(\tau) = \int_0^\infty G(\Gamma) \exp(-\Gamma \tau) \, d\Gamma \]  

(2.7)

where \( \Gamma = Dq^2 \) is the decay constant for a given particle size. The distribution \( G(\Gamma) \) represents the relative intensity of light being scattered with decay constant \( \Gamma \) and will be a function of the number and size of scatterers. Equation 2.7 can be recognized as the Laplace transform of \( G(\Gamma) \) with respect to \( \Gamma \). Hence an inverse Laplace transform on the above equation can be used to obtain \( G(\Gamma) \) but a much simpler method, known as the method of cumulants [27], has been used traditionally to analyze the polydispersity in DLS experiments. Expanding the term \( \exp(-\Gamma \tau) \), in the above equation, about a mean value \( \bar{\Gamma} \) such that

\[ \exp(-\Gamma \tau) = \exp(-\bar{\Gamma} \tau).\exp(-[\Gamma - \bar{\Gamma}] \tau) \]

\[ = \exp(-\bar{\Gamma} \tau).[1-(\Gamma - \bar{\Gamma})\tau + (\Gamma - \bar{\Gamma})^2 \tau^2/2!+....] \]  

(2.8)

(2.9)

When \( G(\Gamma) \) is relatively narrow, terms with order 3 and above can be neglected, as the relative magnitude of contributions of the terms in the bracket falls of rapidly with increasing order and substituting it in equation 2.7, we get

\[ g^{(1)}(\tau) = e^{-\bar{\Gamma} \tau} \left[ \int_0^\infty G(\Gamma)d\Gamma - \tau \int_0^\infty G(\Gamma)d\Gamma + \bar{\Gamma} \tau \int_0^\infty G(\Gamma)d\Gamma + \frac{\tau^2}{2} \int_0^\infty (\Gamma - \bar{\Gamma})^2 G(\Gamma)d\Gamma \right] \]  

(2.10)

\[ = e^{-\bar{\Gamma} \tau} \left[ 1 - \bar{\Gamma} \tau + \bar{\Gamma} \tau + \frac{\mu_2 \tau^2}{2} \right] \]  

(2.11)

where the first cumulant \( \bar{\Gamma} \) and the second cumulant \( \mu_2 \) are defined as
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\[ \bar{\Gamma} = \int_0^\infty G(\Gamma)\Gamma d\Gamma \]  
\[ \mu_2^2 = \int_0^\infty G(\Gamma)(\Gamma - \bar{\Gamma})^2 d\Gamma \]

Thus, we get

\[ g^{(1)}(\tau) = e^{(-\bar{\Gamma}\tau)} \left[ 1 + \frac{\mu_2^2\tau^2}{2} \right] \]

The first and second cumulant respectively give the mean and variance of the distribution \( G(\Gamma) \) and by fitting the experimentally observed \( g^{(1)}(\tau) \) to a quadratic in \( \tau \) we can get the mean, \( \bar{\Gamma} \) and the variance, \( \mu_2^2 \). The ratio of variance to the square of the mean is a measure of the polydispersity of the diffusion coefficient and this is represented as the polydispersity index in DLS experiments.

In the present work Dynamic light scattering measurements were performed using a Malvern 4800 Autosizer employing a 7132 digital correlator. The light sources used were either an Ar-ion laser operated at 514.5 nm with maximum power output of 2 W or a 5 mW helium-neon laser at a wavelength of 633 nm. All measurements were carried out at 25.0 ± 0.1 °C using a circulating water bath. Cylindrical quartz cells of 10 mm diameter were used in all of the light scattering experiments. Measurements were made at an angle of 130°.

2.2 Small angle neutron scattering (SANS)

Thermal neutrons have found out to be an ideal probe for investigating the structure and dynamics of materials at microscopic level [28-30]. Neutron scattering consists of a whole family of techniques and small angle neutron scattering (SANS) is one of these techniques [31-33]. Different neutron scattering techniques give complementary information
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about any material. For example, coherent elastic scattering (diffraction) of neutrons gives the crystallographic or the magnetic structure of the material substance, coherent inelastic scattering experiments determine phonon dispersion curves, and incoherent inelastic and quasielastic measurements on hydrogenous samples give the nature of random molecular motions (translational and/or rotational) in solids and liquids.

The technique of SANS is used for studying the structure of a material in the length scale of 10-1000 Å. SANS is a neutron diffraction technique which involves scattering of a beam of neutrons from the sample and measuring the scattered neutron intensity as a function of the scattering angle. The wave vector transfer \( q = \frac{4\pi \sin \theta}{\lambda} \), where \( 2\theta \) is the scattering angle and \( \lambda \) is the neutron wavelength) in these experiments is small, typically in the range of 0.001 to 1.0 Å\(^{-1}\) and the wavelength of neutron used for these experiments is usually 4-10 Å. Since the small q values occur at small scattering angles, therefore this is known as the small angle neutron scattering. The SANS experiment determines the differential cross-section \( \frac{d\Sigma}{d\Omega} \) since it is this which contains all the information on the shape, size, and interactions of the scattering bodies (assemblies of scattering centres) in the sample. It is seen that the scattering cross-section from a collection of particles consists of two terms, the first of which depends on the intraparticle scattering and the second on interparticle scattering.

The differential cross-section is given by

\[
\frac{d\Sigma}{d\Omega}(q) = n\left(\rho_p - \rho_m\right)^2V^2P(q)S(q)
\]  \hspace{1cm} (2.15)

where \( n \) is the number of particles per unit volume of the sample, \( V \) is the volume of one scattering body, \( \left(\rho_m - \rho_s\right)^2 \) is known as contrast factor, \( P(q) \) is a function known as the form or
shape factor, $S(q)$ is the interparticle structure factor and $q$ is the scattering vector. The differential scattering cross-section has dimensions of \((\text{length})^{-1}\).

### 2.2.1 Contrast factor

The square of the difference between the average scattering length density of the particle and the average scattering length density of the solvent $(\rho_m - \rho_s)^2$ is known as the contrast factor. As mentioned above, the scattered neutron intensity in a SANS experiment depends on $(\rho_m - \rho_s)^2$. The contrast factor in SANS experiments is equivalent to the contrast term in optics, where it is decided by the difference in the refractive indices of the particle and the solvent. The scattering length of hydrogen is negative $(= -0.3723 \times 10^{-12} \text{ cm})$ and for deuterium, it is positive $(= 0.6674 \times 10^{-12} \text{ cm})$. Hence it is possible to have a good contrast between the hydrogenous particle and the solvent by deuterating either the particle or the solvent.

### 2.2.2 Determination of Intraparticle Structure

In the previous section we have seen that the differential scattering cross-section is related to the intraparticle structure factor $P(q)$ and the interparticle structure factor $S(q)$. The intraparticle structure factor is the square of the particle form factor. It depends on the shape and size of the particles and can be denoted for any geometry.

$$ P(q) = \left( |F(q)|^2 \right) $$  \hspace{1cm} (2.16)

Dilute systems are ideally suited for studying the shapes and sizes of the particles as the concentration of particles is very low. The interparticle distances are much larger than the particle size in these systems, the interparticle interference is negligible and $S(q) \approx 1$. Thus the
scattering distribution depends on the functionality of \( P(q) \) and the equation for dilute systems becomes

\[
\frac{d\Sigma}{d\Omega} = n\left(\rho_r - \rho_m\right)^2 V^2 P(q)
\]  

(2.17)

The expressions for \( P(q) \) for different structures of the particles are available [34, 35]. The expressions for some of the shapes are given below.

(i) Sphere of radius \( R \)

\[
P(q) = \left[ \frac{3(\sin qR - qR \cos qR)}{(qR)^3} \right]^2
\]  

(2.18)

(ii) Ellipsoid of major axis \( 2a \) and minor axis \( 2b = 2c \)

\[
P(q) = \int_{0}^{1} \left[F(q, \mu)\right]^2 d\mu
\]  

(2.19)

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

(iii) Cylinder of radius \( R \) and length \( L = 2l \)

\[
P(q) = \int_{0}^{\pi/2} \frac{\sin^2(q l \cos \beta)}{q^2 l^2 \cos^2 \beta} \frac{4J_1^2(q R \sin \beta)}{q^2 R^2 \sin^2 \beta} \sin \beta d\beta
\]

(2.20)

where \( \beta \) is the angle between the axis of the cylinder and bisectrix, \( J_1 \) is the Bessel function of order unity. The disk being special case of cylinder when \( L \ll R \). It can be shown that for...
cylindrical particle \( P(q) \) varies as \( 1/q \) in the \( q \) range of \( 1/L < q < 1/R \) and as \( 1/q^2 \) for disk-like particle in the \( q \) range of \( 1/R < q < 1/L \).

It has been observed that \( P(q) \) has a slope of \(-1\) for cylindrical particles and \(-2\) for disk-like particles in the intermediate \( q \) range. Irrespective of the shape of the particles, \( P(q) \) can be written in simple forms for limited \( q \) ranges. The most important of these simple forms are the Guinier approximation and the Porod law.

### 2.2.3 Polydisperse particles

In practice, colloidal systems typically contain a distribution of particle sizes. Hence in the case of polydisperse systems (particles having different sizes), the scattering cross-section is given by

\[
\frac{d\Sigma}{d\Omega}(q) = n\left(\rho_p - \rho_m\right)^2 \int V^2(R)P(q,R)f(R)dR
\]

where \( f(R) \) is the particle size distribution. Usually, log normal or Schultz distribution is assumed for the polydispersity.

### 2.2.4 Guinier Approximation

When there are no interparticle interactions (i.e. in the dilute limit) it is possible to obtain important structural information from the scattering spectra. In the small \( q \) region (\( qR_g < 1 \)), \( P(q) \) decreases in an exponential manner and is known as Guinier approximation [34]

\[
P(q) = \exp\left(-\frac{q^2R_g^2}{3}\right)
\]

where \( R_g \) is the radius of gyration of the particle and is defined as
\[ Rg^2 = \frac{\int \nu r^2 dv}{V} \quad (2.23) \]

Thus a plot of logarithm of the scattering intensity versus \( q^2 \) will be a straight line in the small \( q \) region and the slope can give the radius of gyration of the particle.

### 2.2.5 Porod law

As already discussed, Guinier limit mainly focuses on the scattering observed at low or intermediate values of \( q \). Structural information can also be obtained from the higher \( q \)-region without the need of any assumptions. At large \( q \) values, for homogenous particles with sharp boundaries, \( P(q) \) varies as \( 1/q^4 \) and is known as the Porod law [36]. For a spherical particle at large \( q \), \( P(q) \) can be defined as

\[ P(q) = \frac{2\pi S}{V} \frac{1}{q^4} \quad (2.24) \]

where \( S \) is the surface area and \( V \) is the volume of the scattering particle.

### 2.2.6 Determination of Interparticle structure factor

The structure factor \( S(q) \) can have a pronounced effect on the appearance of the SANS experimental data, particularly at the scattering intensity in the low \( q \)-region. The contribution from \( S(q) \) cannot be neglected when the concentration of particles is large and they start interacting with each other. Especially in the case of charged systems the accounting of this contribution is very much necessary for the proper analysis of the data. For concentrated systems, we can say that \( S(q) \neq 1 \). It is not always possible to dilute the system as the particle structure could change on dilution as in the case of micellar solutions. Thus the consideration of \( S(q) \) in the analysis of the scattering data is very important. The nature of \( S(q) \) depends on
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the structure and organization of the particles and the type of interactions existing between the particles. In general, \( S(q) \) shows several maxima and minima of decreasing amplitude and the first peak in \( S(q) \) occurs at \( q_{\text{max}} \sim 2\pi/d \), where \( d \) is the average distance between the particles.

The expression for \( S(q) \) depends on the relative positions of the particles. In the case of isotropic system, \( S(q) \) can be written as

\[
S(q) = 1 + 4\pi \int (g(r) - 1) \frac{\sin qr}{qr} r^2 dr
\]  

(2.25)

Where \( g(r) \) is the radial distribution function i.e. 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 [37]. Thus one has to have the knowledge of \( U(r) \) for calculating \( S(q) \) parameter. This in turn implies that the measured \( S(q) \) can be used to obtain information about the interaction potential \( U(r) \). \( U(r) \) could consists of several terms such as (i) hard sphere term \( U_{hs}(r) \), (ii) van der Waals attractive term \( U_{vw}(r) \), (iii) solvent mediated term \( U_{s}(r) \) and (iv) Coulomb repulsion term \( U_{c}(r) \). \( U_{c}(r) \) is important in ionic micelles, as they are charged. Similarly, it may be noted that \( U_{s}(r) \) is important in nonionic micelles. Colloidal micellar solution is essentially a macrofluid on the length scales probed in SANS. It is thus possible to calculate \( g(r) \) or \( S(q) \) for these solutions using the methods which have been developed for liquids. It can be noted that \( g(r) \) is related to the total correlation \( h(r) \) between the two particles separated by distance \( r \) as

\[
g(r) = 1 + h(r)
\]  

(2.26)

Further, we note that \( h(r) \) consists of two contributions and is given by Ornstein-Zernike equation (OZE) [38]

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\[ h(r) = n \int C(r') h(|r-r'|) d^3r' \]  

(2.27)

where \( C(r) \) is the direct correlation function. To solve OZE, one needs an additional relation (referred as closure relation) between \( h(r) \) and \( C(r) \). Depending on the approximations involved, various closure relations have been proposed in the literature. Some of these are given below.

(a) Mean spherical approximation (MSA) gives

\[ C(r) = -\beta U(r), r > \sigma \]  

(2.28)

\[ h(r) = -1, r \leq \sigma \]  

(2.29)

where \( \beta = 1/kT \) and \( \sigma \) is the particle diameter.

(b) Percus-Yevick approximation (PYA) gives

\[ C(r) = 1 - \exp[\beta U(r)] \]  

(2.30)

(c) Hypernetted chain approximation (HNCA) gives

\[ C(r) = -\beta U(r) + h(r) - \ln[h(r) + 1] \]  

(2.31)

Small angle neutron scattering experiments were carried out using SANS diffractometer at the Dhruva reactor, Bhabha Atomic Research Centre, Trombay. The diffractometer makes use of a beryllium oxide filtered beam with a mean wavelength (\( \lambda \)) of 5.2 Å. The angular distribution of the scattered neutrons is recorded using a one-dimensional position-sensitive detector (PSD). The accessible wave vector transfer (\( q \)) range of the diffractometer is 0.017-0.35 Å\(^{-1}\). The PSD allows simultaneous recording of data over the full
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q. The samples were held in a quartz sample holder of 0.5 cm thickness. In all the measurements the temperature was kept fixed at 30 °C. The measured SANS data have been corrected and normalized to absolute unit (as cross-section per unit volume), using standard procedures.

2.3 Rheology

Surfactant phases have been identified that impart controllable and useful viscous and elastic rheological properties to a wide variety of aqueous liquids. Rheological properties are crucial in food processing, cosmetic formulations, paint formulations, pharmaceutical formulations, etc. Rheological measurements are also essential from a fundamental point of view because they are used to characterize a system. Rheology is the science of deformation and flow of matter. It essentially deals with the response of the materials when it is subjected to some external forces. The behavior can be best illustrated by a simple shear experiment as shown in Figure 2.3.

Figure 2.3 Geometry for a simple shear along y – direction with a velocity gradient along x-direction.

Consider a fluid contained between two parallel plates, each of area A. If a force $F$ is applied to the upper plate such that it moves with a velocity $v$ relative to the lower plate of the
parallel plates. If the displacement of a given element located at \(dx\) is \(dy\), then the shear strain \(\gamma\) and the shear rate \(\dot{\gamma}\) are defined as
\[
\gamma = \frac{dy}{dx} \quad \text{and} \quad \dot{\gamma} = \frac{d\gamma}{dt} = \frac{dv}{dx}
\] (2.32)
Also, the force \(F\) acting at the upper plate produces a shear stress given by
\[
\sigma_s = \frac{F}{A}
\] (2.33)
In the case of a liquid, shear rate is proportional to the shear stress \(\sigma_s\) as shown by the Newton’s equation,
\[
\sigma_s = \eta_0 \dot{\gamma}
\] (2.34)
The proportionality constant \(\eta_0\) is known as the viscosity of the liquid. But in the case of an elastic solid, the shear stress \(\sigma_s\) is proportional to the strain \(\gamma\) as given by the Hooke’s law,
\[
\sigma_s = G_0 \gamma
\] (2.35)
Here the proportionality constant \(G_0\) is called the rigidity modulus or the shear modulus. The Newton's law and the Hooke's law are two limiting cases of the response of a material under shear. The behavior of many materials cannot be described by either Newton’s law or Hooke’s law but they exhibit both viscous and elastic responses and hence, they are designated as viscoelastic materials and for such a fluid the stress-strain relationship can be described by a linear differential equation
\[
\left(1 + \alpha_1 \frac{\partial}{\partial t} + \alpha_2 \frac{\partial^2}{\partial t^2} + \ldots\right) \sigma_s = \left(\beta_0 + \beta_1 \frac{\partial}{\partial t} + \beta_2 \frac{\partial^2}{\partial t^2} + \ldots\right) \gamma
\] (2.36)
where the coefficients \(\alpha_i\) and \(\beta_i\) are material parameters.

In the literature, several mechanical models have been suggested to describe the behavior of linear viscoelastic materials [39, 40]. Majority of the models are approximated by different combinations of one or more viscous elements (i.e., a dashpot) and one or more
elastic elements (i.e., a spring). Whether a viscoelastic material behaves like an elastic solid or viscous liquid depends on a characteristic time known as the relaxation time \( \tau_R \) of the material and is equal to the ratio \( \eta_0/G_0 \). For time scales smaller than \( \tau_R \), the material behaves like an elastic solid while for time scales greater than \( \tau_R \), it behaves like a viscous fluid. Because of this time dependent nature, the dynamic response of these materials becomes important to characterize their rheological behavior.

![Maxwell model](image)

**Figure 2.4** A Maxwell model for viscoelastic fluid.

Dynamic methods of measurements involve looking the response of the material subjected to periodic stress or strain. When a linear viscoelastic material is subjected to a sinusoidal deformation at an angular frequency, \( \omega \), the response of the liquid consists of a sinusoidal stress which is out of phase with the strain, the phase angle being given as \( \delta \). The shear strain exhibited by the liquid can be expressed as

\[
\gamma(t) = \gamma_0 \exp(i \omega t) \tag{2.37}
\]

where \( \gamma_0 \) is the strain amplitude. The corresponding shear rate is given by

\[
\dot{\gamma}(t) = i\omega \gamma_0 \exp(i \omega t) = i\omega \gamma(t) \tag{2.38}
\]

Shear stress developed will always be sinusoidal with the phase angle \( \delta \), thus
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\[ \sigma_s(t) = \sigma_0 \exp(i\omega t + \delta) \]  \hspace{1cm} (2.39)

For an oscillatory shear, we can define a complex shear modulus \( G^* \) as

\[ G^* = \frac{\sigma_s(t)}{\gamma(t)} = \frac{\sigma_0}{\gamma_0} (\cos \delta + i \sin \delta) = G' + iG'' \]  \hspace{1cm} (2.40)

where \( G' \) and \( G'' \) are referred as the storage modulus and the loss modulus respectively. Thus, from the phase angle \( \delta \) and the amplitudes of the shear stress and shear strain, it is possible to calculate the storage modulus and loss modulus, using equations

\[ G' = G_0 \cos \delta \text{ and } G'' = G_0 \sin \delta \]  \hspace{1cm} (2.41)

Similarly we can define the complex viscosity \( \eta^* \) as

\[ \eta^* = \frac{\sigma_s(t)}{\dot{\gamma}(t)} = \frac{G^*}{i\omega} = \eta' - i\eta'' \]  \hspace{1cm} (2.42)

Thus,

\[ \eta'(\omega) = G'/\omega \text{ and } \eta''(\omega) = G''/\omega \]  \hspace{1cm} (2.43)

As mentioned earlier, the rheological properties of viscoelastic materials can be represented by simple mechanical models. One such model is the Maxwell element in which a spring and a dashpot are connected in series as shown in Figure 2.4.

With this combination, the stress is same on both the elements connected in series and strain is the sum of the strain from each elements. i.e.,

\[ \gamma = \gamma_1 + \gamma_2 \text{ and } \dot{\gamma} = \dot{\gamma}_1 + \dot{\gamma}_2 \]  \hspace{1cm} (2.44)

For the viscous element

\[ \dot{\gamma}_1 = \frac{\sigma_s}{\eta_0} \]  \hspace{1cm} (2.45)

And for the elastic component

\[ \gamma_2 = \sigma_s/G_0 \text{ and } \dot{\gamma}_2 = \dot{\sigma}_s/G_0 \]  \hspace{1cm} (2.46)

Hence for the Maxwell model, the differential equation becomes
In terms of the relaxation time, the above equation can be written as

$$\sigma_s + \tau_R \dot{\sigma_s} = \eta_0 \gamma$$

(2.48)

where \(\tau_R = \eta_0 / G_0\) is the relaxation time.

By substituting \(\dot{\gamma} = i \omega \gamma\) and \(\dot{\sigma_s} = i \omega \sigma_s\) in the above equations, we get the complex shear modulus as

$$G^* = \sigma_s \frac{G_0 i \omega \tau_R}{\gamma} = \frac{G_0 i \omega \tau_R}{(1+i \omega \tau_R)}$$

(2.49)

Thus, we have

$$G' = \frac{G_0 (\omega \tau_R)^2}{1+(\omega \tau_R)^2}$$

(2.50)

$$G'' = \frac{G_0 \omega \tau_R}{1+(\omega \tau_R)^2}$$

(2.51)

According to the Maxwell model, the real part of the dynamic modulus \(G'(\omega)\) increases with frequency \(\omega\) at low frequencies and it reaches a plateau value equal to the plateau modulus \(G_0\) at high frequencies. The imaginary part of the modulus \(G''(\omega)\), increases first and passes through a maximum at \(\omega \tau_R = 1\). A convenient way of representing the storage and the loss moduli of a viscoelastic fluid is the so-called Cole-Cole plots in which the loss modulus \(G''(\omega)\) is plotted against the storage modulus \(G'(\omega)\). A Maxwell material can be characterized by a semicircular Cole-Cole plot centered on the \(G'(\omega)\) axis. Thus, with the help of the above mentioned expressions, the Maxwell model can be used to describe the viscoelastic properties of materials.

### 2.3.1 Methods of measurement
There are basically three classes of viscometers that are employed depending on the choice of the particular requirement and on the range of viscosities to be measured. They are known as capillary viscometers, rotational viscometers, and moving body viscometers. Here emphasis is given only to rotational viscometers as they can be used at various shear rates and hence the best suited ones for non-Newtonian fluids.

Rotational viscometer consists of two basic parts separated by the fluid being tested. The parts could be a concentric cylinder, a parallel plate or a cone and plate. In all these cases one of the parts rotates relative to other and this produces a shearing action in the fluid. The resulting couple on the stationary part is measured from which the stress and hence the viscosity can be obtained.

(i) Concentric cylinder viscometer

If the gap between the two concentric cylinders is small enough and the cylinders are in relative motion, the test liquid experiences an almost constant shear rate. If the radii of the outer and the inner cylinders are $R_0$ and $R_i$ respectively and the angular frequency of rotation of outer cylinder is $\Omega$, then the shear rate is given by,

$$\dot{\gamma} = \frac{2\Omega R_0^2}{(R_0 - R_i)}$$

(2.52)

For the gap to be classed as narrow gap and the above approximation to be valid to within a few percent, the ratio of $R_i$ to $R_0$ must be greater than 0.97. If the couple on the inner cylinder is $C$, then the shear stress in the liquid is defined as

$$\sigma_s = \frac{C}{2\pi R_0^2 L}$$

(2.53)

where $L$ is the effective immersed length of the liquid that is being sheared.

(ii) Parallel-plate viscometer
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For torsional flow between parallel plates the shear rate at the rim is given by

\[ \dot{\gamma} = \frac{R \Omega}{h} \quad (2.54) \]

where \( R \) is the radius of the plate, \( h \) is the gap thickness between the plates and \( \Omega \) is the angular velocity of rotation. If \( C \) is the couple on one of the plates, then the shear stress is given by

\[ \sigma_s = \frac{2C}{\pi R^3} \quad (2.55) \]

(iii) Cone-and-plate viscometer

In the cone-and-plate geometry, the shear rate is very nearly the same everywhere in the liquid, provided the cone angle, \( \theta \) is small. The shear rate in the liquid is given by

\[ \dot{\gamma} = \frac{\Omega}{\theta} \quad (2.56) \]

If \( C \) is the couple on the cone, then the shear stress is given by

\[ \sigma_s = \frac{3C}{2\pi R^3} \quad (2.57) \]

Rheological measurements were conducted at 25 °C using an Anton Parr Physica MCR 101 rheometer in a double gap concentric cylinder geometry (DG 26.7) with a Peltier temperature control. Frequency-sweep measurements were done at a constant stress (chosen in a linear viscoelastic range) over a frequency range of 0.01 to 100 rad/s.

2.4 Cryo-TEM

Cryogenic transmission electron microscopy (Cryo-TEM) is a form of electron microscopy where the sample is studied at cryogenic temperatures (usually liquid nitrogen temperatures). Cryo-TEM has become a powerful complementary tool to small-angle X-ray
and neutron scattering, light scattering, nuclear magnetic resonance, and rheological measurements in the study of soft matters. In cryo-TEM, the sample is frozen and the frozen sample grid is then kept at liquid nitrogen temperature in the electron microscope and digital micrographs are collected with a camera.

Cryo-TEM is of great importance to soft matter science due to the fact that direct imaging of liquids is not possible in a high-vacuum TEM chamber because of the vapor pressure of solvents and molecular diffusion. However, most self-assembled structures, with an extensive variety of shapes, such as spherical, worm-like micelles, vesicles, lamellar sheets, sponge-phases, nanotubes, networks, disks, as well as many intermediate and mutative phases are stable only in their native solution conditions including concentration, temperature, pH, and the presence of other molecules such as salts. However, cryo-TEM provides in situ imaging of various delicate structures of soft matter, including liquid systems. In cryo-TEM, a wide range of length scales can be directly imaged, ranging from a few nanometres to several micrometres. Moreover, if several distinctively different aggregate shapes exist in solution, the analysis of scattering spectra is a tedious process. On the other hand, cryo-TEM can easily differentiate between the topologies that are difficult to resolve by scattering such as between small disk objects and spherical micelles, or between narrow ribbon elements and cylindrical micelles.

Cryo-TEM studies were performed using Technai G2 12 BioTWIN at Indian Institute of Technology Bombay, Mumbai. A drop of sample was put on holey carbon grids and then the excess sample was removed by blotting with filter paper. Thereafter, the grids were plunged into liquid ethane using an automated plunger and transferred into a grid holder
kept in liquid nitrogen. The samples were mounted in a cryo specimen holder and observed in
the cryo-TEM instrument.

2.5 UV-vis spectroscopy

Electromagnetic radiation is absorbed or emitted only in discrete packets known as the
photons. The energy $E$ of the photon is defined as

$$ E = h\nu $$

where $h$ is the planck’s constant and $\nu$ is the frequency of the electromagnetic radiation.

Absorption of radiation occurs when a quantum of radiant energy coincides with an allowed
transition to a higher energy level or state of the atom or molecule under study [41, 42].

Transmittance ($T$) is defined as the ratio of the radiant power transmitted by the sample to the
radiant power incident on the sample. Thus

$$ T = I/I_0 $$

The absorbance $A$ is defined as the negative logarithm of transmittance. The fundamental law
underlying the practice of ultraviolet - visible spectroscopy is the Beer-Lambert’s law which
relates the absorbance of the sample with the concentration of the absorbing species as
follows

$$ A = -\log T = \epsilon bc $$

where $c$ is the concentration in moles per litre, $b$ is the length of the path of the light beam in
the sample, and $\epsilon$ is the molar absorptivity, which depends on the wavelength and the nature
of the absorbing species [43].

Spectrophotometer is the instrument which furnishes the ratio of the incident to the
transmitted radiant power as a function of spectral wavelength. The basic components of the
spectrophotometer are (i) source (ii) monochromator (iii) sample compartment (iv) detector
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and (v) recorder. The double beam recording spectrophotometer features a continuous change in the wavelength and an automatic comparison of the light intensities of sample and the reference material. The instrument either plots the transmittance or the absorbance as a function of the wavelength of light [44].

In present work Jasco V-650 spectrophotometer was used for recording the absorbance of the sample at a given wavelength.

2.6 Electrochemical technique

An instrument known as the potentiostat has control of the voltage across the working electrode-counter electrode pair, and it adjusts this voltage to maintain the potential difference between the working and the reference electrode (which it senses through a high-impedance feedback loop) in accord with the program defined by a function generator. One can also view the potentiostat as an active element whose job is to force through the working electrode whatever current is required to achieve the desired potential at any time. Since the current and the potential are related functionally, that current will be unique. Chemically, it is the flow of electrons needed to support the active electrochemical processes at rates consistent with the potential value. Thus in an electrochemical set up, the response from the potentiostat (the current) actually is the experimental observable [45]. There are three basic electrochemical processes that are considered useful in transducers for sensor applications: conductimetry, potentiometry, and (volt)amperometry. After applying a potential, when the current is recorded as a function of time, the technique is called chronoamperometry.

2.6.1 Instrumentation

The conventional voltammetric set up consists of a waveform generator to produce the excitation signal, a potentiostat to apply this signal to an electrochemical cell, a current to
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voltage converter to measure the resulting current, and data recorder to display the voltammogram.

2.6.2 Interdigitated array (IDA) microelectrodes

Silver coated IDA microelectrodes were fabricated. Each electrode had 20 digital pairs with 300 μm digit width. The PCB connector pitch is 2.5 mm wide. The two silver lines at the opposite faces of the membrane were used as two electrodes, which were held by a plastic clip holder with platinum contacts from which connections to the instruments were made.

Electrochemical measurements were performed at room temperature using an Autolab potentiostat / galvanostat, model PGSTAT20 (Eco Chemie, The Netherlands). The equipment was connected to a PC via an Autolab interface and controlled using Autolab GPES. The electrochemical cell was formed by a two-electrode arrangement using silver interdigitated screen-printed electrodes as already described. Current voltage characteristics of the polymer modified electrode were measured in the potential range from −0.5 V to 0 V. Pulsed amperometric measurements were carried out by applying pulse of -0.1 V for 2 seconds.

2.7 Zeta Potential

The liquid layer surrounding the particle exists as two regions; an inner region known as Stern layer where the ions are strongly bound and an outer (diffuse) region where they are less firmly associated [46]. Within the diffuse layer there is a notional boundary inside which the ions and the particles form a stable entity. When a particle moves (e.g. due to gravity), ions within the boundary move with it. Those ions beyond the boundary stay with the bulk dispersant solution. The potential at this boundary (surface of hydrodynamic shear) is called the zeta potential (Figure 2.5).
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The magnitude of zeta potential gives an indication of the potential stability of the colloidal system under study. If all the particles in suspension have a large negative or positive zeta potential then they will tend to repel each other and there will be no tendency for the particles

![Graph showing electrostatic potential](image)

**Figure 2.5** Electrostatic potential near a negatively charged spherical particle.

to come together and hence is considered a stable suspension. However, if the particles have low zeta potential values then there will be no force to prevent the particles from coming together and flocculating. The general dividing line between stable and unstable suspensions is generally taken at either +30 or -30 mV, depending on the surface charge of the particles. Particles with zeta potentials more positive than +30 mV or more negative than -30 mV are normally considered quite stable. However, if the particles have a density different from the dispersant, they will eventually sediment forming a close packed bed. The general method used to estimate the zeta potential is the electrophoresis [47]. The basic theory related to electrophoresis is introduced in the following section.
2.7.1 Electrophoresis

When an electric field is applied across an electrolyte solution, charged particles suspended in the electrolyte are attracted towards the electrode of opposite charge. But the viscous forces acting on the particles tend to oppose this movement. After reaching equilibrium between these two opposing forces, the particles move with constant velocity. The velocity is dependent on various factors such as the strength of the electric field or voltage gradient, the dielectric constant of the medium, the viscosity of the medium, and the zeta potential. The velocity of a particle in a unit electric field is referred to as the electrophoretic mobility of that particle. Zeta potential is related to the electrophoretic mobility by the Henry equation given by

\[ U_E = \frac{2\varepsilon z f(\kappa a)}{3\eta} \]  \hspace{1cm} (2.61)

where \( U_E \) = electrophoretic mobility, \( z \) = zeta potential, \( \varepsilon \) = dielectric constant, \( \eta \) = viscosity and \( f(\kappa a) \) = Henry’s function. The units of \( \kappa \), termed the Debye length, are reciprocal length and \( \kappa^{-1} \) is often taken as a measure of the “thickness” of the electrical double layer. The parameter ‘\( a \)’ refers to the radius of the particle and therefore \( \kappa a \) measures the ratio of the particle radius to the electrical double layer thickness. Electrophoretic determinations of zeta potential are most commonly determined in aqueous media and moderate electrolyte concentration. In this case, the value of \( f(\kappa a) \) is 1.5, and this is referred to as the Smoluchowski approximation. Therefore calculation of zeta potential from the mobility is simple for systems that fit the Smoluchowski model, i.e. particles larger than about 0.2 microns dispersed in electrolytes containing more than \( 10^{-3} \) molar salt. Also, for small particles in low dielectric constant media (e.g. non-aqueous media), \( f(\kappa a) \) becomes 1.0 and allows an equally simple calculation and this is referred to as the Huckel approximation [48].
Zeta potentials were determined with a Nanosizer Z (Malvern Instruments, Malvern, UK) by phase analysis light scattering with applied field strength of $2.5 \times 10^3$ V/m. The light source was He-Ne laser operated at 633 nm operating at 4.0 mW. The zetapotential values are calculated from the electrophoretic mobility data using Smoluchowsky approximation. The experiment was carried out using a quartz cuvette (universal ‘dip’ cell) with 10 mm light pathway. The measurements were performed at 25 °C.

2.8 Materials used

Cetyltrimethylammonium bromide (CTAB), tetradecyltrimethylammonium bromide (TTAB), decyltrimethylammonium bromide (DTAB), sodium 3-hydroxy naphthalene 2-carboxylate (SHNC), and sodium hexametaphosphate (HMP), were obtained from S. D. Fine Ltd. Mumbai (India). Sodium laurate (SL), sodium oleate (NaOL), sodium chloride (NaCl), sodium dihydrogen phosphate, disodium hydrogen phosphate, and bovine serum albumin (BSA), were procured from Sigma aldrich. Sodium 3-hydroxynaphthalene 2-carboxylate (SHNC) was obtained from M/S Atul Products, Mumbai (India). Aniline hydrochloride (AHC) and potassium peroxodisulfate (KPS) were purchased from Fluka, Germany. Progesterone standards in serum and progesterone antiserum from rabbit were obtained from ILCJ, BRIT, Mumbai (India). Solutions of biomolecules were stored in an environment at 4 °C when not in use. All chemicals were used as received without further purification. Deionized water from a Millipore-Milli-Q system (resistivity ~ 18 MΩ cm) was used to prepare all the aqueous solutions. Measurements were done at neutral pH. In case of CTAB/TTAB-SL mixed micelles experiments, the equilibration time was 24 hours at room temperature of 25 °C and for CTAB-NaOL mixed micelles experiments, equilibration time was 48 hours at room temperature of 25 °C and 30 minutes at a temperature of 50 °C.