RAMAN CORRELATION SPECTROSCOPY:
A FEASIBILITY STUDY OF
A NEW OPTICAL CORRELATION TECHNIQUE
AND
DEVELOPMENT OF MULTI-COMPONENT NANOPARTICLES
USING THE REPRECPITATION METHOD

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ABSTRACT 

The feasibility of Raman correlation spectroscopy (RCS) is investigated as a new temporal optical fluctuation spectroscopy in this dissertation. RCS analyzes the correlations of the intensity fluctuations of Raman scattering from particles in a suspension that undergo Brownian motion. Because each Raman emission line arises from a specific molecular bond, the RCS method could yield diffusion behavior of specific chemical species within a dispersion. Due to the nature of Raman scattering as a coherent process, RCS could provide similar information as acquired in dynamic light scattering (DLS) and be practical for various applications that requires the chemical specificity in dynamical information. The theoretical development is discussed, and four experimental implementations of this technique are explained. The autocorrelation of the intensity fluctuations from a β-carotene solution is obtained using the some configurations; however, the difficulty in precise alignment and weak nature of Raman scattering prevented the achievement of high sensitivity and resolution. Possible fluctuations of the phase of Raman scattering could also be affecting the results. A possible explanation of the observed autocorrelation in terms of number fluctuations of particles is also examined to test the feasibility of RCS as a new optical characterization method.
In order to investigate the complex systems for which RCS would be useful, strategies for the creation of a multicomponent nanoparticle system are also explored. Using regular solution theory along with the concept of Hansen solubility parameters, an analytical model is developed to predict whether two or more components will form single nanoparticles, and what effect various processing conditions would have. The reprecipitation method was used to demonstrate the formation of the multicomponent system of the charge transfer complex perylene:TCNQ (tetracyanoquinodimethane) and the active pharmaceutical ingredient cocrystal of CBZ:NCT (carbamazepine:nicotinamide). The experimental results with various characterization methods including DLS, absorption spectroscopy, powder x-ray diffraction, and SEM imaging, verify formation of the multicomponent cocrystals. The observation of the self-assembly of TCNQ crystals is also discussed.
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Part I
Raman Correlation Spectroscopy: A Feasibility Study of a New Optical Correlation Technique

1 Introduction

In the past decade, research and development in nanoscience has significantly contributed in a wide range of innovative applications and fields. Due to the novel phenomena and functionalities arising from the influence of their dimensions, nanoscale objects continue to see interest for further advancement of current technology. Nanoparticle based systems, such as colloids, are one group of nanomaterials that demonstrate remarkable potential and properties that have been used and studied for various applications from commercial products to biomedicine.

Colloids consist of a dispersed phase that is distributed uniformly in a dispersion medium. Both the disperse phase and the dispersion medium of a colloidal system could be any state - gas, liquid, and/or solid. They have always existed in nature. Fogs, mists, and smokes are the dispersion of liquid droplets in air or gas. Biological structures, such as proteins, blood cells, and bacteria, also have colloidal nature. Since ancient times, man has observed and used colloids for daily life. Milk, for example, is a colloidal dispersion of fat droplets in a liquid (aqueous phase). It is not, however, only natural phenomena or commodities of colloids that man has been familiar with. Although it was not until the mid 19th century when Michael Faraday made the first systematic study of gold colloids, the synthesis of this colloid had already been used for creating stained glass\(^1\) and the Lycurgus Cup in Ancient
With the development of colloid science at the beginning of the 20th century, colloids have become indispensable tools in modern living as major components of industrial products. Colloids are often used in food products, paints, cosmetics, and many commercial products. In biomedical fields, there has been a surge of interest in colloidal drug carrier systems for forming aqueous formulations of water-insoluble drugs.

Among all the classes of colloidal systems, this research focuses on colloidal dispersions where fine solid particles are dispersed in a liquid medium so, unlike in the case of suspensions, they are evenly distributed and long-term stable. Therefore, the term colloids in this dissertation is used to describe dispersed nanoparticles.

In terms of physical chemistry, colloids are molecules or polymolecular particles that are large compared to the solvent molecule but small enough to exhibit thermal motion, known as Brownian motion. This motion is the seemingly random movement of particles suspended in a dispersion medium resulting from the bombardment by the neighboring solvent molecules. The general size range for colloidal particles is 10 nm to 10 µm. When a beam of light passes through the dispersion system, strong light scattering can be observed due to the presence of colloids and hence the path of the light can be clearly observed. The effect is known as the Tyndall effect.

The surface area of colloids contributes to their importance as well as their definition. The small particle size of colloids leads to a large surface area per unit volume or mass and high energy from the surface electric charges. Since intermolecular forces act on particle surfaces, unlike the gravitational force that acts on the mass, the high surface-to-volume (or surface-to-mass) ratio prevents the gravitational effect from causing colloids to precipitate. With the effect of Brownian motion, where surrounding solvent molecules hit the colloidal particles from all directions, the colloids stay buoyant for several months up to years. Also, to maintain the colloidal stability
of dispersions, the repulsive forces are required to counterbalance the attractive forces such as van der Waals force. Intermolecular forces from the electrostatic effect and steric effect prevent colloids from aggregation, resulting in sustaining their particle size.

From imaging of a nanoscale object to determining its elemental composition or other properties, a wide range of characterization techniques for such systems has been developed to establish understanding and control of their properties. For example, electron microscopies, such as transmission electron microscopy (TEM) and scanning electrons microscopy (SEM), create an image of species by using electrons. Laser-induced breakdown spectroscopy (LIBS) determines the elemental composition or concentrations by analyzing the laser induced plasma with a spectrometer. Ultraviolet-visible spectroscopy (UV/VIS) analyzes absorbance of the species from electronic transitions to obtain information on their concentration.

To characterize the nanoparticle size, techniques using optical scattering have the advantage of noninvasiveness and simple sample preparation. In particular, optical time correlation spectroscopy methods such as dynamic light scattering (DLS) have long been used for characterization of dynamics of particles in the size range from 10 nm to 1 µm and hence frequently used for studies of colloidal suspensions.

A technique that determines the chemical compositions of materials is also useful in material science. Raman spectroscopy provides a fingerprint of a species by analyzing Raman scattering. When an electromagnetic wave impinges on a material and quasi-elastic light is scattered, simultaneously molecular bonds vibrate and/or molecules rotate, resulting in inelastic scattering. This inelastic scattering has different frequencies depending on the molecular bonds and the motion of vibrations or rotations. Because each band of this scattering identifies the specific chemical bonds and symmetry of the molecules, Raman scattering can be used as an identifier of
molecules. This qualitative identification of the Raman emission line allows one to detect the change in the chemical bonds and hence the chemical composition of the species, including colloidal particles.

If the above two concepts were to be combined, one could make the measurement of the diffusion coefficient of a specific component in a colloidal dispersion system. If this new characterization technique, named Raman correlation spectroscopy (RCS), is successfully developed, it would work as a variation of DLS to provide the chemical specificity in the measurements by analyzing Raman scattering and can be widely used to investigate multicomponent colloidal systems as exemplified in the next chapter (Chapter 2). Therefore, RCS could expand possibilities in colloidal science.

As a novel characterization method for colloidal dispersion, the possibility of RCS is investigated in this part of the dissertation. The theory of RCS is developed and discussed in chapter 4. Several implementations were constructed and tested in chapter 5 and 6. Finally, the results are analyzed to investigate the validity of RCS in chapter 7.

*Some of the contents in this part of the dissertation is currently under preparation as a manuscript to be submitted to Optics Letters.
2 Motivation and Applications

Since each molecular bond has a unique Raman spectrum, RCS could selectively characterize a specific component within a complex mixture. If the possibility of RCS as a variation of DLS can be demonstrated, it has potential to be used in numerous applications in research and development.

2.1 Complex Mixture Characterization

The potential of RCS lies in its specificity that can be used for complex mixture characterization. For example, Van Keuren’s group has been working on the study of the formation of charge transfer nanocrystals, which will be discussed in part II of this dissertation. When a single molecular solution is added to a miscible nonsolvent, nanocrystals can be formed. If the solution is multi-component, phase separation may occur causing different nanocrystals consisting of individual components in the mixture to be formed rather than larger nanocrystals combining both components in the mixture. However, we are interested in the formation of multi-component nanocrystals rather than the single component nanocrystals. RCS could be a great tool for the investigation of the nanocrystal formation because it could distinguish each type of nanocrystals in the complex mixture by analyzing the Raman emission line of each component in the mixture. For instance, if the diffusion coefficient from each species is the same as the other, it would indicate that the multi-component nanocrystals are successfully formed. If the diffusion coefficients were not the same, RCS would confirm that nanocrystals of single species were formed instead; thus, the formation conditions would need to be adjusted to prevent the phase separation.
2.2 Polymer Self-assembly Analysis

RCS would also be a useful tool to study the self-assembly process of polymers. There are two primary kinds of polymerizations: one is addition polymerization that simply adds up monomers without forming byproducts, and the other is condensation polymerization that eliminates some molecules to form a bond with the other monomer or polymer. During polymerization, due to the structural changes, Raman bands observed in monomers may disappear or become shifted in polymer structures, and new Raman bands may arise in polymers. By analyzing Raman bands during the assembly process, RCS would determine the size of the specific polymers so that information of dynamic behaviors and interactions between monomers and polymers during the process can be examined in detail.
3 Light Scattering from Nanoparticles

3.1 Dynamic Light Scattering

DLS is one of the intensity-fluctuation spectroscopies employed to characterize colloidal systems. It is also known as photon correlation spectroscopy (PCS) or quasi-elastic light scattering (QELS). For its simple apparatus and non-invasiveness, this technique has been utilized in structural and dynamical analysis of samples in a wide range of fields. For example, it is often used for quality control of industrial and commercial products, such as pigments, inks, detergents, latexes, agrichemical emulsions, and food products. It also serves in biomedical fields, such as in the study of viruses and the analysis of proteins. The DLS theory is fundamental to RCS. This chapter, therefore, discusses the theory of this technique.

3.1.1 Light Scattering

When electromagnetic radiation impinges on matter, the electric field of the radiation induces an oscillating electric dipole moment in its molecules. Classically, the polarized electrons radiate light, or scatter, at the frequency of their oscillation. This scattered light at the detector at a given time is the superposition of the electric fields radiated from all of the scatterers and so depends on their positions.

The phase of the scattered light at a point with radius vector \( r \) relative to the phase of the scattered light at the origin is \( \mathbf{q} \cdot r \) where \( \mathbf{q} \) is the scattering vector as shown in Figure 3.1:

\[
\mathbf{q} = \mathbf{k}_i - \mathbf{k}_f
\]
where the wave vectors $k_i$ and $k_f$ point in the directions of propagation of the incident light and the scattered light reaching the detector respectively.\cite{6,7} Thus, the phase difference of the scattered light is $\Delta \varphi = q \cdot r = (k_i - k_f) \cdot r$. The magnitudes of $k_i$ and $k_f$ are $2\pi n/\lambda_i$ and $2\pi n/\lambda_f$ respectively, where $n$ is the refractive index of the scattering medium and $\lambda_i$ and $\lambda_f$ are the wavelengths of the incident and the scattered light. The angle between $k_i$ and $k_f$ is the scattering angle $\theta$.

The electric field resulting from scattering by a particle located at position $r$ is

$$E_s = A_j E_o e^{i(q \cdot r - \omega_o t)} \quad (3.2)$$

where $E_o$ is the field amplitude, $\omega_o$ is the angular frequency, and $t$ is time. A term $A_j$ in the above equation contains factors such as the polarizability, the distance $R$ to the detector, and other parameters.\cite{7}
Most of the scattered photons are due to quasi-elastic collisions between the incident photons and molecules of the system, hence the radiation is at almost the same frequency as the incident radiation. Such scattering are described by two models: Rayleigh scattering and Mie scattering. In Rayleigh scattering, the light is scattered by a particle of size much smaller than the light wavelength. The upper limit of the diameter of such a particle is less than 20 to 30 nm or about 1/10 of the wavelength. The refractive index in Rayleigh scattering is close to 1, so that the electric field penetrates through the particle. Hence the scattered photons interfere constructively and the radiation is uniform in all directions. This scattering explains the blue sky. Air molecules undergo Rayleigh scattering with an intensity that has a strong wavelength dependence of $1/\lambda^4$, so the shorter blue wavelengths are scattered stronger than red at large angles with respect to the direction of the sunlight. The intensity of Rayleigh scattering strongly depends on both the particle size and wavelength. When the particles become comparable to the size on the order of the light wavelength and if they are spherical, the scattered photons are described as Mie scattering. Unlike Rayleigh scattering, Mie scattering has a complicated dependence on angle and its intensity is larger in the forward direction than in the reverse direction. The larger the particle size is, the more intense the light is in the forward direction, and because Mie scattering is not strongly wavelength dependent, the white glare observed around the sun is explained by this type of scattering. The white light from mists and fogs where the light passes through clouds of particulate matters is also due to Mie scattering. As the exact solution to Maxwell’s equation, this scattering theory works well with spheres, and although solutions for some other simple shapes like ellipsoids exist, no general solution is known for arbitrary shapes.
3.1.2 Particle Diffusion

During the scattering process, dispersed particles undergo random Brownian motion as a result of bombardment by surrounding solvent molecules due to their thermal energy. The rate of diffusion of the particles is related to their size; small particles move faster than larger particles. Although the particle motion is in a random manner, more particles move from a high concentration region to a low concentration region in a unit time. Diffusion of particles is thus caused by Brownian motion. In diffusion theory, the mean square of the displacement of a particle along a given axis is \( \Delta x^2 = 2D\Delta t \). The phase of the scattered wave is significantly affected when the particle moves over a distance \( \Delta x \approx q^{-1} \). The correlation time \( \tau_c \), representing the characteristics decay time of the particle diffusion, is

\[
\tau_c \sim \frac{1}{Dq^2}
\]  

(3.3)

where \( D \) is the diffusion coefficient and \( q \) is the length of the scattering wave vector \( q \).

Due to the translational diffusion of the particle, the sum of the scattered light from particles at the detector fluctuates in time. If the radiation source, such as a laser, emits monochromatic and coherent light, the coherent scattered photons interfere with each other, and consequently, the interference pattern fluctuates in time. By measuring such fluctuations of scattering, one can obtain dynamical information of the system.
3.1.3 Intensity Autocorrelation Analysis

The time average of the signal is defined by

$$\langle f \rangle = \lim_{T \to \infty} \frac{1}{T} \int_0^T dt f(t). \quad (3.4)$$

When the system is ergodic, this time average equals the ensemble average. Thus, a measure of the correlation of a fluctuating signal is the autocorrelation function of $f$ as

$$\langle f(0)f(\tau) \rangle = \lim_{T \to \infty} \frac{1}{T} \int_0^T dt f(t)f(t+\tau) = \langle f \rangle^2 + \{ \langle f^2 \rangle - \langle f \rangle^2 \} e^{-\tau/\tau_r} \quad (3.5)$$

where $\tau_r$ is the correlation time of the signal. In many cases, because for a long time $\tau$, the signal fluctuation would be totally uncorrelated, the autocorrelation function typically illustrates a single exponential.

The scattering fluctuations from a particle in diffusion take place also in a random manner as explained in the previous section. The autocorrelation of these signals is characterized in DLS to determine the diffusion coefficient of the particle. The autocorrelation function, a measure of the temporal correlation of the electric field of the scattered wave, is defined as:

$$G_1(\tau) = \langle E^\ast(t)E(t+\tau) \rangle \quad (3.6)$$

where $E(t)$ and $E(t+\tau)$ are the electric fields of the scattered light at time $t$ and $t+\tau$ ($\tau$ is delayed time). In practice, the homodyne method, where only the scattered light impinges on the detector, is usually employed, and the autocorrelation function of the
scattered wave is obtained by measuring the temporal fluctuation of light intensity I(t) at the scattering angle.

The intensity I, or the average energy per unit area per unit time, of the scattered light is proportional to the square of the amplitude of the electric field, or $I \propto |E|^2$. The time dependent intensity fluctuation is analyzed using the second order autocorrelation function, or in this case the intensity autocorrelation function that is defined as:

$$G_2(\tau) = \langle I(t)I(t+\tau) \rangle$$  \hspace{1cm} (3.7)

where $I(t)$ and $I(t+\tau)$ are the intensities of the scattered light at time $t$ and $t+\tau$. In experiments, the scattered light intensity is collected and measured by a digital detector and correlator for a long period of time in order to get good photon statistics, which allow an accurate determination of the average in equation (3.7). The comparison of the pairs of all and any data that are separated by the time delay interval $\tau$ is done by using equation (3.7). As $\tau$ increases, the signals become less correlated, hence $G_2$ decreases with $\tau$.

Since the detected signal is the sum of light scattered by many independent particles, it exhibits Gaussian statistics. In this case, a relation between the intensity correlation function $G_2(\tau)$ and the field correlation function $G_1(\tau)$ can be well approximated by the Siegert relation:

$$g_2(\tau) = 1 + \gamma |g_1(\tau)|^2$$  \hspace{1cm} (3.8)

where $\gamma$ is a coherence factor, which depends on the number of coherence areas observed and the sampling time interval, and is unity in the ideal case.$^6$ This equation only holds if the scattering field is a Gaussian process. The coherence factor $\gamma$ is an
important variable for a good signal to noise ratio and will be explained in section 4.2.1 in detail.

In equation (3.8), \( g_1(\tau) \) is the normalized field autocorrelation function, \( g_1(\tau) = G_1(\tau) / G_1(0) \). In a monodisperse particle system where particles are in Brownian motion, the autocorrelation function decays exponentially,\(^6\)

\[
g_1(\tau) = e^{-\Gamma \tau}. \tag{3.9}
\]

\( \Gamma \) in the above equation is the diffusion rate, which is the inverse of the correlation time \( \tau_c \) (equation (3.3)) of the particle diffusion; thereby,

\[
\Gamma = D q^2 \tag{3.10}
\]

where, from equation (3.1),

\[
q^2 = |k_f - k_i|^2 = k_f^2 + k_i^2 - 2 k_f k_i \cos(\theta). \tag{3.11}
\]

For quasi-elastic scattering, \(|k_i| \approx |k_f|\). The length of the vector can then be written as

\[
q = \frac{4 \pi n}{\lambda} \sin \frac{\theta}{2} \tag{3.12}
\]

where \( n \) is the refractive index of the sample, \( \lambda \) is the excitation wavelength, and \( \theta \) is the scattering angle.

In DLS experiments, the intensity fluctuation data is characterized by the normalized autocorrelation function (ACF) of intensity, and then a nonlinear fit algorithm is applied to the ACF obtained. The ideal monodisperse sample yields the single exponential decay of the ACF, but if the data is more complicated, polydisper-
sity of the sample can be analyzed in DLS.

To analyze the data, the cumulants analysis, introduced by Koppel, is often employed in modern DLS instruments. This method defines the expression for the ACFs by calculating the moments or cumulants of the distribution. However, if different numbers of data points are fitted, this traditional cumulant method can produce inconsistent results within the same data. To obtain more robust and satisfactory fits, Frisken reformulated the cumulants method and took into account an arbitrary background signal in the following moment-based expression for the normalized time autocorrelation function of the intensity of scattering $g_2(\tau)$, which is given by

$$g_2(\tau) = \gamma \cdot \exp(-2\Gamma \tau) \times (1 + \frac{\mu_2}{2!} \tau^2 - \frac{\mu_3}{3!} \tau^3 + \ldots)^2 + B \quad (3.13)$$

where $B$ is a baseline, which is the long time value of $g_2(\tau)$, and $\mu_2$ and $\mu_3$ are the second and third moments that are associated with the probability distribution of the diffusion rates. The second moment $\mu_2$ is a measure of the variance of the distribution, and the third moment $\mu_3$ is a measure of the skewness of the distribution. Since no correlation should be observed at long times, $B$ is ideally 1. However, experimental noise can cause the value to deviate from unity. If the obtained data has a value of $B$ that is too far from unity, it indicates a problem in the data for fitting. It could also indicate the presence of larger particles or bad normalization or scaling of data.

Figure 3.2 displays the concept of the ACF. At the delay times $\tau_1$, $\tau_2$, and $\tau_3$ with respect to the reference time $t=\tau_0=0$, a particle moves away in a random manner so the degree of the displacement changes. Hence, at a longer time, the particle has deviated from the original position, and so the correlation decreases.
For a spherical particle in a Newtonian fluid, the diffusion coefficient is inversely proportional to particle radius $R$ and is defined by the Stokes-Einstein relation:

$$D = \frac{k_B T}{6\pi \eta R} \quad (3.14)$$

where $k_B$ is the Boltzmann constant, $T$ the absolute temperature, and $\eta$ the viscosity of the solvent. $R$ is the hydrodynamic radius, which is also known as Stokes radius. This radius is the radius of a hypothetical hard sphere that diffuses at the same rate as the particle under examination, hence it is typically larger than the effective radius of the particle.

Figure 3.3. illustrates the characteristics of ACFs for small and large particles. As mentioned before, the smaller particles move faster so that the intensity fluctuates rapidly and so the ACF decays fast whereas the larger particles with slower intensity fluctuations diffuse more gradually.
The polydispersity can also be characterized in DLS. If only identical size particles are in the suspension, the ACF would show monoexponential decay, and hence the log-linear plot of the ACF will be a straight line, as illustrated in Figure 3.3. However, if there is the presence of non-identical size particles, there will be curvature of the decay in the log-linear plot, which is the result of the sum of exponentials from different sized particles. Hence, polydispersity complicates the data analysis.

The dimensionless polydispersity index (PDI), a measure of the width of the distribution, is calculated from the first and second moments ($\Gamma$ and $\mu_2$) of the ACF as:

$$PDI = \frac{\mu_2}{\Gamma^2}.$$  \hspace{1cm} (3.15)

In the ideal case, if the sample is monodisperse, the value of the PDI is zero. The typical range of PDI for nearly monodisperse samples is between 0.02 to 0.08.\textsuperscript{12} Thereby, the PDI should be less than 0.02 for a monodisperse sample, or the monoexponential decay to the autocorrelation function. If the value is larger than the above range, it indicates significant polydispersity. If PDI is greater than 0.7, the sample is very...
polydisperse so that the interpretation of the fit needs to be analyzed more.

3.2 Raman Scattering

While most of the scattered photons are from the elastic collision process, a very small fraction of the photons are scattered inelastically due to the Raman effect. In this inelastic collision process, the energy transition occurs to the virtual state as in Rayleigh scattering. The time scale of the Raman effect is essentially instantaneous, and hence Raman scattering is a coherent process.\textsuperscript{13–15} This fact is properly described by a Fermi’s golden rule rate, which is linearly proportional to the incident intensity and also proportional to the dipole matrix element products.\textsuperscript{16}

An incident optical electric field $E$, such as from a laser beam, induces an electric dipole in the molecules. The average dipole moment per unit volume is the polarization, described by

$$P(t) = \alpha(t)E(t)$$

where $\alpha$ is the polarizability tensor of electrons in the molecule. The polarizability is modulated by the molecular vibrations. It can be expressed by expanding using a Taylor series expansion with respect to the normal coordinates of vibration, $Q$ as

$$\alpha(t) = \alpha_o + \left( \frac{\partial \alpha}{\partial Q} \right)_o Q(t) + \cdots$$

where $\alpha_o$ is the polarizability at the equilibrium position and $\left( \frac{\partial \alpha}{\partial Q} \right)_o$ is the rate of change of $\alpha$ with respect to change in $Q$ taken at the equilibrium configuration. The forces holding the atoms in the molecule together can be considered as springs so that the nuclei vibrate at frequency $\nu'$. Hence, the induced polarization includes the effect
from such a vibration. The time dependence of $Q$ is given by

$$Q(t) = Q_o \{ e^{-i(\omega_o - \omega')t} + e^{-i(\omega_o + \omega')t} \} \quad (3.18)$$

where $Q_o$ is the normal coordinate amplitude, $\omega_o$ is the frequency of the incident light, and $\omega'$ is the angular light frequency associated with $\nu'$ ($\omega' = 2\pi \nu'$).

Ignoring the higher order terms, equation (3.16) can be expressed as

$$P(t) = \alpha_o E(t) e^{-i\omega_o t} + \left( \frac{\partial \alpha}{\partial Q} \right)_o E(t) Q_o \{ e^{-i(\omega_o - \omega')t} + e^{-i(\omega_o + \omega')t} \}. \quad (3.19)$$

Equation (3.19) illustrates the two types of scattering. The first term is the quasi-elastic scattering ($\omega = \omega_o$ which is the incident light frequency) and the second term describes the inelastic scattering ($\omega = \omega_o \pm \omega'$). The equation also depicts that Raman scattering occurs only when vibrations change the polarizability, or $\left( \frac{\partial \alpha}{\partial Q} \right) \neq 0$. The intensity of Raman scattering is proportional to this polarizability change as well: $I \propto \left( \frac{\partial \alpha}{\partial Q} \right)^2$. When a large concentration of electrons in a molecule is loosely held, the polarizability also becomes large and thus the change of polarizability in the vibration. For example, the stretching mode of multiple bonds yields stronger Raman bands than for single bonds\(^{17}\).

Unlike quasi-elastic scattering, the frequency of photons is shifted from the incident photons in this scattering due to the gain or loss of the vibrational energy of molecules. As a result, there are two types of Raman scattering (Figure 3.4). In one of them, electrons excited to the virtual state relax into a vibrational excited state, or $E_s = h(\nu_o - \nu')$. Since energy is lost to the vibration, the frequency of the scattered light is lower than that of the incident photons. This type of scattering is known as Stokes-Raman scattering. The other type of scattering has a higher frequency due
to the gain of the vibrational energy, or \( E_s = h(\nu_o + \nu') \), and is called anti-Stokes-Raman scattering. To have anti-Stokes scattering, however, molecules need to be in a vibrational excited state prior to the excitation by the monochromatic radiation. Following the Boltzmann distribution, the population of the ground state is much higher than that of the excited state at thermal equilibrium and room temperature. Therefore, the anti-Stokes scattering typically gives much weaker Raman emissions than the Stokes scattering does. Since the Raman effect already has a very weak intensity compared to the quasi-elastic Rayleigh effect due to its low population, the Stokes scattering is measured much more frequently. The relative intensities of the incident light to Rayleigh scattering to Raman scattering is approximately \( 1: 10^{-6}: 10^{-10} \).\(^{17}\)

This weak scattering, nonetheless, provides molecular identification on scattering agents because each energy level of the scattering corresponds to a specific molecular bond structure. Types of vibrations or rotation involving the entire molecule influence the energy level; therefore, each compound produces its own unique Raman spectrum. The typical range of frequency displacements of Raman shift is between

---

**Figure 3.4:** Energy transitions of scattering.
100 and 4000 (cm$^{-1}$). The spectrum is usually presented in units of wavenumber shift (cm$^{-1}$). However, because the monochromator reading in wavelength is mainly used in this research, for convenience, most of the spectra in this dissertation are presented in units of wavelength (nm). The conversion of the two parameters is defined as

$$\lambda_R = \left( \frac{1}{\lambda_o} - \tilde{\nu}_R \right)^{-1}$$

where $\lambda_R$ and $\tilde{\nu}_R$ are wavelength and wavenumber shift of Stokes scattering, respectively, and $\lambda_o$ is the wavelength of the incident laser.

### 3.3 Raman Correlation Spectroscopy

DLS employs quasi-elastic scattering, but because all objects in the scattering volume radiate the light, the source of scattered light cannot be determined. If a multi-component sample were analyzed, DLS would yield information on different particles sizes but without differentiating which component corresponds to which size. The resultant ACF obtained by DLS for the sample is the average decay, but it gives no information on the separate components of the sample. This limits the application of DLS. However, by combining Raman scattering with the intensity correlation analysis, it may be possible to obtain dynamic information of a specific chemical species in a multicomponent system due to the specificity of Raman scattering. The following chapters demonstrate the conditions for Raman Correlation Spectroscopy (RCS) to be feasible, and results from several implementations are used to test the proposed theory.

An earlier version of RCS was developed by Schrof and his colleagues using confocal optics as in fluorescence correlation spectroscopy (FCS). FCS is another temporal intensity fluctuation spectroscopy that employs fluorescence, which is inco-
herently emitted from fluorescent particles, to determine the number of the particles in addition to the diffusion coefficient. In order to analyze the number fluctuations of the incoherent light, confocal optics is required to limit the system size to achieve a small observation volume, and the concentration of the particles also must be very low. Applying this optic setup to RCS is not efficient due to the weak scattering of the Raman effect compared to fluorescence, so the number of materials that this optical setup can characterize is limited. As explained in the previous section, however, Raman scattering is coherent light since the energy transition to a virtual state occurs instantaneously. Therefore, the implementation of RCS could be possible with a similar apparatus as for DLS, which may produce higher sensitivity and be able to characterize a wider range of materials than the confocal optics implementation does.
4 Theoretical Development of RCS

4.1 Derivation of Intensity Correlation Analysis in RCS

As an intensity fluctuation spectroscopy, RCS adapts the intensity correlation analysis described in section 3.1.3. However, because the frequency of Raman scattering is shifted from the incident frequency, the length of the scattering vector $q$ in equation (3.11) is expressed as follows for different sampling geometries:

$$q^2 = \begin{cases} k_i^2 + k_f^2 & \text{for } 90^\circ \text{ geometry,} \\ k_i^2 + k_f^2 - 2|k_i||k_f| & \text{for } 180^\circ \text{ geometry.} \end{cases}$$ (4.1)

Berne and Pecora derived the integrated intensity and the spectral density of Rayleigh scattering for DLS.\(^6\) For the autocorrelation function (ACF), RCS shares many similarities with DLS. We assume that the molecules in the solution are weakly coupled electronically so that the electronic states of the molecules are not perturbed much by the nearby molecules. Thus, the scattered field can be expressed in terms of the sum of all of the polarizability tensors $\alpha$: $E_S \propto \sum_j \alpha^i(t) e^{i\mathbf{q}\cdot\mathbf{r}_j(t)}$ with scattering vector $\mathbf{q}$ and positions $\mathbf{r}$.

The spectral density in terms of polarizability is defined as

$$I^\alpha(\mathbf{q}, \omega) = \frac{1}{2\pi} \int_{-\infty}^{\infty} dt \ e^{-i\omega t} \langle \delta\alpha^*(\mathbf{q}, 0)\delta\alpha(\mathbf{q}, t) \rangle$$ (4.2)

where

$$\delta\alpha(\mathbf{q}, t) = \sum_{j=1}^{N} \alpha_j(t) e^{i\mathbf{q}\cdot\mathbf{r}_j(t)}.$$ (4.3)
\(\delta \alpha\) here is the spatial Fourier component of the polarizability density,

\[
\delta \alpha(\mathbf{r}, t) = \sum_{j=1}^{N} \alpha_j(t) \delta(r - r_j(t)).
\]  

(4.4)

With the Taylor series expansion, equation (4.3) can be rewritten as

\[
\delta \alpha(\mathbf{q}, t) = \sum_{j=1}^{N} \alpha_{jo}(t)e^{i\mathbf{q} \cdot \mathbf{r}_j(t)} + \sum_{j=1}^{N} \frac{\partial \alpha}{\partial Q_j}(t)Q_j(t)e^{i\mathbf{q} \cdot \mathbf{r}_j(t)} + \cdots.
\]  

(4.5)

The first term is for Rayleigh (quasi-elastic) scattering, and the second term is for Raman scattering. The function \(e^{i\mathbf{q} \cdot \mathbf{r}}\) takes into account the translations of the molecules. The polarizability component \(\alpha_j(t)\) varies in time due to the rotations and vibrations of the molecules. In Rayleigh scattering, this factor is only from the instantaneous molecular rotational orientation. However, in Raman scattering, the vibrational modes are also involved. Thus, the Raman term has an additional component for the vibrational displacement dependence in terms of \(Q(t)\).

As previously explained, \(Q_j(t)\) is the nuclear displacement for the vibrating molecule. As Long suggests, the Raman scattering could have an arbitrary phase factor, which makes the phase different from molecule to molecule.\(^{19}\) The detected light wave must be coherent, or otherwise it does not give a meaningful autocorrelation. Therefore, it is important to take into account the phase factor \(\varphi\) in the Stokes Raman scattering, which redefines equation (3.18) to the more general form as following:

\[
Q(t) = Q_0[e^{-i(\omega_0 - \omega)t + \varphi(t)}].
\]  

(4.6)
The Raman term in equation (4.5) can then be rewritten as

$$\delta \alpha_R(\mathbf{q}, t) = \sum_{j=1}^{N} \frac{\partial \alpha_j}{\partial Q_j} Q_{jo} e^{-i\left((\omega_o - \omega') t + \varphi(t)\right)} e^{i\mathbf{q} \cdot \mathbf{r}_j(t)}. \tag{4.7}$$

Both $\frac{\partial \alpha_j}{\partial Q_j}$ and $Q_{jo}$ are constant, but the phase factor $\varphi$, which is relative to the incident field, depends on the phase of the molecular vibration, and hence it is arbitrarily different from molecule to molecule.\textsuperscript{19} That is, each particle acts as an independent source of radiation. However, the Raman scattering process itself is coherent with the incident light. The coherent property of Raman scattering can be explained by the quasi-monochromatic characteristic of each band. Each Raman band has the frequency width $\Delta \nu$ of a few cm$^{-1}$.\textsuperscript{13} The coherence length $l_t$ is defined by the speed of the source over the frequency width ($l_t = c / \Delta \nu$), so $l_t$ of the Raman scattering is on the order of 10$^5$ m, which is very monochromatic, as in quasi-elastic light scattering. Thus, if the ACF only observes the scattering of the same molecule, RCS could work similarly to DLS. In RCS, the spectral density in equation (4.2) would have the same form as in the DLS case but with the Raman scattering frequency ($\omega_o - \omega'$) and a different constant in terms of $\frac{\partial \alpha_j}{\partial Q_j}$ and $Q_{jo}$.

The coherence property is crucial for the intensity fluctuation spectroscopy. Without coherence, no dynamic information can be obtained using the ACF. As explained earlier, although the phase factor of each molecule is different, Raman scattering is a coherent process, and therefore, the scattered Raman fields from the same molecule at different time might produce meaningful correlation functions.\textsuperscript{13,16} If that is the case, this is identical to the situation with Rayleigh scattering. However, if each molecule in the particle emits Raman scattering at a different, rapidly fluctuating phase, the phase change ruins coherence even though Raman process is coherent. A detailed study on Raman scattering from a particle in terms of its phase property has
not been done, and it is beyond the scope of this research. However, \( \varphi(t) \) is the key quantity for RCS in the definition of the Raman scattered field.

When the phase factor is taken into account, since each molecule scatters at different phase\(^{19} \), the scattered field from the \( k^{th} \) molecule in the particle at position \( r \) is

\[
E_k = B_k e^{i q (r + \Delta r_k)} e^{-i \{\omega t + \varphi_k(t)\}}
\]

(4.8)

where the factor \( B_k \) depends on the Raman cross section and the Raman frequency \( (\omega = \omega_o - \omega') \) of the molecules. Each of those molecules could have phase shifts \( \varphi_k \). Raman scattering is insensitive to inhomogeneous broadening, and the Raman line is very narrow (a few cm\(^{-1} \)).\(^{13} \) Because of this narrow Raman linewidth, \( B_k \) is assumed to depend only on the particular Raman band being observed; that is, it is independent of the particular molecule. The sum of exponential terms containing \( \Delta r_k \) then results in a factor for the particle, which is constant in time. On that account, this exponential term and the factor \( B_k \) can be incorporated into constant \( B'_k \) that is defined as

\[
B'_k = B_k e^{i q \Delta r_k}.
\]

(4.9)

The scattered field of the \( j^{th} \) scatterer particle at the position \( r_j \) that has \( M \) molecules can be then defined as

\[
E_j = \sum_{k=1}^{M} A_j(t) B'_k e^{i q r_j} e^{-i \{\omega t + \varphi_k(t)\}}
\]

(4.10)

where the amplitude \( A_j \) depends on the orientation of the scatters.

Since the phase shift of the emitted light, \( \varphi_k \), could be different from molecule to molecule, the total scattered Stokes Raman field from \( N \) scatters (particles) can
then be rewritten as follows:

\[ E_{RS} = \sum_{j=1}^{N} \sum_{k=1}^{M} A_j(t) B'_{jk} e^{i \mathbf{q} \cdot \mathbf{r}_j} e^{-i(\omega t + \varphi_{jk}(t))}. \]  

(4.11)

The subscript of factor \( B'_k \) and \( \varphi_k \) changes to \( B'_{jk} \) and \( \varphi_{jk} \) since each particle has \( M \) molecules.

The average total intensity is given by \( I_{RS} = \langle |E_{RS}^2| \rangle \) as in equation (3.6), and because the positions of the scatterers are not correlated, all cross terms average to zero as

\[ I_{RS} = \langle \sum_j^N |A_j|^2 \rangle = N \langle |A_j|^2 \rangle. \]  

(4.12)

Consequently, the ACF of the Raman scattered fields is

\[ G_1(\tau) = \langle \sum_j^N \sum_{k=1}^{M} A_j^*(t) B'_{jk} e^{-i \mathbf{q} \cdot \mathbf{r}_j} e^{i(\omega t + \varphi_{jk}(t))} \times \sum_{l=1}^N \sum_{p=1}^{M} A_l(t + \tau) B'_{lp} e^{i \mathbf{q} \cdot \mathbf{r}_l} e^{-i(\omega (t+\tau) + \varphi_{lp}(t+\tau))} \rangle. \]  

(4.13)

Because the scatterers, or particles, are in random motion and their translations are not correlated to each other, the cross term from the interactions between different scatterers \( j \neq l \) averages out to zero as in the case of DLS. Hence those terms are disregarded, and the only case where \( j = l \) is considered, which turns the subscripts of the phase factor \( \varphi_{lk} \) (or \( \varphi_{lp} \)) back to the original subscripts \( k \) (or \( p \)). The time scale of translational diffusion should be different from other parameters, including the phase change of scattering from a molecule. For a spherical particle, it is safe to assume that the time scale of functions \( A(t), r(t), \) and \( \varphi(t) \) are not correlated to one another because one is a function of the molecular orientation time scale, the second is a function of the diffusional transition time, and the third is a function in terms of
the phase change. The N scatterer particles are assumed to be identical, so that each particle has M molecules. Therefore, equation (4.13) can be rewritten as

\[
G_1(\tau) = Ne^{-i\omega_R \tau} \left\langle A^*(t) A(t + \tau) \right\rangle \left\langle e^{-i\mathbf{q} \cdot \mathbf{r}(t)} e^{i\mathbf{q} \cdot \mathbf{r}(t+\tau)} \right\rangle \times \\
\left\langle \sum_{k=p}^{M} B'_k e^{i\varphi_k(t)} B_k e^{-i\varphi_k(t+\tau)} + \sum_{k \neq p}^{M} B'_k e^{i\varphi_k(t)} B'_p e^{-i\varphi_p(t+\tau)} \right\rangle.
\]

(4.14)

The last average term has two components: The contributions from the interactions between (a) the same molecules in the same scatterer (\(k=p\)) and (b) the different molecules in the same scatterer (\(k \neq p\)). Since the Raman scattering from different molecules are not in-phase to each other\(^{19}\), then the correlations of the molecules for the case (b) in equation (4.13) averages out to zero. This implies that only the terms with case (a), or the correlation from the same molecule, provide meaningful data. Thereby the case (b) terms can be ignored, and equation (4.14) is rewritten:

\[
G_1(\tau) = Ne^{-i\omega_R \tau} [G_A(\tau)] [G_r(\tau)] [G_\varphi(\tau)]
\]

(4.15)

where

\[
[G_A(\tau)] = \left\langle A^*(t) A(t + \tau) \right\rangle
\]

\[
[G_r(\tau)] = \left\langle e^{-i\mathbf{q} \cdot \mathbf{r}(t)} e^{i\mathbf{q} \cdot \mathbf{r}(t+\tau)} \right\rangle
\]

\[
[G_\varphi(\tau)] = \sum_{k=1}^{M} B'_k \left\langle e^{i\varphi_k(t)} e^{-i\varphi_k(t+\tau)} \right\rangle.
\]

\(\omega_R\) is the particular Raman frequency that is observed and \(B'_k\) is constant in time. The amplitude autocorrelation function \([G_A(\tau)]\) is usually constant, so it does not affect the decay rate of an ACF, but the autocorrelation function \([G_r(\tau)]\) is due to the diffusing position of the scatterer and it depicts the particle translational diffusion.
The intensity ACF in DLS is characterized by these two autocorrelation functions.\textsuperscript{20} However, the contribution of the phase autocorrelation function $[G_{\varphi}(\tau)]$ of the Raman scattering to the correlation is a key factor to the validity of RCS.

If there is a phase shift in Raman scattering as in equation (4.15), the simplest case would be that $\varphi(t)$ is not a function of time but rather constant. This assumption makes the function $[G_{\varphi}(\tau)]$ constant, which makes the derivation of the ACF of Raman fields very similar to the case of the Rayleigh fields. At $\tau = 0$, the time average of the phase also becomes constant. Therefore, it has no effect on the time decay but only contributes as the multiplier of the ACF. Thus, the autocorrelation function of RCS becomes almost the same as that of DLS, as explained by Cummins and Swinney\textsuperscript{20}, except for the additional terms that affect the constant part of the ACF. Since the additional term would not contribute to the diffusional decay rate, the DLS theory directly applies to the RCS method. If this is the case, it can be seen that the coherence of the Raman effect allows the ACF of its intensity fluctuation to yield meaningful data as in DLS. Therefore, equation (3.13) can be applied to RCS with the redefined value of the wave vector $q$ (equation (4.1)).

On the other hand, if $\varphi_k(t)$ of Raman scattering is not constant, the phase changes over time. For a time $T$ that is much longer than the time scale of the phase shift ($T >> t_{\varphi}$), the cross term of the phase contribution would become zero. In this case, the ACF is not useful since the incoherent scattering fields would not be Gaussian so the Siegert relation cannot be applied to the ACF. The correlation rapidly becomes zero that the data would rather appear as noise, so that no dynamic information can be obtained from RCS.

Therefore, the phase autocorrelation function $[G_{\varphi}(\tau)]$ determines if RCS could possibly work as a temporal intensity fluctuation method to characterize the particle diffusion. Although the phase is the crucial factor for RCS, the detailed study of
Raman scattering in terms of incoherence in the phase of each molecule has not been conducted, and it is beyond the scope of this research. However, the above arguments suggest that equation (3.8) could be applied to RCS as in DLS if the phase difference of individual molecule does not affect the autocorrelation. To investigate the theory, the expression of the ACF defined in equation (3.13) is used for the experiments with the redefined value of the scattering vector $q$ in equation (4.1) according to the specific configuration used.

4.2 Resolution and Sensitivity of RCS

To test the RCS technique, careful deliberation for the setup construction is necessary because sensitivity and resolution are crucial in RCS. There are two primary factors that affect the quality of the analysis: coherence and Raman cross section. This section discusses the theoretical optimization of the experiment in order to achieve high sensitivity and resolution for the correlations.

4.2.1 Coherence

Coherence of scattering determines the resolution of intensity fluctuations and consists of two parts: temporal and spatial. The coherence factor $\gamma$ in equations (3.8) and (3.13) incorporates both effects: $\gamma = \gamma_t \gamma_s$ where $\gamma_t$ is a temporal coherence factor and $\gamma_s$ is a spatial coherence factor. In the ideal case, $\gamma$ is unity.

To obtain meaningful ACF data, the time interval of the light waves should have constant relative phase, known as the coherence time $t_c$ which is proportional to the inverse of the range of frequency, or linewidth, $\Delta \omega$. Since the typical $\Delta \omega$ of common lasers is on the order of GHz, they are distinguished as monochromatic light sources. Therefore, they maintain temporal coherence and are effective for exciting
molecules to scatter.\textsuperscript{22}

In light scattering, there are two kinds of linewidth broadening: inhomogeneous and homogeneous broadenings. In inhomogeneous broadening, different particles may scatter at slightly different wavelengths, resulting in a broader linewidth. However, the Raman effect generally has little inhomogeneous broadening.\textsuperscript{13} Homogeneous broadening of the linewidth occurs when stimulated atoms in the system are all affected in the same manner. A Raman scattering peak is described using the central value $\nu'$ of a distribution of wavenumbers that is associated with the uncertainty of the energy transition level. With the uncertainty, this broadening contributes to the Raman line shape. The measured linewidth could also be affected by the condition of instrumentation.\textsuperscript{19} Although the broadening of Raman scattering is typically very small (a few cm$^{-1}$)\textsuperscript{13}, in case of a broad Raman band, the slit insertion in the monochromator increases the resolution of the system by restricting the range of the Raman wavelength/frequency that the detector observes, and consequently helps the correlation. Thus, we expect no effect from Raman broadening.

Since the integration time or sampling time of the intensity fluctuation measurement is typically much smaller than the diffusion decay, it can be considered that the value of $\gamma_t$ is unity. That implies that the coherence factor in the ACF only depends on $\gamma_s$, or $\gamma \approx \gamma_s$. Hence, the term coherence factor usually refers to the spatial coherence factor.

Spatial coherence depends on the experimental geometry. It is a measure of the correlation between the phases of the light wave at different points in space perpendicular to the propagation of the light. To have good spatial coherence, the maximum phase difference in the scattered light at the detector plane should be limited to $\leq \pi$, or the path-length difference should be limited to $\leq \lambda/2$. The scattered light will be spatially coherence if a detected area is within $A_c$, the coherence area, which is
generally calculated as:

\[
A_c = \frac{(r\bar{\lambda})^2}{A_D} \approx \frac{\bar{\lambda}^2}{\Omega_s}
\]  

(4.16)

where \(\lambda\) is the effective light wavelength, \(A_D\) is the area of the scattering volume observed by the detector, \(r\) is the distance between the scattering source and the detector, and \(\Omega\) is the solid angle.\(^{23-24}\) Increasing the size of a light collection aperture beyond \(A_c\) may increase the total intensity measured but does not improve the signal-to-noise ratio because temporal fluctuations in the intensity would be averaged out. When the collection area is \(J\) times \(A_c\), \(\gamma_s \approx 1/J << 1\), it results in losing coherence and hence lowering resolution. However, if a light-collection aperture in the setup has the same size as \(A_c\), the correlation of the phase of the light is maintained on the detector. As a result, a high resolution ACF is obtained for more accurate analysis.

### 4.2.2 Raman Intensity and Cross Section

The Raman signal intensity measured at the detector is proportional to the Raman cross section. It is given in units of electrons by\(^{25}\)

\[
S = (P_D\beta D_n K)(\Omega A_D T Q)t
\]  

(4.17)

where \(P_D\) is the laser power density (photons \(s^{-1} cm^{-2}\)), \(\beta\) is the differential Raman scattering cross section (\(cm^2\) molecule\(^{-1}\) sr\(^{-1}\)), \(D_n\) is the number density of the analyte (molecules \(cm^{-3}\)), \(K\) is a geometric factor depending on observation geometry, \(\Omega\) is the solid angle of collection, \(A_D\) is the sample area monitored by the spectrometer or detector (\(cm^2\)), \(T\) is the spectrometer transmission, \(Q\) is the quantum efficiency of the detector (\(e^-\) photon\(^{-1}\)), and \(t\) is the measurement time (s). Equation (4.17) illustrates that a sample with a large Raman cross section produces a high intensity Raman scattering, enhancing the sensitivity of the measurement. Therefore,
\( \beta \)-carotene, which is known for its strong Raman intensity, is used as a test sample in constructing the RCS setup. However, a large Raman intensity does not necessarily mean that it provides a high signal-to-noise ratio. As explained in the previous section, coherence of scattering must be maintained to analyze the intensity fluctuations of the scatters. \( A_D \) must be equal or less than \( A_c \), the coherence area, in order to keep good coherence of the scattered signals.

The value of the differential Raman cross section \( \beta \) may be determined by comparison to that of a reference material with the following equation:

\[
\beta^* = \beta \frac{S^*}{S} \frac{\rho}{\rho^*} (\frac{n^*}{n})^2 \frac{F}{F^*}
\]

where \( \rho \) is the molar concentration, \( n \) is the refractive index, and \( F \) is the sensitivity of the detection system at the scattered wavelength.\(^{26}\) The asterisk symbol (*) indicates the sample parameters, and the non-asterisked parameters correspond to the reference material.
5 Experimental Implementation

In order to investigate the feasibility of RCS as an intensity temporal fluctuation characterization technique, several configurations were constructed in this research. The general setup for RCS is as follows: a monochromatic light laser is used to induce light scattering from sample particles after passing through focusing optics. The focusing optics generally helps to enhance the coherence factor $\gamma$ since a well-focused beam produces the same total scattering power as the unfocused beam does but from a smaller scattering volume. Raman scattered light from the molecules of a sample is then collected by optics and sent through a monochromator. As in Raman spectroscopy, a monochromator is indispensable in the RCS apparatus in order to permit the scattered light only at certain wavelengths to reach the detector. The scattered light is then focused onto a light detector, such as an avalanche photodiode detector (APD) or a photomultiplier tube (PMT). The fluctuations of the detected signals are measured and analyzed by a hardware autocorrelator. In this research, an avalanche photodiode single photon counting module (Perkin Elmer SPCM CD2882) is used for the detection, and the intensity fluctuations of the scattered light signals are collected and measured by an ALV-5000 Multiple Tau Digital Correlator (ALV-GmbH ALV-500/E-Win V.2.7.0 11/99). The laser used in most cases in this research is an Argon laser at 514.5 nm excitation (Spectra-Physics Beamlok 2060).

The obtained data can be analyzed using various methods. In this research, the data is exported to the MATLAB program (in Appendix A) to analyze the ACFs. This program allows a user to define the range of the data set to be analyzed, and with appropriate parameters, the data is fitted to equation (3.13), which defines the exact expression to the second order, by solving the nonlinear least-squares curve-fitting (or data-fitting). This fitting takes the difference between the data and the
exact expression, and determine the parameters that gives the least-squares difference between the data \( g_{\text{data}} \) and the expression \( g_{\text{fit}} \):

\[
\min_x \| f(x) \|_2^2 = \min_x (f_1(x)^2 + f_2(x)^2 + \cdots + f_n(x)^2) \tag{5.1}
\]

where

\[ f(x) = g_{\text{data}} - g_{\text{fit}}. \]

The initial guess for parameters are set according to each data, and the lower and upper limits of the fit parameters are also inputs to the program. With the acquired decay rate of the data, this program calculates the hydrodynamic radius of particles.

In the following sections, the characterization of each part of the apparatus is described. The determination of the various optical systems for focusing and collection to obtain high sensitivity and resolution are also explained.

## 5.1 Instruments

### 5.1.1 Monochromator

As in Raman spectroscopy, a monochromator is indispensable in the RCS apparatus. A monochromator has a movable dispersive element inside, either a diffraction grating or dispersive prism, with slits at the entrance and the exit to limit the diffracted light. By adjusting the angle of the dispersive element, only a narrow range of wavelengths of the incoming light is directed to the exit slit.

A double monochromator consists of two single monochromators connected in series with a mechanical system operating both the gratings to select the same wavelength simultaneously. Since the light is dispersed twice, the double monochromator rejects elastic scattering about \( 10^5 \) times more than a single monochromator.
so it permits very low stray light, allowing low Raman shifts to be observed easily.\textsuperscript{25} However, high stray light rejection generally reduces the sensitivity due to its lower transmission and collection efficiencies. Because the sensitivity is crucial to carry out RCS measurements, a single-grating monochromator is used in all of the setups in this research.

Typically, the single monochromator passes stray light in a region of approximately $10^{-5}$ relative to the transmitted light. However, the intensity of quasi-elastic scattering is $10^4$ to $10^6$ higher than that of Raman scattering, so that the quasi-elastic scattering as stray light masks Raman scattering, especially for low wavenumber Raman emission lines. To minimize this problem when using a single grating monochromator, an edge filter, or a long wave pass filter, is employed to reject the incident wavelength. The step edge of transmission at the incident wavelength, in this case 514.5 nm, enables a better signal-to-noise ratio and detection of short wavelength Raman lines (Figure 5.1). A spectrophotometer (CHEM2000-UV-VIS) with operating software OOIBase 32 from Ocean Optics was used to obtain the edge filter spectrum.

![Figure 5.1: Transmission spectrum of the 514.5 nm edge filter.](image)

To maintain the light throughput maximum, the F-number (f/#) of the input lens and that of the monochromator ideally should match. The typical f/# of a
monochromator is between 3.9 and 6.0.\textsuperscript{25} The f/# of the single monochromator used in this research (Jarrell Ash #82-410 Monochromator) is about 3.4 according to the geometric calculation of the configuration of the monochromator.\textsuperscript{27} In this research, the input optical components are matched and positioned with the monochromator experimentally such that the beam size on the grating mirror of the monochromator is within the size of the grating without underfilling it too much (Figure 5.2). This is done especially for those setups with the single-mode optical fiber input. The SM fiber has very small NA (\(~0.13\)) hence the beam size is adjusted manually.

To determine the transmission rate of the monochromator, the laser power at its entrance slit and at exit slit are measured. The monochromator used in the experiment has 40\% transmission rate.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig52.png}
\caption{Beam spot size on a grating mirror of a monochromator. Figure (a) is too underfilled and (c) is overfilled. The beam spot size should be the size like figure (b).}
\end{figure}

5.1.2 Avalanche photodiode detector

For the signal detection, an avalanche photodiode (APD) is used in RCS as in other light scattering techniques. The APD is a highly sensitive semiconductor electronic device that is used to detect the Raman scattered photons.

By the light absorption, the generation of an electron/hole pair occurs in the semiconductor heterojunction. Because an APD is operated at a high reverse bias, the generated primary free electrons are accelerated by the high electric field, causing
the generation of the secondary free electrons via impact ionization. This multiplies each carrier by the avalanche breakdown, resulting in amplifying the signal before entering an external circuit. The quantum efficiency of the APD depends on the photosensitive silicon layer, but the typical APD has between 80 to 90% efficiency in the response range of 300 to 1050 nm. The quality of the APD used in the experiments was checked during the course of setting up different configurations, since the device could be degraded over time or by exposure to strong intensity of light.

The APD is a digital photocount detection device. Thus, it counts the number of pulses in a given time, or in this research the count rate of the scattered photons $n(t)$. With the sampling interval $\delta t$, $n(t)$ is related to the instantaneous light intensity $I(R_d,t)$ by the following relation,

$$n(t) = \xi \int_{A_p} d^2 R \int_t^{t+\delta t} dt' I(R_d,t') \quad (5.2)$$

where $\xi$ is a constant related to the quantum efficiency of the photocathode. $\delta t$ is the sampling interval. The spatial integration is over the illuminated area of the photocathode $A_p$. The digital photocounter computes the time-autocorrelation function of the measured photocounts that is given by

$$C(t) \equiv \langle n(t)n(t+\tau) \rangle. \quad (5.3)$$

Hence, in the Gaussian approximation for a homodyne experiment,

$$C(t) = \langle n \rangle^2 (1 + \gamma(A_c)e^{-2t/\tau}) \quad (5.4)$$
where $\langle n \rangle$ is the average number of photocounts in the time interval $\delta t$, $\gamma(A_c)$ is the spatial coherence factor depending on the number of coherence areas observed, and the correlation time $\tau$. This is equivalent to equation (3.8) in the Brownian motion case. Because $\langle n \rangle \equiv \xi(\delta t)\langle I \rangle$, or the average number of photocounts in the time interval $\delta t$, it is directly measured from the total count rate. However, $\gamma(A)$ and $\tau$ (or the diffusion rate $\Gamma$ in equation (3.8)) are obtained from the experimental time-correlation function decay time.

### 5.1.3 Raman microscope

In order to substantiate the Raman emission lines obtained by the RCS setups, a Raman microscope (Renishaw RM 1000 Confocal Research Raman Microscope) is used to obtain Raman spectra of samples. The incident laser beam, provided by the 514.5 nm line of an Ar ion laser or the 785 nm line of a diode laser, is focused onto a sample on a stage. Scattering from the sample is gathered through the microscope objective and split from the incident beam direction by a cube beam splitter to a diffraction spectrometer for the Raman spectrum to be recorded by the Renishaw WiRE™ software. The confocal microscope has the ability to measure high resolution Raman spectra due to its small scattering volume and high rejection of stray light.

### 5.2 Materials

#### 5.2.1 Monodisperse Testing Materials

Among a variety of sample solutions, $\beta$-carotene in aqueous solution is mainly used due to its well known strong Raman emissions. $\beta$-carotene powder (Lucarotin 10 CWD G/Y) was prepared by BASF using the carotenoid micronization method to achieve a stable colloidal size. This particular powder can be dispersed in water
as nanoparticles with about 140 nm (± 10) diameter. The strong Raman bands of β-carotene are at 1005 cm$^{-1}$, 1150 cm$^{-1}$, and 1520 cm$^{-1}$; the first shift is from the out-of- and in-phase combinations of the in-plane rockings of the CH$_3$ groups, and the latter two are assigned to C-C stretching and C=C stretching bonds respectively.\textsuperscript{28,30} The C=C bond is especially known for its strong Raman effect.\textsuperscript{31} Figure 5.3 shows the Raman spectrum of β-carotene measured by the Raman microscope for the 514.5 nm excitation.

Ethanol is also used to test the sensitivity and the resolution of the Raman spectrum of each setup. Raman emissions of ethanol are found at 433.5 cm$^{-1}$, 888.3 cm$^{-1}$, 1051.6 cm$^{-1}$, 1095.2 cm$^{-1}$, and 1453.2 cm$^{-1}$.\textsuperscript{27} It also has three C-H vibrational stretching modes at 2877 cm$^{-1}$, 2927 cm$^{-1}$, and 2971 cm$^{-1}$.\textsuperscript{32} All sample solutions are held in a clean plastic or glass cuvette.

![Raman spectrum of β-carotene measured by the Raman microscope. An Ar laser with the 514.5 nm excitation was used.](image)

**Figure 5.3:** Raman spectrum of β-carotene measured by the Raman microscope. An Ar laser with the 514.5 nm excitation was used.
5.2.2 Surface Enhanced Raman materials in RCS

The surface enhanced Raman scattering (SERS) method was considered also to be used in preparation of testing samples. In SERS, when some types of molecules adsorb on metallic surface structures, the compounds show a large enhancement factor for Raman signals by as high as $10^{14}$ to $10^{15}$. Field enhancement is one of the two contributors to the increase in the Raman intensity. When a metal surface is exposed to the incident laser at the appropriate wavelength, the electric field of the incident light is increased at the particle surface, resulting in an increase of the Raman signal. Although the enhancement significantly depends on several variables, the high SERS signal is due to the field enhancement factor. The conditions depend on each substrate; for example, a silver surface provides strong field enhancement when excited in the midvisible wavelength region, such as 514.5 nm, whereas gold and copper surfaces are most effective if excited at longer wavelengths, such as red region wavelengths.

Another enhancement contributor is chemical enhancement. It results from the bond formation, or the charge transfer, of metal and absorbates that increases molecular polarizability. The stronger the charge transfer bond is, the stronger the enhancement. Its increase factor is up to $10^2$.

Since Ar lasers, which have been used throughout in this research, have power control capability and silver is known for high enhancement ability with the Ar laser wavelengths, silver particles were considered to be used as metal samples. To prepare silver colloids, several preparation methods have been reported. The two famous methods are the Creighton method, using borohydrate reduction, and the Lee-Meisel method, using citrate reduction. For example, thiol group chemicals could be used as absorbates for the silver colloids because the SH group in thiol
bonds strongly with the silver surface.\textsuperscript{38}

Although the dramatic increase of the signal is very desirable for the development of RCS, SERS samples could not be used due to the fluctuation of the signals both in time and frequency.\textsuperscript{33,39} Nie’s group has observed and studied that a SERS sample yields its signal intermittently, ranging from milliseconds to seconds depending on the intensity of the incident light and the temperature, and different particles produce slightly different Raman frequencies, which suggests that each molecule is absorbed at a different site of a metal colloid. These inconsistent behaviors of SERS particles would destroy the coherence of the scattered light and therefore cannot be utilized in the development of RCS.

5.3 Optical Configuration

5.3.1 Characterization of the Laser Beam

A laser with the lowest TEM\textsubscript{00} mode is a pure Gaussian beam, and in the free space between optical components such as lenses, the Rayleigh range (also known as the Rayleigh length) and its position describe the beam itself. If $\omega_o$ is the beam waist at focus, the Rayleigh range is the length from the focus to the point where the waist equals to $\sqrt{2}\omega_o$, or where the cross-sectional area doubles. The beam waist hence defines the Rayleigh range. The total angular spread of a Gaussian beam is also defined as $\theta = 4\lambda/2\pi\omega_o$, and it can also be defined in terms of the input diameter of the laser beam $D$ and the focal length of an optical lens $F$ as $\theta = D/F$. That is, the beam waist at the focal spot is

$$2\omega_o = \frac{4\lambda F}{\pi D}. \quad (5.5)$$
With a 1.7 mm diameter Ar laser beam (at $1/e^2$) and a 75 mm focal length lens, the spot size or beam waist diameter would be 28.9 $\mu$m.

The depth of field (DOF) of the beam is twice the Rayleigh length $x_R = \pi \omega_0^2/\lambda$, hence

$$DOF = \frac{8\lambda}{\pi}(\frac{F}{D})^2. \quad (5.6)$$

In the above optical setup, the DOF is 57.8 $\mu$m. That is, if the 90° geometry configuration is used, the collection fiber should be positioned in that range, and the core size of the fiber should be smaller than the DOF to obtain sufficient resolution.

5.4 Challenges in Constructing the RCS Setup

5.4.1 Raman Scattering Intensity

As mentioned earlier, Raman scattering intensity is very weak due to its small cross section. Therefore, RCS is limited to materials that have strong Raman scattering or large cross section. To determine which samples would be useful to try to measure with RCS, the cross sections of several materials were estimated using equation (4.18) in section 4.2.2. The geometry dependence of the cross section was ignored since we were interested only in the obtainable magnitude of the Raman intensity. Since the RCS setup is not specified, equation (4.18) applies to the Raman spectra gained from the Raman microscope measurements. It was also assumed that $F$ and $F^*$ are about the same since the same setup was used. Methanol with a differential Raman cross section of $12.4 \times 10^{-30}$ (cm$^2$ molecule$^{-1}$ sr$^{-1}$) at 2937 cm$^{-1}$ is used as a reference material.$^{26}$

According to equation (4.18) and some assumptions, the differential Raman cross section of 0.4 wt % $\beta$-carotene nanoparticles at 1514 cm$^{-1}$ ($v_{C=C}$) is estimated
Table 5.1: Relative differential Raman cross sections ($\sigma^*$). Each $\sigma^*$ is estimated by equation (4.18). Methanol is used as reference material to estimate the $\sigma^*$ of $\beta$-carotene. The $\sigma^*$ of the rest of materials are measured using the obtained value of $\sigma^*$ of $\beta$-carotene.

<table>
<thead>
<tr>
<th>Material</th>
<th>Raman band (cm$^{-1}$)</th>
<th>$\sigma^*$ (cm$^2$ sr$^-1$)</th>
<th>% with respect to $\beta$-carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$-carotene</td>
<td>1000</td>
<td>$4.226 \times 10^{-25}$</td>
<td>$-$</td>
</tr>
<tr>
<td></td>
<td>1149</td>
<td>$6.152 \times 10^{-25}$</td>
<td>$-$</td>
</tr>
<tr>
<td></td>
<td>1514</td>
<td>$8.451 \times 10^{-25}$</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2668</td>
<td>$5.366 \times 10^{-25}$</td>
<td>$-$</td>
</tr>
<tr>
<td>Al$_2$O$_3$</td>
<td>446</td>
<td>$1.653 \times 10^{-27}$</td>
<td>0.450</td>
</tr>
<tr>
<td></td>
<td>464</td>
<td>$7.398 \times 10^{-27}$</td>
<td>2.013</td>
</tr>
<tr>
<td>TiO$_3$</td>
<td>444</td>
<td>$1.450 \times 10^{-27}$</td>
<td>0.395</td>
</tr>
<tr>
<td></td>
<td>464</td>
<td>$3.724 \times 10^{-27}$</td>
<td>1.013</td>
</tr>
<tr>
<td></td>
<td>915</td>
<td>$1.945 \times 10^{-27}$</td>
<td>0.529</td>
</tr>
<tr>
<td></td>
<td>1821</td>
<td>$1.128 \times 10^{-27}$</td>
<td>0.307</td>
</tr>
<tr>
<td>Polystyrene†</td>
<td>992</td>
<td>$28 \times 10^{-30}$</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>3060</td>
<td>$45 \times 10^{-30}$</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Table 5.1: Relative differential Raman cross sections ($\sigma^*$). Each $\sigma^*$ is estimated by equation (4.18). Methanol is used as reference material to estimate the $\sigma^*$ of $\beta$-carotene. The $\sigma^*$ of the rest of materials are measured using the obtained value of $\sigma^*$ of $\beta$-carotene. †For polystyrene, the absolute Raman cross section of benzene is listed as a reference. 

Therefore, a $\beta$-carotene suspension has a larger Raman cross section than ethanol does, resulting in the emission of a higher intensity of scattered light.

The derived Raman cross-section of $\beta$-carotene is used as a reference value for other materials. Using the counts at Raman bands of each material after background subtraction, relative differential Raman cross-sections are estimated by comparing $\beta$-carotene data at 1514 cm$^{-1}$ that are taken under the same setting. Table 5.1 shows this estimation. Besides the ones that are listed, PVA, PMMA, tungsten carbide-cobalt (WC-Co), Polyethylene glycol (PEG), and titanium nitride (TiN) were also tested but no Raman bands were observed in those materials. Since polystyrene pro-

†This Raman frequency value of $\beta$-carotene is slightly lower compared to section 5.2.1 (1520 cm$^{-1}$) due to the calibration error of the Raman microscope used in this experiment. However, this Raman line is coming from the C=C stretching bond of $\beta$-carotene as in section 5.2.1.
duces the Raman spectrum of benzene,\textsuperscript{41} the absolute Raman cross section of benzene is used as a reference for the Raman cross section of polystyrene.\textsuperscript{40} Some of the results were later used to estimate signal strength for several configurations.

Another challenge of RCS related to Raman scattering intensity is the coherence area of the optical configuration. In order to prevent losing spatial coherence of the Raman scattering, the size of the coherence area must be obtained, but for weak Raman scattering, a smaller aperture size to maintain good coherence is challenging since it results in lowering the Raman intensity monitored by the detector. To counteract the problem, high-efficiency collection optics is essential.

\subsection{5.4.2 Fluorescence Background}

Another complication of RCS is due to background noise. When a material is excited, Rayleigh scattering and Raman scattering are not the only light emission processes of a molecule. Fluorescence could also occur when the incident photon is absorbed and causes electron transitions to a higher electronic energy state in impurities of polluted samples and/or from the surrounding. After a certain lifetime in the excited electronic level, the molecule relaxes to the ground state. It may lose some energy through heat or vibration, or it can emit a photon, which is referred to as fluorescence. Since the emitted photon has less energy than the excitation energy, the fluorescence wavelength is longer than the incident wavelength. The emission line is typically a few hundred cm\textsuperscript{-1} so it is much broader than Raman scattering, which typically has a narrower linewidth of a few cm\textsuperscript{-1}.

In the presence of fluorescence, Raman emission lines are likely to be masked because fluorescence is more intense than Raman scattering since the cross section of fluorescence can be about 14 orders of magnitude larger than that of the Raman scattering.\textsuperscript{33} In RCS, fluorescence signals behave as background noise that interferes...
with the analysis of the Raman signals. As the incident wavelength increases, its energy decreases so that fluorescence that is due to an electronic transition to the excited state becomes less intense relative to Raman scattering, which is a transition to the virtual state. Thus, using longer wavelength laser excitation is one way to avoid fluorescence emission. Limiting the collection volume is another way to avoid fluorescence emission, but this also decreases Raman intensity.\footnote{42}

The fluorescence effect can be taken into account in the data analysis because the incoherence of fluorescence signals affects the coherence factor $\gamma$ of the intensity correlation function of RCS (equation (3.8)).\footnote{13} The ideal coherence factor is unity, and Flammer and Ríčka have shown that in DLS it can be obtained by using a single mode fiber.\footnote{43} Their analysis holds for RCS as well. As they claim, when a single mode fiber is used, the predicted signal transmitted through the fiber to the detector is $J(t) = \mathcal{E}(t)\mathcal{E}^*(t)$ where $\mathcal{E}(t)$ is the amplitude of the single mode that is equivalent to the electric field $E(t)$. The spontaneous light emission signal that RCS detects may be a sum of two independent contributions to the signal amplitude: $\mathcal{E}(t) = \mathcal{E}_R(t) + \mathcal{E}_F(t)$ where $\mathcal{E}_R(t)$ is from Raman scattering and $\mathcal{E}_F(t)$ is from fluorescence emission.\footnote{43} That is, in RCS, the signal $J(t)$ can be expressed as

$$J(t) = J_R(t) + J_F(t) + \mathcal{E}_R(t)\mathcal{E}_F^*(t) + \mathcal{E}_F(t)\mathcal{E}_R^*(t)$$ \hspace{1cm} (5.7)

where $J_R(t)$ and $J_F(t)$ are the signals from Raman or fluorescence emission respectively as functions of time. Both Raman and fluorescence contributions are assumed to obey complex Gaussian statistics, but while the fluctuations in the Raman scattering are much longer than the sampling time of the detector, the fluorescence is incoherent and thus produces fluctuations faster than the sampling time.\footnote{13,15,43} Its emission also occurs at random times. Thus, the property of the fluorescence al-
lows $J_F(t)$ to be replaced with its average $J_F$, and the cross terms with $E_F(t)$ then also average out. With these approximations, the normalized ACF, which is defined as $g_2(\tau) = \langle I(t)I(t+\tau) \rangle/\langle I(t)^2 \rangle$ (the normalized version of equation (3.7)), can be rewritten as in the Siegert relation;

$$g_2(\tau) = 1 + j_R^2 [g_1(\tau)]^2. \tag{5.8}$$

The factor $j_R$ is equivalent to a coherence factor $\gamma$ and is defined as

$$j_R = \frac{\langle J_R \rangle}{J_F + \langle J_R \rangle}. \tag{5.9}$$

Therefore the fluorescence reduces the SNR in a known way and can be accounted for in the data analysis in the fiber optical system. For example, in the single-mode fiber collection setup explained in section 5.5.1, the expected $\gamma$ can be calculated from the obtained Raman spectrum illustrated in figure 5.5. With an ideal apparatus and the measured fluorescence effect, $\gamma \approx 0.45$ at the 558 nm Raman band. If, however, $j_R$ is too small or fluorescence strongly appears, the ACF would become nearly constant, so that the accuracy of the fitting on the decay rate of the particle diffusion would be lost. Therefore, the fluorescence must be minimized experimentally.

5.5 Preliminary Experiment

5.5.1 Preliminary Fiber Optics RCS Setup

Fiber optic coupling to a DLS system has been shown to give a high signal-to-noise ratio. Wiese et al have demonstrated that the multiple scattering effect is negligible to obtain the meaningful ACFs in DLS when an optical fiber with a core size that is smaller than the scattering mean free path in the sample is used, and it can be
applied to a sample with up to 50% concentration. Since Raman scattering is weak, using a concentrated sample is desirable. Therefore, in the preliminary configuration, an optical fiber was used for the collection optics. This fiber optics setup of RCS (FORCS) is illustrated in Figure 5.4. An Ar laser is used as an excitation source. A plano-convex (PCX) lens was placed so that the focal point of the lens was close to the inside wall of the cuvette. The single-mode fiber immersed in the sample was positioned at the side of the wall in the cuvette where it was believed to be in the focused area of the incident beam. The small collection cone of the single mode fiber with a core radius of 2 µm yields a high signal-to-noise ratio. The fiber was mounted on a three-axis flexure stage in order to align it right on top of the focal point of the incident beam. Although intensity was relatively low, the Raman emission lines of β-carotene were observed at 542.5 nm ($\nu_{CH}$), 547.1 nm ($\nu_{C-C}$), and 558.2 nm ($\nu_{C=C}$), which all are the expected wavelength for 514.5 nm excitation (Figure 5.5).‡

For each detected Raman emission line, the intensity ACF was collected for 10 minutes for several runs. Although the data is noisy at shorter decay (lag) time, for all Raman lines of β-carotene, decay was observed in the ACF graphs as shown

‡ In frequency, Raman lines at 542.5 nm, 547.1 nm, and 558.2 nm are 1005 cm$^{-1}$, 1150 cm$^{-1}$, and 1520 cm$^{-1}$ respectively.
Figure 5.5: Raman spectrum of β-carotene measured by the preliminary FORCS setup (with 150mW excitation).

![Raman Spectrum of β-carotene](image)

**Figure 5.6:** ACFs of β-carotene at different Raman lines obtained by the preliminary FORCS setup (with 150mW excitation).

![ACFs of β-carotene](image)

In Figure 5.6. The ACFs from both the $\nu_{C=C}$ line at 558.2 nm and the $\nu_{C-C}$ line at 547.1 nm have almost the same decay while the overall data from $\nu_{CH}$ line at 542.5 nm are more noisy. This is due to its very weak signal that is almost comparable with noise. Although this is not the ideal case, the data seems to show it has a similar decay rate as the stronger Raman lines. Hence, it can be concluded that all data are coming from the same sample. Because each type of bond is from the same molecule, according to the RCS theory the information that each ACF provides should also be the same.
5.5.2 Results and Discussions of Preliminary FORCS Setup

Each of the obtained data set was fit to the normalized nonlinear correlation function according to Frisken using the MATLAB program (Figure 5.7). Because of noise at the short decay time, the data set from the longer decay time range was used for the analysis. The results from the radius calculations using the diffusion coefficients obtained from the fits are shown in Table 5.2.

![Figure 5.7: ACF analysis of Raman line at 558.2 nm (νC=C) of β-carotene obtained by the preliminary FORCS setup.](image)

<table>
<thead>
<tr>
<th>Raman emission line</th>
<th>γ</th>
<th>Γ  (ms⁻¹)</th>
<th>Experimental Radius (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>558.2 nm (C=C)</td>
<td>0.023</td>
<td>0.022</td>
<td>5649.75 ± 521.52</td>
</tr>
<tr>
<td>547.1 nm (C-C)</td>
<td>0.019</td>
<td>0.022</td>
<td>5620.74 ± 585.62</td>
</tr>
<tr>
<td>542.5 nm (C-H)</td>
<td>0.006</td>
<td>0.010</td>
<td>11431.1 ± n/a</td>
</tr>
</tbody>
</table>

Table 5.2: Experimental analysis results of the preliminary FORCS. Radius of β-carotene nanoparticle is calculated from the decay rate (Γ=Dq²) obtained by the fitted ACFs. PDIs of all data were also determined to be very close to zero, suggesting the monodispersity of the sample with the fit.
To verify the resulting radius, the actual hydrodynamic radius of $\beta$-carotene was determined by the DLS technique. A diluted solution of the same $\beta$-carotene sample solution was excited by a HeNe laser, and its quasi-elastic scattering was detected by an avalanche photodiode detector, placed at a 90° scattering angle from the sample. The radius of this sample was determined to be 70±5 nm. Therefore, the obtained radius results from the RCS analysis is too large to be correct, indicating either the fitted equation cannot be applied for RCS or the correlation is not from the intensity fluctuations from the particles.

Although the analysis of the two stronger Raman lines (C=C and C-C bond lines) yielded the similar radius values, the data from the weakest C-H bond Raman line is close to the noise level, so the result of the autocorrelation data may not be reliable. Similarly, the spatial coherence factor of the obtained intensity ACF in this setup was very low ($\sim$0.02-0.03), and noise interfered with data collection. The origin of the correlation cannot be confirmed with only these data. Because better sensitivity and resolution are needed in order to characterize weak Raman scattering, other optical setups were developed and are explained in chapter 6. Analysis of the experimental results and other possibilities of the origin of the autocorrelation are discussed in chapter 7.
6 Experimental Results and Discussion

6.1 Free Space Optics RCS Setup

Since the preliminary experiment described in section 5.5.1 produced a low sensitivity result even though fiber optics was used, instead of the fiber, a free space optics input setup was tested. In this setup, the pinhole is used because it sets the effective coherence area. The schematic of the setup is illustrated in Figure 6.1. As shown in the figure, the scattering volume is determined by the length of the cuvette and the optics of the setup. Since the detected scattering volume extends over almost the entire cuvette-length (1cm), theoretically more scattered photons can be detected and hence higher sensitivity is achieved than in the FORCS setup. A long focal length lens is preferred for an excitation focusing to avoid losing angular definition in the
sampling path length. The lenses are positioned according to the thin lens equation. The monochromator has an f/# of 3.4, but to underfill the grating mirror and thereby avoid the light intensity loss, the input lens combination with an f/# of 6.5 is used.

To adjust the position of lenses, the pinhole, and the APD, the XYZ translators are mounted to each section. To guide the positions of the optical components, a cage mounting system is used.

After repeated adjustments of the position of the optical components of the system were made in order to improve its alignment, the Raman spectra of 0.4 wt % \( \beta \)-carotene aqueous solution and pure ethanol were measured. Even though some Raman emission lines could be observed with a 150 \( \mu \)m pinhole, the Raman spectrum of \( \beta \)-carotene showed considerable fluorescence background (Figure 6.2a). Despite the expectation, the sensitivity decreased as well, compared to the result from the preliminary FORCS experiments. Since Raman scattering intensity of \( \beta \)-carotene is already low, the pinhole size could not be reduced lower than 150 \( \mu \)m, which is too large compared to the coherence area (or coherence length which should be around \( l_c \approx 4.3 \, \mu \)m for \( \beta \)-carotene Raman lines in this setup). The peaks in the obtained Raman spectrum of ethanol match the expected values, but the emission lines from the three C-H vibrational stretching modes around the 2900 cm\(^{-1}\) range (or the 605 nm range in Figure 6.2b) appear as a single peak in this setup. Thus, the spectrum of ethanol illustrates wide linewidth of each Raman emission line with very weak scattering intensity, suggesting low resolution.

The spectra of \( \beta \)-carotene and ethanol depict that both sensitivity and resolution decreased in the free space optics system; that is, the implementation was much less effective than the fiber optics system. Therefore, further measurements were not pursued with this setup.
6.2 Fused Fiber Coupler RCS Setup

The spatial coherence factor $\gamma_s$ of the pinhole setup varies from 0.5 to 0.8 whereas the single mode fiber collection has $\gamma_s$ very close to 1, which is the optimum value. It also has been demonstrated that the fiber optics probe can be used at significantly higher concentration, which is useful for sensitive samples that do not have high scattering intensity, for DLS applications. Therefore, instead of free space optics that relies on a classical pinhole detection method, a fiber optics setup was investigated again due to its high spatial coherence factor and high light-collection efficiency. The use of fiber optics is more promising to obtain a high correlation signal-to-noise ratio for RCS.

The modified version of the FORCS setup employs a fused fiber coupler; hence, both the focusing and collection optics are based on fiber optics. The laser beam is focused into one branch of the fused fiber coupler using a lens. The fused fiber coupler merges two fibers together, and one end of the fiber is used as the emitting and collecting optics simultaneously. Because the fiber is split, the intensity of the
output beam is reduced; however, this type of fiber has been used successfully for the DLS technique applied on concentrated systems. Another end of the fiber is immersed in an index-matching material (Nye curing gel OCK 451) that has the same refractive index as the core \( n = 1.51 \) to prevent backreflected light from entering the monochromator (Figure 6.3). The index material needs to be changed every six months. The detected area is small, as in the preliminary FORCS setup, but this time, the setup has 180° or backscattering geometry. The collected beam through the fused fiber, which is collimated at a fiber coupler, is focused on to the monochromator with a lens. The output optics of the monochromator uses the lens to focus the beam to the detector. This lens and the detector were positioned according to the thin lens equation. To make the alignment of the light path at the output easier, first a multi-mode fiber was used between the focusing and collection optics before the fused coupler fiber was used. The multi-mode fiber provides a much higher signal throughput than the single-mode fiber does, hence it makes easier to find the Raman signal.

A 1wt % \( \beta \)-carotene aqueous solution and a sample of pure ethanol were again tested to acquire their Raman spectra (Figure 6.4). However, the fluorescence light
from the fiber itself, where the peak was around 526 nm, was too strong so that no Raman emissions line could be observed for either sample. Thus, this setup did not show any improvement on sensitivity or resolution at all.

![Raman spectra](image)

**Figure 6.4:** Raman spectra measured by the fused fiber coupler RCS setup. Raman spectra of (a) β-carotene obtained with 29 mW excitation and (b) ethanol measured with 75 mW excitation.

Since a longer excitation wavelength is likely to reduce fluorescence, a diode laser (785 nm wavelength) was also tested as the excitation source in place of the Ar laser. However, the diode laser did not give a sufficiently stable intensity, so it could not be used for the measurement. Although the fiber alignment was very simple once the Raman signal was obtained, due to the unavoidable fluorescence effect, the use of this setup was terminated.

### 6.3 Fiber-to-Fiber Optics RCS Setup

A modified version of the fused fiber optics coupler setup was constructed by following the apparatus that Van Keuren et al. had developed for DLS. Instead of the fused coupler fiber, this setup requires two single mode fibers immersed in a sample solution: one for injecting the incident light and the other for collection optics. Thus, this configuration is named fiber-to-fiber optics RCS (FTFORCS) setup. The
fibers are set perpendicular to each other to construct a 90° observation geometry (Figure 6.5). To have the fiber separation very small (roughly 50 µm), the edges of the fibers are polished at an angle, following the grinding procedure explained by Van Keuren et al. While the position of the excitation fiber is fixed, the collection fiber is mounted on a three-axis translation platform to position the fiber. To align the fibers, the collection fiber is manually positioned such that the count rate reaches a maximum at the incident laser wavelength. The laser beam was focused through the excitation fiber using a microscope objective lens (10X, NA = 0.25). A lower magnification objective that matched with the NA of a single mode fiber (~0.13) would work better for coupling, but an objective lens with sufficient working distance that was available was used to be coupled with the fiber attached to the adapter. This focusing optics reduces the laser beam intensity at the sample. The laser transmission of the objective lens was 28%. A quality check was done for each fiber for its transmission.
rate and the output beam quality. The transmission rate of the single mode fibers was 26 to 50%. This is a disadvantage of the setup, but with such a fiber assembly, the scattering volume will be small so that the multiple scattering effect is minimized.

In an experiment done by Van Keuren et al. for DLS, a 100 kHz count rate of quasi elastic scattering from a 94 nm polystyrene latex dispersions at weight fraction 20% was obtained with a HeNe laser excitation (∼5 mW) and photomultiplier tube (PMT) detection. In our apparatus, an Ar laser is used as an excitation source, which has more than 20 times higher power than a HeNe laser. For detection, using an APD instead of a PMT yields an increase in the quantum efficiency of the detector by a factor of 4. Since Raman scattering is weaker than quasi-elastic scattering by a factor of 10⁻⁴, the ratio of the count rates of Raman to quasi-elastic scattering using this setup would be about 2% according to the ratio from equation (4.17). As a result of this higher power excitation and more efficient detection, it is expected to have approximately a 2 kHz count rate for β-carotene Raman scattering, so high sensitivity may be achievable.

In the setup, several multi-axis translator stages were used to optimize the beam alignment. The laser beam must go straight through the fiber cable to prevent power loss, and the collection fiber should be positioned in such a way that the scattered beam is guided to the monochromator. If the beam is not aligned perfectly, Raman lines are not detected by the APD. Furthermore, the two fiber alignment requires extra care in each experiment because it is very sensitive to any vibration from the environment or from the manual positioning and adjustment. The stages can drift mechanically, so the setup often needs to be tightened time to time. Also, since the two fibers are immersed in a solution, both fibers need cleaning periodically with isopropyl alcohol (IPA) and sonication. Otherwise sample particles could attach to the end face of the fiber core causing dispersion of the output beam. Therefore,
each experiment requires careful alignment of the fibers in this setup. This was one of the drawbacks of the setup and made it difficult to observe the Raman spectra due to the need for optimum alignment of multiple components.

Before the use of the single mode fiber for excitation and collection optics, multimode fibers were used to test the setup, since this type of a fiber allows higher transmission rate of the beam compared to the single mode as mentioned earlier. Since the edges of the fibers needed to be grinded and polished, one side of the fiber was stripped and epoxied into a stainless steel tube, and the other side was coupled to a standard FC type connector.

The efficiency of the monochromator was tested after the input optical system was constructed using quasi-elastic scattering detection. It was determined that, in this setup, the monochromator efficiency was reduced from 40% to 20%. This implies that the laser alignment was not the most effective. The difficulty of the alignment resulted in the setup not reproducing the same result each time since the alignment had to be adjusted each time when the fiber was cleaned.

Nonetheless, with a 0.5 wt % $\beta$-carotene solution, a Raman spectrum was obtained and the main three Raman lines of $\beta$-carotene were observed, as shown in figure 6.6. The ACF of the line was analyzed for a 10 minutes run. Due to the low over-all intensity of the sample, only the strongest Raman line at 558.2 nm from the C=C stretching bonds seemingly yields the autocorrelation decay. The fiber alignment, which was manually done, often required readjustment to ensure the maximum intensity is detected. Since the alignment was very sensitive, perfect alignment of fibers may not be obtained in each experiment. Due to this difficulty of the implementation, each autocorrelation obtained with this implementation shows slightly different quality (Figure 6.7). However, all data seem to have a similar decay rate. Detailed discussion of these results is continued in section 7.1.
Figure 6.6: Raman spectrum of $\beta$-carotene measured by the FT-FORCS setup (with 110mW excitation).

![Raman spectrum of $\beta$-carotene](image)

Figure 6.7: ACFs of Raman line at 558.2 nm ($\nu_{C=C}$) of $\beta$-carotene obtained by the FT-FORCS setup. Each data was taken after readjustment of the fiber alignment. Orange data is using 0.1 wt % concentration and both blue and green data are using 0.5 wt % concentration. Although all data is for the same sample at different concentration, the ACFs did not reproduced the same decay due to the need for fiber cleaning after each run as well as the difficulties of the fiber alignment.

![ACFs of Raman line at 558.2 nm](image)
6.4 Modified Fiber Optics RCS Setup

After reconsideration of several possible configurations for RCS, I decided to modify the very original setup with free space excitation and the single-mode fiber collection. An advantage of using this setup is that the collection angle can be easily changed. By using free optics for focusing, the setup would not suffer extreme incident power loss like the previous setups with fiber excitation. However, because of the use of fiber optics for the collection optics, fiber losses are still unavoidable and the alignment can be tedious since the collection fiber must be positioned very close to the location of the excitation beam entering the solution.

Figure 6.8: Schematic of the modified FORCS setup (L1, L4 = achromatic lenses, L2 = ball lens for fiber coupler, L3 = microscope objective lens, FO = grinded fiber).

To build this setup, an achromatic lens was used for laser focusing to the sample to limit the effect of chromatic aberration in case different excitation wavelengths are used in the future. The laser beam focused on the inner edge of the glass sample cuvette where the polished single-mode fiber is positioned at a 90° angle. To control the position of fiber collection, the fiber cable was attached to a XYZ translator. The other end of the fiber was directly attached by a customized fiber coupler to the monochromator via a microscope objective lens. To control the NA matching
of the input system to the monochromator, the objective lens was manually aligned by observing the beam size at the grating mirror as explained in section 5.1.1. The schematic of this setup is shown in Figure 6.8.

For this setup, the transmission ratio of the apparatus is measured to check the quality of the setup. Using a quasi-elastic light scattering signal, the signal intensity obtained with this RCS setup is compared with that of a simple DLS setup. For both cases, the 514.5 nm Ar laser line is used for excitation, and the single-mode fiber is immersed in the same sample (0.1 wt % β-carotene) against the cuvette wall in such a way that maximum signals are measured. For the DLS setup, the collection fiber is directly attached to an APD with a fiber adaptor, and for the RCS setup, the collected beam passed through the input focusing lens, the monochromator, and the output focusing lens to the APD. The characterization shows that the modified FORCS setup has 18% transmission compared to the DLS setup. The transmission rate at each component of the setup is also measured to the check the quality of the APD by directly collecting laser light with the collection fiber.

The characterization of the setup is also done by comparing the above result with the same setup, except with no fiber immersion in the sample. The laser beam that is focused on to a fiber is directed to the monochromator through the microscope objective lens and to the detector instead of measuring the scattered light. This system yields 25% transmission, close to the above result.

The estimated Raman signals for this setup are obtained using equation (4.17). The solid angle Ω for the fiber is defined as

$$\Omega = \frac{\pi a^2}{4\pi d^2} = \left(\frac{a}{2\omega_0}\right)^2$$  \hspace{1cm} (6.1)

where \(a\) is the single mode fiber radius, and \(d\) is the height of the acceptance cone.
and in this case it is assumed to be the beam waist $\omega_0$ at the focal point for ideal detection. The geometric parameter $K$ is the path length, which can be defined as the base diameter of the acceptance cone of the fiber,

$$K \approx 2a + 2d\theta_m$$  \hspace{1cm} (6.2)

where $\theta_m$ can be obtained from the numeral aperture definition, $NA \approx \theta_m$, for the single mode fiber. Using the estimated Raman cross section in Table 5.1, the signal strength for several materials is roughly estimated (Table 6.1). In the ideal setting, this setup should be able to obtain a $\beta$-carotene Raman signal from C=C bonds on the order of 10 kHz at 10mW input power.

<table>
<thead>
<tr>
<th>Material</th>
<th>Raman band ($cm^{-1}$)</th>
<th>Estimated Raman signal (kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$-carotene</td>
<td>1514</td>
<td>16.90</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>3060</td>
<td>0.01</td>
</tr>
<tr>
<td>Al$_3$O$_2$</td>
<td>464</td>
<td>0.34</td>
</tr>
<tr>
<td>TiO$_2$</td>
<td>464</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Table 6.1: Estimated Raman signals for the modified FORCS setup for different materials (at 10mW incident power for 1% concentration).

However, when a Raman spectrum for a 0.25 wt % $\beta$-carotene aqueous solution was obtained, the strongest C=C bond Raman line only had 0.65 kHz with 0.25 kHz background noise at 128 mW. Even considering the concentration and the incident power differences, this is much lower (approximately 100 times) than the estimated value, and even though no fluorescence background is observed with this configuration, the count rate is too low to obtain an ACF. The direct collection input to the monochromator (without NA matching with the objective lens, which is the same configuration as the preliminary FORCS setup) was also performed with ex-
pectation of a higher transmission rate. Although the Raman lines are observed for β-carotene, the intensity was only slightly higher (0.71 kHz) and no correlation was observed. Since the signal strength calculation assumes the perfect condition of the alignment with the maximum detection probability, the difficulty of detecting weak Raman signal demonstrates the necessity of precise alignment to attain the signal at the estimated order of magnitude.
7 Feasibility of RCS

In spite of the careful construction of several implementations of RCS, no satisfactory signals were obtained. In this chapter, the obtained RCS data are analyzed to determine the feasibility of RCS according to the theory discussed in chapter 4, and possible causes of the autocorrelations observed are discussed.

7.1 Analysis of RCS Theory Using Experimental Data

Among the several implementations of RCS, only the FTFORCS setup was capable of producing good autocorrelations. Hence, the data obtained was carefully analyzed. Compared with the result from the preliminary FORCS configuration experiments in section 5.5, the coherence factor seems reduced by less than half and much noisier, as shown in Figure 7.1. Noise is partially due to the lower resolution of the implementation since the Raman spectra (Figure 6.6) had noticeable fluorescence background. If the autocorrelations were from the intensity fluctuations of scattering particles as predicted, this could also partially be due to the more precise fiber alignment that is required in this configuration than the preliminary FORCS configuration, since fibers are used for both the excitation and the collection optics. However, the light collection efficiency decreases with an increase of the excitation and the observation beam size since the beam divergence determines the solid angle of the light collection. If the fibers are aligned perfectly.

The fit analysis, however, yields the same decay rate as the preliminary FORCS configuration did as shown in Table 7.1. I calculated the hydrodynamic radius to be 5 to 10 µm, much larger than the known radius of the particles. Although the concentrations of the samples are slightly different, the ACF of two data should give a
Figure 7.1: ACF analysis of Raman line at 558.2 nm ($\nu_{\text{C=C}}$) of $\beta$-carotene obtained by the preliminary FTFORCS setup. (a) is 0.5 wt % concentration sample and (b) is 0.1 wt% concentration. The fiber alignment was done before each data.

![Figure 7.1: ACF analysis of Raman line at 558.2 nm ($\nu_{\text{C=C}}$) of $\beta$-carotene obtained by the preliminary FTFORCS setup. (a) is 0.5 wt % concentration sample and (b) is 0.1 wt% concentration. The fiber alignment was done before each data.](image)

Table 7.1: Experimental analysis results of the FTFORCS setup. Two RCS data corresponds to data in Figure 7.1. PDI of both data was close to 0, suggesting the monodispersity of the sample with the fit.

<table>
<thead>
<tr>
<th>Data</th>
<th>$\gamma$</th>
<th>$\Gamma$ (ms$^{-1}$)</th>
<th>Radius (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>0.0092</td>
<td>0.021</td>
<td>5534.38</td>
</tr>
<tr>
<td>(b)</td>
<td>0.0048</td>
<td>0.010</td>
<td>11660.33</td>
</tr>
</tbody>
</table>

similar result since the diffusion rate is not concentration dependent at this level. It is interesting to note, however, that the data in Figure 7.1a is fitted better than data in 7.1b. Prior to measurement, the fiber alignment was done each time to obtain a stronger signal for the analysis. Although the experimental data is steeper than the fit, the data in Figure 7.1a shows a relatively better fit than the other since there is less noise. This suggests that the system alignment of data in Figure 7.1a was better than that of data in Figure 7.1b. Their fit results of the diffusion rate $\Gamma$ also resemble the data from the stronger C=C Raman line and from the much weaker and noisier C-H Raman line in the preliminary experiment. Hence, the two different configura-
tions produced results that are in agreement. To determine if the correlation signals are certainly coming from Raman scattering of $\beta$-carotene, the ACF measurements were also taken at wavelength slightly off (a few cm$^{-1}$ off) from the Raman line peak. The experiments showed no correlation in intensity fluctuation as expected, hence it can be confirm that the ACFs observed at the Raman line are from the Raman scattering of $\beta$-carotene.

However, because the coherence factor is much lower for the RCS measurement compared to the DLS measurement (Figure 7.2), the correlation seen in Figure 7.2a could be the noise from the laser itself. To investigate this, the ACF of the Ar laser at 514.5 nm is measured directly. With the laser line filter, the fiber coupled APD is placed right in front of the laser beam. So as not to damage the sensitive APD, the laser power was cut down to 8.15 $\mu$W with ND filters. As shown in Figure 7.3, at the lag time when the correlation decay was observed in the RCS measurement (around 50 ms), there is no decay for the laser test. The correlation of the laser noise is random as expected, and in general, it is constant and very close to zero. Hence, we can conclude that the ACF observed in Figure 7.2a is not from the laser noise.

![Figure 7.2: ACFs of a 0.5 wt % $\beta$-carotene solution by the FT-FORCS setup for RCS and DLS measurements. (a) is a RCS measurement observed at 558.2 nm ($\nu_{C=C}$) and (b) is a DLS measurement observed at 514.5 nm.](image-url)
However, the decay times between the RCS measurement and the DLS measurement were different for the same setup (Figure 7.2). Since the wavevector factor $q^2$ is only slightly different between the two techniques, the diffusion rates $\Gamma$ should not be different in the order of the magnitude, as shown in Figure 7.4. Hence, the decay rate difference in Figure 7.2 suggests that our analysis in terms of ACFs similar to that of DLS may not be correct.

The radius calculations demonstrate that the RCS theory predicted in chapter 4 does not provide an accurate diffusion measurement. However, the observed autocorrelations from the $\beta$-carotene solution sample do not prove that they are definitely not the intensity fluctuations from the diffusing particles either. Low signal intensities
that were obtained in the experiments could be producing deceiving results since the data were close to the noise level. An optical system configuration that gives higher sensitivity and resolution, which would require better instrumentation, would better reveal the feasibility of RCS as a variation of DLS. However, if the phase factor as described in equation (4.15) and as discussed in section 4.1 would affect the autocorrelation, no correlation should be observed since incoherence of the phase of Raman scattering should average the correlation to zero.

7.2 Fiber Backscattering Optics RCS Setup

One possible way to improve the sensitivity and more definitively confirm the feasibility of RCS would be by using another type of fiber excitation/collection configuration. Unlike the FTFORCS setup, this configuration uses two fibers aligned almost next to each other in such a way that collected signals would be close to backscattering as shown in Figure 7.5. This arrangement has been reported to be an effective setup in a number of DLS instruments.\textsuperscript{52–53} Although the input power will be limited compared to the free space optics as already demonstrated with the FTFORCS setup, unlike the modified FORCS setup where the fibers are aligned perpendicularly, the detected volume in this implementation will be larger because of near the backscatter configuration. Since the beam size would be tighter than in the modified FORCS setup, its solid angle would become larger, so that the signal sensitivity could improve by more than a factor of 10. The sensitivity of this configuration would enable a wider range of materials to be characterized. Table 7.2 lists the

\textbf{Figure 7.5:} Schematic of the fiber backscattering setup.
<table>
<thead>
<tr>
<th>Material</th>
<th>Raman band (cm(^{-1}))</th>
<th>Estimated Raman signal (kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-carotene</td>
<td>1514</td>
<td>186.0</td>
</tr>
<tr>
<td>Al(_3)O(_2)</td>
<td>464</td>
<td>3.72</td>
</tr>
<tr>
<td>TiO(_2)</td>
<td>464</td>
<td>1.86</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>3060</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Table 7.2: Estimated Raman signal for the fiber backscattering optics RCS setup for different materials (at 10mW incident power for 1% concentration sample).

The estimated signals for this all-fiber setup are based on the calculated collection efficiency and the measured Raman cross-section from different materials in the similar manner used in the signal estimation for the modified FORCS setup.

Although preparing and aligning fibers immersed in a sample is challenging, as in the FTFORCS setup, this configuration has an advantage of automatic spatial coherence due to its near backscatter configuration, hence better resolution if single-mode fibers are used for both excitation and collection.\(^{50}\) The successful construction of this configuration could produce sufficient Raman intensity, which would contribute to obtaining better autocorrelations of the intensity fluctuation. Therefore, it could verify RCS’s versatility as a variation of DLS if the phases from different Raman molecules do not destruct the autocorrelation.

### 7.3 Data Analysis in Terms of Number Fluctuations

Another possible explanation of the observed autocorrelation is that it could be due to number fluctuations of particles. That is, instead of following the theory of DLS, RCS could be working based on the principles of fluorescence correlation spectroscopy (FCS) as reported by Schrof et al.\(^{18}\) In FCS, the temporal evolution of fluorescent signals from the particles in the observation volume is analyzed.\(^{54}\) For fluctuations \(\delta F(t)\) of fluorescence signal \(F(t)\), the normalized autocorrelation function
is defined as

\[ G(\tau) = \frac{\langle \delta F(t) \delta F(t + \tau) \rangle}{\langle F(t) \rangle^2} \]  

(7.1)

where \( \delta F(t) = F(t) - \langle F(t) \rangle \) and \( \delta F(t + \tau) = F(t + \tau) - \langle F(t + \tau) \rangle \).

The typical FCS measurement uses a confocal microscope configuration with a high numerical aperture objective, where the observation volume is the prolate ellipsoid that is determined by the objective and the diameter of the confocal pinhole. Schrof et al. used scattering from the C=C bond Raman line of \( \beta \)-carotene to test RCS with the confocal optics setup. With a viscosity dependence test, they demonstrated that the FCS theory seems to apply for their data although the experimental data is always steeper than the best fit from equation (7.2) and the sensitivity of the configuration is problematic too, as in our case of RCS as a variation of DLS.

It has been recently demonstrated that a single-mode fiber can be used as an excitation/collection optics in place of the traditional confocal setup for FCS.\(^{55}\) Balaji et al examined and demonstrated that the single-mode fiber has similar spatial resolution as the confocal detection.\(^{56}\) Although the high background fluorescence from the fiber limits the sensitivity and the fluorescence intensity detected by the fiber-optics implementation is low, Garai’s group demonstrated the potential of such implementation in detecting aggregate particle sizes of 100 nm or greater.\(^{57}\) Therefore, the correlation observed in our fiber optics setup could possibly be due to number fluctuations as in the FCS case. To investigate such a possibility, several approaches were considered.

In fiber optics FCS, because the focused beam still is larger than the geometry of photon collection by a fiber, the diffusion along the longitudinal direction may be neglected. The same should apply to the fiber optics RCS configurations. Assuming the observation volume is approximated by a Gaussian volume extending out from
the fiber, in the 2 dimensional model, the autocorrelation function \( G(\tau) \) then becomes

\[
G(\tau) = \frac{1}{N} \left( 1 + \frac{\tau}{\tau_D} \right)^{-1}
\]

(7.2)

where \( N \) is the average number of particles in the observation sample volume, and \( \tau_D \) is the diffusion time related to the diffusion coefficient \( D \) and the observation volume with axial dimension or the beam radius \( w_0 \), \( \tau_D = w_0^2 / 4D \).\(^{55,58-60}\) For spherical fluorescent particles undergoing Brownian diffusion, \( D \) is defined by the Stokes-Einstein relation (equation (3.14)).

With previously obtained correlations of the \( \beta \)-carotene dispersion sample using the FTFORCS configuration, equation (7.2) was used in the modified version of the MATLAB analysis for DLS-like data fitting given in appendix A to determine if the correlations were from the number fluctuations of particles. Using the hydrodynamic radius of \( \beta \)-carotene nanoparticles obtained with DLS (\( \sim 70 \) nm), \( D \) was calculated to be \( 3.49 \times 10^{-12} \text{ m}^2/\text{s} \). With the least-square curve fitting method, the MATLAB program determined the values of \( N \) and \( \tau_D \) and calculated \( w_0 \) from the fit result.

The fit plots are shown in Figure 7.6, and the analysis results are shown in Table 7.3. Since data (a) is from a more concentrated sample than data (b), \( N \) of (a) should be larger than (b) if the autocorrelations were from the number fluctuations. However, the quality of data is low due to the noise, so no conclusion can be made with the result even though the fitted value of \( N \) is realistic. Also, the calculated value of \( w_0 \) at \( \sim 0.5 \mu \text{m} \) is smaller compared to the manufacture-supplied mode field radius, \( 1.7 \mu \text{m} \). Although a definite conclusion cannot be made based on this analysis, it shows that the ACFs obtained from the experiments could be due to the number fluctuations.
Figure 7.6: Number fluctuation analysis of β-carotene at the 558.2 nm Raman line ($\nu_{C=C}$) obtained with the FTFORCS setup. The experimental ACFs are from (a) 0.5 wt % sample and (b) 0.1 wt % sample.

![Graphs showing ACFs for different concentrations of β-carotene](image)

Table 7.3: Results of the number fluctuations analysis for the β-carotene data obtained with the FTFORCS setup (Figure 7.1).

<table>
<thead>
<tr>
<th>Data</th>
<th>Sample Concentration</th>
<th>N</th>
<th>$\tau_D$ (ms)</th>
<th>$w_0$ (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>0.5 %</td>
<td>107</td>
<td>14.061</td>
<td>0.428</td>
</tr>
<tr>
<td>(b)</td>
<td>0.1 %</td>
<td>160</td>
<td>18.965</td>
<td>0.514</td>
</tr>
</tbody>
</table>

Another way to test the applicability of FCS theory on fiber optics RCS is to compare the number of particles $N$ calculated from the concentration of the sample and that from the magnitude of $G(0)$ from the data fitting analysis. Although the background noise affects the accuracy of the analytical value of $N$ from $G(0)$, which is the case of our data, the order of magnitude of the particle number can be estimated. In terms of concentration $C$, $N = CV$, where $V$ is the effective observation volume. For the β-carotene sample used in data (a) of Table 7.3, $C = 5.67 \times 10^{-18}$ particles/m$^3$. Using the definition of the fiber observation volume that Balaji et al estimated,$^{55}$ $V = 150 \mu$m$^3$, the estimated number of particles is $N = 851$. The actual observation volume of our setup would be smaller than the case of Balahji’s work, because the free
optic focusing is used for the excitation and the probability of detecting the signal within the acceptance cone of the fiber would presumably be lower for our case. The smaller volume leads to a lower \( N \), so that the actual \( N \) in this configuration could be closer to the analytical value of \( N \) from the data fitting, which is determined as \( N = 107 \). The values are roughly a similar order of magnitude, based on the crudeness of the analysis. However, the accuracy of the value cannot be established with this method.

If the correlations from Raman scattering were easy to obtain, changing the condition parameters such as concentration, viscosity, and temperature, could be useful to investigate the fiber optics RCS configuration for its possibility as an FCS-like implementation. However, since sensitivity and resolution are the issues of RCS, the preliminary fiber optics RCS configuration was tested with a fluorescence dye, Rhodamine 6G (Acros Organics), which is often used to calibrate FCS. With several concentration samples (10\(^{-7}\) M, 10\(^{-8}\) M, and 10\(^{-9}\) M), the fluorescence signal was examined for its autocorrelation at 556 nm, near the fluorescence emission peak, at 150mW excitation power of the Ar laser at 514.5 nm for a 10 minutes run. Rhodamine 6G has \( D = 2.8 \times 10^{-6} \text{ cm}^2 \text{s}^{-1} \), \(^{59}\) and if RCS functions as FCS, the correlation should show such decay. However, no meaningful autocorrelations were observed. Although fluorescence was detected, the background noise, presumably from the collection fiber itself is reducing the sensitivity dramatically so that the autocorrelation of the fluorescence signals could be masked.

Although Garai et al have demonstrated the potential of backscattering fiber optics FCS, our configurations of RCS involve the fiber optic collection configuration with the separate fiber optic focusing of the excitation beam, so that the observation profile is different. From the data obtained from the FTFORCS setup, it could not be demonstrated that this implementation could function as a variation of FCS.
8 Conclusions and Future Research

In this part of this dissertation, the possibility of using temporal fluctuations of Raman scattering intensity to characterize nanoparticles in a dispersion was investigated. Raman scattering is a unique identifier of molecular bonds. Thus, using Raman scattering, with its property of chemical specificity, RCS would enable one to obtain dynamics of individual chemical species of colloidal systems providing a detailed picture of both simple and complex systems. It would also enable the determination of colocalization of two or more species within single nanoparticles. RCS has great potential to serve in a wide range of fields as a variation of dynamic light scattering (DLS) or possibly as a variation of fluorescence correlation spectroscopy (FCS).

However, for RCS to produce meaningful temporal fluctuations of Raman scattering, Raman scattering must be coherent. Raman scattering is a coherent process, but each molecule could have a different phase when it scatters. I have shown that if the phase fluctuates rapidly in time, it would destroy temporal coherence of the intensity fluctuations that the correlation requires. On the other hand, if the coherent property of Raman scattering is maintained in the intensity fluctuations, RCS would be possible with a similar apparatus as for DLS, which generally has a simple setup. The RCS theory in this dissertation is derived based on the assumption that the Raman scattering phase would not affect the autocorrelation of the intensity fluctuations; thus, several configurations were implemented to test the theory.

Due to the low efficiency of Raman scattering, the sensitivity and resolution in the RCS instrumentation is very crucial for the intensity correlation analysis and for the investigation of the possibility of this technique. While a larger detection area allows one to obtain a higher intensity of Raman scattering, this area needs
to be minimized to maintain spatial coherence, which enables us to obtain a good correlation function. Therefore, maintaining the balance between the detection area and coherence area is a key in constructing a setup that optimizes both parameters since signal strength and coherence are inversely proportional to each other. Even though the manual alignment of the system components requires extreme precision and so could be very difficult, Raman spectra from a β-carotene nanoparticle dispersion sample were obtainable with different implementations.

From the β-carotene Raman scattering lines, some correlations were observed with low coherence, but no ACFs that match the prediction were obtained. It is confirmed that these correlations are linked to the individual Raman emission line of a sample species because no correlations were observed at the non-Raman emission wavelength. Therefore, RCS does yield a correlation from Raman scattering even with low coherence but at longer decay times compared to DLS-like measurement. The discrepancy between the experimental results and the theoretical derivation of the ACF based on the DLS theory could be due to the sensitivity and resolution issues of the current implementations. The correlations that are obtained in this project are close to the noise level. Thus, the improvement in sensitivity and resolution in the implementation will help obtaining higher Raman signal and thereby a better signal-to-noise ratio. Employing better apparatus, such as a higher power laser and a monochromator with a higher transmission efficiency, could improve the coherence factor and confirm the potential of RCS as a variation of DLS. The cross correlation technique similar to two-color dynamic light scattering (TCDLS)\textsuperscript{61,62} could also be integrated to RCS. In this type of correlation technique, for the DLS measurement, two beams are focused into a sample at an appropriate small crossing angle, and the cross correlated scattering signal is analyzed. This correlation is due to only the singly scattered light. Segré et al. successfully demonstrated that TCDLS can be
applied to turbid samples, where strong multiple scattering is present. With the RCS instrument, two distinct Raman bands of a sample could be measured simultaneously and the photon count signals cross correlated. When the corresponding bonds are both located in the same nanoparticle, the fluctuations in the signals would be correlated. If the two species are diffusing in separate particles, no correlation would be measured. This would have several effects. One would be to give further specificity by simultaneously observing two distinct bonds. Another would be to suppress multiple scattering, as it does in the case of DLS. Finally, cross correlation would help avoid the drop in single-to-noise ratio due to fluorescence. Since the fluorescence signals will be completely uncorrelated, they would no effect on the cross correlation. Carrying out cross correlation would require splitting the output and sending it through two monochromators/APDs. This technique would be especially useful for multi-component particles, such as nanocomposite particles, to investigate their properties.

If the coherence cannot be improved with a better implementation, this could imply that the correlation of the scattered photons is affected by a phase change of the Raman scattering from individual molecules. If the phase change were rapidly occurring, the decay could be happening faster than the detector can observe the change. Thus, a much faster response detector could be used to support the understanding of the photon correlations in RCS. In addition, the phase behavior of Raman scattering from a molecule is not well studied. If the behavior of Raman scattering phase change could be well established, an appropriate description could be applied to the theoretical derivation of an intensity ACF for RCS by taking into account the phase factor, resulting in more accurate prediction of the intensity fluctuation. Therefore, the theoretical research in the Raman scattering behavior of a molecule would support the development of RCS.
However, from the data I obtained, the most likely possibility is that the correlations that were observed could be coming from the number fluctuations from the particles in the detected area defined by the fiber. To further test this hypothesis, there are number of experiments that can be conducted by changing the sample conditions. For example, by changing the viscosity of the solvent, the resulting ACFs would reveal whether the correlation is coming from particle diffusion or not. If nanoparticles are in a more viscous environment, they diffuse slower; thus, if the correlation were from the number fluctuations, the diffusion coefficient would be lower. In this case, the y-intercept of the ACF should remain the same as in the case of a less viscous environment because the intercept should be inversely proportional to the number of particles in the sampling volume. Similar tests can be done by changing other parameters of the environment, such as temperature, which the diffusion coefficient is proportional to, and the hydrodynamic radius of the sample. Because the number fluctuation analysis requires the hydrodynamic radius to be known, a sample preparation method that prepare nanoparticles in different sizes from the same species would be useful in this testing.

Even though this research did not fully established the source of the correlations, the fact that there is a correlation observed provides some promise for RCS as a characterization method. Although understanding the origin of correlation will require more study theoretically and experimentally, I believe that the improved setup would help establish the technique, which could be an important addition to the nanoparticle characterization techniques for studying colloidal dispersions.
Part II
Development of Multi-component Nanoparticles Using the Reprecipitation Method

9 Introduction

Room temperature solution-based synthetic methods have been an important option for the production of a wide range of nanomaterials. Composite nanoparticles produced by such methods can contain multiple chemical species or structures with different functionalities or with cooperative properties of constituents. Raman correlation spectroscopy (RCS) could be useful for studying such multi-component nanocomposites because of its ability to selectively characterize a specific component within complex mixture. RCS would yield the same diffusion coefficients from the Raman bands of different species that are within a multi-component material since both of the species form the material. To demonstrate such ability of RCS, strategies for the creation of multi-component nanocomposite particles are explored as the second part of this dissertation.

This part explains the development of such a strategy and models for predicting the ability of individual components to form multicomponent nanoparticles. One method of synthesis is a reprecipitation method in which nanoparticle nucleation and growth is induced by the rapid injection of a molecular solution into a miscible non-solvent. This method has been studied for single-component particles for more than three decades, but it has not been extended to multiple components. Here, we demonstrate that the method can be extended to multicomponent nanopar-
articles by synthesizing nanocrystals of the charge transfer complex perylene:TCNQ (tetracyanoquinodimethane). The conditions for multicomponent nanoparticle formation are investigated, and Hansen solubility parameter theory is applied to model the reprecipitation method for a multicomponent system. Interesting observations on the formation process of TCNQ nanocrystal structures are also discussed in a later chapter. This research would be useful to a wide range of fields due to the multi-functionality that nanocomposites could posses. §

§ Some of the contents of this part of the dissertation has been published in CMC (Computers, Materials, & Continua). 53
10 Background on Nanoparticle Formation

10.1 Nanomaterial Synthesis

Nanomaterials synthesis has progressed beyond the creation of single component, monofunctional substances. Complex multifunctional materials are being developed, for example, nanoparticle drugs that contain surface receptors for targeting and contrast agents for imaging.\textsuperscript{64,65} Multiple component nanoparticles can also be important in enabling the combination of species that do not exist in isolated form, for example, the endohedral fullerene \( \text{C}_{80}:\text{M}_3\text{N} \) (\( \text{M}=\text{transition metal} \)); neither \( \text{C}_{80} \) nor the trimetallic nitrides are stable on their own.\textsuperscript{66}

In order to create specific particle architectures that are formed by certain interactions of molecules, molecular self-assembly and self-organization have been extensively looked at as a useful means of creating nanoparticles and other nanomaterials.\textsuperscript{67} In these methods, a detailed understanding of interactions at the molecular level is needed in order to be able to predict the success of specific processing as well as tailor the properties of the resulting materials. These interactions can be in many forms: van der Waals, covalent, hydrogen bonds etc. To further complicate matters, the strength of these interactions can depend strongly on many variables such as concentration, temperature, \( \text{pH} \), polarity, surface modification, solubility etc.

The challenge is to develop general methods for making nanoparticles that enable a broad range of different types of multiple components to be combined in a predetermined way. In addition, there should be a straightforward way to scale the method up to production of industrial quantities. Along with this is the need for a better understanding of the relation between the molecular properties of the individual components, the specific synthesis and processing parameters and the final
bulk material properties, so that materials can be engineered to yield desired functions. Among numerous synthetic methods, the formation of nanoparticles using the reprecipitation method\textsuperscript{68} is studied and developed in this research.

### 10.2 Reprecipitation Method

The reprecipitation method\textsuperscript{68} is also known as solvent shifting\textsuperscript{69}, micronization\textsuperscript{70}, or drowning out crystallization\textsuperscript{71}. In this method, nanoparticle nucleation and growth is induced by the rapid injection of a molecular solution, such as an acetone or ethanol solution, but usually organic and nonpolar, mixed into a non-solvent, such as water. The solubility of the solute decreases rapidly, usually inhomogeneously and over various time scales, and the molecules aggregate and start forming nanoparticles. Despite the lack of precise control over the molecular self-organization, the nanoparticles synthesized by this method usually have a narrow size distribution. The reprecipitation method has been used in various forms to produce optical materials\textsuperscript{72}, polymers\textsuperscript{73}, organic nanocrystals\textsuperscript{74}, and provitamins\textsuperscript{75}. The last of these is important because it demonstrates the scalability of the method to industrial levels; BASF produces large quantities of beta carotene nanoparticles and related materials for use as vitamin additives.

The method has been used and works well for single component nanoparticles. However, creating nanoparticles containing multiple components using this method is not as simple, although the many potential applications for multifunctional nanoparticles make this a promising area of research.\textsuperscript{76} A comprehensive understanding of the nucleation and growth of nanoparticles in the reprecipitation method requires a consideration of both kinetics and thermodynamics. The fact that the formation occurs in an inhomogeneous and rapidly varying environment complicates the analysis.
At a basic level, many of the same issues are faced by crystal growers attempting to co-crystallize materials. While inducing a phase transformation requires only a reduction in the free energy of one phase with respect to the other, co-crystallization of two or more components involves the interactions between the two components as well as each with the solvent.

10.3 Classical Nucleation Theory

The nucleation and growth of a solid phase from a solution can be approached from a number of perspectives. Classical nucleation theory (CNT) treats the microscopic activities with a macroscopic approach; in CNT, the nucleation is simply a first order phase transition in a thermodynamic picture.\textsuperscript{77} When a solution is slowly brought to supersaturation, it enters a metastable region in which separation into two phases is energetically favorable, but where an energy barrier, which is exponential in the nucleation rate, prevents immediate nucleation of particles. This barrier is due to a positive free energy contribution for molecules at the particles surface, and results in a critical radius, below which clusters of molecules may form, but they will not be large enough to be kinetically stable. As the concentration is further increased, this energy barrier will be overcome by the higher solute concentration and at some point, the system undergoes a first order phase transition and stable particles will form.\textsuperscript{78} This description is a basic starting point for the development of the thermodynamics of crystallization.\textsuperscript{79}

On a molecular level, as the two solvents are mixed during the reprecipitation, the solute sees fewer and fewer favorable solvent molecules in its vicinity with which it can associate, and eventually begins to self organize with similar species. For a slow increase in the supersaturation, this should happen according to the standard models
of phase transitions. However, in reprecipitation the system is far from equilibrium. Despite this, it been shown by Mori et al. that the nucleation and growth can be accurately described using CNT, which gives the rate of nanoparticle formation as a function of the degree of supersaturation.\textsuperscript{80} It is likely that while the initial nucleation is heterogeneous, the later stages of growth of the nanoparticles occur under quasi-equilibrium conditions, since the nucleation and growth of particles reduces the concentration of free (dissolved) solute. In these later stages, the solution approaches a homogeneous system nearly in equilibrium with the solid nanoparticle phase, with growth by molecular diffusion to the particle surface.

This model of a rapid nucleation burst followed by slower diffusion-limited growth was originally proposed by LaMer and Dinegar as a way to understand the very narrow size distributions that result from these types of synthetic methods.\textsuperscript{81} It was later then shown that that diffusional growth itself resulted in a further narrowing of size distributions.\textsuperscript{82} Although the basic ideas are applicable to a general understanding of the nucleation and growth of nanocrystals in the reprecipitation method, the additional complexity introduced by the interactions between the different species must be taken into account for co-crystallization of multiple components. The following section discusses a starting point to attack this complex problem in co-crystallization.

10.4 Co-crystallization and Solubility

10.4.1 Hildebrand Solubility Parameter

To consider whether two components co-crystallize, the use of solubility parameters is explored. The Hildebrand solubility parameter is a value related to the free energy required to remove single molecules of a material from a crystal to an
isolated state, and is defined as the cohesive energy density in term of the energy of vaporization $E$ and the molar volume of the pure solvent $V$. In the ideal case, the Hildebrand solubility parameter is written as

$$\delta = \sqrt{\frac{E}{V}} = \sqrt{\frac{\Delta H - RT}{V_m}}$$

(10.1)

where $\Delta H$ is the heat of vaporization, $R$ is the gas constant, $T$ is the temperature and $V_m$ is the molar volume of the mixture. The miscibility of two materials is often related to how close their solubility parameters are to each other. The temperature dependence can be used to adjust the values of the two parameters in order to increase the likelihood of co-crystallization.

The initial formation of a particle will depend critically on the relative interactions between the two or more solutes, the solvent(s) and solute, and the solvent molecules with each other. On the microscopic scale, the solubility of a particular chemical species can be considered in terms of the interaction strength with the solvent and with other solute molecules, both relative to the thermal energy $kT$. If the thermal energy is larger than both interaction energies, the solute should be soluble. If $kT$ is smaller, and if the interaction between solute and solvent is stronger than between solute molecules, the solubility will also be large, since the solute molecules will preferentially associate with the solvent rather than each other. If the opposite is true, the solute molecules will self associate and lead to phase separation. This is all for the case of very dilute samples. As the concentration increases, the average strengths of the solute-solute and solute-solvent interactions will be altered.

The formation of single component nanoparticles in these terms is straightforward. After mixing, the solubility of the solute in the new solvent is greatly reduced, causing the molecules to associate into particles. For more than two components
(two solutes and solvent), there are multiple interaction strengths to consider. As was the case for the single solute, if $kT$ is larger than all interaction energies, then there should be mutual solubility (single phase).

These are all considerations in the thermodynamic limit. The heterogeneous particle formation during mixing may affect the actual types of particles that form. There is also the assumption that the remaining amount of good original solvent can be ignored, but this may not be valid. It needs to be considered, for example, whether the good solvent can form a solvent cage around one or both species and what the affect would be.

For the particle formation, temperature and initial concentration are two obvious factors that affect solubility. As in equation (10.1), temperature affects the value of the solubility parameter directly. It also indirectly influences the solubility of the individual components in the solvent as it has been shown that the temperature plays a significant role in the growth of nanoparticles of perylene prepared using the reprecipitation method.\(^8^4\)

Concentration has also been shown to play a large role in the size of single component nanoparticles prepared with reprecipitation.\(^7^0\) Van Keuren’s group has shown a strong nonlinear dependence of particle size on concentration of Magnesium phthalocyanine nanoparticles prepared with reprecipitation.\(^8^5\) In the case that nanocrystals can be formed from multiple components, there will be the additional matter of obtaining the correct stoichiometry of the crystal - depending on the interactions, this may not result from a strict molar ratio in the initial solutions.

Other conditions will also likely play a role in the reprecipitation of composite nanoparticles as they do for single component ones. Besides the van der Waals forces, which are the primary interactions at long range, hydrogen bonding and electrostatic (charge and dipole) interactions will influence the formation and stability of
10.4.2 Hansen Solubility Parameters

More complex analysis of the solubility leads to multivalued solubility parameters such as Hansen solubility (cohesion) parameters (HSP)\textsuperscript{86}, which consider separate cohesion energy contributions from dispersion interactions ($E_D$), dipolar intermolecular interactions ($E_P$), and hydrogen bonding molecular interactions ($E_H$),

$$E = E_D + E_P + E_H. \quad (10.2)$$

There are other sources of cohesion energy, such as induced dipoles, metallic bonds, electrostatic interactions. However, only three major sources that are found in organic molecules are considered in the HSPs. By dividing this total cohesion energy by the molar volume, equation (10.2) becomes the square of the Hildebrand solubility parameters, that is,

$$\delta^2 = \delta_D^2 + \delta_P^2 + \delta_H^2. \quad (10.3)$$

These three parameters can be represented as a point in the three-dimensional space, named Hansen space. This point would be the center of the solubility sphere of a solute with radius $R_o$, which indicates the maximum difference in the solubility parameters of a solvent for the two components (the maximum distance in the Hansen space) to have good miscibility. $R_o$ is considered as an additional, fourth parameter in HSP. It indicates which solvents will dissolve in the solute. Corresponding to the saturation solubility, the measure of distance $R_o$ is concentration dependent. A solute with small concentration would dissolved in many solvents, so $R_o$ would be large, but if the solute is very concentrated, many solvents would not be able to dissolve the solute, thus so $R_o$ would become smaller. It also depends on temperature. If the
temperature increases, the solubility of a solute in a solvent is likely to increase, hence the value of \( R_o \) would increase as more solute can be dissolved.

The above definition of \( R_o \) implies that when the HSP of a solvent falls inside of the solute’s Hansen sphere (or it falls within the \( R_o \) distance from the HSP of the solute), the solvent is a good solvent for the solute. If outside, it cannot dissolve the solute well, thus it is a bad solvent. A measure of distance between the HSPs of two materials is defined by the quantity \( \Delta R_a \):

\[
\Delta R_a^2 = 4(\delta_{D1} - \delta_{D2})^2 + (\delta_{P1} - \delta_{P2})^2 + (\delta_{H1} - \delta_{H2})^2. \quad (10.4)
\]

The factor of 4 was empirically found to be necessary, and this constant was also theoretically derived by the Prigogine corresponding states theory of polymer solutions.\(^{86}\) \( \Delta R_a \) is another key quantity dictating whether there is good miscibility between the two components; the shorter the \( \Delta R_a \) is, the two components become more likely to be mutually soluble. For molecular self-assembly of nanoparticles in the reprecipitation method, a larger value of \( \Delta R_a \) between the solute and the non-solvent is equivalent to a high supersaturation rate. This will have a strong effect on the particle size, although the exact dependence is a nonlinear function of the degree of supersaturation.\(^{85}\)

Hansen determines a value of \( R_o \) mostly by a trial and error approach using the experimental data and the simulation programming codes using the value of the Relative Energy Difference (RED). The RED is a simple composite parameter defined as

\[
RED = \frac{\Delta R_a}{R_o}. \quad (10.5)
\]
When RED is less than 1, two materials are alike hence the solvent is a good solvent, but when it is more than 1, the solute would not dissolve. If RED is equal to 1, it partially dissolves. Figure 10.1 illustrates the reprecipitation method in terms of the Hansen sphere. When a solute with the radius $R_o$ is dissolved in solvent 1 and then rapidly injected to non-miscible solvent 2, the rapid change in solubility yields nanoparticles.

![Figure 10.1](image_url)

**Figure 10.1:** Illustration of the reprecipitation method for a single component material in a Hansen space. The red dashed arrow indicates the rapid change in solubility of a solute with the interaction radius $R_o$ in solvent 1 to solvent 2. $\Delta R_a$ is the distance between the HSP of the solute and the HSP of the solvent 1 or 2.

While these parameters are normally applied to determine the miscibility of two components, they can also be used to determine how multiple components will partition into different phases. For composite nanoparticles, the reprecipitation method can be illustrated as in Figure 10.2. Instead of only one solute, there are two
different materials dissolved in solvent 1 and rapidly injected together in solvent 2. When two constituents with a small value of $\Delta R_a$ are combined with a third medium with a much larger $\Delta R_a$ (vs. both components), they will aggregate and eventually phase separate. If the two are initially completely dissolved, or if there are also stabilizers such as surfactants in the solution, they may aggregate first into a nanoparticle phase, which can be metastable.

![Figure 10.2: Illustration of the reprecipitation method for multi-component materials in a Hansen space.](image)

**Figure 10.2:** Illustration of the reprecipitation method for multi-component materials in a Hansen space.

### 10.5 Derivation of Three Solvent Spheres Model

To synthesize composite nanoparticles, the concept of HSP is useful in studying the conditions of the particle formation. If the HSP of a solute can be determined with $R_o$ written as a function of concentration and temperature, it would contribute to determining whether materials could be induced to form composite nanoparticles
using the reprecipitation method. Because we are applying HSP to a nanoparticle solution system instead of polymers, the regular solution theory was used to estimate $R_o$ as the first step of the modeling. In the theory, the regular solution refers to the ideal solution that forms with an additional enthalpy change and at constant volume. For a solution to form spontaneously, the free energy of mixing $\Delta G_M$ is required to be zero or negative and is given by

$$\Delta G_M = \Delta H_M - T\Delta S_M$$ (10.6)

where $\Delta H_M$ is the heat of mixing, $T$ is the absolute temperature, and $\Delta S_M$ is the entropy change. $\Delta H_M$ was defined by Hildebrandt and Scott, $\Delta H_M = \varphi_A\varphi_B V_m(\delta_A - \delta_B)^2$ (10.7)

where $\varphi_{A,B}$ are the volume fractions of material A and B and $V_m$ is the total volume of the mixture $V_A + V_B$. The above equation was derived with the assumption that the entropy of mixing is due to combinational effects. For mixing of materials A and B, such as solute and solvent, by definition the entropy of mixing is

$$T\Delta S_M = -(n_A RT \ln x_A + n_B RT \ln x_B)$$ (10.8)

where $R$ is the universal gas constant and $n_A$ and $n_B$ are number of moles of materials A and B respectively. $x_A$ and $x_B$ are their mole fractions ($x_{A,B} = \frac{n_{A,B}}{n_A + n_B}$). Since the mole fractions are smaller than 1, the entropy of mixing will turn out to be positive.

If $\Delta G_M$ goes to zero, then equation (10.6) is simply rewritten as equation (10.7) equals to equation (10.8). If material A is a solvent and B is a solute, for a diluted solution where $x_A \gg x_B$, $\varphi_A \approx 1$, which reduces $\varphi_A\varphi_B V_m$ to $V_B$, the total
volume of solute B. Dividing both ∆H_M and T∆S_M terms by n, after rearrangement, the difference in solubility from equation (10.7) can be written as following:

\[
(\delta_A - \delta_B)^2 = \frac{-RT(x_A \ln x_A + x_B \ln x_B)}{V_B}.
\]  

(10.9)

Since R_o can be considered as the maximum difference in δ_A and δ_B such that mixing occurs, equation (10.9) is the definition of R_o. Since V_B is defined in terms of the density ρ and the molar weight mw of solute B, with the simple modification, R_o can be written as

\[
R_o = \sqrt{-\frac{RT \rho_B (x_A \ln x_A + \ln x_B)}{mw_B}}
\]

(10.10)

where ρ_B and mw_B are the density and molar weight of material B. Even though it is not clearly explicit, because the molar fractions are less than zero, the molar fraction terms are negative, so the overall numerator of the right hand side is actually positive. Given the assumptions of the regular solution theory, equation (10.10) is the exact solution for the radius of the Hansen sphere R_o. It shows that R_o is a function of the concentration and temperature. Increasing the concentration of a solute or decreasing the temperature will decrease R_o, and so the system of the set of a solute and a solvent would move from good solubility to insolubility, and the excess solute would precipitate out of solution.

The HSPs of solvents can be found in the literature; however, that of many materials, including the types of materials I used, are still not known, especially since the experimental methods for determining the HSPs are difficult and time consuming without guarantee of accuracy.89 Although many methods have been investigated to determine or predict the HSPs of a material, many are for polymers using the group contribution theory based on the molecular structures.90–95 As a preliminary way
of estimating the HSPs of a material to be used in the syntheses of the composite nanoparticles, the three solvent spheres (TSS) model was developed.

As mentioned earlier, a radius $R_o$ of a Hansen sphere of a solvent can be considered as corresponding to the saturation solubility of the solute. The value of $R_o$ is still unknown; however, if $R_o$ can be defined as a function of the concentration, or solubility, with known HSPs of solvents, one can draw a sphere with its radius $R_o$ centered at the solvent HSPs in the Hansen space. This sphere, named the solvent sphere, is not the same as a Hansen sphere since a Hansen sphere uses the HSP of a solute as the center of the sphere. Nevertheless, the intersections of several solvent spheres would locate the unknown HSP of a solute because the HSP of the solute is a unique point in Hansen space. Thus, instead of starting with a Hansen sphere, the reverse steps of starting with the solvent spheres were taken to derive the definition of $R_o$ in order to find the HSPs of a solute.

In order to study the solubility relation with $R_o$, several solutes with known HSPs as well as solubility in various solvents, such as naphthalene, theophyline, and theobromine, were examined from the reference data. When the data was plotted as in Figure 10.3, the exact value of $R_o$ found using the equation (10.10) with the solubility density from the reference did not quite match with the reference data since it was derived with many assumptions. Instead, however, since the plot depicted an exponential relation, the following empirical, logarithmic relation was derived for the radius of interaction in terms of $C$ as the solubility,

$$R(C) = a \times \ln(C) + b$$

(10.11)

where parameter $a$ is a positive constant, and $b$ is a negative constant. These two constants were determined by fitting the above equation to the reference data. For
each solvent, equation (10.11) is equivalent to the square root of equation (10.4). Although the form of the equation is empirical, it fits better than the exact solution as shown in Figure 10.3 and hence it was used as a starting point to develop the model to estimate the HSP coordinates of an unknown material.

![Figure 10.3](image)

**Figure 10.3:** Solubility of naphthalene in various solvents in terms of the interaction radius. The data was fitted with the exact solution from equation (10.10) and the empirical exponential relation from equation (10.11).

Theoretically, with equation (10.11), if the general values for the constant values $a$ and $b$ are known, one requires only the HSP data of the solvent and the saturation concentration data of a solute in order to determine the HSP and $R_o$ of any solute. To investigate the way to derive the general constant values ($a$ and $b$), I have written a programming code using MATLAB to establish a model (The code in Appendix C). The algorithm of the model is that, for a material, values of constant $a$ and $b$ are assumed using the naphthalene reference data, and the solubility of the material is measured in multiple solvents to calculate the radius $R$. Then, first, three possible solvents are chosen to plot the spheres in Hansen space. If those three spheres intersect with each other, the distance between the intersection points of the spheres are determined. To do this, the transformation in the coordinate system is
used in the programming code. Detailed derivation of this geometric method for the TSS model is in Appendix B and all of MATLAB codes used for modeling are listed in Appendix C.

Figure 10.4 shows the result of one of the simulations with naphthalene. It should be noted that the figures are not regular Hansen spheres. Green cross mark (×) on the figures indicates naphthalene’s HSP coordinates. Small black plus signs (+) are HSP positions of solvents, and the spheres are solvent spheres with radii defined by equation (10.11), which ideally are $R_o$ at the saturated concentrations. In the perfect system, those radii should be the distance between the HSP positions of solvent and solute, in this case naphthalene, so that the intersections of the three spheres turn out to be at a single point, which would be the same as naphthalene’s HSP coordinates. If in the near perfect situation, the spheres would have two intersection points that are very close to each other with the solute HSP position in between the two. However, because of many assumption made in the derivation of the simple equation (10.11), the radii of the solvent spheres did not get adjusted perfectly, so that the near perfect situation was not able to be achieved.

Table 10.1 lists the averaged results determined with all the possible combinations of known solvent data. Constants a and b are determined the values obtained from the Excel solver implemented on the reference data of naphthalene. As Table 10.1 indicates, the results of naphthalene are closer to the actual HSPs, but not theophylline as expected as the constants are determined using naphthalene as reference points. They, however, are of the right order of magnitude. The next step for this model is to find the way to adjust constants a and b until all the combinations of three spheres have intersection points and to minimize the distance between the points. This is more complicated and will require more information than that used in the model at this point. When further investigations introduce details of the relation
between the solubility and the radius of a Hansen sphere, it would help improve the model to estimate the unknown HSP of a material. This part of the research will be continued to be explored by Van Keuren’s group in the near future.

Figure 10.4: Result from the TSS model simulation. Spheres are three different solvents indicated by small black + with their $R_o$ derived by the model. Red ($\ast$) and blue ($\ast$) asterisks indicate the intersection points of the three spheres, and the green mark ($\times$) indicates the actual HSPs of naphthalene. Ideally the green mark should lies between red and blue asterisk marks in all dimensions.

<table>
<thead>
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<th>HSP</th>
<th>Naphthalene</th>
<th>Theophylline</th>
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<td></td>
<td>Actual</td>
<td>TSS Model</td>
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<tr>
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</tr>
<tr>
<td>$\delta_H$</td>
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</table>

Table 10.1: Results of Three Solvent Spheres Model. The HSPs estimated by the TSS model are compared to the actual values. The TSS model values are the average of the resultant HSPs of the all data.
11 Experimental Methods

11.1 Sample Material

11.1.1 Composite Particles of Charge Transfer Crystals

A candidate to form nanocomposite particles as a model system is a charge transfer material because the transfer creates unique optical and electronic states that do not exist in the individual components. A charge transfer complex is formed by donor-acceptor interactions and can consist of either two organic molecules or an organic molecule with an inorganic molecule. The formation of charge transfer compounds can be monitored using their optical spectra, which make them useful for demonstrating the possibilities of co-crystallization of composite nanoparticles.

The electron acceptor tetracyanoquinodimethane (TCNQ) forms such crystals with a number of aromatic, electron donating molecules such as tetrathiafulvalene (TTF)\textsuperscript{96}, tetracene or perylene\textsuperscript{97}. When perylene, the electron donating organic material consists of polycyclic aromatic hydrocarbons, is crystallized together with the cyano groups (-CN) of TCNQ, the resulting charge transfer crystal is reported to have high mobilities for both holes and electrons and thereby useful as electronic material.\textsuperscript{97} Hence, in addition to as a model system, charge transfer nanocrystals may be suited to various practical applications. For example, the nanocrystal could be useful in organic solar

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{molecular Structures.png}
\caption{Molecular structures of perylene and TCNQ.}
\end{figure}
cells, where charge transport can be as important as energy conversion in obtaining high efficiency devices. Thus, this research can contribute to the energy field in addition to the biomedical field.

11.1.2 Active Pharmaceutical Ingredients

An active pharmaceutical ingredient (API) is an active chemical that provides pharmacological activity as a medication or pesticide. The synthesis and design of cocrystals of APIs have attained considerable interest, especially due to their multifunctionality and their potential applications to a various fields, including pharmaceuticals. Because of the nature of APIs that engage in hydrogen bonding, API cocrystals can modify the composition as well as chemical and/or physical properties without making or breaking covalent bonds. The reprecipitation method could be useful to produce such materials in nanoparticle form.

To demonstrate the possibility of the reprecipitation method, a well-studied API cocrystal was chosen. Carbamazepine (CBZ) is an anti-convulsant drug that has been used for treatments of epilepsy and trigeminal neuralgia. CBZ has been used as an anti-epileptic drug for three decades and also has been used as a favorable candidate in pharmaceutical cocrystals. Nicotinamide (NCT) is a water-soluble vitamin.

Figure 11.2: Molecular structures of CBZ and NCT.

Figure 11.3: Crystal packings of CBZ:NCT.
that has been used for treatment and prevention of diabetes. Both the hydrogen bond donor and acceptors of CBZ interact with the coformer NCT to transform itself to be an API cocrystal.\textsuperscript{99} This CBZ:NCT cocrystal has been used often as a model system in crystal engineering, hence we used it in this research.\textsuperscript{99,103–106} The results will be discussed in section 12.2.

### 11.2 Sample Preparation

![Schematic diagram of the reprecipitation method. The third step is used when a powder sample is required.](image)

Samples are prepared using the reprecipitation method.\textsuperscript{84} Perylene was purchased from Sigma-Aldrich, and 7,7,8,8-Tetracyanoquinodimethane (TCNQ) was purchased from Acros Organics. Both are used without further purification and individually dissolved in acetone at a concentration of 2.5 mM. For the co-crystallization of nanoparticles, a 1:1 molar ratio of perylene and TCNQ are mixed together and the acetone solution of perylene:TCNQ is rapidly injected into distilled water using a micro syringe with a 1:9 ratio (acetone solution:water) and vigorously stirred, usually at room temperature. Figure 11.4 shows the steps of the sample preparation using the reprecipitation method. For comparison, nanoparticles made from individual components, perylene or TCNQ, were also prepared using the same method. Preparation of CBZ:NCT materials is described in detail in section 12.2.
11.3 Characterization Methods

The onset of this self-organization can be monitored in a number of ways, such as in the optical and electronic characteristics. In particular, changes in the optical absorption spectra, especially the appearance of new absorption bands, can be related to the growth of organized structures. For example, new bands appear in the low temperature absorption and fluorescence spectrum of perylene as it dimerizes.\textsuperscript{107}

Much of the early work on nanoparticle synthesis by reprecipitation also used optical spectra as indicators of nanocrystal formation. Kasai et al. used optical absorbance spectra of water dispersions of perylene crystals prepared by the method to show its size dependence and compared them to that of an acetone solution of perylene to show the shift in the excitonic absorption peak of crystals.\textsuperscript{108}

In addition, fluorescence quenching has long been used as a measure of the association of two or more emitting species. For molecules that normally lose excited state energy by fluorescence emission, the association with one or more other molecules provides other pathways for de-excitation, such as intermolecular vibrational states and resonant energy transfer. This results in strong quenching of fluorescence when molecules form dimers or larger assemblies, and this effect has been observed in the study of the self-organization of Magnesium phthalocyanine.\textsuperscript{85}

To characterize the co-crystallized nanoparticles, several different methods are employed. The following are brief descriptions of the techniques used in characterization.

11.3.1 Absorption Spectroscopy

Absorption spectroscopy is used as a major tool in identifying the presence of the charge transfer compound in the co-crystallized nanoparticles. Absorption spectra
of a sample were acquired by a spectrophotometer (Ocean Optics CHEM2000-UV-VIS) in the wavelength range 350 to 1000 nm. A solution of acetone and water with the same ratio as the solvent for the nanoparticle samples (1:9 ratio) is used as a reference.

11.3.2 Dynamic Light Scattering

In order to examine the particle growth, dynamic light scattering (DLS) was used. The detailed explanation of the theory of DLS is already discussed in section 3.1. With excitation from a 10 mW HeNe laser (JDS Uniphase 1202-1), the intensity fluctuation signals of scattered light from a sample are collected by a single mode optical fiber, which is coupled to an avalanche photodiode detector (Perkin Elmer SPCM CD2882), which is oriented to collect scattered light at 90 with respect to the incident laser. The collected signals are input to an ALV-5000 Multiple Tau Digital Correlator and the photon count autocorrelation functions analyzed using a nonlinear fitting routine programmed in MATLAB. The exact expression for the autocorrelation function given by Frisken was used to model the data.\textsuperscript{11} For each run, the sampling time was set to 30 seconds, and the DLS measurements were taken continuously for several hours starting right after mixing.

11.3.3 Raman Spectroscopy

To investigate the change in the charge transfer compounds, Raman spectroscopy is employed as well. Raman spectra of a sample were obtained by a Raman spectrometer (Renishaw Ramanscope system 1000) equipped with CCD detection and with a 514.5 nm Ar laser or a 785 nm diode laser excitation. The sample was dried to have powder form for this characterization.
11.3.4 Scanning Electron Microscopy

To image the crystallized structures of the samples using scanning electron microscopy (SEM), a Zeiss Supra 55VP was used with an in-lens detector at 5kV-15kV beam energy. To prepare for imagining, samples are instantly frozen with liquid nitrogen after a certain time from mixing and then freeze-dried for several hours to sublimate the water. The cooling is more rapid than the reactions of species, so that it is assumed to have no impact on the samples. Since the larger the amount, the longer the sample takes to freeze, I also experimentally tested with different amounts of sample solutions to ensure that rapid freezing with liquid nitrogen would not affect the sample. After freeze drying, powder samples are then deposited in a desiccator to be inserted in the vacuum chamber of SEM for imagining.

11.3.5 WETSEM

WETSEM™, a method for electron imaging of wet samples, was also used to analyze wet samples without freeze drying. The El-mu QuantomiX WETSEM capsule is purchased from Electron Microscopy Sciences. In WETSEM, the sample is enclosed in a small chamber sealed by a membrane that is thin enough for electrons to pass through in order to create the image.\(^{109}\)

For sample preparation, the capsule membrane should be coated with gelatin or other materials for better attachment of samples on its membrane surface. Gelatin type B from Acros Organic was used as an attachment agent in this case. 0.1% w/v gelatin was dissolved in warm distilled water at 37° until no lumps were visible. 15 \(\mu l\) of gelatin solutions was then applied to a capsule, and the coated capsule was incubated for one hour at room temperature. After the gelatin solution was removed and the capsule was washed twice with PBS (hyclone phosphate buffered saline (1X)
from ThermoScientific), it was filled with PBS until seeding the sample solution. This coating process should be done on the day that SEM images are taken. When seeding, 20 µl of sample solution was filled in the liquid dish. The dish was set upside for a while to promote the sample attachment on the membrane. For this method of SEM, the SE2 lens detector was used at 10 kV beam energy. WETSEM was mostly used to study the formations of TCNQ nanocrystals. The detail discussion of the self-organizing progress of TCNQ is discussed in chapter 13.

11.3.6 Energy-dispersive X-ray spectroscopy

Energy-dispersive X-ray spectroscopy (EDS) is used with SEM for chemical characterization and elemental analysis. The samples were prepared in the same way as they were prepared for SEM. The SE2 lens detector was used at 5-15 kV to focus on the sample structure and the INCA X-ray microanalysis system was used to obtain the data. The EDS data was analyzed by INCA software version 4.15 (Oxford Instruments). This technique was used to confirm the element compositions of the cocrystal. To calibrate the data, an empty SEM mount was measured with EDS as well.

11.3.7 Powder X-Ray Diffraction

To confirm crystal formation, a powder X-ray diffraction (PXRD) pattern of a sample of freeze dried powder was obtained using a Rigaku RAPID Curved IP X-ray powder diffractometer with Cu K(α) radiation and an image plane detector. The sample is completely dried with the freeze drying method explained in section 11.3.4.
11.3.8 Differential scanning calorimetry

Diffraction scanning calorimetry (DSC) was measured with a TA instruments 2920 modulated DSC equipped with a refrigerated cooling system (New Castle, DE). DSC was calibrated for temperature using indium, zinc, and tin standard model (TA instruments). To measure melting points and glass transition temperatures, 0.6 to 1.6 mg of sample was placed in a hermetic aluminum pan and sealed with a manual press. An empty sealed pan was also used as a reference sample. All experiments were run in triplicate with a temperature ramping mode of 10 °C from a room temperature to 250 °C. The samples were continuously purged with nitrogen gas at 50 mL/min. Data was acquired with Thermal Advantage (Version 1.1A) and analyzed using Universal Analysis 2000 (Version 4.1D) from TA instruments.
12 Experimental Results

12.1 Optical Characterization Results for Charge Transfer Crystals

12.1.1 Results for the 1:1 molar ratio sample at the room temperature

To investigate the self-organization of 1:1 molar ratio perylene:TCNQ mixture with the reprecipitation method, the particle growth was monitored using DLS and compared to perylene nanoparticles, also prepared by the reprecipitation method at the same concentration. The results in Figure 12.1 shows the rapid formation of perylene:TCNQ particles that increased in size (radius) from roughly 100 nm to 200 nm in the first 30 minutes, and then the size remained stable for several hours. On the other hand, perylene particles grow to a smaller size on the order of 125 nm in 15 minutes after the mixing. The difference in the results suggests that the small sized perylene are not present in the mixed system, which could presumably indicate formation of a different type of particle, such as co-crystals of perylene:TCNQ. The

Figure 12.1: Particle growth of reprecipitated perylene:TCNQ and perylene measured by DLS.
DLS measurements to monitor the particle growth of TCNQ particles were also examined. However, because TCNQ can rapidly form large microcrystalline structures which can intermittently enter the sampling volume and scatter strongly, consistent autocorrelation functions could not be obtained, and hence the results are not included in Figure 12.1.

**Figure 12.2:** Absorption spectra of reprecipitated charge transfer crystals. (a) perylene, (b) TCNQ and (c) a 1:1 molar mixture of perylene:TCNQ were prepared with the reprecipitation method.

The absorption spectrum of nanoparticles of a 1:1 molar ratio of perylene:TCNQ also gives indication of the formation of composite nanocrystals that are composed of the two constituents (Figure 12.2). The broad infrared peak centered around 920 nm appears only in the absorption spectrum of the perylene:TCNQ sample, but it does not exist in the absorption spectra of the sample with perylene or TCNQ individually. Such an absorption peak has already been reported in co-crystals of perylene:TCNQ and is attributed to the charge transfer state. Its appearance in the nanoparticles suggests the formation of the composite nanocrystals, rather than nanoparticles of the individual components or amorphous composite nanoparticles. The charge transfer absorption arises from the dimer of perylene:TCNQ (1:1 molar
ratio), which is likely to be the initial structure formed. This change in the spectrum occurred rapidly (≤1 second) after mixing, suggesting that dimerization occurs quickly. Stop-flow experiments on perylene nanocrystals by Mori et al. indicated that spectral changes due to molecular assembly to form crystals occurs within milliseconds after mixing.\textsuperscript{80}

\textbf{Figure 12.3:} Raman spectra of reprecipitated charge transfer crystals. The measurements were taken with (a) a 1:1 molar mixture of perylene:TCNQ prepared with the reprecipitation method at the 514 nm excitation, (b) perylene powder (no reprecipitation) at 785 nm excitation, and (c) TCNQ powder (no reprecipitation) at 514 nm excitation. The longer wavenumber data in perylene was removed during background subtraction - no spectral features were observed above 1700 cm\textsuperscript{-1}. 
Raman spectra of powder samples of perylene, TCNQ, and perylene:TCNQ were also measured (Figure 12.3). The sensitivity of vibrations to the local environment could cause shifts in IR and Raman lines between the pure compounds and the corresponding lines in the charge transfer crystals. Some lines are unaffected; for example, the 1602 cm\(^{-1}\) line in TCNQ, corresponding to summation frequencies of the C=C ring stretching and CH bending\(^{112}\), does not shift in the nano-co-crystal. However, other lines such as the 1216 cm\(^{-1}\) line in perylene, attributed to in-plane CCH bending\(^{113}\), and the 1207 cm\(^{-1}\) line in TCNQ, due to CH bending\(^{112}\), show significant spectral shifts to lower frequency in the perylene:TCNQ co-crystal (1195 cm\(^{-1}\)) due to interactions with their environment. Table 12.1 lists the major Raman lines of reprecipitated perylene:TCNQ compared with the individual components. The spectrum result seems to agree with previously studied Raman spectra of perylene:TCNQ complexes\(^{114}\).

Scanning electron microscope images corroborate the DLS, absorption, and Raman data and show crystal habits in the co-crystal (Figure 12.4) that are not observed in similar images of individually reprecipitated nanocrystals of perylene (Figure 12.5) or TCNQ (Figure 12.6). Polyhedral structures of perylene:TCNQ nanocrystals have morphologies somewhat similar to perylene nanocrystals in shape, but while perylene generally appears to be cuboid shape, perylene:TCNQ are rhombohedron

<table>
<thead>
<tr>
<th>Material</th>
<th>Perylene</th>
<th>TCNQ</th>
<th>Perylene:TCNQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raman shifts</td>
<td>1216</td>
<td>1207</td>
<td>1195</td>
</tr>
<tr>
<td>(cm(^{-1}))</td>
<td>1303</td>
<td>—</td>
<td>1297</td>
</tr>
<tr>
<td>1365</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1602</td>
<td>1601</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 12.1:** Major Raman shifts observed in reprecipitated perylene:TCNQ compared with reprecipitated perylene and TCNQ.
Figure 12.4: SEM image of reprecipitated perylene:TCNQ nanocrystals.

Figure 12.5: SEM image of reprecipitated perylene nanocrystals.
with some having more faces. In general, perylene:TCNQ is slightly larger than perylene, which agrees with the DLS measurements. TCNQ structures appeared in a wide variety of other forms with a wide variety of size scales, such as rhombic plates and dendritic structures, none of which have any resemblance to the perylene:TCNQ structures. A more detailed discussion on TCNQ structures is in chapter 13.

Energy-dispersive X-ray spectroscopy (EDS) experiments were also conducted to investigate the formation of perylene:TCNQ. Assuming that the 1:1 molecular complex ratio of perylene:TCNQ has a homogeneous composition, in weight percentage terms, perylene:TCNQ is estimated to have 12.27 % nitrogen while TCNQ would have 27.44 % nitrogen (no nitrogen in perylene). Because the X-ray ring in EDS is very large (about 10 µm in diameter) compared to the structures of interest, the data often show contamination elements, presumably from the SEM mount, such as Al, O, Mg, Fe, etc. A few sites of interests in the perylene:TCNQ sample had no N in their EDS spectra, which is possible indication of the formation of perylene structures. Several structures in perylene:TCNQ sample also shows more weight % N than others, which would suggest formation of TCNQ in the sample as well. However,
the overall data shows that the perylene:TCNQ sample had a lower weight % of N compared to the TCNQ sample. For example, in figure 12.7, 5.96 % N was observed in perylene:TCNQ along with several contaminant elements while TCNQ had 15.50 % N. Hence, this is further evidence that the reprecipitation method is forming a multi-component material.

Figure 12.7: EDS results of reprecipitated charge transfer crystals. (a) perylene:TCNQ, (b) perylene, and (c) TCNQ. All prepared by the reprecipitation method for the comparison. The bar graphs show quantitative results of weight percent compositions.
The PXRD pattern of perylene:TCNQ is taken to confirm its crystallization (Figure 12.8). It is also compared to the PXRD patterns of perylene and TCNQ. A new peak at 15° and a shifted peak at 27° confirms crystallization since they are new crystal axis dimensions that do not appear in perylene or TCNQ crystals.

![Figure 12.8: PXRD patterns of reprecipitated charge transfer crystals](image)

12.1.2 Results for samples prepared in various environments

The reprecipitation method was tested in several different environments to examine the effect on co-crystallization. First, different molar ratios of perylene:TCNQ other than the 1:1 ratio were tested to examine the change in the particle interaction. It is known that 1:1 perylene:TCNQ and 3:1 perylene:TCNQ exist. In addition to the 1:1 ratio sample, we have tested samples reprecipitated with 2:1 perylene:TCNQ and 1:2 perylene:TCNQ ratios using DLS and absorption measurements.

In the DLS result, the 2:1 sample showed smaller particles forming compared to the 1:1 ratio. Although no growth was observed, the sample was stable at 100 nm in size (radius). This size resembles the reprecipitated perylene-only sample, but the IR peak appeared in the absorption spectrum of the sample shown in Figure
Figure 12.9: Absorption spectra of reprecipitated perylene:TCNQ at different molar ratios. (a) 1:1, (b) 2:1, and (c) 1:2 molar ratio perylene:TCNQ.

12.9, suggesting that co-crystals are forming. The 1:2 sample also showed a small but definite increase in absorption at the IR wavelength, which indicates co-crystals might be forming. However, the DLS results of the 1:2 sample vary in a wide radius range (300-500nm) over time. Although the ACFs are not distorted as those that the reprecipitated TCNQ-only sample produced, this wide size distribution implies that this sample has more TCNQ features than dual component features. A possible explanation for these results can be formulated in terms of the excess amount of one of the compounds. When both single component and dual component nanoparticles are formed in the solution, the particle size distribution analyzed by DLS is an average of the two. Thus, in the case of 1:2 perylene:TCNQ, although perylene and TCNQ would form the composite nanoparticles, TCNQ in excess forms TCNQ particles, which as mentioned before, have a wide distribution in size in the DLS data. Therefore, this sample displayed a broad size distribution. The same can be said for
the 2:1 perylene:TCNQ case, which exhibits a smaller particle size distribution than that of 1:1 perylene:TCNQ, closer to that of the perylene-only nanoparticles. Also as shown in Figure 12.9, the IR peak of 2:1 perylene:TCNQ is not as obvious as the 1:1 perylene:TCNQ since more perylene-only nanoparticles form.

Temperature is another factor that can considerably affect the synthesis process and hence the resulting particles. To control the environment, a temperature-controlled cuvette connected to a water pump was used. In Figure 12.10, the absorption spectroscopy measurements shows the spectra in a cool (7°) and warm (40°) environment compared to the result at room temperature. In both the measurements at the cold temperature and room temperature, the IR peak was observed, but at the warm temperature, it did not appear, suggesting no formation of co-crystals. Reprecipitated particles formed at 40° are smaller (∼200 nm in diameter) than the ones synthesized and kept at room temperature (Figure 12.11a); the result was very close to the perylene-only sample (Figure 12.11b). It is interesting to note that although the absorption peak at the IR wavelength appeared in the sample prepared in the cold temperature, its DLS shows particles are unstable but growing in size for several hours. This could be due to the formation of more TCNQ crystals than the formations of cocrystals. Even though cocrystals form at the cooler temperature (at 7°), the molecules are not active enough to form only cocrystals, so the larger crystal formation of TCNQ is disturbing the DLS measurements. The temperature dependence of the solubility, as mentioned before, affects the relative supersaturation level of a compound. The cooler the temperature is, the smaller the $R_o$ of each compound would be. So one or both compounds could be less soluble in the solvent, meaning they would be more insoluble in the non-miscible solvent; thus, one compound would be more likely to form single component particles with its own kind, affecting the formation of the composite particles.
Figure 12.10: Absorption spectra of reprecipitated perylene:TCNQ at different temperatures (at (a) 7°, (b) 25°, and (c) 40°). All data taken after 2 minutes from mixing.

A perylene:TCNQ sample was also tested with a high viscosity environment. By making the solvent more viscous, the particle growth process would slow down due to the slower flow rate of fluids and the decreased diffusion coefficient. To measure this effect, a perylene:TCNQ acetone solution was reprecipitated in an aqueous glyc-
Glycerol solution instead of just water. When glycerol is mixed in water at 85% weight at 20\(^\circ\), the solution would become about 109 times more viscous than water.\(^{116}\) After the aqueous glycerol solution was stirred for a while with a magnetic stirring bar, perylene:TCNQ acetone solution was rapidly injected in the same way as described in section 11.2. Since the solution was very viscous, it was given a few shakes right after mixing since the injection only separates the acetone solution and the glycerol aqueous solution. The DLS measurements were taken soon after the shake. However, the experiments were not successful since no sufficiently smooth data was observed as reliable ACFs. Absorption spectra were also taken, but the peaks were gone after 2 min and no IR peaks were observed at all. Before further experiments are carried out, a careful study of the material in terms of viscosity needs to be considered.

### 12.2 Optical Characterization Results for API Cocrystals

Beside perylene:TCNQ, another possible co-crystalline nanoparticle was investigated in this research as discussed in section 11.1.2. Both carbamazepine (CBZ) and nicotinamide (NCT) were purchased from Acros Organics and used without further purification. CBZ and NCT were each dissolved in acetone with a concentration of \(1.2 \times 10^{-2}\) M. Because each ingredient should be insoluble in the non-solvent, the solubility of each material in different solvents was checked. Based on the results listed in Table 12.2, hexanes was chosen to be the non-solvent for this experiment instead of water. To prepare a sample, a mixture of an acetone solution with 1:1 molar ratio was injected rapidly in hexanes with a 1:9 acetone:hexanes ratio.

A Raman spectrum of reprecipitated CBZ:NCT is compared with the spectra of powder CBZ and NCT from the manufacturer both without further purification (Figure 12.12). Rodriguez-Hornedo’s group demonstrated the transformation of solid...
<table>
<thead>
<tr>
<th>Solvent</th>
<th>CBZ</th>
<th>NCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>Soluble</td>
<td>Soluble</td>
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<tr>
<td>Cyclohexanone</td>
<td>—</td>
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<tr>
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</tr>
<tr>
<td>Water</td>
<td>Insoluble</td>
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</tr>
</tbody>
</table>

**Table 12.2:** Solubility check for CBZ and NCT in different solvents.

phase CBZ Form III to CBZ:NCT at room temperature that occurred in 3 hours of mixing; in their work, the stretching mode of a C-N-C Raman band shifted from 722 to 718 cm$^{-1}$. The shift was also observed in our experiment as listed in Table 12.3. The shift of the bending mode of a C-H bond to 1047 cm$^{-1}$ from 1039 cm$^{-1}$ is also similar to the result of the work of Seefeldt et al. These shifts suggest the co-crystallization of CBZ:NCT. The 1:1 molar ratio is chosen not only because of simplicity but also because it has been reported that a 1:2 ratio of CBZ:NCT would not form. With a cocrystal solubility product of their mathematical model as well as the binding constants of complexes formed in solution, Nehm et al. has explained that the solubility of cocrystals depends on concentration of each component in solution.

The DSC result shown in Figure 12.13 also confirms the co-crystal nature of the sample. The melting point of CBZ:NCT is at 159°C, which differs from those of CBZ (127°C) or NCT (191°C), and the result agrees with previously reported papers that confirms cocrystal formation of CBZ:NCT.

These results, matching with previously reported results using other methods, show that the reprecipitation method has potential to be a synthetic method for API cocrystals that can be utilized in various fields, including pharmaceutical areas.
Figure 12.12: Raman spectrum of reprecipitated CBZ:NCT compared with that of CBZ and NCT powder. Three plots on the bottom are the specific ranges of wavelength where Raman band shifts were observed.

<table>
<thead>
<tr>
<th>Reprecipitated CBZ:NCT (cm$^{-1}$)</th>
<th>CBZ (cm$^{-1}$)</th>
<th>$\Delta \nu$ (cm$^{-1}$)</th>
<th>Vibration assignments of CBZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>576.14</td>
<td>580.33</td>
<td>-4.19</td>
<td>$\nu$(C-N-C)$^{118,119}$</td>
</tr>
<tr>
<td>715.65</td>
<td>717.30</td>
<td>-1.67</td>
<td>$\delta$(C-H)$^{119}$</td>
</tr>
<tr>
<td>1047.30</td>
<td>1039.61</td>
<td>+7.69</td>
<td></td>
</tr>
</tbody>
</table>

$\nu$: stretch, $\delta$: bend

Table 12.3: Raman band shifts of reprecipitated CBZ:NCT compared to CBZ powder.
Figure 12.13: DSC of reprecipitated CBZ:NCT compared with CBZ and NCT powder.
13 Formation and Progression in the self-assembly of TCNQ crystals

During the study of the synthesis of nanocomposite perylene:TCNQ particles using the reprecipitation method, the unique self-assembly characteristic of 7,7,8,8-Tetracyanoquinodimethane (TCNQ) crystals was observed. Molecules that self-assemble in solution may pass through multiple phases and morphologies before reaching a thermodynamically stable state. This section demonstrates such a metastable transition process in TCNQ, which could be the possible evidence for Ostwald’s rule of stages in crystallization. The observation also shows that this material self-assembles in numerous morphologies at early growth stages, from nanocoils to polyhedral crystals. SEM imaging on freeze-dried samples reveals the crystal growth of TCNQ starting from seed-shaped nucleation sites, progressing through flower-like structures and finally forming polyhedral micro-crystals. These results are supplemented by absorption spectroscopy as well as PXRD characterization of a sample.

13.1 Background

Nucleation and growth are the underlying basis for the formation of many materials. Processes such as solution crystallization, self-assembly, protein aggregation, biomineralization and many types of nanoparticle formation all begin with the initial dimerization of molecules to form nucleation sites, followed by addition of monomers. Both the kinetics and the thermodynamics are important for understanding the properties of the resulting materials, and the details of intermediate processes can have a critical effect on the characteristics of the final materials.

*Much of the contents of this chapter is currently under preparation as a manuscript to be submitted to Langmuir.*
In the classical point of view, crystallization is described as a single step process with a parent phase directly transforming to the thermodynamically stable crystalline phase. However, crystal morphology (phase and habit) can pass through various metastable transient regions before reaching the stable one. For the phase, Ostwald’s rule of stages describes such a process by predicting that the metastable phase that requires the smallest free energy change will form before the most thermodynamically stable form is reached (Figure 13.1). Although Ostwald’s rule is not universal, some experimental evidence exists for this rule; for example, Chung et al. have demonstrated a progression of phases in a metal phosphate nanocrystal using in situ high-resolution electron microscopy. Numerous studies of the kinetics of the transition process in crystal nucleation have been used to establish analytical models that confirm the existence of the metastable phase. Likewise, crystal habit may change during the crystallization as more stable forms emerge and as the local level of supersaturation changes.

It has been observed that nucleation and crystal growth in the reprecipitation method occur on several different time scales. Using dynamic light scattering (DLS), Van Keuren et al showed the slow growth of organic nanoparticles of reprecipitated naphthalene and perylene solutions, which took place over 20-50 minutes. However, work done by Mori et al using UV-visible absorption spectroscopy with the stopped-flow technique on several types of reprecipitated nanoparticles demonstrated...
a fast growing process occurring in the range of milliseconds. Prudhommes group has also shown extremely rapid polymer nanoparticle formation times in double jet mixers. These results, confirmed by fluorescence quenching measurements in combination with DLS in Magnesium phthalocyanine, suggest that the initial formation of nucleation sites is extremely rapid, with the growth of larger particles in the hundreds of nanometers to micron size range occurring much more slowly.

This slower growth is due to monomer addition to the surface of growing particles as well as coalescence of primary particles. The monomer supply comes from both dissolved molecules as well as those that desorb from other particles. The adsorption/desorption of molecules at the surface of a particle is a kinetic process that depends on the local level of saturation as well as the surface energy of the adsorbed molecules. It can lead to the phenomenon of Ostwald ripening, in which larger particles grow at the expense of smaller ones due to the fact that the surface tension dependence on particle size favors adsorption on larger particles. The details of how these monomers adsorb and what, if any, crystal structures form is not always clear.

13.2 Experimental Methods

TCNQ is an organic electron-acceptor compound, which is often used in charge transfer salts, for example, tetrathiafulvalene-tetracyanoquinodimethane (TTF-TCNQ), for molecular electronics applications. The material described here is prepared using the reprecipitation method, described in section 11.2. In the reprecipitation method, the growth of crystals typically becomes stable after 20-30 minutes of mixing. To study the morphology of the particles formed at this stage of the growth, we rapidly froze the samples, followed by freeze drying, in order to obtain samples for scanning electron microscopy (SEM) and powder X-ray diffraction (PXRD). 40 minutes after
the initial mixing, the samples were immersed in liquid nitrogen. Compared to the
typical rates of particle formation at this stage of growth, immersion in liquid nitrogen
provides a sufficient cooling rate to lock in the morphologies of the nanoparticles.\textsuperscript{131}
The frozen sample was then freeze dried at -80 °C under vacuum for several hours to
days in order to sublimate the water.

\textbf{13.3 Characterization and Results}

To characterize the crystallized structures of TCNQ, several characterization
methods were used, including SEM, WETSEM, PXRD, and absorption spectroscopy.
The detailed description of each method is written in section 11.3.

The absorption spectra of the reprecipitated TCNQ crystals were compared to
that of a TCNQ acetone solution. As shown in Figure 13.2, the reprecipitated sample
has broader absorption peaks, blue-shifted about 10 nm with respect to the solution
peaks, which suggests a broader distribution of molecular energy levels of TCNQ and
an increased bandgap. This may be due to the formation of H-aggregates, giving rise
to excitonic absorption.\textsuperscript{132}

In Figure 13.3, the PXRD patterns of the freeze dried reprecipitated sample
confirms the formation of TCNQ crystals as the observed pattern follows the PXRD
pattern of TCNQ in the Cambridge Structural Database. We also attempted to
observe the particle growth of TCNQ crystals vs. time using dynamic light scat-
ttering. However, TCNQ can rapidly form large microcrystalline structures, which can
intermittently enter the sampling volume and scatter strongly, so consistent autocor-
relation functions of the scattered photon count could not be obtained.
13.4 SEM Observations and Results

Micro- and nano-crystals of TCNQ have been previously observed with several different structures; for example, helical nanowires and dendrites of complexes with TTF and Ag-TTF have been reported in several works,\textsuperscript{133,134} and, depending on solute concentration, TCNQ films on Cu and Al form structures that are dendritic or rhombic\textsuperscript{115}. However, as shown in Figure 13.4, we observed an extremely wide variety of TCNQ structures at several different size scales in samples prepared using reprecipitation. Within the same sample, helical structures and rectangular parallelepipeds in the 200-400 nm size range were observed, but also 10-20 micron-sized polyhedral structures appeared in multiple forms as seen in Figure 13.4c. To confirm that the structures observed were due to the nucleation and crystallization growth in the reprecipitation method, SEM images of a TCNQ acetone solution (no freeze
drying, only vacuum sublimation) were also taken. In the acetone solution sample, plate structures with warped edges were mostly observed but not the multitude of structures seen in the reprecipitated sample. We also used WETSESM technology to image nanocrystals dispersions in situ in order to ensure that the structures observed in the SEM images were not artifacts of the freeze drying process. As shown in Figure 13.4d, the same polyhedral structures of TCNQ were observed in WETSEM as in the regular SEM imaging. Due to the nature of WETSEM, a sample crystal needs to be attached on the membrane of the wet sample capsule to be observed, and the beam energy must be maintained low not to damage the membrane. Therefore, the smaller structures could not be observed.

Figure 13.4: SEM images of various shapes of reprecipitated TCNQ. Various shapes were observed such as (a) coiling structures, (b) rectangular parallelepipeds, and (c) different polyhedra. (d) WETSESM image also shows polyhedral structures.
In general, the variety in structure forms can be explained in terms of the bond weakness of TCNQ. Molecular crystals of an organic compound are usually held together with weak van der Waals forces.\textsuperscript{135} The energy minima are likely to be shallow, with multiple closely spaced metastable energy states of the crystals nearby that can coexist with the thermodynamically stable one, especially at short times. The closeness of the energy levels may result in the transformation of such structures to the most stable state being too slow to observe.

Despite the variety of morphologies, some of the TCNQ structures seemingly show the growth process of polyhedron plates starting as seed-like structures. The images in Figure 13.5 are representatives of a large number of similar structures observed in the SEM images. As shown in Figure 13.5a, there are many 100-200 nm wide seed-like structures, with 30-50 nm wide branches growing out of them. In structures that seem to be further along in the growth pathway, these branches have extended out in all directions and start resembling dandelion flowers (Figure 13.5c). The flower-like structures then connect to their neighbors, and as they bundle together, they become the polyhedral shapes shown in Figure 13.5e. At some point, the flower-like structures on the outmost layers of the polyhedral formations get flattened and smoothed out. The resulting structures then become 10-20 $\mu$m polyhedra (Figure 13.5h). As the structures reach this stable state, smooth surfaces are formed. The flatness of the polyhedra seems to indicate it is the final, thermodynamically stable morphology.

If these structures do represent a progression of nanocrystals morphologies, they reveal a complex process of formation. Ostwald’s rule of crystallization stages maintains that crystals can pass through a series of metastable phases with successive phases being those that require the lowest amount of free energy to form. While we are not able to determine whether the different morphologies represent different crystal
phases, some of the transitions in the formation of microcrystals from nanostructures seem to adhere to this rule. For example, the change from the flower-like structures in Figures 13.5e and 13.5f to the final polyhedra in 13.5h seems to represent an actual change of phase. An interesting aspect of these observations is that metastable forms remain for considerable times even after the stable structures have formed; the seed-like nucleation sites coexist with flattened polyhedra. Current efforts are aimed at further investigations of the progression of these forms. Ostwald ripening will provide a further mechanism for the disappearance of the smaller structures and growth of the larger ones. Thereby, if unstable, the smaller structures should eventually disappear.

The polyhedral TCNQ structures showed a dependence on preparation conditions as well. At ten times higher concentration of the initial TCNQ acetone solution, smaller polyhedra formed, around a few microns in size, as expected from classical nucleation theory (CNT) (Figure 13.6). In CNT, as the concentration increases, so does the rate of crystal formation. This leads to a larger number of initial nucleation sites, which in turn results in smaller particles.80

The shapes and sizes of the TCNQ nanocrystals also had a strong temperature dependence. When the samples were reprecipitated in an ice cold bath, polyhedral structures were formed, mainly in the form of 30 micron long sword-like structures in which the crystals grew much more along one axis as shown in Figure 13.7a. In contrast, samples reprecipitated in a hot bath showed a wide variety of shapes, including polyhedra that were often near perfect rhombic shapes (Figure 13.7b).
Figure 13.5: SEM images of progression in self-assembly of reprecipitated TCNQ. Starting as (a) 100-300 nm size seed-like structures, TCNQ seems to extend (b) 30-50 nm wide branches from the seed-like structures, and (c) such branch structures elongated in all direction. (d) Branches are connected to neighbors, resembling dandelion flowers a few microns in size. (e) Flower-like structures bundle together to (f) seemingly form a polyhedral plate as they bloom more. (g) Flower-like structures on the outmost layer of a polyhedron become flattened as the progression goes, and eventually (h) form smooth surfaces. The resulting polyhedral structure is formed with sizes ranging from 10 to 20 microns.
Figure 13.6: SEM images of reprecipitated TCNQ at a high concentration (1.5mM). (a) SEM image of polyhedra shapes forming 1-4 µm in size. (b) WETSEM image.

Figure 13.7: SEM images of reprecipitated TCNQ at different temperatures. Reprecipitated TCNQ crystals prepared in (a) cold and (b) hot bath formed different shapes of polyhedral crystal structures than that of the sample prepared at room temperature. The sample reprecipitated in cold bath formed 30 µm long sword-like structures, and the other synthesized in hot bath had 10-30 µm nearly perfect rhombic structures.
14 Conclusions and Future Research

In this part of the dissertation, the development of the strategy for the creation of multicomponent nanoparticles using the reprecipitation method is discussed. In spite of the challenge in establishing the model, the successful preparation of mixed charge transfer nanocrystals of perylene:TCNQ using the reprecipitation method was demonstrated in this research. This is an example of the type of system that would benefit greatly from the successful development of RCS. The onset of this self-organization was monitored by a number of characterization methods. These nanocrystals form and stabilize quickly, within approximately 30 minutes. Nanocrystal sizes in the range of several hundred nanometers were found in the mixed system by DLS and SEM. The presence of the charge transfer crystal is inferred from the absorption and Raman spectra. The element analysis was tested with EDS, and the crystal formation was confirmed with PXRD. The environment dependence was also investigated by changing the molar ratio and temperature, and the experiments showed their influence on the cocrystal nature of the sample. An API cocrystal consisting of CBZ:NCT was also prepared using the reprecipitation method to demonstrate the versatility of the method. Hence the reprecipitation method could be used in numerous applications in the chemical and pharmaceutical industries. The experimental results confirm that the reprecipitation method successfully enables two species to form into the same multi-component nanoparticle.

For further establishment of the reprecipitation method for a multi-component nanoparticle synthesis, it would be useful to develop a general model to be used in predicting the possibility of forming composite particles from the individual components and parameters of the processing conditions. A preliminary model was developed using the regular solution theory along the concept of the Hansen solubility parameters.
in this research, but to fully develop the model, it would require further investigation of a general rule of solubility conditions. The statistical design of experimental method that determined critical parameters that influence nanoparticle formation could aid development of the model. Additional experiments on multi-component particle formation would provide information on how different species would co-self-assemble and how their solubility would change according to environmental changes. One set of experiments can be done using perylene:TCNQ. In this dissertation, several condition dependent experiments were conducted, but a more careful study of the material is required. For example, how the formation varies with temperature, solvent, or concentration. Hence, future research can continue to investigate the reprecipitation method using this material. Using other species to form the multi-component nanoparticle would indeed help determining the condition dependency in solubility, or the theoretical expression for solubility in general. Therefore, a next step of this research would be to conduct more experiments with varieties of materials and solute and solvent conditions. Once the model is fully established, it would contribute to determining whether materials could be induced to form composite nanoparticles using the reprecipitation method, or under which conditions those materials can be forced to self-assemble. Therefore, this research could contribute to developing more novel nanoparticles containing multiple functionalities due to contributions from individual ingredient species. It would be applicable to any nanomaterial science, including both industrial and biomedical fields.

During the course of the study of the reprecipitated perylene:TCNQ, a unique self-assembly characteristic of TCNQ crystals was observed. Based on findings from SEM imaging, a model was proposed of crystal formation via intermediate metastable states and multiple progressions of crystal morphologies of TCNQ prepared by the reprecipitation method. Starting as seed-like structures, TCNQ crystals bloomed like
flowers and eventually formed polyhedron shapes. This self-organizing progression seems to follow Ostwald’s rule of stages. The observations seemingly following this rule were circumstantial, so further investigation of the specific phase changes is required to confirm if such is the case. For example, \textit{in situ} high-resolution electron microscopy would help for more detailed atomic-level observation of the structure. Besides the evidence the images provide relating to Ostwald’s rule, the results also suggest a complex process of nanoparticle formation in the reprecipitation method. If the different structures could be isolated, crystallographic study of the structures would also prove the crystal progression. Understanding this process provides valuable insights for the application of the reprecipitation method for industrial use.
Appendices

A MATLAB Codes for Intensity Fluctuation Analysis in RCS

A.1 Main Routine: RCSascfit.m

```matlab
% %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% PROGRAM: RCSascfit
% %
% This program analyzes the ACF data of Raman Correlation Spectroscopy
% and determine the hydrodynamic radius of particle.
% %
% The nonlinear equation to be fitted is:
% 
% \[ g = \beta \exp(-2\gamma t)(1+\mu^2/2)t^2)^2 + B; \]
% 
% In the program x is a vector: x(1)=\beta, x(2)=\gamma, x(3)=\mu^2, x(4)=B;
% The initial guessed values are set in the array, x0.
% By using lsqcurvefit, x is automatically optimized under the lower
% and upper bounds (lb and ub). Restricting helps the fit.
% The collected data (i.e. \beta, \gamma, \mu^2, B, and particle radius) are
% saved to the data file: fitdata.txt.
% 
% In addition to fit program feature, this program also determines
% particle radius using the Stokes–Einstein relation and plot
% the autocorrelation function with the fit.
% For radius calculation, be sure to check constant values
% (i.e. viscosity, temperature, refractive index).
% Individual graph is saved under the same name of the source file
% in jpeg.
% 
% This program requires the lsqcurvefit function (Optimization Toolbox)
% and function file fun2.m for the exact expression of
% the autocorrelation function. The program optimize 3 times, defined
% by num_inner_loop.
% 
% ***Important source file format issue***:
% %
% (1) if the time in the source date file is not in quotation mark,
% use the line with "q_issue1" comment and make the line below a
% comment line in the Matlab program ascfit.m, and vice versa;
% (2) if the word Correlation in the source date file is not in
```

132
clear;

format long;

%%%Constant Assignments:
nu = 0.89454e−3; % viscosity in Pas
Temperature = 298.16;
kB = 1.38e−23; %Boltzmann Constant (J/K)
ref_index = 1.33; %refractive index

disp('It is important to check if the time and/or the word Correlation');
disp('in the source data file is in quotation mark,');
disp('because it affects if the program runs correctly.');
disp('press ''ctrl+c'' to stop the program and type ''help RCSascfit''');
disp('for help about the quotation issue.');
disp(' ');
disp('Initial parameters and limits are set in the program.');
disp('Read the end of the program to change the setting.');

disp('Input the format of file name: XN.asc,')
disp('here X is a set of text characters for the base file name, and');
disp('N is a 4-digit number,');
disp('but not necessarily;')
disp('N is a range if a series of data files are to be fitted.');
filenameX=input('Input the base file name: ','s');
disp(' ');
disp('Specify the range of the data files, usually from 1 ~ 9999');
filenameN_min=input('Input the beginning number of the data files N_min:','s');
filenameN_max=input('Input the ending number of the data files N_max >= N_min:','s');
disp(' ');
disp('Specify the data points to be fitted');
M_first1=input('Input the first data point, 1 ~ 30: ','s');
M_last1=input('Input the last data point, 120 ~ 175: ','s');
disp(' ');

%%%RCS:
%lamda_input1 =input('Input the incident wavelength (nm): ','s');
%lamda_temp1 = str2num(lamda_input1);
%lamda_inc = lamda_temp1*10.^-9;
   lamda_inc = 514.5*10.^-9;
% lamda_inc = 488.0*10.^-9;
fprintf('---Incident Wavelength is set at %.2f', lamda_inc/10.^-9);
fprintf('nm. 
');
lamda_input2 =input('Input the scattering(Raman) wavelength (nm): ','s');
lamda_temp2 = str2num(lamda_input2);
lamda_scat = lamda_temp2*10.^-9;
disp(' ');

%%%!!!!!!!!!!!!-------------------------------!!!!!!!!!!!!%%% % Initial Guess parameter for fits:
%This can be changed according to Data
guess1=input('Input guess for beta: ','s');
guess2=input('Input guess for Gamma: ','s');
guess3=input('Input guess for mu2: ','s');
guess4=input('Input guess for B: ','s');
guess1=str2num(guess1);
guess2=str2num(guess2);
guess3=str2num(guess3);
guess4=str2num(guess4);
x0temp = [guess1 guess2 guess3 guess4];
%x0temp0 = [0.02 0.02 0 0];
%%%!!!!!!!!!!!!-------------------------------!!!!!!!!!!!!%%%
xguess=x0temp;

disp('##########################################################################');
disp('The data variables from the fit (i.e.,beta, gamma, mu2 and B),');
disp('are saved to the data file: fitdata.txt. ');
disp('##########################################################################');
disp(' ');
disp('You have finished specifying parameters. ');
disp('If you want to change them, press "ctrl-c" to stop the program, ');
disp('and run it again. ');
disp('Otherwise, press "Enter" to run the program. ');
pause;

num_total_points=175;
M_first=str2num(M_first1);
M_last=str2num(M_last1);

N_beginning_remove=M_first-1;
N_ending_remove=num_total_points-M_last;
%file name numbers
N_min=str2num(filenameN_min);
N_max=str2num(filenameN_max);
num_of_runs=N_max-N_min+1;

num_inner_loop=3; % # of loop needed to obtain optimized coefficients

fid1=fopen('fitdata.txt','w');

%%%%%RCS:
fprintf(fid1,'Incisdent Wavelength (nm): %.2f \n', lamda_inc/10.^-9);
fprintf(fid1,'Scattering Wavelength (nm): %.2f \n', lamda_scat/10.^-9);
fprintf(fid1,'Temperature (K): %f \n', Temperature);
fprintf(fid1,'Visicosity: %f \n', nu);
fprintf(fid1,'Refractive index: %f \n', ref_index);
fprintf(fid1,'Data points taken between: %s', M_first1);
fprintf(fid1,' to %s 
',M_last1);

fprintf(fid1,'Initial GUESS PARAMETERS for fit:\n');
fprintf(fid1,'beta Gamma mu2 B\n');
fprintf(fid1,'%.3f %.3f %.3f %.3f
', xguess(1),xguess(2),xguess(3),xguess(4));

fprintf(fid1,'FIT RESULT(S):\n');
fprintf(fid1,'%s %s %s %s %s %s
','filename','beta','Gamma','mu2','B','residue','PDI','Radius(nm)');

for ir=1:num_of_runs %big loop, number of runs
    filename_N=ir+N_min-1;
    filename_N_string=num2str(filename_N);
    filenameN=['000', filename_N_string];
    if filename_N<10
        filenameN=['00', filename_N_string];
    end
    if filename_N<100
        filenameN=['0', filename_N_string];
    end
    if filename_N<1000
        filenameN=[filename_N_string];
    end
    if filename_N<10000
        filenameN=[filename_N_string];
    end
    filename=[filenameX, filenameN,'.asc'];

for it=1:inf
    temp0=textread(filename, '%s', it);
    if strcmp(temp0(it), 'Time');
        it_time=it;
    end

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% const=strcmp(temp0(it), 'Correlation'); % q
const=strcmp(temp0(it), '"Correlation"');
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
if const==1
    it
    start0=it; %Where "Correlation" is.
    break;
end
end

time1=temp0(it_time+2);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% time2=time1{1}(1:end); % q_issue1
    time2=time1{1}(2:end);
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

it_end0=it_start0+num_total_points*2+1; %where "Count Rate" is.
temp1=textread(filename, '%s', it_end0);

it_start=it_start0+2*N_beginning_remove;
it_end=it_end0-2*N_ending_remove;

it_total_xy=it_end-it_start-1;
it_total=round(it_total_xy/2);

for it=1:it_total_xy
    temp2(it)=temp1(it+it_start);
end

temp3=str2double(temp2);

for it=1:it_total
    t(it)=temp3(2*it-1);
    g(it)=temp3(2*it);
end

x0=x0temp;

% Set Lower and upper bounds of parameters x(i) used lsqcurvefit
% [beta Gamma mu2 B]
lb = [0 0 0 0]; % defining lower band
ub = [1 1 0.001 0.001]; % defining upper band

% Fit the data with the autocorrelation function defined in fun2.m
for is = 1:num_inner_loop
    [x,resnorm]=lsqcurvefit(@fun2,x0,t,g,lb,ub);
    x0=x;
end

%%% RCS: Radius Calculation from ascfit result for RCS measurement:
qq = (2*pi*(ref_index/lambda_inc))^2+(2*pi*ref_index/lambda_scat)^2;
Gamma = x(2)*1000; % convert x(2) in 1/ms to 1/s
D = Gamma/qq; % from Gamma = Dq^2 Stoke–Einstein Relation
PDI = x(3)/Gamma/Gamma;
Radius = kB*Temperature/(6*pi*nu*D)/10^-9; % Particle Radius in nm

fprintf(fid1,'%s %f %f %.3e %.3e %.3e %.3e %f
',filename,x(1),Gamma,x(3),x(4),resnorm,PDI,Radius);

gfit=x(1)*exp(-2*x(2)*t).*(1+x(3)*t^2+0.25*x(3)^2*t^4)+x(4);

%%% RCS: ACF Graph
graphname = [filenameX, filenameN,'pt',M_first1,'-',M_last1];
semilogx(t,g,'b.',t,gfit,'m');
xlabel('Lag time (ms)');
title(sprintf('Autocorrelation function of %s', graphname));
legend('source data','Nonlinear Fit');
print ('-djpeg', graphname); % save each graph as jpeg
end % big loop, number of runs

disp(' ');
disp('----------------------------------------------------------------------------------------------------');
disp(' ');
disp('The initial Guessed parameters were set in variable x0 as below.');
disp('Fit were done using the initial guess');
disp('Initial Guessed parameters were set in variable x0:');
fprintf('coherence factor (beta) = %.3f
',xguess(1));
fprintf('decay rate (Gamma in 1/ms)= %.3f
',xguess(2));
fprintf('2nd moment (mu2) = %.3f
',xguess(3));
fprintf('Baseline (B) = %.3f
',xguess(4));
disp('----------------------------------------------------------------------------------------------------');
disp(' ');
disp('This program used the second order term for the ACF fit.');
disp('Fitting parameters were restricted in following condition.');
disp(' [beta Gamma(1/ms) mu2 B ]');
fprintf('Lower Bound(lb): [%.2f %.2f %.2f %.2f]
',lb(1),lb(2),lb(3),lb(4));
fprintf('Upper Bound(ub): [%.2f %.2f %.2f %.2f]
',ub(1),ub(2),ub(3),ub(4));
disp(' ');
disp('See filedata.text under work directory for the results.');
disp('Graphs are saved under the same directory in jpeg format.');
disp(' ');
disp('If Guess (initial) parameters should be changed,');
disp('change the array x0 in RCSascfit.m and rerun the program with');
disp('the new initial parameters.'');
disp('If that does not help, try restricting lower and upper bounds.');
fclose(fid1);

A.2 Subroutine: fun2.m

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% fun2.m
% This is function for intensity autocorrelation function fit using the
% modified cumulant method.
% %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
function G_fun=fun2(x,xdata)

G_fun=x(1)*exp(-2*x(2)*xdata).*(1+x(3)*xdata.^2+0.25*x(3)^2*xdata.^4)+x(4);
B Geometric Method of the Three Solvent Spheres Model

This section presents the derivation of the Three Solvent Spheres Model using the geometric method. At the saturated concentration, the interaction radius $R_o$ is the distance between the HSPs of a solvent and a solute in Hansen space. An unknown HSPs of a solute can be then determined if HSPs and $R_o$ of solvents are known by drawing spheres of solvents with their HSPs being center and $R(C)$ in equation (10.11) being the radii. The intersection of those spheres reflects the HSPs of the solute. To acquire the solute HSPs, three solvents were chosen so that three spheres are used to find the intersection (Figure B.1).

The geometric algorithm of this model is as follows. In the three dimensional space, assume that three spheres are intersecting with one another. If we consider only two spheres, the intersection of the two is a circle. The third sphere must intersect along the circle, and consequently, the intersections of three spheres are two points along that circle (Figure B.2). To determine those points, the transformations of the coordination system are applied for easy navigation of the derivation. Let the centers of the spheres 1, 2, and 3 with radii $r_1$, $r_2$, and $r_3$ respectively be defined as

![Figure B.1: Schematic of three solvent spheres in a Hansen space. The spheres represent different solvents. The center of a sphere is its HSP position and the radius of the sphere is $R_o$ of the individual solvent.](image)
Figure B.2: Diagrams of the intersections of spheres. (a) Intersection of two spheres is a circle. (b) Circle A is an intersection between two spheres that lie on plane A, and circle B is an intersection between another pair of spheres on plane B. Two intersection points of these circles are the intersection points of three spheres.

the following in the original space:

\[ \vec{S}_1 = (x_1, y_1, z_1) \]
\[ \vec{S}_2 = (x_2, y_2, z_2) \]
\[ \vec{S}_3 = (x_3, y_3, z_3) \] (B.1)

The centers of the paired spheres 1 and 2 lie on a plane with one of the axes along the vector \( \vec{d}_{12} = \vec{S}_1 - \vec{S}_2 \) from the origin of Hansen space. The same applies for the pair of spheres 1 and 3 on a plane along the vector \( \vec{d}_{13} = \vec{S}_1 - \vec{S}_3 \).

First, when only the pair of sphere 1 and 2 is considered, it is convenient to use a new, temporary coordinate system (coordinate system A) where the center of sphere 1 is considered as the origin of this temporary space with one of the axes along \( \vec{d}_{12} \). Thus, the equations to describe the spheres in this new coordinate system are

\[ x_A^2 + y_A^2 + z_A^2 = r_1^2 \] (B.2a)
\[ (x_A - d_{12})^2 + y_A^2 + z_A^2 = r_2^2 \] (B.2b)
where $d_{12}$ is the magnitude of $\vec{d}_{12}$, or the distance between the centers of the two spheres.

The intersection of the two spheres is a circle (circle A) that lies on a plane perpendicular to $\vec{d}_{12}$ at $x_A$. Thus, by solving equations (B.2) for $x_A$, we find that the intersecting circle A lies at

$$x_A = \frac{d_{12}^2 - r_1^2 - r_2^2}{2d_{12}}. \quad (B.3)$$

This is the x coordinate of the intersection points of the two spheres in the coordinate system A. The similar situation is also said for the pair of spheres 1 and 3, which lie together in a temporary coordinate system B where the center of sphere 1 is still the origin of the space but the axis is along $\vec{d}_{13}$ this time.

Since the coordinate systems A and B use the center of sphere 1 as the origin, it can be easily seen that system B is the rotated version of system A by angle $\alpha$. The angle is defined by the dot product of $\vec{d}_{12}$ and $\vec{d}_{13}$:

$$\cos(\alpha) = \frac{\vec{d}_{12} \cdot \vec{d}_{13}}{|\vec{d}_{12}||\vec{d}_{13}|}. \quad (B.4)$$

The intersection of spheres 1 and 3 that is a circle (circle B) lies on a plane at $x_B$, which can be written in terms of the coordinate system A instead of B as

$$x_B = \frac{d_{13}^2 - r_1^2 - r_3^2}{2d_{13}} = x_A\cos(\alpha) + y_A\sin(\alpha). \quad (B.5)$$

Solving the above equation for $y_A$ provides the y coordinate for the intersection points of three spheres in system A:

$$y_A = \frac{\frac{d_{13}^2 - r_1^2 - r_3^2}{2d_{13}} - x_A\cos(\alpha)}{\sin(\alpha)}. \quad (B.6)$$
With equations (B.3) and (B.6), the equation of spheres gives the \( z \) coordinate of the intersection points in the system \( A \):

\[
z_A^\pm = \pm \sqrt{r_1^2 - x_A^2 - y_A^2}.
\]  

(B.7)

Therefore, in the temporary coordinate system \( A \), the two intersection points can be written in terms of the following vector from the center of sphere 1:

\[
\vec{I}_1 = (x_A, y_A, z_A^+) \quad \quad \vec{I}_2 = (x_A, y_A, z_A^-).
\]  

(B.8)

Definition of the intersection points must be redefined in the original Hansen space coordinate system. Since \( \vec{d}_{12} \) and \( \vec{d}_{13} \) are in the same plane in the temporary coordinate system, the cross product of them gives the vector perpendicular to the plane. Thus, the following derivations transform back \( \vec{I} \) in the Hansen space:

\[
\begin{align*}
\vec{x}_i &= x_A \frac{d_{12}}{|d_{12}|} \\
\vec{y}_i &= y_A \left( \frac{d_{12} \times d_{13}}{|d_{12} \times d_{13}|} \times d_{12} \right) \\
\vec{z}_i^+ &= z_A^+ \frac{d_{12} \times d_{13}}{|d_{12} \times d_{13}|} \\
\vec{z}_i^- &= -z_i^+.
\end{align*}
\]  

(B.9)

The above definitions of the interception points do not take into account the position of sphere 1. Hence, the interception points \( T_{i1} \) and \( T_{i2} \) need to be shifted; therefore, the two intersection points of three spheres can be defined as

\[
\begin{align*}
\vec{T}_{i1} &= \vec{S}_1 + \vec{x}_i + \vec{y}_i + \vec{z}_i^+ \\
\vec{T}_{i2} &= \vec{S}_1 + \vec{x}_i + \vec{y}_i + \vec{z}_i^-.
\end{align*}
\]  

(B.10)
with all terms defined in equations (B.1) and (B.9). This geometric algorithm is applied using the HSP data of three solvents. The two intersection points of three spheres were determined using the MATLAB function code (intersection3sphere.m), which is listed in Appendix C.2.

The mid point of the two intersection points is ideally the HSP of a solute sphere if the interaction radius $R_o$, which was derived in equation (10.11) as a preliminary guess, was a nearly perfect fit (In the perfect fit situation, the spheres only intersect at the single point; hence, the algorithm described above is not necessary).

The main MATLAB code (TSSmodel.m in Appendix C.1) will run all the possible combinations of three different solvents that are listed in a data file. The coordinates of the mid points of the intersection distances are averaged out. The resultant value is used as an estimate of HSP of a solute. Appendix C lists the all MATLAB codes used to demonstrate this model.

The code can also pick only the combinations that have the distance between the intersection points smaller than the preset value to gives the average values for the HSP. To optimize the HSP coordinates by adjusting the value of $R(C)$, this algorithm should also use the minimization method to have the distance between the intersection points shortest in the end. However, it would require more complex arguments and assumptions to establish the relation with the general constant values described in equation (10.11). Therefore, before exploring possible ways to do so, the simple way was chosen to demonstrate. The results for three different materials are shown in section 10.5.
C MATLAB Codes for Three Solvent Spheres Model

C.1 Main Routine: TSSmodel.m

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% TSSmodel.m
%
% This program determines the estimated Hansen solubility parameters of a
% solute using the solubility parameter data of solvents. It will pick all
% possible 3 combinations of solvents. If the combo spheres intersect, the
% intersection points, length of them, and its mid points are recorded in
% data file. If If do not interact, the code moves to next combination.
%
% Solvent solubility parameters should be formatted in tab delimited txt
% file (.txt) with the strings marked with quotation marks(" ").
% "Sample" "D" "P" "H" "Concentration"
% "Solvent 1"
% "Solvent 2"
% "Solvent 3"
% ...
%
% "Concentration here means solubility..."
%
% The program also requires separate function files:
% intersect3spheres.m
% checkintersectibility.m
% objfundistance.m
% calcintlengh.m
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Written by Maki Nishida
% Department of Physics, Georgetown University
% 2010
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%Concentration is molar ratio so it must be less than 1
% R^2 = A*ln(C)+B
% where A is negative and B is positive always.
% or r(i)=sqrt(1/B*log(A)-1/B*log(concentration(i)));

clear;
format long;

%Read a txt file of the solubility parameters
disp('');
disp('The txt file should contain solubility parameters of all solvents');
disp('The format of the txt file(tab delimited) should be the following:');
disp('______________________________');
disp('"Sample" "D" "P" "H" "Concentration"');
disp('"Solvent 1" "%f" "%f" "%f" "%f"');
disp('...');
disp('--------------------------------------------------------------------------------------------------');
disp('All strings should be in quotations. %f is a numerical value.');
disp(' ');
fileName = input('Enter the txt file of solubility data with all solvents: ','s');
fileName = [fileName, '.txt'];

fileName = ['HSPNaphthaleneHighSolvents.txt'];
soluteName = ['Napthalene'];

[solute,soluteD,soluteP,soluteH] = textread(fileName, '%q %f %f %f',
1,'headerlines', 1);
[solName,dSol,pSol,hSol,concentrationSol] = textread(fileName, '%q %f %f %f %f %f',
'headerlines', 3);
m = size(solName,1);

m = size(solName,1);

% To pick all possible combinations, % use permutations for 3 solvents of all data.
solvCombo = nchoosek(1:1:m,3);
combsize = size(solvCombo,1);

resultfileName = ['solParaResult', fileName];
fid1=fopen(resultfileName,'w');
fprintf(fid1,'Hansen Solubility Parameter Estimation 
');
for j = 1:combsize
    fprintf(fid1,'Combo#','Dispersion', 'Polar', 'Hydrogen', 'interx D', 'interx P', 'interx H', 'mDispersion', 'mPolar', 'mHydrogen');
end

% Define constant for radius calculation
constB = 0.00765; %b
constA = 0.340; %a
k = 1;
thereisimaginary = 0;
for j = 1:combsize
    % Define each parameters of 3 solvents
for i=1:3;
    disper(i)= dSol(solvCombo(j,i));
    polar(i) = pSol(solvCombo(j,i));
    hydro(i) = hSol(solvCombo(j,i));
    concentration(i) = concentrationSol(solvCombo(j,i));
end

%Set radius of solvent sphere
for i = 1:3;
    rSolv(i)=sqrt(1/constB*log(constA)−1/constB*log(concentration(i)));
end

[intLength,intPoints] = calcintlength(disper,polar,
         hydro,concentration,rSolv);

for ii = 1:2
    for jj = 1:3
        if abs(imag(intPoints(ii,jj)))>0
            thereisimaginary = thereisimaginary+1;
        end
    end
end

if thereisimaginary == 0
    intmidPts(k,1) = abs(intPoints(1,1)+intPoints(2,1))/2;
    intmidPts(k,2) = abs(intPoints(1,2)+intPoints(2,2))/2;
    intmidPts(k,3) = abs(intPoints(1,3)+intPoints(2,3))/2;
    interxlength(k) = intLength;
    fprintf(fid1,'%2d %2d %2d |t %s |
',
            solvCombo(j,:),solName{1},disper(1),polar(1),
            hydro(1),concentration(1),intPoints(1,:),intLength,
            intmidPts(k,1),intmidPts(k,2),intmidPts(k,3));
    fprintf(fid1,'%2d |t %s |
',
            solName{2},disper(2),polar(2),hydro(2),concentration(2),intPoints(2,:));
    fprintf(fid1,'%2d |t %s |
',
            solName{3},disper(3),polar(3),hydro(3),concentration(3));
    k=k+1;
end

thereisimaginary = 0; %Reset counter
end

estimSolute = mean(intmidPts);
fprintf(fid1,'Estimated HSP of Solute: %3.3f %3.3f %3.3f n',
        estimSolute);
% Find estimated HSP of solute only the combo that gives smaller
% intersection length (maxLength)
maxLength = 5;
count = 0;
interxlength = interxlength';
umInterx= size(interxlength,1);
interxD = 0;
interxP = 0;
interxH = 0;
for i = 1:numInterx
    if interxlength(i) < maxLength
        count = count+1;
        interxD = interxD+intmidPts(i,1);
        interxP = interxP+intmidPts(i,2);
        interxH = interxH+intmidPts(i,3);
    end
end
fprintf(fid1,'\n|t|t|t|t|t|t|t For interx Length less than%d\n', maxLength);
fprintf(fid1,'|t|t|t|t|t|t|t Estimated HSP for Solute:|t %.3f\t %.3f\t %.3f',interxD/count,interxP/count,interxH/count);
fclose(fid1);

disp(' ');
disp('Estimated HSP of this solute:');
disp([estimSolute(1),estimSolute(2),estimSolute(3)]);
disp(['With limitation of intersection length with ', int2str(maxLength)]);
disp([interxD/count,interxP/count,interxH/count]);
disp(' ');
disp('Open the result file "TSSresult_(Name of Data file).txt" in Excel
to see the result');

C.2 Subroutine: intersect3sphere.m

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% intersect3spheres.m
% This is function to determine the intersecting points of 3 spheres. It
% will return the intersecting points as two vectors.
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
function [intPoints] = intersect3spheres(d,p,h,r)

%Define each solvent sphere with its parameters
Sphere1 = [d(1) p(1) h(1)];  %d has extra factor of 2 because it is for
% radius of Hanson solubility parameters

Sphere2 = [d(2) p(2) h(2)];
Sphere3 = [d(3) p(3) h(3)];

% vectors from Sphere 1 to 2 and 1 to 3
distS12vect = Sphere2 - Sphere1;
distS13vect = Sphere3 - Sphere1;

% angle between distantS12 and distant13
distS12 = norm(distS12vect);
distS13 = norm(distS13vect);
alphaAngle = acos(1/(distS12*distS13)*dot(distS12vect,distS13vect));

% Intersection coordinates in the temporal coordinate system
    tempX = (distS12^2 + r(1)^2 - r(2)^2)/(2*distS12);
    tempXprime = (distS13^2 + r(1)^2 - r(3)^2)/(2*distS13);
    tempY = (tempXprime - tempX^2 - tempX^2 - tempY^2);
% tempZ_plus = sqrt(r(1)^2 - tempX^2 - tempY^2);
% tempZ_minus = -1*tempZ_plus;

% Transform the coordinate system back to the original
    cPlanevect = cross(distS12vect,distS13vect);
cPlane = norm(cPlanevect);
x = tempX*distS12vect/distS12;
z_plus = tempZ_plus*cPlanevect/cPlane;
z_minus = -1*z_plus;
y = tempY*cross(cPlanevect,distS12vect)/norm(cross(cPlanevect,distS12vect));

% Shift back to the original positions in the original coordinate system.
    intersect_plus = x+y+z_plus+Sphere1;
    intersect_minus = x+y+z_minus+Sphere1;

% Return the two intersecting points as vectors
intPoints = [intersect_plus; intersect_minus];

end

C.3 Subroutine: plotsphere.m

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% plotsphere.m
% This is function to plot a sphere with given center coordinate, radius, % and color and to be used in solPara.m.
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

function plotsphere(center, radius, color)
phi=linspace(0,pi,50);
theta=linspace(0,2*pi,50);
[phi,theta]=meshgrid(phi,theta);
x=radius*sin(phi).*cos(theta);
y=radius*sin(phi).*sin(theta);
z=radius*cos(phi);

alphaNumEdge = 0.15; % 0.3;
alphaNumFace = 0;
mesh(x+center(1), y+center(2), z+center(3), 'facecolor',color,
     'edgecolor', color,'facealpha', alphaNumFace,
     'edgealpha',alphaNumEdge);
camlight;
lighting gouraud;

C.4 Subroutine: calcintlength.m

function [intLength, intPoints]= calcintlength(d,p,h,concentration,r)

% Determine the intersections of 3 spheres
[intPoints] = intersect3spheres(d,p,h,r);

%distance between intersection points
intLength = objfundistance(intPoints);

return;

C.5 Subroutine: checkintersectibility.m

function

% checkintersectibility.m
% This function checks if 3 spheres intersect. If they do not intersect, it
% will increase radius of each sphere by decreasing A.
%
function [A]=checkintersectibility(intPoints,d,p,h,concentration,A)
for i = 1:2
    for j = 1:3
        if abs(imag(intPoints(i,j)))>0
            thereisimaginary = thereisimaginary+1;
        end
    end
end

if thereisimaginary > 1
    while thereisimaginary > 0
        A = A+1;
        for i = 1:3;
            r(i) = sqrt(A*(1-log(concentration(i))));
        end
        [intLength,intPoints] = calcintlength(d,p,h,concentration,A);
        thereisimaginary = 0;
        for i = 1:2
            for j = 1:3
                if abs(imag(intPoints(i,j)))>0
                    thereisimaginary = thereisimaginary+1;
                else
                    thereisimaginary = thereisimaginary;
                end
            end
        end
    end
else
    [intLength,intPoints] = calcintlength(d,p,h,concentration,A);
end
end
References


35. J. Creighton, C. Blatchford, and M. Albrecht, “Plasma resonance enhancement


95. P. Choi, T. Kavassalis, and A. Rudin, “Estimation of hansen solubility parameters for (hydroxyethyl) and (hydroxypropyl) cellulose through molecular simulation,” Industrial & Engineering Chemistry Research, vol. 33, pp. 3154–3159,


104. S. Nehm, B. Rodríguez-Spong, and N. Rodriguez-Hornedo, “Phase solubility diagrams of cocrystals are explained by solubility product and solution complexation,” *Crystal Growth & Design*, vol. 6, pp. 592–600, Jan 2006.


111. M. Michaud, N. Hota, and J. Zauhar, “Temperature dependent electronic absorption spectrum of k(tcnq), ba(tcnq)2, ca(tcnq)2 and perylene-tcnq,” *Chemical Physics*, vol. 36, pp. 79–84, Jan 1979.


