AN INVESTIGATION OF THE AGGREGATION BEHAVIOR OF CARNATION RING SPOT VIRUS USING DYNAMIC LIGHT SCATTERING.

by

LLOYD WILLIAMS

B. Sc., Imperial College, London, 1983

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of MASTER OF SCIENCE in The Faculty of Graduate Studies Department of Physics

We accept this thesis as conforming to the required standard

The University of British Columbia

April 1986

© LLOYD WILLIAMS, 1986
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Physics

The University of British Columbia
1956 Main Mall
Vancouver, Canada
V6T 1Y3

Date 29 May 86
The technique of digital autocorrelation of intensity fluctuations of scattered laser light is used to investigate the aggregation behavior of carnation ringspot virus. It is hypothesized by the author that the aggregation rate \( R \) is given by

\[
R(T, \nu_0) = \begin{cases} \frac{2\nu_0 k_B T}{3\eta} \exp[-E_{agg}/k_B(T - T_c)] & \text{if } T > T_c \\ 0 & \text{if } T < T_c \end{cases}
\]

\( R \) is measured for a range of values of the dependent experimental variables, temperature \( T \), and virus concentration \( \nu_0 \). And the dependence of the two aggregation parameters, the temperature of aggregation \( T_c \) and the energy of aggregation \( E_{agg} \), on the experimental variables is ascertained.

Also certain physical properties of the virus are measured: size, molecular weight, and diffusion coefficient.

The experimental accuracy of the apparatus is determined by performing a series of experiments on a known system of Latex spheres.
# TABLE OF CONTENTS

## ABSTRACT

- ii

## TABLE OF CONTENTS

- iii

## LIST OF TABLES

- v

## LIST OF FIGURES

- vi

## ACKNOWLEDGMENTS

- viii

## CHAPTER 1: INTRODUCTION

- 1

## CHAPTER 2: THEORY

- 4

  - 2.1 Introduction ........................................... 4
  - 2.2 Synopsis ............................................... 5
  - 2.3 The Theory of Light Beating Spectroscopy .......... 7
  - 2.4 Light Scattering From Macromolecules .......... 10
  - 2.5 Calculation of the Self Intermediate Scattering Function 16
  - 2.6 Koppel Method of Cumulants ...................... 19
  - 2.7 Theory of Brownian Aggregation ............... 21

## CHAPTER 3: EXPERIMENT

- 31

  - 3.1 The Correlator ........................................ 31
  - 3.2 The Digital Correlator Computer Interface .... 34
  - 3.3 The Index Matched Cell and Temperature Control Device 36
  - 3.4 The Optics ............................................ 37
  - 3.5 The Preparation of the Sample .................. 39
  - 3.6 The Experimental Procedure ..................... 40

## CHAPTER 4: DATA ANALYSIS

- 43

  - 4.1 Monodispersive Data Analysis .................... 43
  - 4.2 Polydispersive Data Analysis .................... 45

## CHAPTER 5: RESULTS

- 54

  - 5.1 Introduction .......................................... 54
LIST OF TABLES

V–I Table of the results of measurements on Latex spheres ....................... 55
V–II Table of the results of measurements on CRSV ............................... 60
V–III Values of the aggregation energy and temperature of aggregation .... 69
# LIST OF FIGURES

1-1 Effect of temperature on two strains of CRSV. ........................................... 3

2-1 Schematic representation of the light scattering experiment. .......................... 5

2-2 Schematic representation of the light scattering experiment. .......................... 6

2-3 Scattering vector geometry. ............................................................................. 12

3-1 Digitization of fluctuating light intensity. ....................................................... 32

3-2 Schematics of a digital clipped correlator. ...................................................... 33

3-3 Digital correlator interface block diag. ......................................................... 35

3-4 Index matched cell. ....................................................................................... 37

3-5 A diagram of the apparatus. .......................................................................... 38

4-1 CORR.AG output Pg.1 .................................................................................. 47

4-2 CORR.AG output Pg.2 .................................................................................. 48

4-3 CORR.AG output Pg.3 .................................................................................. 49

4-4 CORR.AG output Pg.4 .................................................................................. 50

4-5 Average temperature of cell once in I.M.C. .................................................. 52

5-1 Variation in $\Gamma$ as a function of $q^2$. ....................................................... 56

5-2 Variation in $\xi$ as a function of $q$. ............................................................... 57

5-3 Variation in $\xi$, for CRSV, as a function of temperature. .............................. 61

5-4 Variation in $\xi$, for CRSV, as a function of time. ......................................... 63

5-5 Theoretical fit by ANALYSIS to aggregation data. ....................................... 66

5-6 Alternative fit by ANALYSIS to aggregation data. ....................................... 67

5-7 Final fit by ANALYSIS to aggregation data. .................................................. 68

5-8 Log of the aggregation rate as a function of temperature ............................... 70

5-9 Energy of aggregation as a function of concentration ................................... 71

5-10 Temperature of aggregation as a function of concentration .......................... 72

A1-1 Correlator front panel. ................................................................................. 82
ACKNOWLEDGMENTS

It has been a pleasure to work in the U.B.C. physics department critical phenomena lab. I would like to thank Dr. David Balzarini for his assistance and guidance, and Jack Tremaine and Bill Ronald of the Vancouver Agriculture Canada Station, for providing me with the virus samples and advice on the virological aspects of the experiment. I am also greatly indebted to John de Bruyn for many helpful discussions, and to Ulrike Närger for her co-operation in sharing dark-room facilities.

I would also like to thank Alex Harper for proof reading this thesis, and Mary-Anne Potts for writing the original version of COR.AG—CORR.S.

Finally, and most importantly, I wish to thank Urmil whose support and encouragement was of great value to me.
The intention of this thesis is to present the results of an investigation of certain physical properties of carnation ringspot virus (CRSV): size, molecular weight, diffusion coefficient, and aggregation behavior. An approach based on Smoluchowski's theory of Brownian aggregation is used to model the aggregation phenomenon. This is combined with a temperature dependent probability of aggregation that is hypothesized by the author. As a result a variable can be defined which characterizes the rate of aggregation. This is then measured for various combinations of the experimental variables, temperature and concentration, allowing the dependence on concentration of the aggregation parameters to be determined. Light scattering spectroscopy was the experimental technique used. Its capability to make rapid and accurate measurements of particle size, diffusion coefficient, and polydispersity make it an ideal tool for such an investigation. Prior to the study of CRSV, a control system of polystyrene latex spheres was examined to ascertain the characteristics and limits of accuracy of the apparatus.

Samples of Carnation Ring Spot Virus were provided by Dr J.H. Tremaine of the Agriculture Canada research station in Vancouver. There are three reported strains of CRSV: N, A, and R. These three strains show no differences in host reactions, but do differ in such properties as particle aggregation, amino acid composition and serological properties. Particles are stabilized by a pH dependent
protein–protein interaction, and are stored in a pH 5 sodium acetate buffer to prevent swelling. Particles of the A strain can form six–particle aggregates, and linked aggregates. Particles of the R and N strain aggregate in a temperature–reversible manner: first investigated by J.H.Tremaine and W.P.Ronald\textsuperscript{3,4,5}. The purpose of this study is to investigate the behavior of CRSV-N.

Experiments by Tremaine and Ronald have consisted of measuring the absorbance of virus preparations in different concentrations, at a wavelength of 340nm, as the samples were heated at a rate of 0.25°C per min. Some of their results\textsuperscript{4} are shown in Fig. 1-1. It can be seen that the temperature required to induce aggregation in each of the strains increases with decreasing virus concentration. It was also observed that the maximum absorbance on heating CRSV was directly proportional to the logarithm of the virus concentration. Tremaine and Ronald\textsuperscript{4} also raised the question of whether the molecular weight of the aggregates formed at maximum absorbance was similar for all concentrations.

The use of light scattering spectroscopy has the advantage over absorbance studies that it directly measures the particle size. It also provides information as to whether the viruses are still aggregating once the system has become turbid. This is when the extinction coefficient as measured by the absorbance studies has become a constant.

The process of aggregation is endothermic, and therefore driven by entropy. It is also reversible. It is thought that hydrophobic bonds along with conformational changes in the protein are involved in the aggregation process.
Figure 1-1 Effect of temperature on two strains of CCSV at various concentrations (measured in mg/ml) illustrating the increase in absorbance due to aggregation caused by heating.
CHAPTER 2

THEORY

2.1 Introduction

The technique of digital autocorrelation of intensity fluctuations of scattered laser light has been shown to afford precise and rapid measurements of the translational diffusion constants of macromolecules. This information can be used, with the Einstein relation for the diffusion constant, to give the hydrodynamic radius for the molecule. If this is combined with the Koppel method of cumulants approach to an analysis of the correlation function, information on the polydispersity of the sample can be obtained. This technique can then be used to investigate the aggregation properties of a system, as reflected in the increase in the mean radius and polydispersity of the scattering particles in that system.

Chapter 2 will begin with a synoptic view of light scattering experiments. The theory of light beating spectroscopy is then discussed in terms of the relationship between the optical spectrum of light scattered by macromolecules, and the spectrum of the measured photoelectric current. In the next section the concept of a self-intermediate scattering function will be introduced, and its relationship to the optical spectrum of a dilute system of macromolecules will be examined. A derivation of the self-intermediate scattering function of such a system will then be undertaken. This will yield an analytical expression describing the spectrum and corresponding correlation function of a monodisperse system. The Koppel method
of cumulants approach to the analysis of correlation data will then be outlined, and
and an analytical expression for the correlation function of a polydisperse system
will be derived. The last section will review the theory of Brownian aggregation.

2.2 Synopsis

In a light scattering experiment, light which has been passed through a
polarizer to define the polarization of the incident beam impinges on a scattering
medium. The scattered light then passes through an analyser which selects a certain
polarization and finally enters a detector. The position of the detector defines
the scattering angle $\theta$. In addition, the intersection of the incident beam and the
beam intercepted by the detector defines a scattering region of volume $V$. This is
illustrated in Fig. 2-1.

![Schematic representation of the light-scattering experiment.](image)

**Figure 2-1** Schematic representation of the light-scattering experiment.
In modern light scattering experiments a photomultiplier is the main detector, but the pre- and post- photomultiplier systems will differ according to the frequency spectrum of the scattered light. The three different methods—filter, homodyne (or self-beat), and heterodyne are schematically illustrated in Fig 2–2.

Figure 2-2 Schematic illustration of the various techniques used in light-scattering experiments: a) filter methods; b) homodyne; c) heterodyne.

The spectral characteristics of the scattered light depend on the time scales characterizing the motions of the scatterers. The quantities measured in light scattering experiments are derived from the time correlation functions of either the scattered electromagnetic field or the scattered intensity. Consequently the core of this chapter is devoted to the derivation of an analytical expression that describes the correlation function of the intensity of light scattered by a dilute system of spherical particles. This is an adequate model for the system in the experiment.
Since certain time scales crucial to the understanding of this subject are mentioned during this chapter it will be instructive to explain them at the outset. $T_r$ is defined as the relaxation time of the field correlation function. $\tau_s$ is defined as the characteristic time of the velocity autocorrelation function of the scattering particle. In other words, the 'memory' of the molecule's velocity during a given interval of time—say between $t$ and $t + dt$—is 'completely lost' after a lapse of time large in comparison with $\tau_s$. Likewise $\tau^*$ is defined as the characteristic correlation time for the fluctuating force on the scattering particles that arises from the incessant and random bombardment of the solvent molecules. It is found that $T_r \gg \tau_s \gg \tau^*$. 

2.3 The Theory of Light Beating Spectroscopy 

Consider a classical coherent field at the photocathode of the photomultiplier specified by the vector 

$$E(t) = E_0 \exp(-i\omega t). \quad (2-1)$$

The probability per unit time of photoelectron emission from the photocathode is found to be 

$$W^{(1)}(t) = \sigma E^*(t)E(t), \quad (2-2)$$

where $\sigma$ is the quantum efficiency. The corresponding photocurrent is 

$$i(t) = eW^{(1)}(t) = e\sigma E^*(t)E(t). \quad (2-3)$$

The Wiener-Khinchin theorem expresses the power spectrum of the photocurrent as 

$$P_i(\omega) = \frac{1}{2\pi} \int_{-\infty}^{+\infty} e^{i\omega \tau} C_i(\tau) \, d\tau, \quad (2-4)$$
where \( C_i(\tau) \) (the current autocorrelation function) is

\[
C_i(\tau) = \langle i(t) i(t + \tau) \rangle = e^2 \left\langle W^{(1)}(t) W^{(1)}(t + \tau) \right\rangle.
\] (2-5)

The photocurrent actually consists of a series of discrete pulses. Therefore \( C_i(\tau) \) has two distinct contributions: if the electrons at \( t \) and \( t + \tau \) are distinct,

\[
\left\langle W^{(1)}(t) W^{(1)}(t + \tau) \right\rangle = \left\langle W^{(2)}(t, t + \tau) \right\rangle
= \sigma^2 \langle I \rangle^2 g^{(2)}(\tau),
\] (2-6)

where

\[
\langle I \rangle = \langle E^*(t) E(t) \rangle
\]
(2-7)

\[
g^{(2)}(\tau) = \frac{\langle E^*(t) E(t) E^*(t + \tau) E(t + \tau) \rangle}{\langle E^* E \rangle^2},
\] (2-8)

while if the same electron occurs at \( t \) and \( t + \tau \)

\[
\left\langle W^{(1)}(t) W^{(1)}(t + \tau) \right\rangle = \left\langle W^{(1)}(t) \right\rangle \delta(\tau)
= \sigma \langle I \rangle \delta(\tau).
\] (2-9)

Therefore

\[
C_i(\tau) = e^2 \sigma \langle I \rangle \delta(\tau) + e^2 \sigma^2 \langle I \rangle^2 g^{(2)}(\tau)
= e \langle i \rangle \delta(\tau) + \langle i \rangle^2 g^{(2)}(\tau).
\] (2-10)

The two commonly used detection schemes are the homodyne and heterodyne schemes. In the heterodyne scheme the light scattered from the system of interest is mixed with a local oscillator, whereas in the homodyne scheme—which is used in this experiment—only the scattered light impinges on the photocathode.
The electromagnetic field scattered by the system is characterized by the autocorrelation function

$$G^{(1)}(\tau) = \langle E'(t)E(t + \tau) \rangle = \langle I \rangle g^{(1)}(\tau). \quad (2-11)$$

The electromagnetic field correlation function $g^{(1)}$ is related to the intensity correlation function $g^{(2)}$, for random Gaussian fields, by the Siegart relation

$$g^{(2)}(\tau) = 1 + |g^{(1)}(\tau)|^2. \quad (2-12)$$

Accordingly, the current autocorrelation function becomes

$$C_i(\tau) = e(i)\delta(\tau) + \langle i \rangle^2 \left[ 1 + |g^{(1)}(\tau)|^2 \right]. \quad (2-13)$$

The experiment was performed with a digital correlator that uses the method of photocount autocorrelation in which the current is sampled at discrete intervals of time $t_i$ and recorded as a number of photons $n(t_i)$ detected in the sampling interval $i$ centred at time $t_i$. Accordingly, $(i(t)i(t - \tau))$ would be constructed as a sum of products

$$\sum_{i=M}^{N} n(t_i)n(t_{i-m}), \quad (2-14)$$

for a range of delay times $mT_s$ where $m = 1, 2, 3 \ldots M$. $M$ is the total number of channels built into the autocorrelator, $N$ is the number of sample times, and $T_s$ is the autocorrelator sample time.

In practice, computation of Eq. 2–14 requires an excessive amount of digital electronics for the data acquisition and multiplication processes. A method of clipping the signal is used, therefore, in which a clipped count $n_k(t_i)$ is measured so that

$$n_k(t_i) = \begin{cases} 1 & \text{if } n(t_i) > k \\ 0 & \text{if } n(t_i) \leq k \end{cases}$$
where the non-negative integer $k$ is the clipping level. A single clipped correlator then measures

$$\sum_{i=m}^{N} n(t_i) n_k(t_{i-m}). \tag{2-15}$$

The advantage of clipped over full correlation is that it allows the past history of photocounts to be stored as a linear chain of 1's and 0's. This leads to a considerable simplification in the circuitry of the correlator, with little loss of physical information. The single clipped correlation function is given by\textsuperscript{10,11}

$$C_{ik}(\tau) = \langle n(t_i) n_k(t_{i-m}) \rangle = \langle n \rangle \langle n_k \rangle \left[ 1 + \frac{(1 + k)}{(1 + \langle n \rangle)} \beta |g(mT)|^2 \right]. \tag{2-16}$$

with

$$|g(mT)| = \frac{\langle E'(t) E(t + mT) \rangle}{\langle |E(t)|^2 \rangle}. \tag{2-17}$$

The factor $\beta$ in Eq. 2-16 takes into account such effects as the incomplete spatial coherence of the light over a finite size detector and detector dark current\textsuperscript{11}. It is a complicated function of $\langle n \rangle$, $k$, $mT$, and the geometrical arrangement of the optical components. However, it is not seen to be a function of $m$ for the correlation functions encountered in the experiment, but can be regarded as a constant parameter to be determined experimentally for a given experimental run.

2.4 Light Scattering From Macromolecules

First let us consider what happens when an incident monochromatic beam described by

$$E_i(r, t) = n_i E_0 \exp i[k_i \cdot r - \omega_i t] \tag{2-18}$$
impinges on a single molecule which has a polarizability specified by the tensor \( \alpha \).

The incident light will induce a dipole moment \( \mu \) given by

\[
\mu(t) = \bar{\alpha} \cdot E_i(t).
\]

(2 - 19)

If the fluctuation of the dipole moment is given by \( \bar{\alpha}(t) = \alpha_0 + \delta \alpha(r, t) \), the electric field of the scattered light at the detector, at position \( R \) with polarization \( n_f \) and frequency \( \omega_f \) is\(^{12}\),

\[
E_s(R, t) = \frac{E_o}{R \epsilon_o} \exp ik_f R \int \exp i(q \cdot r - i\omega_f t)
\]

\[
[n_f \cdot [k_f \times (k_f \times (\delta \alpha(r, t) \cdot n_i))]] \, dr,
\]

(2 - 20)

where the subscript \( V \) indicates that the integral is taken over the scattering volume.

The vector \( q \) is defined in terms of the scattering geometry as

\[
q = k_i - k_f.
\]

(2 - 21)

Which for quasi-elastic light scattering i.e., \( |k_i| \approx |k_f| \), is

\[
q = \frac{4\pi n}{\lambda_i} \sin \frac{\theta}{2},
\]

(2 - 22)

where \( n \) is the refractive index of the medium. This is illustrated in Fig. 2-3.

Eq. 2-15 can be expanded in terms of spatial Fourier transforms of the dielectric fluctuations, and the vector cross products can be evaluated to give

\[
E_s(R, t) = \frac{-k_f^2 E_o}{R \epsilon_o} \exp i(k_f R - \omega_i t) \delta \alpha_{i,f}(q, t),
\]

(2 - 23)

where

\[
\delta \alpha_{i,f}(q, t) \equiv n_f \cdot \delta \alpha(q, t) \cdot n_i,
\]

(2 - 24)
Figure 2-3 Light of polarization $\mathbf{n}_i$ and wave vector $\mathbf{k}_i$ is scattered in all directions. Only scattered light of wave vector $\mathbf{k}_f$ and polarization $\mathbf{n}_f$ arrives at the detector. The scattering vector $\mathbf{q} = \mathbf{k}_i - \mathbf{k}_f$ is defined by the geometry. Since the scattered wave has essentially the same wavelength as the incident wave $k_f \approx (2\pi n)/\lambda_i = k_i$, it follows from the law of cosines that $q = 2k_i \sin \theta/2$

is the component of the dielectric constant fluctuation tensor along the initial and final polarization directions.

For our purposes we are interested in the time correlation function

$$
\langle E_z^*(R,0)E_z(R,t) \rangle = \frac{k_i^4|E_0|^2}{R^2 \epsilon_0^2} \langle \delta \alpha_{zf}(q,0)\delta \alpha_{zf}(q,t) \rangle \exp -i\omega_i t,
$$

which we shall herein refer to as $G^{(1)}(t)$. By application of the Wiener–Khinchin
CHAPTER 2: THEORY

Theorem, the spectral density of the light scattered into the detector is given by

\[ I_{if}(q, \omega_f, R) = \frac{E_0^4 k_f^4}{\pi^2 R^2 \varepsilon_0^2} \int_{-\infty}^{\infty} dt \langle \delta \alpha_{if}(q, 0) \delta \alpha_{if}(q, t) \rangle \exp \left( i \omega_f - \omega_i \right) t. \quad (2 - 26) \]

\( I_{if}(q, \omega_f, R) \) is therefore proportional to the spectral density of the fluctuations of the molecular dipole moments \( I_{if}^\circ(q, \omega) \) :

\[ I_{if}(q, \omega_f, R) \propto I_{if}^\circ(q, \omega), \quad (2 - 27) \]

where

\[ I_{if}^\circ(q, t) = \langle \delta \alpha_{if}^\circ(q, 0) \delta \alpha_{if}(q, t) \rangle, \quad (2 - 28) \]

\[ \delta \alpha_{if}(q, t) = \sum_{j=1}^{N} \alpha_{ij}^f(t) \exp i q \cdot r(t), \quad (2 - 29) \]

and

\[ \delta \alpha_{if}(r, t) = \sum_{j=1}^{N} \alpha_{ij}^f(r - r(t)). \quad (2 - 30) \]

It should be noted that this molecular approach to light scattering theory is clearly an approximation as it ignores collision induced effects. However, these effects are temporally separable from the motions of the molecules that are responsible for Rayleigh scattering, and thus only give insignificant unresolvable contributions to the correlation functions. For the purpose of this study our system can be modeled as spherical molecules with isotropic polarizabilities. Thus the tensor \( \tilde{\alpha} \) becomes proportional to \( \alpha \mathbf{I} \) where \( \mathbf{I} \) is the \( 3 \times 3 \) identity matrix and \( \alpha \) is a scalar. The elements \( \alpha_{if}(q, t) \) in Eq. 2-28–30 can be written as

\[ \alpha_{if} = n_i \cdot \tilde{\alpha} \cdot n_f = \alpha (n_i \cdot n_f), \quad (2 - 31) \]
\[ \delta \alpha_{ij}(q, t) = (\mathbf{n}_i \cdot \mathbf{n}_j) \alpha \sum_{j=1}^{N} \exp i(q \cdot r_j(t)). \]  \hspace{1cm} (2-32)

Thus \( G^1(t) \) is proportional to \( F_1(q, t) \) where

\[ F_1(q, t) = \left( \sum_{i,j} \exp i q \cdot [r_i(t) - r_j(t)] \right) \]  \hspace{1cm} (2-33)

is the dynamic structure factor of the system.

It should be made clear at this point that the summation is restricted to molecules in the scattering volume \( V \). To emphasize this point we can introduce the following quantity for the \( j^{th} \) particle:

\[ b_j(t) = \begin{cases} 1 & j \in V \\ 0 & j \notin V \end{cases} . \]

Thus

\[ F_1(q, t) = \left( \sum_{i,j=1}^{N} b_i(0)b_j(t) \exp i q \cdot [r_i(t) - r_j(0)] \right) . \]  \hspace{1cm} (2-34)

The summation now extends over all \( N \) molecules in the system.

In a typical solution of macromolecular Brownian particles it is found that the polarizability of the macromolecules is much greater than that of the solvent molecules and therefore they scatter more light. The macromolecules also move much more slowly than the solvent molecules, and consequently contribute a slowly fluctuating field at the detector as compared to the solvent molecules. The macromolecular motion should therefore be temporally separable from the solvent motion. And as macromolecules dominate the long time behavior, we need to only sum over these molecules.
In a sufficiently dilute solutions the macromolecules so rarely encounter each other we can assume their positions to be statistically independent. In this case Eq. 2-34 simplifies to

$$F_1(q, t) = \left\langle \sum_{j=1}^{N} b_j(0) b_j(t) \exp{iq \cdot [r_j(t) - r_j(0)]} \right\rangle. \quad (2 - 35)$$

This is an example of a self-correlation function.

As the time scale for variation of $b_j(0)b_j(t)$ is typically a factor of $10^6$ longer than that of the argument of the exponent, it is permissible to set $b_j(0)b_j(t)$ equal to its initial value $b_j(0)b_j(0)$. This is because $b_j(0)$ can only have two values 0, or 1, $b_j^2(0) = b_j(0)$, and

$$F_1(q, t) = \left\langle \sum_{j=1}^{N} b_j(0) \exp{iq \cdot [r_j(t) - r_j(0)]} \right\rangle. \quad (2 - 36)$$

The quantity $\exp{iq \cdot [r_j(t) - r_j(0)]}$ is statistically independent of whether the particle $j$ is in the scattering volume or not. Thus

$$F_1(q, t) = \sum_{j=1}^{N} \langle b_j(0) \rangle \langle \exp{iq \cdot [r_j(t) - r_j(0)]} \rangle. \quad (2 - 37)$$

The quantity

$$F_s(q, t) = \langle \exp{iq \cdot [r_j(t) - r_j(0)]} \rangle, \quad (2 - 38)$$

the dynamic single particle structure factor of the system or the self-intermediate scattering function (S.I.S.F) as it is often called$^{12}$, is identical for each particle as it represents an ensemble average. $F_s(q, t)$ can therefore be factored out of the above
sum. Moreover, \( \langle \sum_{j=1}^{N} b_j(0) \rangle \) is simply \( \langle N \rangle \) (the average number of particles in \( V \)) so that \( F_1(q, t) \) becomes

\[
F_1(q, t) = \langle N \rangle F_s(q, t), \tag{2-39}
\]

the mean number of particles in \( V \) times the S.I.S.F. To obtain an analytical expression for the correlation function, therefore, the S.I.S.F. for the system must be calculated.

2.5 Calculation of the Self–Intermediate Scattering Function

To calculate \( F_s(q, t) \) we first note that the argument of the exponential can be expressed as

\[
r(t) - r(0) = \int_0^t v(\tau) \, d\tau. \tag{2-40}
\]

If \( q \) is taken in the \( x \) direction Eq. 2–38 can then be written as

\[
F_s(q, t) = \left\langle \exp \left( i q \int_0^t v_x(\tau) \, d\tau \right) \right\rangle \tag{2-41}
\]

where \( v_x(\tau) \) is the \( x \) component of \( v \). If the exponential in Eq. 2–41 is then expanded, we get

\[
F_s(q, t) = 1 + ik \int_0^t \langle v_x(\tau) \rangle \, d\tau \\
- \frac{k^2}{2} \int_0^t \int_0^t \langle v_x(\tau_1) v_x(\tau_2) \rangle \, d\tau_1 \, d\tau_2 \\
+ \frac{ik^3}{3!} \int_0^t \int_0^t \int_0^t \langle v_x(\tau_1) v_x(\tau_2) v_x(\tau_3) \rangle \, d\tau_1 \, d\tau_2 \, d\tau_3 \\
- \frac{k^4}{4!} \int_0^t \int_0^t \int_0^t \int_0^t \langle v_x(\tau_1) v_x(\tau_2) v_x(\tau_3) v_x(\tau_4) \rangle \, d\tau_1 \, d\tau_2 \, d\tau_3 \, d\tau_4. \tag{2-42}
\]
This expression can then be simplified by employing the following two assumptions: firstly, that the randomly fluctuating particle velocity \( v(t) \) has a mean value of zero and that the higher odd moments are all zero; secondly, that due to random collisions between the particles and the solvent molecules \( v(t) \) will change rapidly in magnitude and direction over the time scale of interest. If we denote the autocorrelation function of the particle's velocity \( K_v(t_1, t_2) \) as

\[
K_v(t_1, t_2) = \langle v(t_1)v(t_2) \rangle, \tag{2 - 43}
\]

a consequence of the second assumption is that

\[
K_v(t_1, t_2) = 0 \text{ for } |t_1 - t_2| \gg \tau_v,
\]

where \( \tau_v \) is the characteristic relaxation time of the velocity autocorrelation function, and is seen to be much smaller than the relaxation time of the electric field \( (T_r) \).

Applying these assumptions to Eq. 2-42 we see that from the first assumption all the terms which contain an odd power of \( k \) are zero; and from second assumption that the multiple integrals corresponding to the higher moments are only non-zero when pairs of times lie within the characteristic time for the velocity autocorrelation function. Thus, for example, in the \( k^4 \) term the integral can be split into three pairs of integrals, one corresponding to \( t_1 \approx t_2 \) and \( t_3 \approx t_4 \), another to \( t_1 \approx t_3 \) and \( t_2 \approx t_4 \), and the third to \( t_1 \approx t_4 \) and \( t_2 \approx t_3 \). Eq. 2-41 then reduces to

\[
F_s(q, t) = 1 - \frac{k^2}{2} \left[ 2\tau \int_0^\infty \langle v_z(0)v_z(t) \rangle \, dt \right] + \frac{k^4}{4!} \left\{ 3 \left[ 2\tau \int_0^\infty \langle v_z(0)v_z(t) \rangle \, dt \right]^2 \right\} + \ldots
\]

\[
= 1 + \sum_{m=1}^{\infty} \frac{(-k^2)^m}{(2m)!} (2m - 1)(2m - 3) \ldots 3 \cdot 1 \left[ 2\tau \int_0^\infty \langle v_z(0)v_z(t) \rangle \, dt \right]^m
\]
The diffusion coefficient of the system can consequently be identified as

\[ D = \int_0^\infty \langle v_z(0)v_z(t) \rangle \, dt. \]  

(2-45)

The substitution of Eq. 2-45 into Eq. 2-44 gives

\[ F_s(q,t) = \exp(-Dq^2\tau), \]  

(2-46)

and Eq. 2-16 becomes,

\[ C_{ik}(\tau) = \langle n_i \rangle \langle n_k \rangle \left[ 1 + \frac{(1 + k)}{(1 + \langle n \rangle)} \beta \exp(-2Dq^2\tau) \right]. \]  

(2-47)

The relaxation time of the field autocorrelation function can therefore be defined as

\[ T_r = \frac{1}{Dq^2}. \]  

(2-48)

The reciprocal of \( T_r \) is known as the Rayleigh linewidth (\( \Gamma \)) of the corresponding spectrum. Computer analysis of the correlation data can then yield the diffusion constant if the data are fitted to the function

\[ C_{ik}(\tau) = \langle n \rangle \langle n_k \rangle \left[ 1 + \gamma \exp(-2\Gamma\tau) \right], \]  

(2-49)

where \( \Gamma \) and \( \gamma \) are treated as constant experimental parameters in a data fit. If the hydrodynamic diameter of the scattering particles (\( \zeta \)) is desired, this can be obtained by utilizing the Einstein relation for the diffusion coefficient

\[ D = k_B T / 6\pi \eta \zeta, \]

\* see appendix A
where $\eta$ is the viscosity of the solvent. Hence

$$\zeta = \frac{k_B T}{3\pi \eta} \cdot (q^2 / \Gamma). \quad (2-50)$$

### 2.6 Koppel Method of Cumulants.

For samples in which there is significant aggregation there can be a great diversity in the diameters of the scattering particles, which means that the correlation function can no longer be described by a single exponential. Aggregates of mean diameter $\zeta_i$ will have a diffusion coefficient $D_i = k_B T / 6\pi \eta \zeta_i$ and a corresponding relaxation parameter $\Gamma_i$. Consequently, the distribution of particle aggregates of various sizes in a polydispersive sample can be characterized by a distribution function $P(\Gamma)$. Thus the correlation function is given by

$$|g^{(1)}(\tau)| = \int_0^\infty P(\Gamma) \exp(-\Gamma\tau) \, d\Gamma. \quad (2-51)$$

The remainder of this section illustrates how the correlation can be expressed in terms of the moments of the distribution function $P(\Gamma)$. It is noted the correlation function obtained from a polydispersive solution corresponds to the moment generating function

$$\mathcal{N}(-\tau; \Gamma) \equiv \langle \exp(-\Gamma\tau) \rangle \quad (2-52)$$

$$= |g^{(1)}(\tau)|. \quad (2-53)$$

This suggests analysis in terms of moments or cumulants. The moments of the distribution are related to the derivatives of $\mathcal{N}(-\tau; \Gamma)$ with respect to $(-\tau)$

$$\mu_m(\Gamma) \equiv \langle \Gamma^m \rangle \quad (2-54)$$

$$= \left| \frac{d^m}{d(-\tau)^m} \mathcal{N}(-\tau; \Gamma) \right|_{-\tau=0}$$
Similarly, one can define the cumulant generating function as the natural logarithm of the moment generating function:

\[ \Psi(-\tau; \Gamma) \equiv \ln N(-\tau; \Gamma) \]
\[ = \ln |g^{(1)}(\tau)|, \]

which thus takes the form of a power series in \( \tau \). That is,

\[ \psi_m(\Gamma) \equiv \left[ \frac{d^m}{d(-\tau)^m} \right] \Psi(-\tau; \Gamma) \bigg|_{-\tau=0} \]

and

\[ \Psi(-\tau; \Gamma) = \sum_{m=1}^{\infty} \psi_m(\Gamma) \frac{(-\tau)^m}{m!}. \]

The cumulants can be written explicitly in terms of the moments

\[ \psi_m(\Gamma) = \sum_{l=1}^{m} (-1)^{l-1}(l-1)! \sum_{\{a\}} c(l; a_1 \ldots a_m) \]
\[ |\mu_1(\Gamma)|^{a_1} \cdot |\mu_m(\Gamma)|^{a_m} \]

where

\[ c(l; a_1 \ldots a_m) = \frac{m!}{\prod_{j=1}^{m} a_j!(j!)^{a_j}}. \]

The sum over \( \{a\} \) includes the set of non negative integers \( a_1 \ldots a_m \) for which

\[ \sum_{j=1}^{m} j a_j = m, \]

and

\[ \sum_{j=1}^{m} a_j = l. \]
Accordingly, by combining Eq. 2–56 and 59 the cumulant generating function can be written as a series expansion in terms of the moments. Therefore:

\[ \ln |g^{(1)}(\tau)| = -\tilde{\Gamma} \tau + \frac{\mu_2(\tilde{\Gamma} \tau)^2}{2! \tilde{\Gamma}^2} - \frac{\mu_3(\tilde{\Gamma} \tau)^3}{3! \tilde{\Gamma}^3} + \frac{(\mu_4 - 3\mu_2)(\tilde{\Gamma} \tau)^4}{4! \tilde{\Gamma}^4}. \]  

(2 – 63)

For a single exponential correlation function Eq. 2–63 is linear in \( \tau \). The power of this approach derives from the fact that for many \( g^{(1)}(\tau) \)'s of interest the terms in Eq. 2–63 fall off rapidly with increasing order of \( \tilde{\Gamma} \). In the current analysis only the first two terms were kept. Consequently, the correlation function for polydisperse systems is

\[ C_{ik}(\tau) = \langle n \rangle \langle n_k \rangle \left[ 1 + \frac{(1 + k)}{(1 + \langle n \rangle)} \beta \exp(-2\tilde{\Gamma} t + \mu_2 t^2) \right]. \]  

(2 – 64)

\( \tilde{\Gamma} \) can be related to an average diffusion coefficient. Assuming \( \Gamma_j = D_j q^2 \), we get

\[ \tilde{D} \equiv \tilde{\Gamma}/q^2 \]

\[ \sum_j \langle I_j \rangle D_j = \frac{\sum_j \langle I_j \rangle}{\sum_j \langle I_j \rangle}. \]  

(2 – 65)

A quality parameter \( Q \) can also be defined as

\[ Q = \frac{\mu_2}{(\mu)^2}. \]  

(2 – 66)

Only if \( Q \) is zero to within experimental error can a system be considered monodisperse.

2.7 Theory of Brownian Aggregation

The following approach to be discussed was first considered by Smoluchowski \(^2\) and has been reviewed by S. Chandrasekhar \(^13\). In this approach the system is
supposed to consist initially of single particles that describe Brownian motion at a
given rate determined by temperature, their size, and the viscosity of the solution
that they are in. Each particle is considered to be surrounded by a sphere of
influence of radius \( R \), so that if the spheres of influences of two particles overlap the
two particles will join and become a double particle. This double particle will then
continue to describe Brownian motion but at a reduced rate, due to its increased
size, until such time as it encounters another single, or double particle. An overlap
in the spheres of influence of the two particles will then result in the creation of a
triple, or quadrupole particle. This process will continue until all the particles have
coagulated to form one single aggregate.

The problem that we wish to solve is the specification of the concentrations
\( \nu_1, \nu_2, \nu_3 \ldots \) of single, double, triple,(etcetera) aggregates at time \( t \)—given that at
time \( t = 0 \) there were \( \nu_0 \) single particles.

Before tackling this problem, however, it is illuminating to consider the fol­
lowing more elementary problem. What is the rate at which surrounding Brownian
particles are deposited on the surface of a particle fixed at the origin and of ra­
dius \( R \)? The dynamics of the particles are governed by the diffusion equation, the
solution of which represents the number of Brownian particles per unit volume at
position \( r \) and time \( t \), given some initial distribution of an ensemble of particles.
The problem can therefore be formulated as the solution of the diffusion equation,
with the initial condition of a uniform distribution of particles for \( |r| > R \), and the
boundary condition that at \( |r| = R \) for time \( t > 0 \) we have a perfect absorber.
Thus

\[
\frac{\partial \omega}{\partial t} = D \nabla^2 \omega, \tag{2-67}
\]

with boundary conditions

\[
\omega \equiv \nu = \text{constant, at } t = 0 \text{ for } |r| > R. \tag{2-68}
\]

\[
\omega \equiv 0 \text{ at } |r| = R \text{ for } t > 0, \tag{2-69}
\]
where \( \omega \) is the concentration of particles at position \( r \) and time \( t \). As the problem has spherical symmetry the appropriate form of the diffusion equation is

\[
\left( \frac{\partial}{\partial t} \right) (r\omega) = D \left( \frac{\partial^2}{\partial r^2} \right) (r\omega).
\]  

(2 - 70)

The solution of this problem is

\[
\omega = \nu \left[ 1 - \frac{R}{r} + \frac{2R}{r \sqrt{\pi}} \int_0^{(r-R)/2(Dt)^{1/2}} \exp(-z^2) \, dz \right].
\]  

(2 - 71)

From the above it follows that the rate at which the particles arrive at the surface is given by

\[
4\pi D \frac{\partial \omega}{\partial r} \bigg|_{r=R} = 4\pi DR \nu \left( 1 + \frac{R}{(\pi Dt)^{1/2}} \right).
\]  

(2 - 72)

Accordingly, Eq. 2-72 gives the rate at which particles describing Brownian motion will arrive at the surface of the sphere of influence of radius \( R \) of a particle situated at the origin. If, however, this particle is not stationary, but also describes Brownian motion, what would be the corresponding generalization of Eq. 2-72? It is clear that we must deal with the relative displacements between the two particles, and it can be readily shown that the relative displacements between two particles describing Brownian motion independently of each other and with the diffusion coefficients \( D_1 \) and \( D_2 \) also follow the laws of Brownian motion with diffusion coefficient \( D_{12} = D_1 + D_2 \). The probability that the relative displacements of two particles, initially together at \( t = 0 \), lies between \( r \) and \( r + dr \) is

\[
W(r) \, dr = dr \int_{-\infty}^{\infty} W_1(r_1)W_2(r_1 + r) \, dr_1
\]  

(2 - 73)

\[
= \frac{dr}{(4\pi D_1 t)^{3/2}(4\pi D_2 t)^{3/2}} \int_{-\infty}^{\infty} \exp(-|r_1|^2/4D_1 t) \exp(-|r_1 + r|^2/4D_2 t) \, dr_1
\]
or
\[ W(r) = \left( \frac{1}{4\pi(D_1 + D_2)t^{3/2}} \right) \exp\left(-|r|^2/4(D_1 + D_2)t\right). \] (2-74)

Consequently, we conclude that the relative displacements do follow the laws of Brownian motion with the diffusion coefficient \((D_1 + D_2)\). The subsequent required generalization of Eq. 2-72 is

\[ 4\pi(D_1 + D_2)R\nu \left( 1 + \frac{R}{\pi(D_1 + D_2)t^{1/2}} \right). \] (2-75)

We now generalize this further and consider two sorts of particles with concentrations \(\nu_i\) and \(\nu_k\), with respective diffusion coefficients \(D_i\) and \(D_k\). Let \(R_{ik}\) denote the separation distance within which two particles join to form a multiple particle. The rate of formation of the multiple particles by the coagulation of the particles of the kind considered is then given by

\[ J_{i+k} dt = 4\pi D_{ik} R_{ik} \nu_i \nu_k \left( 1 + \frac{R_{ik}}{(\pi D_{ik} t)^{1/2}} \right) dt, \] (2-76)

where

\[ D_{ik} = D_i + D_k. \]

From here on in we shall ignore the second term in the parenthesis on the right hand side of Eq. 2-76, which implies that we restrict ourselves to time intervals \(\Delta t \gg (R^2/D)\). In most cases of practical interest this is justifiable as \(R^2/D \approx 10^{-3} - 10^{-4}\) second. With this understanding we can write

\[ J_{i+k} dt \approx 4\pi D_{ik} R_{ik} \nu_i \nu_k dt. \] (2-77)

Using this we can now write down the fundamental differential equations which govern the variations in \(\nu_1, \nu_2, \ldots, \nu_k\ldots\) (of single, double, \ldots k-fold \ldots particles)
with time. So, considering the variation of the number of \(k\)-fold aggregates with time, we have in analogy with the equations of chemical kinetics,

\[
\frac{dv_k}{dt} = 4\pi \left( \frac{1}{2} \sum_{i+j=k} \nu_i \nu_j D_{ij} R_{ij} - \nu_k \sum_{j=1}^{\infty} \nu_j D_{kj} R_{kj} \right) \quad (k = 1, 2, \ldots). \tag{2 - 78}
\]

In this equation the first summation on the right hand side represents the increase in \(\nu_k\) due to the formation of \(k\)-fold particles by the joining of an \(i\)-fold and a \(j\)-fold particle (with \(i + j = k\)). While the second summation represents the decrease in \(\nu_k\) due to the formation of \((k + j)\)-fold particles in which one of the interacting particles is \(k\)-fold. A general solution of this equation is not feasible but a special case considered by Smoluchowski appears sufficiently illustrative of the general solution. We first assume that \(R_{ik} = \frac{1}{2}(R_i + R_k)\). Secondly, we note that the diffusion coefficient is inversely proportional to the radius of the particle. And since, on the basis of experimental evidence, it appears that the radii of the spheres of influence of various multiple particles are proportional to the radii of the respective particles, we can therefore make the additional assumption

\[
D_i R_i = DR \quad (i = 1, 2, \ldots). \tag{2 - 79}
\]

This leads to

\[
D_{ik} R_{ik} = \frac{1}{2} (D_i + D_k)(R_i + R_k)
= \frac{1}{2} DR (R_i^{-1} + R_k^{-1})(R_i + R_k)
= \frac{1}{2} DR (R_i + R_k)^2 R_i^{-1} R_k^{-1}. \tag{2 - 80}
\]
CHAPTER 2: THEORY

Finally, for the sake of mathematical simplicity we make—although not very plausible—the assumption that $R_i = R_k$. With all these assumptions

$$D_{ik}R_{ik} = 2DR,$$  \hfill (2-81)

and Eq. 2-78 becomes

$$\frac{d\nu_k}{dt} = 8\pi DR\left(\frac{1}{2} \sum_{i+j=k} \nu_i \nu_j - \nu_k \sum_{j=1}^{\infty} \nu_j \right) \quad (k = 1, 2, \ldots).$$  \hfill (2-82)

If we now let

$$\tau = 4\pi DRt,$$  \hfill (2-83)

Eq. 2-82 takes the more convenient form of

$$\frac{d\nu_k}{d\tau} = \sum_{i+j=k} \nu_i \nu_j - 2\nu_k \sum_{j=1}^{\infty} \nu_j \quad (k = 1, 2, \ldots).$$  \hfill (2-84)

This leads to

$$\frac{d}{d\tau} \left( \sum_{k=1}^{\infty} \nu_k \right) = \sum_{i=1}^{\infty} \sum_{j=1}^{\infty} \nu_i \nu_j - 2 \sum_{k=1}^{\infty} \sum_{j=1}^{\infty} \nu_k \nu_j$$

$$= - \left( \sum_{k=1}^{\infty} \nu_k \right)^2$$  \hfill (2-85)

or,

$$\sum_{k=1}^{\infty} \nu_k = \frac{\nu_0}{1 + \nu_0 \tau}.$$  \hfill (2-86)
Thus using Eq. 2–84 we can successively obtain solutions for \( \nu_1, \nu_2, \) etc. Considering the equation for \( \nu_1 \) we have

\[
\frac{d\nu_1}{dt} = -2\nu_1 \sum_{k=1}^{\infty} \nu_k = \frac{-2\nu_1 \nu_0}{(1 + \nu_0 t)}, \tag{2–87}
\]

or in other words

\[
\nu_1 = \frac{\nu_0}{(1 + \nu_0 t)^2}, \tag{2–88}
\]

again using the boundary condition that \( \nu_1 = \nu_0 \) at \( t = 0 \). Proceeding in this manner we can prove by induction that

\[
\nu_k = \nu_0 [(\nu_0 t)^{k-1} / (1 + \nu_0 t)^{k+1}]. \tag{2–89}
\]

Extensive comparison with experiment\(^{14,15}\) has lead to the general conclusion that Smoluchowski’s theory gives a fairly satisfactory account of the broad features of the coagulation phenomenon. With this result we can predict that the average size of aggregate in the solution, \( \bar{k} \), will grow with time in a manner governed by

\[
\bar{k}(t) = \frac{\sum_{k=1}^{\infty} k \nu_k}{\sum_{k=1}^{\infty} \nu_k}. \tag{2–90}
\]

This will be reflected in an increase in the average size of scattering particles obtained by measuring the average diffusion coefficient of the system. However, since this average diffusion coefficient—which can be obtained from the first moment of the distribution of decay rates of the correlation function \( G^{(1)}(t) \)—is an average in which each contribution to the sum is weighted by the relative intensity of the
scattered light from that contribution,

\[ D = \frac{\Gamma}{q^2} \] (2-91)

\[ = \frac{\sum_k (I_k) D_k}{\sum_k (I_k)}. \] (2-92)

For solutions of particles small compared to \( q^{-1} \) with equal polarizability per unit mass

\[ \langle I_k \rangle \propto \nu_k M_k^2 \propto \nu_k k^2; \] (2-93)

thus

\[ D = \frac{\sum_k D_k \nu_k k^2}{\sum_k \nu_k k^2} \] (2-94)

and

\[ \bar{k}_{zz} = \frac{\sum_k k^3 \nu_k}{\sum_k k^2 \nu_k}. \] (2-95)

Consequently, by substitution of Eq. 2-89 in Eq. 2-95 we see that

\[ \bar{k}_{zz} = \frac{\sum_k k^3 \nu_0 \left[ \frac{(\nu_0)^{k-1}}{(1 + \nu_0)^{k+1}} \right]}{\sum_k k^2 \nu_0 \left[ \frac{(\nu_0)^{k-1}}{(1 + \nu_0)^{k+1}} \right]} \] (2-96)

This summation can be evaluated in the following manner. If we make the substitution \( a = \nu_0 \tau/(1 + \nu_0 \tau) \),

\[ \sum_k k^3 \nu_0 (\nu_0 \tau)^{k-1} \frac{1}{(1 + \nu_0 \tau)^{k+1}} \]

\[ = \frac{\nu_0 a^2}{(1 + \nu_0 \tau)^2} \sum_k k^3 a^{k-3} \]

\[ = \frac{\nu_0 a^2}{(1 + \nu_0 \tau)^2} \frac{\partial^3}{\partial a^3} \sum_k a^k \]

\[ + \frac{3\nu_0 a}{(1 + \nu_0 \tau)^2} \frac{\partial^2}{\partial a^2} \sum_k a^k \]
\[ + \frac{\nu_0}{(1 + \nu_0 \tau)^2} \frac{\partial}{\partial a} \sum_k a^k \]

\[ = \frac{\nu_0 a^2}{(1 + \nu_0 \tau)^2} \frac{\partial^3}{\partial a^3} \left( \frac{1}{1 - a} \right) \]

\[ + \frac{3\nu_0 a}{(1 + \nu_0 \tau)^2} \frac{\partial^2}{\partial a^2} \left( \frac{1}{1 - a} \right) \]

\[ + \frac{\nu_0}{(1 + \nu_0 \tau)^2} \frac{\partial}{\partial a} \left( \frac{1}{1 - a} \right) \]

\[ = \nu_0 \left[ 6(\nu_0 \tau)^2 + 6(\nu_0 \tau) + 1 \right]. \quad (2-97) \]

Similarly,

\[ \sum_k k^2 \nu_k = [1 + 2(\nu_0 \tau)]. \quad (2-98) \]

Therefore

\[ k_{ex} = \frac{[6(\nu_0 \tau)^2 + 6(\nu_0 \tau) + 1]}{[1 + 2(\nu_0 \tau)]}. \quad (2-99) \]

This relationship, though, does not completely describe the virus that is of interest (CRSV), as it assumes that whenever two viruses or aggregates of viruses come to within a distance \( R \) of each other they always join to form a multiple particle. However this is not always observed to be true. Previous studies by Tremaine et. al.\(^3,4,5\) indicate that there is a temperature dependent probability that upon collision a multiple particle will be formed. We therefore hypothesize that this temperature dependent probability is of the form

\[ P(T) = \begin{cases} \exp[-E_{agg}/k_B(T - T_c)] & \text{if } T > T_c; \\ 0 & \text{if } T < T_c; \end{cases} \quad (2-100) \]

where \( E_{agg} \) and \( T_c \) are termed the energy of aggregation (E.A.) and temperature of aggregation (T.A.). The rate of formation of multiple particles is then given by

\[ J_{i+k} dt = 4\pi D_{ik} R_{ik} \nu_i \nu_k \exp[-E_{agg}/k_B(T - T_c)]. \quad (2-101) \]
CHAPTER 2: THEORY

Following the same procedure as before we arrive at the conclusion that

\[
\kappa_{zz} = \frac{6(\nu_0 \tau)^2 + 6(\nu_0 \tau) + 1}{1 + 2\nu_0 \tau},
\]

and

\[
\tau = 4\pi DR \exp\left[-\frac{E_{agg}}{k_B(T - T_c)}\right] t.
\]
CHAPTER 3

EXPERIMENT

3.1 The Correlator

The correlator used in these experiments was a Malvern K7023 digital correlator. Its operation and the method of photocount autocorrelation have been discussed in detail by many others\textsuperscript{10,11,16,17,18,19}. Here I shall assemble the important concepts relevant to macromolecular solutions.

Prior to processing by the correlator the signal is digitized. An example of this process is illustrated in Fig. 3-1, which shows a typical trace of light of fluctuating intensity. About three coherence times are spanned in this figure. At any instant the probability (per unit time) of detecting a photon is proportional to the intensity\textsuperscript{20}. The second line shows a typical distribution of detected photons, or photocounts, per small time interval of length $T_t$. The bunching of photons due to fluctuating intensity is evident: when the intensity is high many photons are detected per interval, and when it is low few are detected. The third line shows the number of photons detected in the sample time (S.T.) $T_s$, centered at time $t_i$; defined to be the number of photocounts, $n(t_i)$.

The photomultiplier, discriminator and amplifier assembly used was an E. M. I D307K which produced pulses of height $-1.2$ V and width 30ns, with a uniform rise and fall time; each pulse corresponding to a single photon. The method of integrating the total signal arriving between two sample time clock pulses has the
advantage that no photocounts are lost due to dead time between S.T.’s, therefore no information is lost.

Figure 3-1 Typical trace of fluctuating light intensity, and typical distribution of detected photons (photocounts). The unclipped photon number, \( n \), and clipped photon number, \( n_k \), for clipping levels 0-4, are also shown.

The operation of the correlator is illustrated in Fig. 3-2. The object of operation is to construct the sum

\[
C_m = \sum_{i=M}^{N} n(t_i) n_k(t_i-m),
\]

where \( t_{i+1} - t_i = T_s \).
and $M$ is the number of correlator channels. The correlator uses a shift register to store the delayed counts $n_k(t_{i-m})$ in a single bit quantized form. Those that arrive during the S.T., $T_s$, centered at, $t_i$ are counted in the scaler. Thus for the duration of this sample time the scaler will store the value $n(t_i)$.

If the pre-set clip level, $k$, is exceeded a logical 1 is stored in the first element of the shift register. Otherwise, the input is zero. At the next S.T., at $t_{i+1}$, the scaler counts the new number of photocounts $n(t_{i+1})$. Within this sample time the contents of the shift register are shifted one place to the right, and the first element of the shift register is set equal to the clipped value of $n(t_{i+1})$. Thus at time $t_i$, the contents of the $r^{th}$ element of the shift register are represented by $n_k(t_{i-rT})$. As the register counts only 1's and 0's multiplication can be performed by using the register to control 'and gates' that lead to separate storage channels. The instantaneous
signal pulses, \( n(t) \), are thus applied to each 'and gate' and the resulting product \( n(t) \cdot n_k(t - rT_s) \) is stored in the \( r \)th storage channel. By repeating the process for \( N \) samples where \( N \) is a large number, the contents of the store form the average of the product \( G^{(r)}(rT_s) = N \langle n(t)n_k(t - rT_s) \rangle \). The progress of the correlation function is monitored on an oscilloscope screen, and data are collected until a correlation function of sufficient accuracy is achieved. Thus the correlator store contains \( M \) correlation coefficients for delays between \( T_s \) and \( MT_s \). For the Malvern K7023, \( M \) is 24, and the S.T.\( (T_s) \) can be varied from 1 s to 50 ns.

There is facility for two input signals A and B; and in addition to the 24 channels the total number of counts in A and B, \( \langle n^A(t) \rangle \) and \( \langle n^B(t) \rangle \), are monitored, as are the number of clipped counts \( \langle n_k(t) \rangle \) and the total number of sample times \( N \). The aforementioned four quantities are stored in channels 0 to 3 of the correlator output and the correlation function occupies channel 4 to 27. When the correlator is being used in single clip mode, input B is unused and correspondingly channel 3 will always contain zero. A detailed description of the controls is contained in appendix 2.

3.2 Digital Correlator Computer Interface

To extract the data from the correlator in a form that would facilitate ease of manipulation, a microcomputer (built by the U.B.C. electronics shop) was used. This interfaces the output of the correlator to the U.B.C. Amdahl 5850 mainframe computer via a control terminal. This was done in such a way as to allow the terminal to be used in its normal fashion when the correlator was not being used. A block diagram of the interface is shown in Fig. 3-3.

The hardware consists of 4 circuit boards, the CRT terminal, a Cromemco single card computer (based on the Z80 microprocessor), an Industrial Micro systems 8k static RAM, a Cromemco TU-ART serial communication board, a UBC
Figure 3-3 Digital correlator interface block diagram.

The Cromemco single card computer has 8k of programmable ROM which contains the interfacing program and Z80 Assembler routines to control the interface. This board also contains a serial communications device (UART) to allow communications between the terminal and the microcomputer. The 8k static RAM is used to store the data from the digital correlator prior to sending it to the Amdahl 5850. The Cromemco TU-ART is used as an RS-232 communications device to allow communications between the microcomputer and the Amdahl 5850.
CHAPTER 3: EXPERIMENT

The Parallel Interface board was constructed by the Electronics shop to interface the Malvern Digital Correlator's calculator output to the Z80 Microcomputer. It consists of 42 data and status signals and 1 output command signal to correlator.

The communication line to the Amdahl 5850 is a leased line from the computing center running under Hardcopy support. Transmission speed to the Amdal 5850 is at 300 Baud while reception is at 9600 Baud. This means that while the terminal is used in normal mode the communications appear to take place at the typing speed of the operator. The transmission speed is only apparent when sending a block of data.

3.3 The Index Matched Cell and Temperature Control Device

The sample cell for the experiment was a 1 cm bore pyrex test tube that held approximately $3 \text{cm}^3$ of solution. The cell was mounted in a Malvern Systems Index Matched Cell (I.M.C.). This is illustrated in Fig. 3-4.

Ease of optical alignment is facilitated by a refractive index matching fluid—water in this case, that surrounds the specimen cell, filling the internal capacity of the I.M.C. For temperature control the central block of the I.M.C. contained a heating coil that was connected to a Malvern temperature control unit RR56. With this unit the temperature of the specimen cell could be set to an accuracy of ±0.05\% absolute with a temperature stability of 0.05°C. Prior to the experiment the Malvern temperature control unit was calibrated against a thermistor embedded in a small copper block and subsequently inserted in the I.M.C. in place of the sample cell. The thermistor was then used as one arm of a Wheatstone bridge and balanced against a decade box using a Hewlett-Packard 419A D.C. nullmeter. The thermistor was calibrated against a Hewlett-Packard 2804A quartz thermometer, which had previously been calibrated by comparison with a Jarrett triple point of water cell.
3.4 Optics

A diagram of the apparatus is illustrated in Fig. 3-5. The laser source was a Hughes 10 mW helium-neon laser. The first two polaroids that the beam encounters are mounted in such a way as to allow independent rotation. The axis of polarization of the second defines the incident polarization of the beam ($\mathbf{u}_i$) while the angle between the axes of polarization of the two defines the incident intensity. The beam is focused, by a microscope objective, onto an optical filter (consisting of a 10 $\mu$m pinhole) which is at the focal point of a 50 mm lens, which collimates the emergent beam. A 400 mm lens focuses the beam to a diameter of $\approx 1/10^{th}$ mm at the scattering volume.

The polaroid in front of the P.M.T. defines the polarization of the scattered light collected: $\mathbf{n}_f$. The entrance aperture of the P.M.T. was variable from 1/2 mm
to 6 mm in diameter. For all experiments it was set to 1/2 mm for maximum angular resolution.

Two screws in the photomultiplier tube (P.M.T.) position the P.M.T.'s lens so as to form an image of the scattering volume on the pinhole aperture of the P.M.T. To ensure that this was done accurately a long necked pipette of inside diameter \( \approx 1 \text{mm} \) was filled with spheres and placed inside the I.M.C. and aligned by two locating holes. The intersection of the beam with the pipette provided a bright target for alignment of the P.M.T. lens. The two screws in the P.M.T. were adjusted to achieve a maximum count rate observable on the correlator's rate meter with this target. To ensure that the scattering volume was at the focal point of the P.M.T. lens the P.M.T. was swung around to the zero degree position, and the pinhole aperture of the P.M.T. was illuminated by a desk lamp shone through an observation window in the P.M.T. The P.M.T. was then slid back and forth on its
rail until a sharp image of the pinhole could be seen through a telescope placed between lens number 1 and lens number 2 and focussed at infinity.

3.5 Preparation of Sample

The Spheres

The sample cells used were Corning 10 × 75 mm Pyrex test tubes. The Latex Spheres used were manufactured by the Dow Chemical Company and marketed by Seragen diagnostics. They were made of styrene butadiene, and were of diameter 0.087 μm, with a standard deviation of 4.6 nm.

The samples were prepared as follows. The concentrated stock suspension of 10% solids was shaken and allowed to settle for a few hours. Then 0.05cc of the suspension was withdrawn by pipette from just below the surface, in the hope that any impurities or coagulated spheres would have settled out of this region faster than the particles of interest. This drop of suspension was added to a sample cell which contained distilled deionized water which had been passed through a 2 μm Millipore filter. This cell was then centrifuged for 20 minutes to remove further impurities or coagulated spheres, and decanted into a second sample cell. This sample was then used as a base solution to be diluted down to the required concentration for a particular run.

The Viruses

The sample of CRVS used was provided by the Agriculture Canada Station, Vancouver, Canada. The sample was prepared in 0.1M sodium acetate buffer (pH 5.0). The acidity of the buffer was necessary to avoid the virus swelling. To avoid the accidental destruction of the virus sample, which was in short supply, the sample was split into four aliquots, and placed into four separate sample cells. Glass beads were added to the cells to raise the level of the solution to the level of the laser
beam. The concentration of the sample was measured, by Agriculture Canada, spectrophotometrically using the extinction coefficient of $6.5 \text{ cm}^2/\text{mg}$ at 260 nm$^4$, after each experiment.

Cleaning the Cells

The cleaning procedure for the cells was as follows.

(i) The cells and all pipettes, syringes, and any other equipment that was to come into contact with the sample were immersed in a solution of equal parts of alcohol and a 30% concentrated hydrochloric acid solution, and thereafter left for 4 to 6 hours.

(ii) The equipment was then thoroughly rinsed with distilled water until no trace of acid remained.

(iii) The equipment was then flushed with acetone squeezed out of a wash bottle.

(iv) Further washing was subsequently done with acetone that had been freshly distilled, using it as it fell from the still into a beaker.

(v) Drying was done in a clean oven for 20 min at a temp of 75 °C.

3.6 The Experimental Procedure

Each repetition of one of the following experimental procedures constitutes a run. Two experimental procedures were followed. Which one was chosen depended on whether the sample was in a dynamic state or an equilibrium state. An Equilibrium state is one in which the correlation function is not changing with time—Latex spheres is an example. A dynamic state is one in which the correlation function is changing with time. An example of this could be a system that was undergoing a chemical reaction, or in this case a system that is aggregating.

An Equilibrium Run

An equilibrium run is a process by which correlation data are taken on a system that is in an equilibrium state. Prior to the run the sample was placed in
the I.M.C. which had previously been raised to the desired temperature for the run. The desired sample time, clip rate, and total number of sample times to be taken were set using the correlator controls. For most of the runs the number of photons per sample time \( r \) was in the range 0-1 to 1 and the correlation functions were of the form \( \exp(-t) \), with a small correction term for the polydispersity. The minimum error was obtained when the sample time \( T_s \) was chosen so that \( MTT_s \sim 2 - 3 \). It should be noted that when \( r \) lies in the range 10-100, \( T_s \) should be chosen so that \( MTT_s \sim 1 - 2 \). The clip rate was chosen by performing a trial run before the experimental run and adjusting the clip level so that the second channel accumulates at no more than half the rate of the fourth channel. For the vast majority of sphere runs the clip rate was zero due to the low count rate. As has been previously mentioned the total number of sample times was chosen, from experience, to ensure a high signal to noise ratio.

Prior to each experimental run a computer file was created that subsequently contained the data from the run. The creation of the file was facilitated by the program CORRFILECRE which created a file and named it with the date and time that it was created, eg JAN21-113145. The command $RUN CORRFILECRE is coded to be activated by the CTRL-F key. Information about the particular run is then written on the first line of the file: date, scattering angle, sample time, clip rate, temperature, and multiplication factors for the first four channels. The run is then initiated by clearing the correlator store of all previous runs and pushing the start button. The correlator will stop automatically after \( N \) sample times. The PUNCH readout mode is then selected on the correlator and the data are then loaded into the computer file via the Z80 by depressing the ESC-G the ESC-S keys at the terminal. The run is now complete and data are ready to be processed.

Non-Equilibrium or Dynamic Runs

A dynamic run is essentially a succession of short equilibrium runs on a
single sample that is in a dynamic state: aggregating, in this case. Such a run gives information on the progress of the dynamic process: the aggregation. More specifically, a measure of the mean scattering particle diameter ($\bar{\sigma}$) is a measure of the mean number of viruses in an aggregate ($\bar{k}$).\(^*\) The rate of aggregation can be measured by sampling $\bar{k}_{ee}$ at regular intervals of time. Thus each 30 min dynamic run consisted firstly, of a two minute period to allow the sample cell to reach the temperature of the I.M.C. Correlation data was then taken over ten one minute periods, each being followed by a two minute period during which the data that had just been collected would be processed, and, depending on the results, certain adjustments would be made to the correlator settings in preparation for the next one minute of data collection. Consequently, statistical accuracy could be maintained despite the changing intensity of the scattered light and increase in particle size.

Each dynamic run therefore makes a measurement of the aggregation rate that corresponds to the two experimental variables, temperature and concentration. By preparing samples of various concentrations and performing a series of dynamic runs at different temperatures the dependence of the aggregation rate on temperature and concentration could thus be investigated.

\(^*\) As explained in Sec. 4.2
4.1 Monodispersive Data Analysis

The desired system parameter—the diffusion coefficient—can be obtained by fitting the data obtained from the correlator to the expected analytical form of the correlation function, and extracting the material parameters from the fit parameters.

For equilibrium runs the basic equation for data analysis is

\[
\langle C_m \rangle = N \langle n \rangle \langle n_k \rangle [1 + \gamma \exp (-2\Gamma m T)],
\]

\[\text{(4 - 1)}\]

where \( \gamma = \beta \frac{1 + k}{1 + \langle n \rangle} \), \[\text{(4 - 2)}\]

and \( \Gamma = Dq^2 \). \[\text{(4 - 3)}\]

Eq. 4-1 relates the average correlation store contents, \( \langle C_m \rangle \), and the average single channel counting rates, \( \langle n \rangle \) and \( \langle n_k \rangle \), to the decay rate \( \Gamma \). However, due to the random nature of both the intensity fluctuations and the photodetection process one obtains only an approximation of the above quantities.
A set of experimental signals can be defined by

\[ S_m = \frac{C_m}{N \langle n \rangle_{\epsilon z} \langle n_k \rangle_{\epsilon z}} - 1, \quad (4-4) \]

where \( \langle n \rangle_{\epsilon z} = \frac{\sum_{i=1}^{N} n(t_i)}{N} \), \( (4-5) \)

and \( \langle n_k \rangle_{\epsilon z} = \frac{\sum_{i=1}^{N} n_k(t_i)}{N} \). \( (4-6) \)

which must be fit to the function \( \gamma \exp(-2\Gamma mT_s) \) in order to obtain an experimental value for \( \Gamma \). In practice it is more convenient to fit \( \ln S_m \) to the straight line \( (\ln \gamma - 2\Gamma mT_s) \) than to fit \( S_m \) directly to an exponential. The data analysis thus consists of a two parameter minimization of the sum

\[ \sum_{m=1}^{N} (\ln S_m - \ln \gamma + 2\Gamma mT_s)^2 \chi_m, \quad (4-7) \]

where the \( \chi_m \)'s are weight factors. A reasonable fit is obtained by taking the weight \( \chi_m \) to be inversely proportional to the variance in the measured quantity \( \sigma_m^2 \), i.e.

\[ \chi_m \propto \frac{1}{\langle (\delta \ln S_m)^2 \rangle} \approx \frac{S_m^2}{\langle (\delta S_m)^2 \rangle}, \quad (4-8) \]

where \( \langle (\delta S_m)^2 \rangle \) is the expected variance in \( S_m \). One can, however, neglect the relatively weak \( m \) dependence \( \sigma_m^2 \) of \( \langle (\delta S_m)^2 \rangle \) and use

\[ \chi_m \propto S_m^2. \quad (4-9) \]
4.2 Polydisperse Data Analysis

Polydisperse data analysis consists of fitting to the function

\[ S_m = \ln \gamma - 2\Gamma m T_s + \mu_2 (m T_s)^2, \]

with the same weighting as before. A system of aggregated particles will contain many different size particles. The Einstein relation therefore generalizes to

\[ D_k = \frac{k_B T}{3\pi \eta \zeta (f/f_0)}, \]

where \((f/f_0)\) is a shape factor, and \(\eta\) is the viscosity of the solvent. In this case the solvent was sodium acetate, which has approximately the same viscosity as water, given as a function of temperature by the relation\(^{23}\),

\[ \log_{10} \frac{\eta}{\eta_{20}} = \frac{1.3272(20 - T) - 0.001053(T - 20)^2}{T + 105}. \]

where \(\eta_{20}\) is the viscosity of water at 20°C and \(T\) is measured in °C. It is assumed that the viruses aggregate along no preferred axis and therefore form approximately spherical aggregates. This being so, then \((f/f_0) \approx 1\). It is also possible to calculate an experimental estimate of the average number of particles in an aggregate \((\bar{k}_{zz})\).

As the diameter of a single virus has been found to be 34nm\(^1\) then

\[ \bar{k}_{zz} = \left( (\xi/d)^3 - 1 \right) P_f + 1, \]

where \(d\) is the diameter of a single virus, \(P_f\) is the packing fraction, and \(\xi\) is the mean diameter of the aggregates in the system. It was assumed on consultation with Tremaine and Ronald that the aggregates formed some sort of close packing structure, and so the packing fraction was estimated to be 0.7.
The program CORR.AG was used to fit the data, and is listed in appendix 3. From the parameters of the fit, values of $D$, $Q$, and $k_{tz}$ were calculated, using Eq. 2-64, 65, 66, and Eq. 4-13. Fig. 4-1 through 4-4 give an example of the output of CORR.AG. By thus using the program CORR.AG to analyse the data produced by each dynamical run it is possible to measure the rate of increase of $k_{tz}$ with time. It is expected that this will be governed by Eq. 2-99. If this is rewritten as

$$k_{tz}(t) = \frac{[6(Rt)^2 + 6(Rt) + 1]}{[1 + 2(Rt)]}, \quad (4 - 14)$$

where

$$R = \nu_0(r/t), \quad (4 - 15)$$

then as R is a constant for each particular concentration and temperature, it can be thought of as the rate of aggregation. The next stage of the analysis consists of fitting the values of $k_{tz}$ obtained from the correlator runs to Eq. 4-14 to obtain the dependence of the rate constant on temperature and initial concentration. The program ANALYSIS was used for this. ANALYSIS performed a two parameter fit to the function,

$$k_{tz}(t) = \frac{[6x^2 + 6x + 1]}{[1 + 2x]} \quad (4 - 16)$$

with $x = P_1(t + P_2). \quad (4 - 17)$

$P_1$ and $P_2$ are the two parameters in the fit, representing $R$, and $t_s$ respectively, where $t_s$ is the time that has elapsed between the system commencing aggregation and the experiment starting *. The timing of the runs commenced two minutes after placing the scattering cell inside the I.M.C. This is because it was calculated that the contents of the scattering cell would take two minutes to reach the temperature of the I.M.C. This conclusion was arrived at by solving the heat equation for a

* see below
cylindrically symmetric tube 1cm in diameter which is initially at temperature \( T = T_0 \) with wall held at a constant temperature \( T = T_{\text{cell}} \). The series of Bessel functions that results is plotted in Fig. 4-5. This, however, is the maximum time. If there is convection in the cell the contents will reach the temperature of the I.M.C. faster. This approach does have the limitation that some aggregation may occur before \( t = 0 \). Indeed, experiments carried out a good deal below the temperature at which aggregation commences indicate that there is some residual aggregation left from some previous passage of the system past the temperature of aggregation. To allow for this eventuality we therefore performed a two parameter fit on the data. The parameter \( P_2 \) takes into account any aggregation in the sample at \( t = 0 \) in the following manner. It is assumed that all aggregation present at \( t = 0 \) had taken place at the rate \( R \) from time \( t = (-P_2) \). In other words, we are assuming that at the time \( t = -P_2 \), the initially monodispersive system was instantly raised to the temperature of the I.M.C. The system then proceeded to aggregate at the rate \( R \) until \( t = 0 \), when the experiment is commenced with the system in a partially aggregated state. It was also noted that for large values of \( k \), this equation was often not a good description of the data. This was because as \( \zeta \) approaches \( q^{-1} \), Eq.2-93 is not true and \( \langle I_k \rangle \) is no longer proportional to \( \nu_k k^2 \). Thus

\[
D \neq \frac{\sum_k D_k \nu_k k^2}{\sum_k \nu_k k^2}.
\]  

(4 - 18)

It was further noted that once the system had reached this stage of aggregation, it becomes somewhat turbid. We shall therefore herein refer to data collected while the system was in this state as data collected while in the turbid régime. Data taken in this régime gives an indication as to whether the system is still aggregating, but values of \( k_{xz} \) so obtained have no precise correspondence to \( \tilde{k} \). The program ANALYSIS can thus perform a fit to any particular range of \( t \) values to ensure a
good fit. The relationship between the aggregation rate \( R \), and \( \nu_0 \) and \( T \) is governed by

\[
R = \exp[-E_{agg}/k_B(T - T_c)] \cdot \left( \frac{3\nu_0 k_B T}{2\eta(T)} \right). \tag{4 - 19}
\]

If this is rearranged it becomes

\[
\frac{k_B}{E_{agg}}(T - T_c) = \frac{1}{\ln\left(\frac{R\eta(T)}{3\nu_0 k_B T}\right)}. \tag{4 - 20}
\]

The program LEASTSQUARE then performs a least squares fit to the function,

\[
\frac{1}{\ln\left(\frac{R\eta(T)}{3\nu_0 k_B T}\right)} = P_1 T + P_2, \tag{4 - 21}
\]

for the values of \( R \) and \( T \) corresponding to each concentration tried. Values of \( E_{agg} = E_{agg}(\nu_0) \) and \( T_c = T_c(\nu_0) \) can then be extracted from the two parameters, and the functional form of their dependence on \( \nu_0 \) can be investigated.
FILE = sep16.112430

THE EXPERIMENTAL PARAMETERS
RUN#=16098501  THETA= 90.000  STIME= 28.50
CLIPRATE= 0  TEMP= 30.00  MULT.FACTS.= 0 0 0 0

THE FIRST 4 CORRELATOR CHANNELS ARE
.NCOUNT= 1256642  NCLIP= 1131904
.ZERO= 0  NSTIME= 1000918

XN = NCOUNT/NSTIME = 0.1257
BL = XN * NCLIP = 0.1422E+06
CF = (XN+1)/(CLIPRATE+1) = 1.126
B = EXPERIMENTAL PARAMETER (DARK NOISE)

INPUT = DATA READ IN;
X = MULTIPLE OF STIME;
Y1 = G(T) = (INPUT - BL)/(YMAX - BL);
Y2 = LN G(T) + LN(B/CF) = LN( INPUT/(BL - 1) );
WT = ( INPUT/(BL - 1) ) ** 2.0 = STAT WEIGHT

<table>
<thead>
<tr>
<th>INPUT</th>
<th>X</th>
<th>Y1 OR G(T)</th>
<th>LN G(T) + LN(B/CF)</th>
<th>WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>228898</td>
<td>0.0</td>
<td>1.00000000</td>
<td>-0.4952995</td>
<td>2.590168</td>
</tr>
<tr>
<td>219415</td>
<td>28.50</td>
<td>0.89058661</td>
<td>-0.6111743</td>
<td>2.379996</td>
</tr>
<tr>
<td>211147</td>
<td>57.00</td>
<td>0.79519171</td>
<td>-0.7244727</td>
<td>2.204091</td>
</tr>
<tr>
<td>203121</td>
<td>85.50</td>
<td>0.70258898</td>
<td>-0.8482835</td>
<td>2.039640</td>
</tr>
<tr>
<td>197531</td>
<td>114.00</td>
<td>0.63809246</td>
<td>-0.9455734</td>
<td>1.928920</td>
</tr>
<tr>
<td>191543</td>
<td>142.50</td>
<td>0.56900382</td>
<td>-1.0591698</td>
<td>1.813743</td>
</tr>
<tr>
<td>185916</td>
<td>171.00</td>
<td>0.50408041</td>
<td>-1.1803198</td>
<td>1.708746</td>
</tr>
<tr>
<td>181398</td>
<td>199.50</td>
<td>0.45195240</td>
<td>-1.2894773</td>
<td>1.626704</td>
</tr>
<tr>
<td>177679</td>
<td>228.00</td>
<td>0.40904319</td>
<td>-1.3892355</td>
<td>1.560686</td>
</tr>
<tr>
<td>172154</td>
<td>256.50</td>
<td>0.34529662</td>
<td>-1.5586510</td>
<td>1.465136</td>
</tr>
<tr>
<td>169597</td>
<td>285.00</td>
<td>0.31579435</td>
<td>-1.6479654</td>
<td>1.421936</td>
</tr>
<tr>
<td>167373</td>
<td>313.50</td>
<td>0.29013419</td>
<td>-1.7327156</td>
<td>1.384887</td>
</tr>
<tr>
<td>163745</td>
<td>342.00</td>
<td>0.24827486</td>
<td>-1.8885193</td>
<td>1.325499</td>
</tr>
<tr>
<td>162187</td>
<td>370.50</td>
<td>0.23029894</td>
<td>-1.9636812</td>
<td>1.300396</td>
</tr>
<tr>
<td>159012</td>
<td>399.00</td>
<td>0.19366628</td>
<td>-2.1369247</td>
<td>1.249981</td>
</tr>
<tr>
<td>157633</td>
<td>427.50</td>
<td>0.17775559</td>
<td>-2.2226458</td>
<td>1.228395</td>
</tr>
<tr>
<td>155836</td>
<td>456.00</td>
<td>0.15702206</td>
<td>-2.3466730</td>
<td>1.200546</td>
</tr>
<tr>
<td>154589</td>
<td>484.50</td>
<td>0.14263439</td>
<td>-2.4427805</td>
<td>1.181410</td>
</tr>
<tr>
<td>153990</td>
<td>513.00</td>
<td>0.13572323</td>
<td>-2.4924459</td>
<td>1.172717</td>
</tr>
<tr>
<td>152876</td>
<td>541.50</td>
<td>0.12287009</td>
<td>-2.5919371</td>
<td>1.155372</td>
</tr>
<tr>
<td>151029</td>
<td>570.00</td>
<td>0.10155970</td>
<td>-2.7824173</td>
<td>1.127623</td>
</tr>
<tr>
<td>150345</td>
<td>598.50</td>
<td>0.09366781</td>
<td>-2.8633137</td>
<td>1.117432</td>
</tr>
<tr>
<td>149426</td>
<td>627.00</td>
<td>0.08306450</td>
<td>-2.9834442</td>
<td>1.103814</td>
</tr>
<tr>
<td>148913</td>
<td>655.50</td>
<td>0.07714558</td>
<td>-3.0573654</td>
<td>1.096248</td>
</tr>
</tbody>
</table>

Figure 4-1 CORR.AG output Pg.1.
Figure 4-2 CORR.AG output Pg.2.
FITTING POLYNOMIAL: $Y = P_1 + P_2X + P_3X^2$.

INTERMEDIATE ESTIMATES OF PARAMETERS, SUM OF SQUARES

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>115.77</td>
<td></td>
</tr>
<tr>
<td>-0.48848</td>
<td>-0.41372E-02</td>
<td>0.28945E-06</td>
<td>0.16555E-01</td>
<td></td>
</tr>
<tr>
<td>-0.48848</td>
<td>-0.41372E-02</td>
<td>0.28937E-06</td>
<td>0.16555E-01</td>
<td></td>
</tr>
</tbody>
</table>

FINAL ESTIMATES OF PARAMETERS

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.48848</td>
<td>-0.41372E-02</td>
<td>0.28937E-06</td>
</tr>
</tbody>
</table>

SUM OF SQUARES 0.16555E-01

<table>
<thead>
<tr>
<th>E1</th>
<th>E2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.382567E+00</td>
<td>0.304686E-02</td>
<td>0.475851E-05</td>
</tr>
<tr>
<td>0.107414E-01</td>
<td>0.855470E-04</td>
<td>0.133605E-06</td>
</tr>
</tbody>
</table>

$K =$ SCATTERING VECTOR (/cm)
$D =$ DIFFUSION COEFFICIENT (cm/sec)
$R_L =$ RAYLEIGH LINEWIDTH (hz)
$M_2 =$ 2nd MOMENT OF LINE WIDTH DISTRIBUTION (hz**2)
$Q =$ THE QUALITY FACTOR (dimensionless)
$DIA =$ THE AVERAGE DIAMETER OF THE PARTICLES (nm)
$K_BAR =$ THE MEAN NUMBER OF PARTICLES PER AGGREGATE

$K = 187320.$
$D = 0.589532E-07$
$R_L = 2068.60$
$M_2 = 289373.$
$Q = 0.676246E-01$
$DIA = 94.4532$
$K_BAR = 11.22$

Figure 4-3 CORR.AG output Pg.3.
CHAPTER 4: DATA ANALYSIS

PLOT OF LOG OF CORRELATION FUNCTION

Figure 4-4 CORR.AG output Pg.4.
Figure 4-5 The average temperature of the cell after it has been placed in the I.M.C.
5.1 Introduction
This chapter is organized into four sections. The first section will cover experiments in which the scattering sample was polystyrene latex spheres. The diffusion coefficient is measured, and its dependence on temperature and scattering angle is discussed. For the second and third sections the scattering sample was CRSV. In the second section the runs were performed at a temperature below which the virus is expected to aggregate for that particular concentration. The diffusion coefficient is measured and its dependence on temperature and scattering angle is discussed, along with the possibility of residual aggregates being present from previous aggregations. In the third section the aggregation behaviour of CRSV is studied. The dependence of the aggregation rate upon virus concentration and temperature is discussed and the temperature of aggregation (T.A.) and aggregation energy (A.E.) are calculated for each concentration sample. In the fourth section a few suggestions for further investigations are made.

5.2 Latex Sphere Results
The latex sphere samples were purchased from Seragen Diagnostics\textsuperscript{24}, and were 0.087 \( \mu \)m in diameter. They were suspended in deionized distilled water at a concentration of \( 3.35 \times 10^{-5} \) g/ml, which corresponds to a mean spacing between...
particles of twenty-five times their diameter. Experiments were performed at scattering angles of 30° to 140° at 10° intervals. The entrance and exit apertures of the I.M.C. prevented readings outside this range. For each run $\Gamma$ and $\mu_2$ were extracted from the fit to the correlation data, and the corresponding values of $\bar{D}$, $\xi$, and $Q$ were calculated.

In Fig. 5-1 a graph of the Rayleigh linewidth, $\bar{\Gamma}$, versus the square of the scattering vector $q^2$, is plotted, and a straight line is sketched through the data points corresponding to the same temperature. As $\bar{\Gamma} = \bar{D} q^2$, the increase in slope of each line occurs as a consequence of the increase in $\bar{D}$ with temperature. To check that this is consistent with the Einstein relation for the diffusion coefficient the diameters corresponding to each run are plotted in Fig. 5-2, and in Table V-I the values of the mean diameter $\bar{\xi}$, the standard deviation from $\bar{\xi}$, and the mean value of the quality factor $Q$ are listed for each temperature.

<table>
<thead>
<tr>
<th>temp $(^\circ \text{C})$</th>
<th>$\bar{\xi}$ (nm)</th>
<th>$\bar{\xi}\sigma^{n-1}$ (nm)</th>
<th>$Q$</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>98.4</td>
<td>3.7</td>
<td>0.03</td>
</tr>
<tr>
<td>30</td>
<td>100.7</td>
<td>3.2</td>
<td>0.02</td>
</tr>
<tr>
<td>34</td>
<td>99.7</td>
<td>2.8</td>
<td>0.02</td>
</tr>
</tbody>
</table>

The scatter in the diameters in Fig. 5-2 shows firstly, that any systematic deviation from the Einstein relation for the diffusion coefficient as the temperature is increased is less than the uncertainty in the measurement; and secondly, that the diameter seems to be independent of the scattering angle, within the accuracy of the experiment. The $Q$ values were all ±30%. These non zero $Q$ values suggest that the system was not entirely monodispersive. Possible reasons for non-zero $Q$ values would be dust in the sample, or particles in the solvent which was only filtered to 2 $\mu$m. It has been also reported$^{25}$ that samples of latex spheres of this size have a
Figure 5-1 Variation in $\bar{\Gamma}$ with $q^2$ for latex spheres.
RAYLEIGH LINEWIDTH vs SCATTERING VECTOR SQUARED FOR THREE DIFFERENT TEMPERATURES

Figure 5-2 Variation in $\xi$ with $q$ for latex spheres.
tendency to form into larger aggregates over long periods of time. Both these eventualities would lead to a larger value of \( \bar{\xi} \) than expected. An inspection of Table V–I also indicates that the mean diameter, \( \bar{\xi} \) is \( \approx 13\% \) larger than the values quoted by Seragen diagnostics, although the standard deviation is approximately the same as that quoted by Seragen: 4.6 nm. Possible reasons for this are that Seragen are incorrect in their measurement, or that the dust or particles in the solvent are leading to a larger average diameter of particle in the system, or that the hydrodynamic radius of the particles is larger than the dry radius of the particles that is measured by inspection of the spheres under an electron microscope (which is how they are measured by Seragen); or that they are somewhat aggregated as mentioned before, or—more likely a combination of all four reasons. If the large value of \( \bar{\xi} \) was due to the aforementioned impurities in the solvent, one would have expected a larger standard deviation than was measured, but the actual measurement was smaller than that quoted by Seragen. The same can be also said if the spheres were aggregated. A second measurement of the spheres is therefore clearly required. Evidence for an increase in the hydrodynamic radii with increasing temperature has been reported\(^2\), but this increase in the mean hydrodynamic radius with temperature was not seen.

Having examined the results of the measurements on Latex spheres, the next step is to discuss the error in the results. CORR.AG provides an estimate of the error in the three parameters \( (\bar{\Gamma}, \mu_2, \text{ and } \gamma) \) of the data fit to the Eq. 2.64.

\[
C_{ik} = \langle n \rangle \langle u_k \rangle \left[ 1 + \gamma \exp(-2\bar{\Gamma} t + \mu_2 \tau) \right]. 
\] (5 – 1)

For each parameter \( P_i \), CORR.AG produces a value

\[
P_i = P_i \pm E_i, \tag{5 – 2}
\]
where,

\[ E_i = \left[ \sum_{k=1}^{N} w_k \delta^2(N - M) \right]^{\frac{1}{2}} b_{ii} \quad \text{for } i = 1, 2, 3. \quad (5-3) \]

\( N \) is the number of data points (twenty eight), \( M \) is the number of parameters in the fit (three), \( w_k \) is the statistical weight given to the \( k \)th point, \( \delta \) is the residual in the least squares minimization,

\[ \delta = \left( y_k - f(x_k, P_1, P_2, P_3) \right), \quad (5-4) \]

and \( b_{ii} \) is the diagonal element of the covariance matrix corresponding to the \( i \)th parameter. The errors in the parameters \( \gamma, \bar{\Gamma}, \) and \( \mu_2 \) were \( \pm 1\% \), \( \pm 3\% \), and \( \pm 30\% \) respectively. As \( \zeta \) is given by

\[ \zeta = \frac{k_B T q^2}{6 \pi \eta \bar{\Gamma}}, \quad (5-5) \]

we can calculate the error in \( \zeta \) to be

\[ \frac{\Delta \zeta}{\zeta} = \left[ \left( \frac{\Delta \bar{\Gamma}}{\bar{\Gamma}} \right)^2 + \left( \frac{\Delta q^2}{q^2} \right)^2 + \left( \frac{\Delta \eta}{\eta} \right)^2 + \left( \frac{\Delta T}{T} \right)^2 \right]^{\frac{1}{2}}. \quad (5-6) \]

\( \Delta \bar{\Gamma}/\Gamma \) is given by CORR.AG to be 0.03. The only significant contribution to \( \Delta q^2/q^2 \) comes from uncertainty in the measurement of the scattering angle \( \theta \). Allowing for alignment of photomultiplier tube (P.M.T.), and adjustment of the lens in the P.M.T. that focuses the image of the scattering volume onto the pinhole aperture of the P.M.T., \( \Delta \theta \) could be as large as 1°. Consequently the error in \( q \) can be obtained by differentiating the square of Eq. 2-22 with respect to \( \theta \), giving

\[ \frac{\Delta q^2}{q^2} = \frac{\Delta \theta}{57 \tan(\frac{\theta}{2})}, \quad (5-7) \]
where $\theta$ is in degrees. For $\theta = 90^\circ$, then, $\Delta q/q \approx 0.0175$. The temperature was known to an accuracy of $\approx 0.05\%$, thus the uncertainty in $\eta$ is about $0.1\%$. Consequently, $\Delta r/r \approx 3.2\%$. As the standard deviation in the sample provided by Seragen is $\approx 5\%$ and the scatter in our data, from Fig. 5–2 is $\approx 6\%$, the results seem to be within experimental error.

5.3 CRSV Results - I- Nonaggregating Régime

Nonaggregating samples consisted of solutions of CRSV that were either at temperatures below the temperature at which aggregation commences, or at a temperature close enough to T.A. that aggregation took place very slowly. (slowly implies $k_{ex}$ changes by less than 50%, from 1 to 1.5, over a period of an hour — aggregation much slower than this is too slow to measure). Experiments were performed from 30°C to 50°C in 5°C intervals. At the higher temperatures some aggregation was seen, but it is left for the next section to examine the aggregation in detail. The concentration used was 0.87 mg/ml, and angles $\theta$ chosen for collecting data were: 7.5°, 90° and 105°. For each run $D$ was extracted from the correlation data and the Einstein relation was used to calculate $\xi$. The results are plotted in Fig. 5–3. In table V–II the mean and standard deviation diameters, along with the mean value of $Q$, are listed. The results were seen to be independent of scattering angle and therefore the results are displayed grouped by temperature only.

<table>
<thead>
<tr>
<th>temp (°C)</th>
<th>$\bar{\xi}$ (nm)</th>
<th>$\bar{\xi} \sigma^{-1}$ (nm)</th>
<th>$Q$</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>34.5</td>
<td>0.55</td>
<td>0.016</td>
</tr>
<tr>
<td>35</td>
<td>34.5</td>
<td>0.89</td>
<td>0.02</td>
</tr>
<tr>
<td>40</td>
<td>36.4</td>
<td>0.43</td>
<td>0.03</td>
</tr>
<tr>
<td>45</td>
<td>37.0</td>
<td>1.25</td>
<td>0.03</td>
</tr>
<tr>
<td>50</td>
<td>40.3</td>
<td>1.96</td>
<td>0.10</td>
</tr>
</tbody>
</table>
Figure 5-3 Values of $\xi$ in CRSV for different temperatures.
The mean diameter of the results performed at 30°C is 1.5% higher than the expected diameter of CRSV and is within the calculated experimental error. Indeed, the range of scatter of the results from the mean is only ±2\%\%, which is also within experimental error. As the apparatus was not changed from the experiments on latex spheres and the buffer in which the virus was suspended was unfiltered, it seems likely that the results obtained in Sec. 5.2 were an accurate description of the size of the particles.

If the results at 30°C are assumed to accurately represent the monodisperse state of the system, the diffusion coefficient of CRSV at 20°C ($D_{20w}$) can be calculated to be $1.24 \times 10^{-7}$ cm$^2$/s, which is 20% lower than the previously published result$^1$. But if the present result is used—along with the sedimentation coefficient—to calculate the molecular weight ($M$) of the virus, a result is obtained that is consistent with theoretical calculations which assume that the viruses consist of 180 protein subunits of 38,000 daltons and RNA-1 of $1.5 \times 10^6$ daltons, giving $M = 8.3 \times 10^6$ daltons. Using the Svedburg relation

\[
M = \frac{RTS_{20w}}{D_{20w}(1 - \rho \phi)},
\]

where $\phi$ is the specific volume of the virus (0.693 ml/g), $\rho$ is the density of water (0.998 g/ml), and $S_{20w}$ is the sedimentation coefficient$^1$ ($133 \times 10^{-13}$ s$^{-1}$), $M$ is calculated to be $8.4 \times 10^6$ daltons—using the above value of $D_{20w}$.

The results at higher temperatures suggest that some aggregation is taking place. This is reflected not only in the higher mean values of particle diameter, but also in the increase in the standard deviation in $\bar{f}$ and the higher values of $Q$.

A series of runs of one minute duration were performed on a sample at 50°C at 20 minute intervals. The results are plotted in Fig. 5-4. An increase in the mean diameter can be noted. The large scatter in the data are due to the short
run time of 1 min, and the increase in $\zeta$ is small as the virus is aggregating relatively slowly. The Q values, averaged over three runs, for each consecutive hour were: 0.02, 0.03, and 0.05. This increase reflects the increase in polydispersity in the system during the aggregation processes.

5.4 CRSV Results -II- Aggregating Régime

In this section of the experiment the dependence on temperature and virus concentration of the aggregation rate (A.R.) of CRSV is determined. This is achieved by first measuring the A.R. as a function of these variables and then fitting the results to the previously hypothesized form of the aggregation rate. In doing so the dependence of the two aggregation parameters T.A. and E.A. on the experimental variables is ascertained.

Experimental data was gathered by taking four samples of differing concentrations and measuring the aggregation rate for temperatures at approximately 1°C intervals through the range of temperatures over which this was possible. Experimental runs were limited to 30 minutes to avoid damage to the virus. The temperature range was limited by two factors. First, its lower limit was determined by the fact that if $k_{rz}$ did not increase by more than the average uncertainty in any one measurement during the 30 minutes of the experiment, the aggregation rate was deemed to slow to measure. Second, its upper limit was restricted by rate of aggregation. If the aggregation resulted in the formation of an average size of aggregate comparable to $q^{-1}$ before the first measurement could be made, the aggregation rate was deemed to be too fast to measure. This is because the system was in the turbid régime where Eq. 2-93 to 95 are no longer valid.

It was noted that as the system aggregated the amount of scattered light increased, as can be seen by reinspecting Eq. 2-93. Although the number of scattering centers decreases with time for an aggregating system, the number of particles in
Figure 5-4 Increase in $\zeta$ with time as CRSV aggregates.
Each aggregate \( \bar{k} \) increases, and as this appears as the second power the total intensity of scattered light will increase. If the amount of scattered light is increasing then the average number of photons per sample time \( \langle n \rangle \) recorded by the correlator will increase, so between each one minute run the mean number of clipped counts per sample time must be checked. If it is much greater than 0.5 then the clip rate must be adjusted accordingly.

As the system aggregates \( \zeta \) will increase and \( \bar{\Gamma} \) will decrease since \( \zeta \propto D^{-1} \) and \( D \propto \bar{\Gamma} \). Therefore, as statistical error is minimized when \( M \bar{\Gamma} T_s \sim 2 - 3 \) \( T_s \) must be adjusted accordingly.* \( M \bar{\Gamma} T_s \) is reflected in the fourth column of page one of the output of CORR.AG. ** To achieve maximum accuracy, \( \ln G(24 T_s) - \ln G(0) \sim 2 - 3 \). Column four must therefore span a range of 2 - 3. This was checked after each 1 minute run and \( T_s \) adjusted accordingly.

Each 30 minute run produced 10 values of \( \bar{k}_{xx} \) at 180 sec. time intervals. These points were used by ANALYSIS as a data set to fit to the function

\[
\bar{k}_{xx}(t) = \frac{[6(Rt)^2 + 6(Rt) + 1]}{[1 + 2(Rt)]}.
\]  

(5 - 9)

An example of a bad fit to this function is shown in Fig. 5-5. The last three points correspond to mean aggregates much larger than \( q^{-1} \), where the system is in the turbid régime, and therefore Eq. 2-93 does not apply. Accordingly ANALYSIS is rerun excluding these points from its data fit. Fig. 5-6 shows the new fit. Here the fit is much better, but now on this expanded scale it can be seen that only the first three points fall on a straight line. Consequently ANALYSIS is run again with just those points, see Fig. 5-7. Now it can be seen that there is a good fit. The maximum point on this graph \( \bar{k}_{xx} \approx 25 \) corresponds to a mean aggregate size of \( \approx 0.1 \mu m \), which is approximately the same magnitude as \( q^{-1} \approx 0.06 \mu m \). The three

* as explained in Sec. 3.6
** see Fig. 4.1
values of $R$ obtained from the fits were: 4.1, 2.7, and $1.7 \times 10^{-2}$ s$^{-1}$ respectively. For the majority of experimental runs it was found that all ten data points could be used. The previous example is that of a system nearly at the maximum aggregation rate one could measure.

Having obtained the aggregation rates in this manner, the next stage of the analysis was to fit them to Eq. 4-19

$$R = \exp\left[-\frac{E}{k_B(T - T_c)}\right] \cdot \left(\frac{3\nu_0 k_B T}{2\eta(T)}\right). \quad (5 - 10)$$

In Fig. 5-8 we therefore plot $1/\ln\left(\frac{R_{\text{agg}}(T)}{3\nu_0 k_B T}\right)$ versus $T$ for the four concentrations. The data fit was done by the program LEASTSQUARE which performed a two parameter fit to Eq. 4-20. The four values of the energy of aggregation and temperature of aggregation are plotted in Figs. 5-9, and 10, and listed in Table V-III.

<table>
<thead>
<tr>
<th>conc. (mg/ml)</th>
<th>$E_{\text{agg}}$ (K)</th>
<th>$T_c$ (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.31</td>
<td>214 ± 16.5</td>
<td>289.8 ± 1.4</td>
</tr>
<tr>
<td>3.16</td>
<td>186 ± 22.0</td>
<td>295.1 ± 1.8</td>
</tr>
<tr>
<td>1.90</td>
<td>166 ± 20.0</td>
<td>300.2 ± 1.6</td>
</tr>
<tr>
<td>0.975</td>
<td>138 ± 16.0</td>
<td>333.4 ± 1.3</td>
</tr>
</tbody>
</table>

From Figs. 5-9 and 10 it appears, firstly, that the aggregation energy increases in a logarithmic fashion with concentration, and secondly, that the temperature of aggregation decreases exponentially with concentration. This is in good agreement with the results of Tremaine and Ronald. If we refer to Fig. 1-1 we can see as a consequence of the latter result aggregation commences at a lower temperature for higher concentrations.

The first result implies there is a higher rate of change of the aggregation rate with temperature for higher concentrations. This is reflected in Fig. 1-1. As
Figure 5-5 Theoretical fit by ANALYSIS to aggregation data.
Figure 5-6 Alternative fit by ANALYSIS to aggregation data.
Figure 5-7 Final fit by ANALYSIS to aggregation data.
LOG OF THE AGGREGATION RATE vs TEMP FOR FOUR DIFFERENT CONCENTRATIONS

Figure 5-8 Graph of $1/\ln\left(\frac{R_2 n(T)}{\bar{z} u k_B T}\right)$ versus $T$ for the four concentrations.
Figure 5-9 Graph of energy of aggregation vs concentration.
Figure 5-10 Graph of temperature of aggregation vs concentration.
each specimen was heated at a rate of 0.25 C/min the start of each curve represents the commencement of measurable aggregation, and the near-vertical part of each curve corresponds to the temperature and time at which the virus is aggregating so rapidly that the solution has become turbid. The first result is reflected in the decrease in the time interval between these two stages that is apparent in Fig. 1–1.

Measurements taken over long periods in the turbid régime show \( k_{xz} \) continually increasing, and it is assumed that if left for long enough at a temperature greater than \( T_c \) the virus would aggregate into one large mass.

5.5 Suggestions for Further Study

Further study could well be aimed at trying to understand the bonding process, through which the aggregation takes place, by varying the environment of the viruses. The following further suggestion for investigations were arrived at through consultation with Dr. Tremaine.

(i) An investigation of the effect of various chemicals on the aggregation of CRSV-N, particularly those that were studied by Tremaine and Ronald in their absorbance studies\(^5\).

(ii) An investigation of the swelling of CRSV-N with pH adjustments, from pH 5 to pH 7, and the effects of divalent cations on the swelling. This might include a study of any hysteresis effects similar to those reported by Tremaine\(^27\) and Harrison\(^28\).

(iii) The size of twelve particle aggregates, and linked aggregates, are known in CRSV-A, as are their sedimentation coefficients. However, their non-spherical shape means that one could investigate their diffusion coefficient.
CHAPTER 6

CONCLUSIONS

The aggregation rate of CRSV was measured for a range of temperatures and concentrations. The function

\[ R(T, \nu) = \begin{cases} \frac{2\nu_0 k_B T}{3\eta} \exp\left[-E_{agg}/k_B(T - T_c)\right] & \text{if } T > T_c \\ 0 & \text{if } T < T_c \end{cases} \]

was found to fit the results well. It was also found that the aggregation parameters, \( E_{agg} \) and \( T_c \), were a function of concentration. \( E_{agg} \) increased with concentration. However, the rate of increase decreases. \( T_c \) decreased with concentration. Similarly, the rate of decrease lessens.

The size of the virus was confirmed to be 34 nm. The diffusion coefficient was found to be \( 1.24 \times 10^{-7} \) cm\(^2\)/s and the molecular weight \( 8.3 \times 10^6 \) daltons. The results were in good accord with the work of Dr. Tremaine.

The measurements on the Latex spheres were consistent with an expected experimental accuracy of \( 3\frac{1}{2}\% \) in the hydrodynamic radius of the scattering particle.
BIBLIOGRAPHY


22. Instruction Manual, Type K7023 Malvern Digital Correlator.


24. Seragen Diagnostics, Indianapolis, IN 46225, U.S.A.


If we consider the motion of the macromolecules in the solution to arise from incessant random bombardment by the solvent molecules, then the theories of Brownian motion can be used to calculate the integral of the velocity autocorrelation function

\[ I = \int_0^\infty \langle v_x(0)v_x(t) \rangle \, dt \]

To do this one makes the observation that

\[ r(t) = \int_0^t v(u) \, du. \tag{A1.1} \]

From which it follows that,

\[ \langle r^2(t) \rangle = \int_0^t \int_0^t \langle v(u_1)v(u_2) \rangle \, du_1 \, du_2, \tag{A1.2} \]

If we denote the autocorrelation function of the velocity by the symbol \( K_v(u_1, u_2) \) and note the following properties:

(i) In a stationary ensemble the function \( K_v(u_1, u_2) \) depends only on the time interval \( (u_1 - u_2) \). Denoting this by the symbol \( \varepsilon \), we have

\[ K_v(u_1, u_1 + \varepsilon) = K_v(\varepsilon). \]
APPENDIX 1: EVALUATION OF VELOCITY AUTOCORRELATION FUNCTION

(ii) The quality $K_v(0)$, which is equal to the mean square value of the variable $v$ at time $u_1$ must be positive definite, and, in a stationary ensemble, it must be constant.

(iii) For any value of $s$, the magnitude of the function $K_v(s)$ cannot exceed the value at $K_v(0)$.

(iv) The function $K_v(s)$ is symmetric about the value $s = 0$.

(v) As $s$ becomes large in comparison with the characteristic time $\tau$, the values of $v(u_1)$ and $v(u_1 + s)$ become uncorrelated, that is

$$\lim_{s \gg \tau} K_v(s) = 0.$$ 

To evaluate Eq. 1.2 then, make the following change of variables

$$S = \frac{1}{2}(u_1 + u_2) \quad \text{and} \quad s = (u_2 - u_1)$$

giving

$$I = \int_0^{1/2} \int_{-2S}^{+2S} K_v(s) \, dS \, ds + \int_{1/2}^t \int_{-2(t-S)}^{+2(t-S)} K_v(s) \, dS \, ds. \quad (A1.3)$$

But in view of property (v) the function $K_v(s)$ will only be non-zero in the narrow region of the order of $\tau$ around the central value of $s = 0$. Therefore if $t \gg \tau$ the limits of integration for $s$ may be replaced by $-\infty$ and $+\infty$ with the result

$$\langle \tau^2(t) \rangle = t \int_{-\infty}^{+\infty} \langle v(0)v(s) \rangle \, ds. \quad (A1.4)$$

An established result of the Einstein and Langevin theories of Brownian Motion is that for $t \gg \tau$

$$\langle \tau^2 \rangle = 6Dt. \quad (A1.5)$$
Equating Eq. A1.5 and Eq. A1.4 we arrive at

\[ 6D = \int_{-\infty}^{+\infty} \langle v(0)v(s) \rangle \, ds, \]  

(A1.6)

or

\[ D = \int_{0}^{+\infty} \langle v_x(0)v_x(s) \rangle \, ds. \]  

(A1.7)
APPENDIX 2

DESCRIPTION OF THE CORRELATOR CONTROLS

A diagram of the front panel of the correlator is illustrated in Fig. A1-1. A description of the controls is as follows.

Input Channels A and B

The input channels have an input impedance of 50Ω.
Logical 1 -1 volt
Logical 0 0 volt
Absolute maximum input levels +1 volt to -3 volts
Pulse width 25ns
Rise time not to exceed 1μs
Channels A and B have internal selection of positive or negative going triggering edges.

Function Switch

Provides selection of the following modes of operation:-
Autocorrelation Single Clipped
Autocorrelation Double Clipped
Crosscorrelation Single Clipped
Probability Density
Probability Distribution
Signal Averaging multiscaling Internal Trigger

Signal Averaging multiscaling External Trigger

Figure A1-1 Correlator front panel.

Clipping Level

The decade switch determines the clip level. The clipped signal then being monitored on channel (01).
Sample Time

Three decade switches set up the sample time in steps of $0.05\mu s$ up to a maximum of $9.95\mu s$. Another decade switch increases the sample time by multiples of 10 up to the maximum sample time of $9.95 \times 10^5 \mu s$.

Number of Samples

This decade switch determines the number of samples in multiples of 10. Up to $10^9$.

Readout Mode

The contents of the dynamic store may be presented in four forms.

These are as follows:

Scope

Here the presentation is on the C.R.T display whose X deflection represents the channel address, and whose Y deflection is proportionate to the number of counts in the dynamic store. The Y outputs F.S.D. can be adjusted by means of the switch immediately below the readout mode selector switch, such that the Y sensitivity is variable from 50 counts per centimeter up to a maximum of $5 \times 10^6$ (5M) counts per centimeter.

Plot

In this mode the Z80 microcomputer is used to load the data into a computer file *.

Punch

In this mode the START button is used to start the high speed punch FACIT Type 4070 75 CHAR/SEC.

* see section 3.2
Step

In the plot position, with the Z80 disconnected the contents of each of the store channels can be stepped through and read from the display window.

Monitor Channel Overflow Indicators and Multipliers

With the Monitor Channel Multipliers in the $\times 1$ position the count capacity of the store of any monitor channel is $33,554,431$. Any counts in excess of this number will cause the Monitor Channel Indicator for the particular channel in question to illuminate. The effective capacity for any channel can be extended by using the monitor channel multipliers up to a maximum count of $3,355,443,100$.

- Monitor Channel 00 accumulates the total unclipped counts in A.
- Monitor Channel 01 accumulates the total clipped counts in A.
- Monitor Channel 02 accumulates the total unclipped counts in B.
- Monitor Channel 03 accumulates the total samples count.

Rate

The signal from this socket is used to examine the pulse rate of either input channel.
APPENDIX 3

LISTING OF CORR.AG

EXTERNAL AUX
C DATA TO BE READ IN FROM UNIT 5
REAL*4 STIME, THETA, TEMP
INTEGER*4 NRUN, RCLIP, MULTS(4), NCOUNT, NCLIP
INTEGER*4 ZERO, NSTIME, VALUES(100)

C VARIABLES FOR FITTING A STRAIGHT LINE
REAL*4 YF(100), WT(400), E1(3), E2(3), P(3)

C VARIABLES FOR 2ND PLOT
REAL*4 XINT(100), YINT(100)
COMMON /PLOT2/ XINT, YINT, WT

C LOCAL VARIABLES
REAL*4 X(100), Y(100), YLOG(100)
REAL*4 SM(100), FACTR, KD, Q, M2, RL, D20, VISC, DI
LOGICAL*1 YLABEL(10), EOF, FD(40)

C COMMON M
COMMON /PLTPAR/ NRUN, THETA, STIME, NSTIME, RCLIP, TEMP, MULTS,
+ XFIRST, XLAST, YFIRST, YLAST, D, LABELN, YLABEL

C FACTR = 2.64911E05
CALL GFNAME(5, FD, LEN, & 500)
WRITE(6, 59) (FD(I), I=1, LEN)
59 FORMAT(1X, 'FILE = ', 40A1)

C READ IN FIRST 5 LINES FROM UNIT 5
READ(5, 50) NRUN, THETA, STIME, RCLIP, TEMP, MULTS, NCOUNT, NCLIP,
+ ZERO, NSTIME
50 FORMAT(I10, G10.4, G10.4, I2, G10.4, 4I1, 4(/, 3X, 19))
WRITE(6, 60) NRUN, THETA, STIME, RCLIP, TEMP, MULTS, NCOUNT, NCLIP,
+ ZERO, NSTIME
60 FORMAT(/,
+ 1X, 'THE EXPERIMENTAL PARAMETERS ', /,
+ 1X, 'RUN#=', I8, 5X, 'THETA=', F7.3, 11X, 'STIME=', G10.4, /,
+ 1X, 'CLIPRATE=', I2, 11X,
APPENDIX 3: LISTING OF CORR.AG

+ ' TEMP=',G10.4,1X,'MULT.FACTS.=',4I2,/, /
+ ,1X,'THE FIRST 4 CORRELATOR CHANNELS ARE ',/,
+ 1X,' NCOUNT=',I9,4X,' NCLIP=',I9,/, 
+ 1X,' ZERO=',I9,4X,'NSTIME=',I9)
C
C READ IN REST OF DATA
N = 1
1 CONTINUE
READ(5,51,END=100) NUM,VALUES(N)
51 FORMAT(I3,I9)
IF(NUM.LT.0)GO TO 100
N = N + 1
GO TO 1
100 N = N - 1
C
C EMPLOY MULTIPLICATION FACTORS
NCOUNT = NCOUNT * 10.0**MULTS(1)
NCLIP = NCLIP * 10.0**MULTS(2)
ZERO = ZERO * 10.0**MULTS(3)
NSTIME = NSTIME * 10.0**MULTS(4)
C
C CALCULATE THE VISCOSITY OF WATER
VISC=(( 1 .3272M20-TEMP))
+-((0.001053*((TEMP-20.0)**2.0)))/(TEMP+105)
VISC=1.002E-02M
10.0**VISC)
C
C CALCULATE XN,BL AND CF
XN = FLOAT(NCOUNT) / FLOAT(NSTIME)
BL = XN * FLOAT(NCLIP)
CF = (XN+1.0) / (FLOAT(RCLIP)+1.0)
WRITE(6,61) XN,BL,CF
61 FORMAT(/,1X,'XN = NCOUNT/NSTIME =',T30,G10.4, 
+ ,/1X,'BL = XN * NCLIP =',T30,G10.4, 
+ ,/1X,'CF = (XN+1)/(CLIPRATE+1) =',T30,G10.4, 
+ ,/1X,'B = EXPERIMENTAL PARAMETER (DARK NOISE)'/)
C
C CALCULATE YMAX
YMAX = 0.0
DO 150 I* 1 ,N
YMAX = AMAX1(YMAX, FLOAT(VALUES(I)))
150 CONTINUE
YNORM = YMAX-BL
C
C FILL X,Y AND YLOG ARRAYS
WRITE(6,662)
662 FORMAT(IX,'INPUT = DATA READ IN;',/, 
1X,'X = MULTIPLE OF STIME;',/, 
1X,'Y1 = G(T) = (INPUT - BL)/(YMAX - BL);',/,
1X,'Y2 = LN G(T) + LN(B/CF) = LN( INPUT/(BL - 1) );',/,
1X,'WT = ( INPUT/(BL - 1) ) ** 2.0 = STAT WEIGHT ',/,
+ ' INPUT',T20,'X','T29','Y1 OR G(T)', 
+ T42,'LN G(T) + LN(B/CF)',T65,'WT')
DO 200 I=1,N
X(I) = (I-1) * STIME
Y(I) = (FLOAT(VALUES(I))-BL)/YNORM
YLOG(I) = ALOG(FLOAT(VALUES(I))/BL - 1)
SM(I)=FLOAT(VALUES(I))/(BL - 1)
WT(I)=SM(I)**2.0
WRITE(6,62) VALUES(I),X(I),Y(I),YLOG(I),WT(I)
APPENDIX 3: LISTING OF CORR.AG 86

62 FORMAT(110,4X,F10.3,4X,F10.8,4X,F10.7,8X,F10.7)
200 CONTINUE

C
C MAKE FIRST PICTURE
CALL MOVEC(4,'G(T)',YLABEL)
LABLEN = 4
D = 99999.99
CALL PICT(N,X,Y)
EOF = .FALSE.

C
C FIT POLYNOMIAL ORDER 2
P(1) = 0.0
P(2) = 0.0
P(3) = 0.0
WZ = 1.0
D = 99999.99
M = 3
NI = 6
EPS = 0.001
WRITE(6,64)
64 FORMAT(1X,'FITTING POLYNOMIAL : Y = PI + P2*X + P3*X^2. ')
CALL LQF(X,YLOG,YF,WT,E1,E2,P,WZ,N,M,NI,ND,EPS,AUX)
WRITE(6,63) (E1(I),I=1,M),ND
63 FORMAT(/,1X,'E1=',E12.6,2(4X,E12.6),6X,'ND=',14)
WRITE(6,663) (E2(I),I=1,M)
663 FORMAT(1X,'E2=',E12.6,2(4X,E12.6),///)
IF(ND.NE.1)GO TO 300
C
RADS = (3.14159265 * THETA )/360.
KD = FACTR * SIN(RADS)
D=ABS(P(2))*1.0E+06/(2.0*KD**2)
M2=P(3)*1.OE+12
RL=KD*KD*D
Q=M2/(RL**2.0)
DI = 10E+7*(1.3807E-16*(273.16+TEMP))/(3.0*3.1415926*VISC*D)
FRPAC=0.5
VNUM=(((DI/34.0)**3.0)-1)*FRPAC+1.0
WRITE(6,66)
66 FORMAT(1X,'K=SCATTERING VECTOR (/cm) ',/,
+1X,'D=DIFFUSION COEFFICIENT (cm/sec) ')
WRITE(6,67)
67 FORMAT(1X,'RL=RAYLEIGH LINEWIDTH (hz)',/,
+1X,'M2=2nd MOMENT OF LINE WIDTH DISTRIBUTION (hz**2) ')
WRITE(6,68)
68 FORMAT(1X,'Q=THE QUALITY FACTOR (dimensionless)',/,
+1X,'DIA=THE AVERAGE DIAMETER OF THE PARTICLES (nm) ')
WRITE(6,69)
69 FORMAT(1X,'KBAR=THE MEAN NUMBER OF PARTICLES PER AGGREGATE ')
WRITE(6,65) KD,D,RL,M2,Q,DI,VNUM
65 FORMAT(1X,/,/,1X,'K = ',G16.6,5X,'D = ',G16.6,6X,'RL = '
+,G16.6,5X,'Q = ',G16.6,5X,'DIA = ',G16.6
+,/1X,'KBAR = ',F11.2)
C
C MAKE SECOND PICTURE
300 CONTINUE
CALL MOVEC(7,'LN G(T)',YLABEL)
LABLEN = 7
CALL PICT(N,X,YLOG)
C
500 STOP
APPENDIX 8: LISTING OF CORR.AG

FUNCTION AUX(P,D,X,L)
DIMENSION P(1),D(1)
COMMON M
D(1) = 1.0
AUX = P(1)
DO 10 J=2,M
  D(J) = D(J-1) * X
  AUX = AUX + P(J) * D(J)
10 CONTINUE
RETURN
END

SUBROUTINE PICT(N,X,Y)

TYPE OF ARGUMENTS
*************************
INTEGER*4 N,NRUN,NSTIME,RCLIP,MULTS(4),LABLEN
REAL*4 X(N),Y(N),THETA,STIME,TEMP,YFIRST,YLAST,D,RVAR
LOGICAL*1 YLABEL(10)
COMMON /PLTPAR/ NRUN,THETA,STIME,NSTIME,RCLIP,TEMP,MULTS,
+ XFIRST,XLAST,YFIRST,YLAST,D,LABELN,YLABEL

RANGE OF X AND Y AXES
*************************
XMIN=X(1)
XMAX=X(N)
YMIN=99999.99
YMAX=-99999.99
DO 1 J=1,N
  YMIN=AMIN1(Y(J),YMIN)
  YMAX=AMAX1(Y(J),YMAX)
1 CONTINUE
EXTRA = (YMAX-YMIN)/20.0
YMAX = YMAX + EXTRA
YMIN = YMIN - EXTRA

PICTURE ORIGIN
****************
XO=0.5
YO=0.5

AXES LENGTHS & SCALE FACTORS
******************************
XLEN=11.0
YLEN=9.0
XSCALE=XLEN/(XMAX-XMIN)
YSCALE=YLEN/(YMAX-YMIN)

TICK MARKS
************
XTIC=STIME* 2.0
YTIC=(YMAX-YMIN)/10.0
XGAP=XSCALE*XTIC
YGAP=YSCALE*YTIC
TIC=0.1

X-AXIS DRAWING
**************
CALL PLOT(XO,YO,3)
XX=XO
REALT=XM
BIT = 0.00001
NDIGIT=2
HT=0.1
XSHIFT=0.3
YSHIFT=0.2
CONTINUE
CALL PLOT(XX,YO,2)
CALL PLOT(XX,YO+TIC,2)
CALL NUMBER(XX-XSHIFT,YO-YSHIFT,HT,REALN,0.0,NDIGIT)
CALL PLOT(XX,YO,3)
REALN=REALN+XTIC
IF(REALN.GT.XMAX+BIT)GO TO 15
XX=XX+XGAP
GO TO 10
15 CALL PLOT(XLEN+XO,YO,2)
HT=0.14
NLAB=7
XX=(XO+XLEN)/2.0 - (6/7 * HT * NLAB)
CALL SYMBOL(XX,0.0,HT,'DELAY T',0.0,NLAB)
Y-AXIS DRAWING **************
CALL PLOT(XO,YO,3)
YY=YO
REALT=YMN
NDIGIT=3
HT=0.1
XSHIFT=0.6
YSHIFT=0.05
CONTINUE
CALL PLOT(XO,YY,2)
CALL PLOT(XO+TIC,YY,2)
CALL NUMBER(XO-XSHIFT,YY-YSHIFT,HT,REALN,0.0,NDIGIT)
CALL PLOT(XO,YY,3)
REALN=REALN+YTIC
IF(REALN.GT.YMAX+BIT)GO TO 25
YY=YY+YGAP
GO TO 20
25 CALL PLOT(XO,YLEN+YO,2)
HT=0.14
NLAB=LABLEN
YY=(YO+YLEN)/2.0 - (6/7 * HT * NLAB)
XX=XO - XSHIFT - 0.1
CALL SYMBOL(XX,YY,HT,YLABEL,90.0,NLAB)
IF(D.GT.99999.0)GO TO 29
FITTED POINTS *************
XX = (XFIR-S-XMIN)*XS + XO
YY = (YFIRST-YMIN)*YS + YO
CALL SYMBOL(XX,YY,0.14,0,0.0,-1)
XX = (XLAST-XMIN)*XS + XO
YY = (YLAST-YMIN)*YS + YO
CALL SYMBOL(XX,YY,0.14,0,0.1,-1)
PLOTTING THE DATA *************
APPENDIX S: LISTING OF CORR.AG

29 NSYMB=3
HT=0.14
DO 30 I=1,N
   XX=(X(I)-XMIN)*XSCALE+XO
   YY=(Y(I)-YMIN)*YSCALE+YO
   CALL SYMBOL(XX,YY,HT,NSYMB,0.0,-1)
30 CONTINUE

C
C PICTURE TITLE

400 HT=0.14
XX=XO
YY=YO+YLEN+2.5*HT
C
CALL SYMBOL(XX,YY,HT,'RUN#=',0.0,5)
CALL WHERE(XX,YIGNRE)
RVAR = FLOAT(NRUN/100)
CALL NUMBER(XX+HT,YY,HT,RVAR,0.0,-1)
CALL WHERE(XX,YIGNRE)
CALL SYMBOL(XX,YY,HT,'-',0.0,1)
RVAR = NRUN - IFIX(RVAR)*100
CALL WHERE(XX,YIGNRE)
CALL NUMBER(XX,YY,HT,RVAR,0.0,-1)

C
CALL WHERE(XX,YIGNRE)
CALL SYMBOL(XX+2.0*HT,YY,HT,'THETA=',0.0,6)
CALL WHERE(XX,YIGNRE)
CALL NUMBER(XX+HT,YY,HT,THETA,0.0,2)

C
CALL WHERE(XX,YIGNRE)
CALL SYMBOL(XX+2.0*HT,YY,HT,'STIME=',0.0,6)
CALL WHERE(XX,YIGNRE)
CALL NUMBER(XX+HT,YY,HT,STIME,0.0,3)

C
CALL WHERE(XX,YIGNRE)
CALL SYMBOL(XX+2.0*HT,YY,HT,'#STIMES=',0.0,8)
CALL WHERE(XX,YIGNRE)
RVAR = FLOAT(NSTIME/100)
CALL NUMBER(XX+HT,YY,HT,RVAR,0.0,-1)
RVAR = NSTIME - IFIX(RVAR)*100
IF(RVAR.GE.10.0)GO TO 299
   CALL WHERE(XX,YIGNRE)
   CALL SYMBOL(XX,YY,HT,'0',0.0,1)
299 CALL WHERE(XX,YIGNRE)
   CALL NUMBER(XX,YY,HT,RVAR,0.0,-1)

C
CALL WHERE(XX,YIGNRE)
CALL SYMBOL(XX+2.0*HT,YY,HT,'CLIPRATE=',0.0,9)
CALL WHERE(XX,YIGNRE)
RVAR = FLOAT(RCLIP)
CALL NUMBER(XX+HT,YY,HT,RVAR,0.0,-1)

C
XX = XO
YY = YO + YLEN + HT

C
CALL SYMBOL(XX,YY,HT,'TEMP=',0.0,5)
CALL WHERE(XX,YIGNRE)
CALL NUMBER(XX+HT,YY,HT,TEMP,0.0,2)

C
CALL WHERE(XX,YIGNRE)
CALL SYMBOL(XX+2.0*HT,YY,HT,'MULTS=',0.0,6)
DO 301 I=1,4
   CALL WHERE(XX,YIGNRE)
   RVAR = FLOAT(MULTS(I))
   CALL NUMBER(XX+HT,YY,HT,RVAR,0.0,-1)
301 CONTINUE
C
IF(D.GT.99999.0)GO TO 500
CALL WHERE(XX,YIGNRE)
RVAR = D*10**9
CALL SYMBOL(XX+2.0*HT,YY,HT,'D=',0.0,2)
CALL WHERE(XX,YIGNRE)
CALL NUMBER(XX+HT,YY,HT,RVAR,0.0,3)
CALL WHERE(XX,YIGNRE)
CALL SYMBOL(XX+(HT/2.0),YY,HT,'x10**-09',0.0,8)
C
C
500 XX=XLEN+XO+2.0
CALL PLOT(XX,0.0,-3)
RETURN
END