Assignment 2 –Estimates of Hydrodynamic Radii from Dynamic Light Scattering Data.
ANSWERS

Objective: Analyze experimental correlation functions of scattered light to determine the mean hydrodynamic radius and distribution width of a monomodal population of large unilamellar vesicles (LUVs).

Introduction: The time scales of fluctuations in scattered light from a sample can be used to estimate the diffusion coefficients, and from that the hydrodynamic radius (radii), of particles (or a collection of particles) in a liquid sample. As we discussed in class, particles that have refractive indices different from the solvent, scatter light. The light scattered intensity depends on the number of particles in the scattering volume (the volume of sample from which the scattered light is detected) and the (intrinsic) scattering strength of the particle. In a DLS experiment we are interested in scattering from particles that are typically much smaller than the wavelength of light, which exhibits Rayleigh scattering (see the Wikipedia page for an explanation). The scattering cross section of a particle depends, among other things, on the size and refractive index of the particle.

The correlation function is simplest to analyze for a simple sample, *e.g.* one that consists of a non-aggregating protein. The correlation function for light scattered from such a *monodisperse* solution of a macromolecule decays by a single exponential:

$g^2(\tau) = y_0 + y_1 * \exp[-2\Gamma\tau]$

where τ is the delay time and the decay rate, Γ, is related to the diffusion coefficient, D, by

$D = \Gamma/q^2$

The scattering vector, q, is given by

$$q = \frac{4\pi n_0}{\lambda_0} \sin\left(\frac{\theta}{2}\right)$$

From D, the hydrodynamic radius is estimated for the equivalent sphere by the Stokes equation.

Lipid vesicles (and most synthetic polymers) do not have a single molecular weight. Instead the samples are comprised of distribution of weights, and therefore a distribution of hydrodynamic radii. The analysis of these samples then requires an analysis of this underlying distribution. If it is well described by a single Gaussian distribution, then the method of cumulants can be used (Koppel, 1972). Such samples are *polydisperse* and *monomodal*, which is to say the distribution is characterized by a mean and a distribution width (variance).

$g^2(\tau) = y_0 + y_1 * \exp[-2\Gamma\tau] * (1 + \mu_2\tau^2/2)^2$

where μ_2 is the variance of the distribution and all the other variables have the previous definitions.

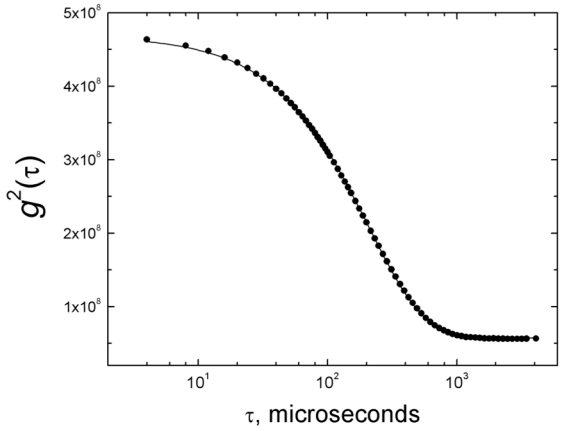
Assignment Tasks

1. Login to the course website (http://people.umass.edu/rmweis/chem728/). Navigate to assignments.
2. Download the data in the Excel file: "Assgnmnt2_data_DLS.xls". The file has three data sets, one experimental correlation function for sonicated unilamellar vesicles (SUVs) and two sets obtained with large unilamellar vesicles (LUVs).
3. Open Origin. Copy and paste the SUV data set from the Excel file into an Origin worksheet. (Eventually, you will analyze all three sets.)
4. Plot the data as a *Scatter* plot.
5. Fit the Data to a single exponential decay with the nonlinear least squares 'fitting engine'. **cps**(x) = y_0 + y_1*exp[-G*x]
6. Make a note of the fit parameters (y_0, y_1, G).
7. Create a new column in the worksheet and use the fit parameters generate a column of values that is given by **cps**(x) = y_0 + y_1*exp[-G*x].
8. Create another new column in the worksheet, fill the column with residuals, where residuals = **cps**(data) - **cps**(fit), and plot the residuals.
9. Repeat steps 5-8. Plot the data in a new graph window and fit the data to **cps**(x) = y_0 + y_1*exp[-G*x]*(1+(mu/2*x^2))^2 (where mu is an additional variable related to the width of the particle size distribution)
10. Plot the residuals generated with the two different functions in the same window. How do they compare? Is either fit satisfactory?
11. Compute the diffusion coefficient from the two different fits and the corresponding hydrodynamic radii. From the second fit, compute the distribution width. In these calculations, you will need to use the following parameters from the scattering experiment:

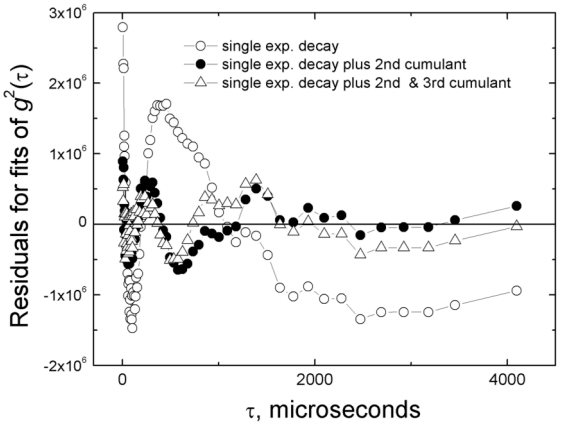
Scattering angle, $\theta = 90^o$
Wavelength of incident radiation, λ_0 = 685 nm
refractive index, n_0 = 1.333
viscosity, η = 1.002 cP (centiPoise)
Temperature = 293 K

12. Repeat these manipulations for the other two data sets (LUV50 and LUV100). What do learn from an analysis of all these data?

5. Fit the SUV data to a single exponential decay with nonlinear least squares.



8. Create another new column in the worksheet, fill the column with residuals, where residuals = **cps**(data) - **cps**(fit), and plot the residuals.



9. Repeat steps 5-8. Plot the data in a new graph window and fit the data to **cps**(x) = $y_0 + y_1*\exp[-G*x]*(1+(mu/2*x^2))^2$ (where mu is an additional variable related to the width of the particle size distribution)

10. Plot the residuals generated with the two different functions in the same window. How do they compare? Is either fit satisfactory?

The fit improves with the introduction of the second cumulant, but does not improve significantly with the provision for a third cumulant. There is systematic error in all the fits, indicating that the models do not fully explain all the features in the data.

11. Compute the diffusion coefficient from the two different fits and the corresponding hydrodynamic radii. From the second fit, compute the distribution width. In these calculations, you will need to use the following parameters from the scattering experiment:

First compute the scattering vector $\boldsymbol{q}$.

$$\boldsymbol{q} = \frac{4\pi \boldsymbol{n}_0}{\lambda_0}\sin\left(\frac{\theta}{2}\right) = \frac{4 * 3.141593 * 1.333}{685}\sin\left(\frac{\pi}{4}\right) = 0.01729\,\mathrm{nm}^{-1}$$

Calculate the diffusion coefficient (D) *for the SUVs from the decay rate of the correlation function* (Γ).

$D = \Gamma/q^2 = (0.002405\ \mu s^{-1})/(0.01729\ nm^{-1})^2 = 8.045\ nm^2/\mu s$
$D = (8.045\ nm^2/\mu s)*(10^{-14}\ cm^2/nm^2)*(10^6\ \mu s/s) = 8.045\ x\ 10^{-8}\ cm^2/s$

Then, the Einstein-Smoluchowski and Stokes equations are combined to determine from D the radius of the hydrodynamically equivalent sphere.

$D = k_B T/f \qquad f = 6\pi\eta r$

$D = k_B T/6\pi\eta r \quad \rightarrow \quad r = k_B T/6\pi\eta D$

Viscosity has units of pressure and time. The mks units of viscosity are Pa·s (kg·m⁻¹s⁻¹), cgs units are Poise, and 1 Poise = 0.1 Pa·s

Example Calculation for the SUV:

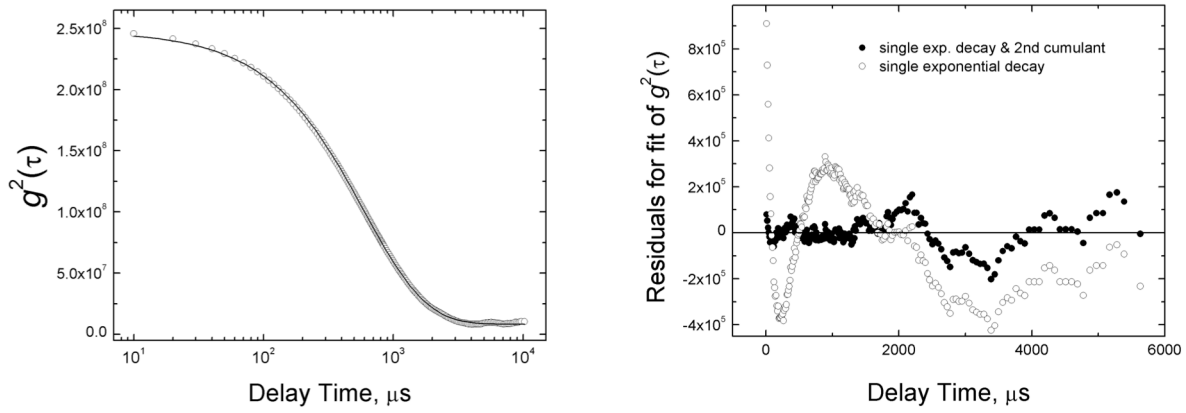
$r = k_B T/6\pi\eta D = (1.38\ x\ 10^{-16}\ g{\cdot}cm^2 s^{-2} K^{-1})(293\ K)/(6*3.14159*(0.01002\ g{\cdot}s^{-1}cm^{-1})*(8.045\ x\ 10^{-8}\ cm^2 s^{-1}))$

$r = 2.661\ x\ 10^{-6}\ cm = 26.64\ nm$

Data Set	Model	Γ	μ	Radius (nm)	Dist. Width (nm)
SUV	Exp	0.00241		26.64	
		0.00247	$5.762\ x\ 10^{-7}$	25.94	25.62

12. Repeat these manipulations for the other two data sets (LUV50 and LUV100). What do learn from an analysis of all these data?

Correlation Decay Function & Residuals for LUVs extruded through filters 50 nm diameter pores.



Summary of DLS analysis of Vesicle Diffusion Coefficients and Hydrodynamic Radii

Data Set	Model	Γ	μ	D cm^2/s	radius (nm)	Dist. Width (nm)
SUV	Exp	0.00241		8.045×10^{-8}	26.64	
		0.00247	5.762×10^{-7}	8.262×10^{-8}	25.94	25.62
LUV50	Exp	0.00158		5.285×10^{-8}	40.55	
		0.00169	4.616×10^{-7}	5.670×10^{-8}	37.80	84.80
LUV100	Exp	0.000790		2.643×10^{-8}	81.10	
		0.000835	1.262×10^{-7}	2.793×10^{-8}	76.73	236.55

A provisional estimate for the distribution of vesicle sizes was generated using 'mu', in which the range of values for D is manifested. 'mu' is proportional to the distribution variance of Γ, and $2\mu^{1/2}$ is the width. Therefore, the mean and spread in D is given by $\Gamma/q^2 \pm 2\mu^{1/2}/q^2$. From this, the minimum and maximum radii are calculated (from the maximum and minimum diffusion coefficients) to give the size range, and the spread is approximated as ½ the range.

D (cm^2/s)	radius (nm)	μ	δD	D_{min}	D_{max}	r_{max}	r_{min}	δr
8.045E-08	26.64							
8.262E-08	25.94	5.76E-07	5.08E-08	3.18E-08	1.33E-07	67.31	16.06	25.62
5.285E-08	40.55							
5.670E-08	37.80	4.62E-07	4.55E-08	1.12E-08	1.02E-07	190.57	20.98	84.80
2.643E-08	81.10							
2.793E-08	76.73	1.26E-07	2.38E-08	4.16E-09	5.17E-08	514.55	41.45	236.55

Vesicles prepared by sonication, SUVs, are the smallest in their size, ~53 nm in diameter. Vesicles prepared by extrusion through filters with pores that were 50 and 100 nm in diameter yielded vesicles with mean diameters of ~80 and ~160 nm, respectively, somewhat larger than the pore diameter of the filter.

Summary

- Sonication produced vesicles of the smallest size.
- Extrusion produced vesicles with mean diameters in proportion to the pore diameter.
- Inspection of the data, plot as the intensity autocorrelation function versus logτ revealed a moderately homogeneous population characterized by a single decay rate, *even before fitting was started*.
- By inspection of the residuals, the fits of the data improved when a term that allowed for sample polydispersity (via the 2nd cumulant) was included.
- The change in the mean diameter, generated by the inclusion of the 2nd cumulant, was rather small, which was ~10% reduction in mean diameter.
- Including the 3rd cumulant did not improve the fit substantially. (*data not shown*)
- Estimates of the size distribution are probably *not* realistic for the following reasons (and others):
    - (i) The widths are unrealistically large.
    - (ii) The method does not account for differences in scattering strength as a function of particle size.
- The method is simple and provides a decent estimate of particle size (model based) for a monomodal sample.
- As an alternate method, the constrained inverse Laplace transform technique can be used to estimate the mean size and size distribution in monomodal and multimodal systems.