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DYNAMIC LIGHT SCATTERING

to determine the radius of small beads in Brownian motion in
a solution.

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Introduction:

Dynamic Light Scattering is also known as Photon Correlation Spectroscopy. This technique is one of the most popular methods used to determine the size of particles. Shining a monochromatic light beam, such as a laser, onto a solution with spherical particles in Brownian motion causes a Doppler Shift when the light hits the moving particle, changing the wavelength of the incoming light. This change is related to the size of the particle. It is possible to compute the sphere size distribution and give a description of the particle's motion in the medium, measuring the diffusion coefficient of the particle and using the autocorrelation function.

This method has several advantages: first of all the experiment duration is short and it is almost all automatized so that for routine measurements an extensive experience is not required. Moreover, this method has modest development costs.

Commercial "particle sizing" systems mostly operate at only one angle (90°) and use red light (675 nm). Usually in these systems the dependence on concentration is neglected. Using more sophisticated experimental equipment (projector, short-wavelength light source), the methods can be not only considerably extended, but also more complicated and expensive.

Although dynamic scattering is, in principle, capable of distinguishing whether a protein is a monomer or dimer, it is much less accurate for distinguishing small oligomers than is classical light scattering or sedimentation velocity. The advantage of using dynamic scattering is the possibility to analyze samples containing broad distributions of species of widely differing molecular masses (e.g. a native protein and various sizes of aggregates), and to detect very small amounts of the higher mass species ($<0.01\%$ in many cases). Furthermore, one does not have to worry that protein aggregates are being lost within a

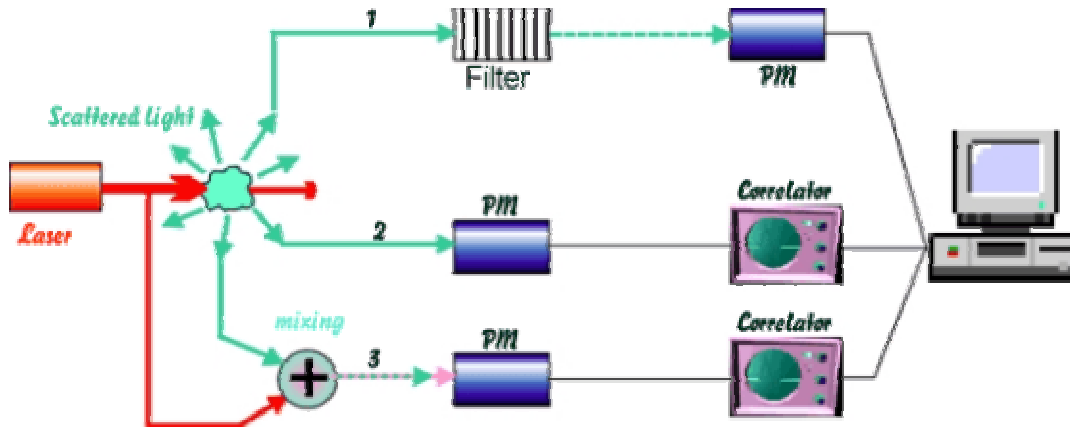
chromatographic column (a common problem in using SEC to characterize aggregates), because there is no chromatographic separation involved.

Moreover, with this technique it is also possible to obtain absolute measurements of several parameters of interest, like molecular weight, radius of gyration, Translational diffusion constant and so on. However, the analysis might be difficult for non-rigid macromolecules. Another limit is that above the zero degree Kelvin molecules fluctuate (i.e. molecules deviate from their average position).

The light scattering:

According to the semi-classical light scattering theory [*Berne and Pecora, "Dynamic Light scattering" John Wiley, 1975*], when light impinges on matter, the electric field of the light induces an oscillating polarization of electrons in the molecules. Hence the molecules provide a secondary source of light and subsequently scatter light. The frequency shifts, the angular distribution, the polarization, and the intensity of the scatter light are determined by the size, shape and molecular interactions in the scattering material. Thanks to this is possible, with the aid of electrodynamics and theory of time dependent statistical mechanics, to get information about the structure and molecular dynamics of the scattering medium through the light scattering characteristics of the system.

Different methods can be used to study the dynamics of a system with particles in Brownian motion, depending on the time scale of the molecular fluctuations. These methods are shown in the following figure [*Berne and Pecora*]



A monochromator or filter (interferometer or diffraction grating) is placed in front of photomultiplier (direction 1) and it is used when the time scale of the fluctuations is above the microsecond (frequency above 1MHz). The average DC output of the photomultiplier is proportional to the spectral density of the scattered light at the filter frequency. The filter is then swept through a range of frequencies.

For processes faster than a picosecond ($f > 10\text{GHz}$), diffraction gratings are used as filter, and for slow fluctuations between picosecond and microsecond, Fabry-Perot interferometers are used.

Optical mixing techniques are used when the fluctuations are slower than microsecond ($f < 1\text{MHz}$).

The theory behind:

The experiment's theory is based essentially on two assumptions. The first condition is that the particles are in Brownian motion (also called 'random walk'); in this situation we know the probability density function, given by the formula:

$$(1) \quad P(r,t|0,0) = (4\pi Dt)^{-3/2} \exp(-r^2/4Dt)$$

where D is the diffusion constant.

The second assumption is that the beads used in the experiment, are spherical particles with a diameter small compared to the molecular dimensions. If it is so, then it is possible to apply the Stoke-Einstein relation and hence have a formula that easily gives the diffusion constant:

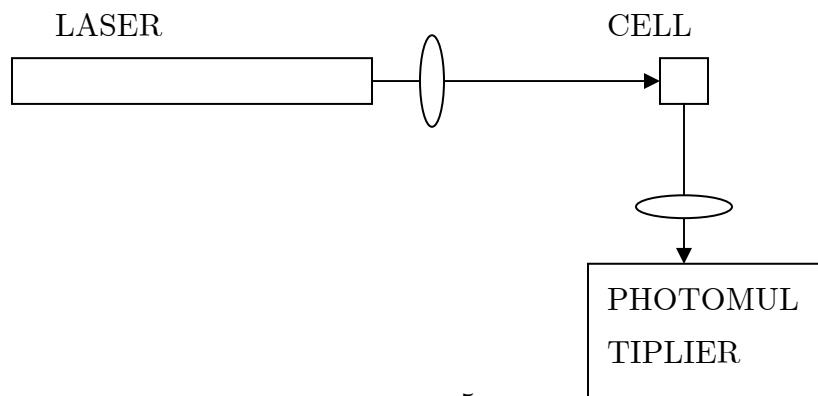
$$(2) \quad D = k_B T / 6\pi\eta a$$

where a is the radius of the beads, k_B is the Boltzmann constant, T is the temperature in Kelvin degrees (in this experiment it will be considered as if it is taking place at room temperature) and η is the viscosity of the solvent.

Since from the light scattering is possible to obtain information about the position of the particles, though the formulas above is easy to get the radius of the beads.

Set up:

The set up is illustrated here after. As it is possible to see from the illustration, the laser passes through a collimator lens and then hits the cell with the solution. The light is scattered and detected by a photomultiplier that transform a variation of intensity into a variation of voltage.



As illustrated in the figure above, there is another collimating lens before the photomultiplier. The use of both the collimating lenses is essential in this experiment: the first lens allows us to focus the beam into the cell, so that the area that we will hit is far enough from the side of the cell. The second lens is used to get an amount of scattered light that is neither too much nor insufficient. The photomultiplier is positioned at a scattering angle of 90 degrees; indeed for this scattering angle it is possible to neglect the nonlinearity of the linewidth with the scattering angle. This is because at this angle $\Delta\Gamma/\Gamma < 0.1$.

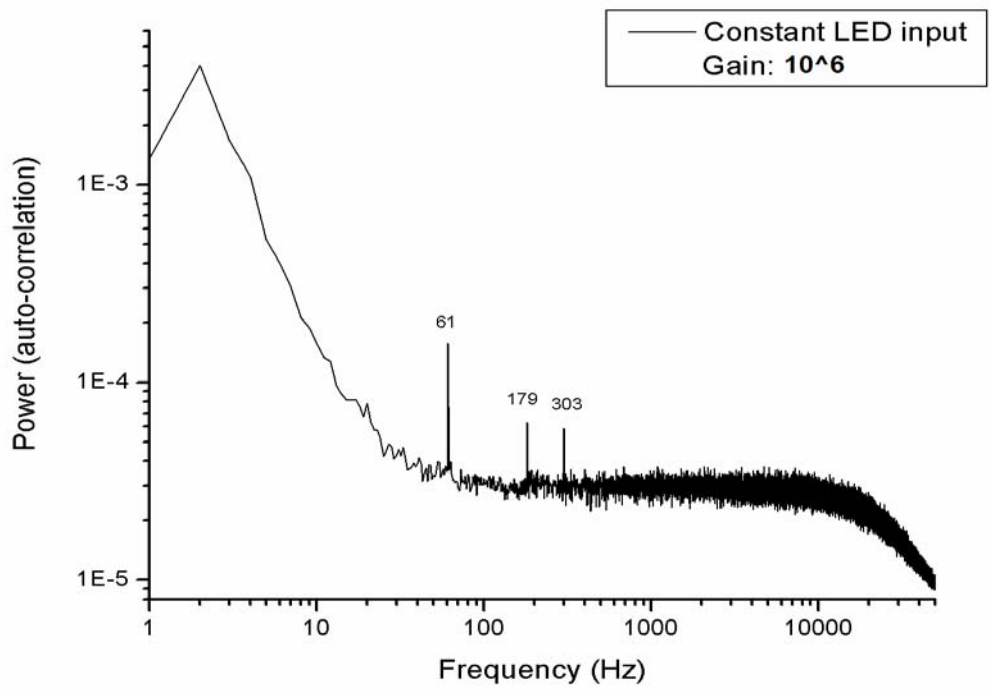
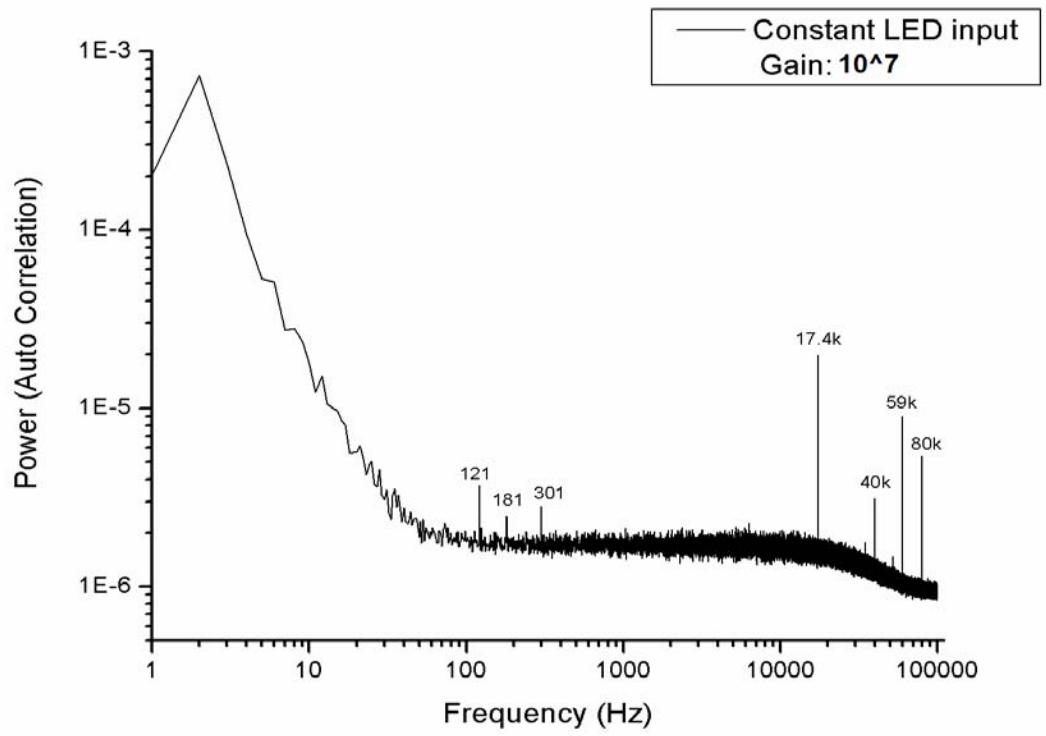
After the photomultiplier, the signal is immediately preamplified and then sent to the computer where the voltage is elaborate through a program in Labview.

Calibration:

The process of calibration is really delicate. The first operation is to check if the laser beam is at the same high in its entire path, then the focus the first lens in the middle of the cell and the second lens into the photomultiplier. A useful control is the one to check if there is incoming light other than the scattered one, reaching the photomultiplier. Once that the laser beam has been set, the second step is controlling the response of the photomultiplier. With a pulsing LED it was possible to observe that in the power spectrum both the peaks and the background were rolling-off¹, it means that there is a roll-off point due to the electronic¹. This was confirmed by the power spectrum of a constant LED light where it is possible to observe a roll-off point at high frequencies (the roll-off point is of the order of the 10 kHz, a value higher than the one of the beads here used). As it possible to observe from the graphs at page 7 an increasing of gain will increase the noise of the signal but its roll-off point will still be high.

¹The roll-off point is the point where the line interpolating the power spectrum at low frequency intersects the one interpolating the power spectrum at high frequency.

²The roll-off point is due to the PMT that contains a shot pulse; a noise caused by random electrons accelerating to dynodes.



All the graphs in this report are normalized, so is possible to compare them and the data extracted from them.

A cell with only deionized water, which will have been the real background, has to show a similar behavior of the LED, even if it will present some noise in the signal (that noise is higher at low frequencies). Moreover, its roll-off point has to be (and in this situation it is) higher than the roll-off point of the solutions (expected to be of the order of 1 kHz), so that it will not interfere in the computation of the diameter of the beads.

The Photomultiplier and more theory:

The photomultiplier translate an intensity variation into a voltage variation that is proportional to it. Because the particles move independently one from the other, the frequency spectrum of the intensity of the scattered light will have the form of a Lorentzian shaped line whose width depends on D and the scattering angle:

$$(3) \quad S_I(\omega) = \frac{\Gamma(\Theta)}{[\omega^2 + \Gamma^2(\Theta)]}$$

Where $\omega = 2\pi f$ with f the roll-off frequency and

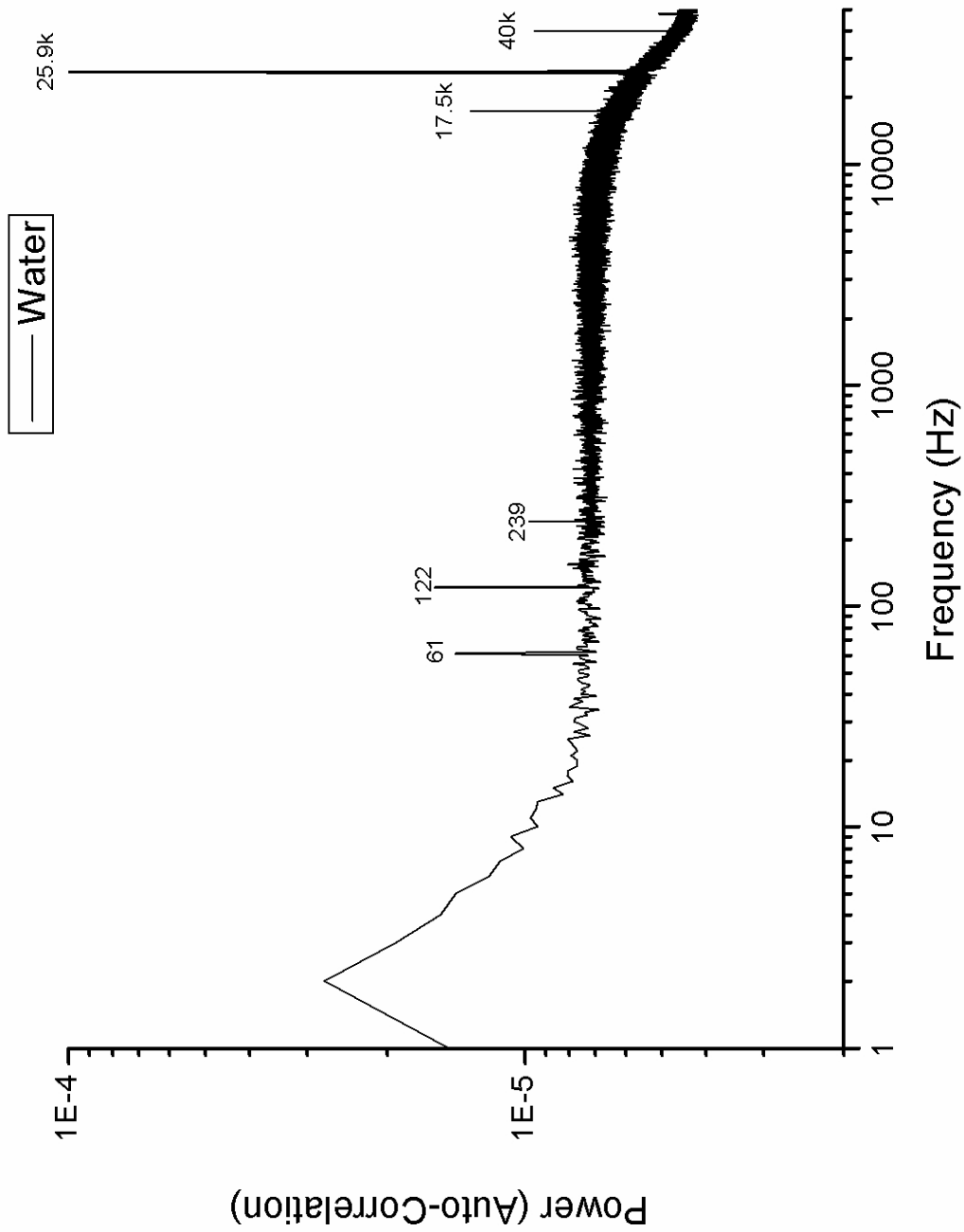
$$(4) \quad \Gamma(\Theta) = 2D \{ [4\pi / (\lambda/n)] \sin^{1/2}\Theta \}^2$$

Where λ is the wavelength of the incident light, n is the index of refraction of the solution and Θ is the scattering angle.

Because the process is random, applying the WIENER-KHINTCHINE theorem gives the spectrum through the correlation function.

In this way spatial correlations are translated into phase correlations according to:

$$(5) \quad S_I(\omega) = \int_{-\infty}^{\infty} e^{i\omega\tau} R_I(\tau) d\tau$$



The half-width at half-height of the Lorentian curve is the correlation time, τ , and represents the time for a particle to move out of phase.

$$(6) \quad \tau = \pi / DK^2$$

$$(7) \quad K = 4\pi / (\lambda/n) \sin(\theta/2)$$

From these formulas it is possible to compute D and hence the radius a from

$$(8) \quad D = kbT / 6\pi\eta a$$

The amplification and the filter-characteristics of the measurement apparatus are determined by four variables:

1. Amplifier gain ($10^4 - 10^{10}$)
2. Amplifier suppression ($10^{-10} - 10^{-3}$) A
3. Amplifier rise time (0.01; 0.03; 0.1; 0.3; ... 300) ms
4. PMT voltage supply [0-2000] V

The Labview program:

The program created for this experiment is really simple and illustrated on the following page.

To the incoming cluster of data is subtracted the DC component of the current. To the resulting cluster is applied the correlation function; from the resulting string of data, the first half can be eliminated and so to the second half is possible to apply the Fourier transform. All the resulting data are averaged by the number of trial. As last step, the program allows to save the resulting string of data as a spreadsheet file that can be converted in any format as .xls or can also be inserted as ASCII file into Origin.

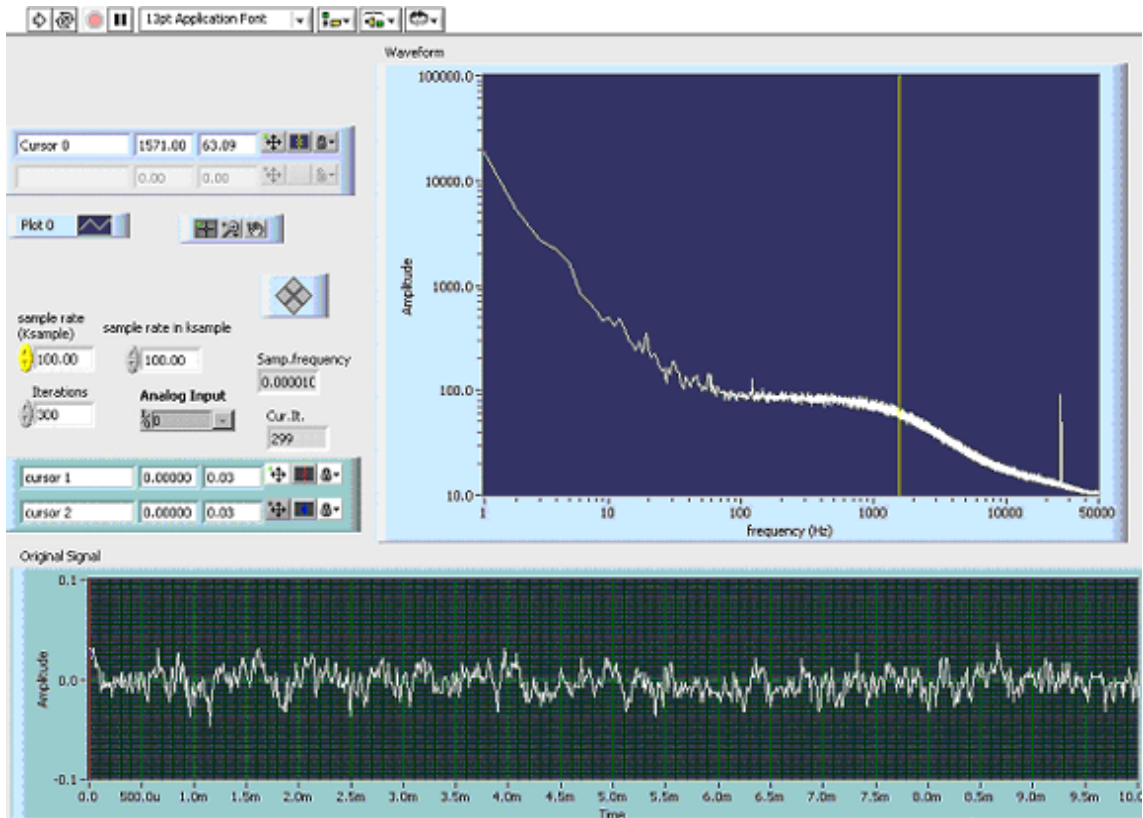
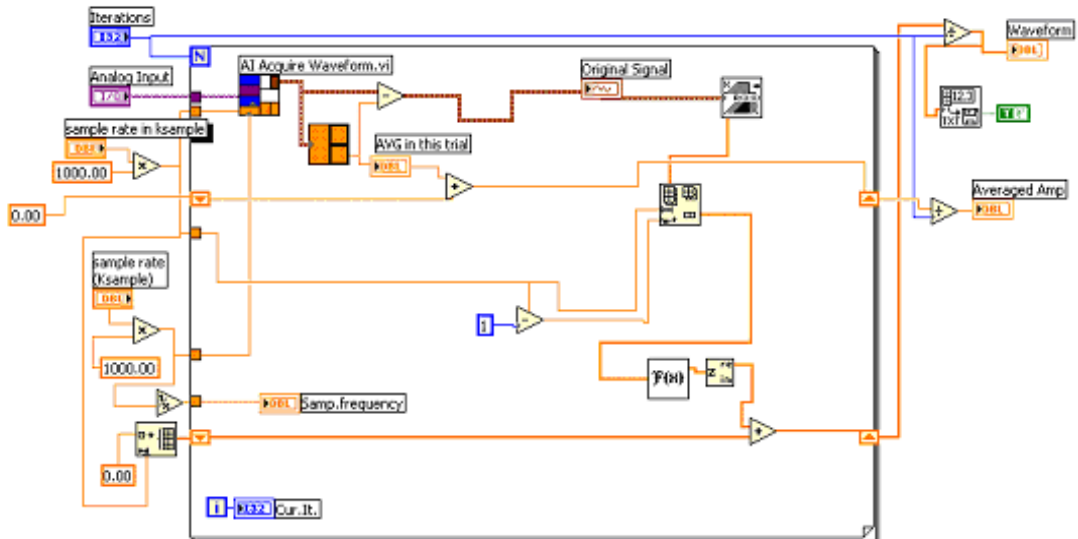


Diagram:



Solutions:

In this particular experiment diameter of the beads were 20 nm and 64 nm. In a first time also diameters of 200nm and 500nm has been taken into consideration, but the roll-off frequency point was too near the low frequencies where the photomultiplier's noise was high².

For the 64 nm beads the concentration used was ranging from 0.15 $\mu\text{l/ml}$ to 12,150 $\mu\text{l/ml}$ with a decreasing rate of three. For the 20 nm beads the increasing rate was the same, but the range was with a different starting point: 1.5 $\mu\text{l/ml}$. this difference is due to the fact that the power spectra of lower concentrations were almost a flat line.

Analysis of data:

For each concentration has been made two trial, possibly in a random order, so that we avoid any kind of influence (like adaptation).

Considering the index of refraction as a parameter on the formula for the radius and averaging the results, it is possible to obtain a more reliable value for it.

Since the 64nm and the 20nm beads are made from two different producers, it was opportune to re-compute the index of refraction separately for the two sizes. We discovered that for the 64nm beads it was 1.22 and for the 20 nm beads it was 1.19. For the 20 nm beads we considered only the first nine points (from 16.5 $\mu\text{l/ml}$ to 450 $\mu\text{l/ml}$), indeed for higher concentration the data was deviating from the expected value³.

The computed values are summarized on the following tables.

² At low frequency there are a lot of external factor coming into and the system wasn't isolated from that.

³ further consideration will be done later on

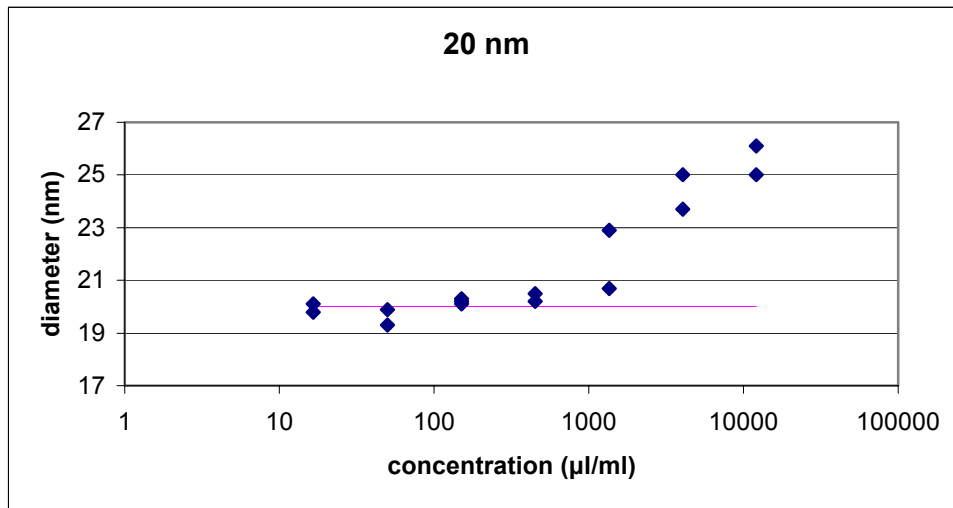
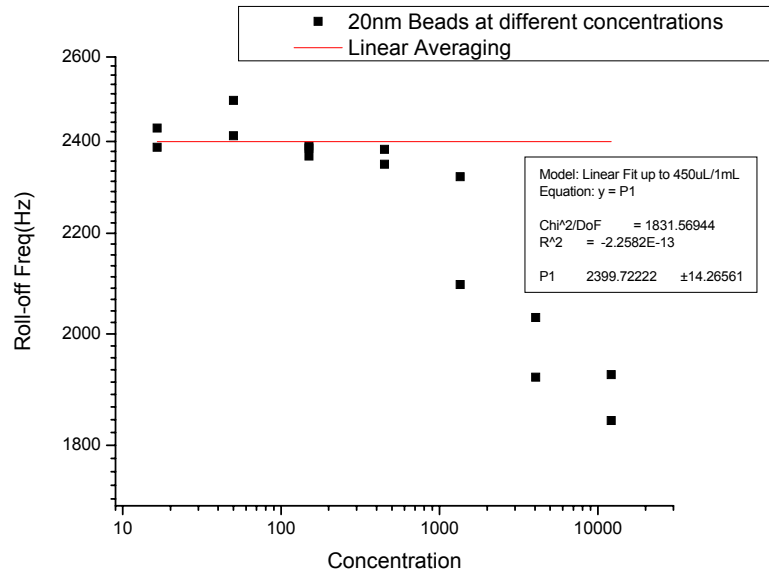
The graphs at pages 14 and 16 show the variation of the roll-off point versus the concentration⁴ and the variation of the diameter versus the concentration.

20nm

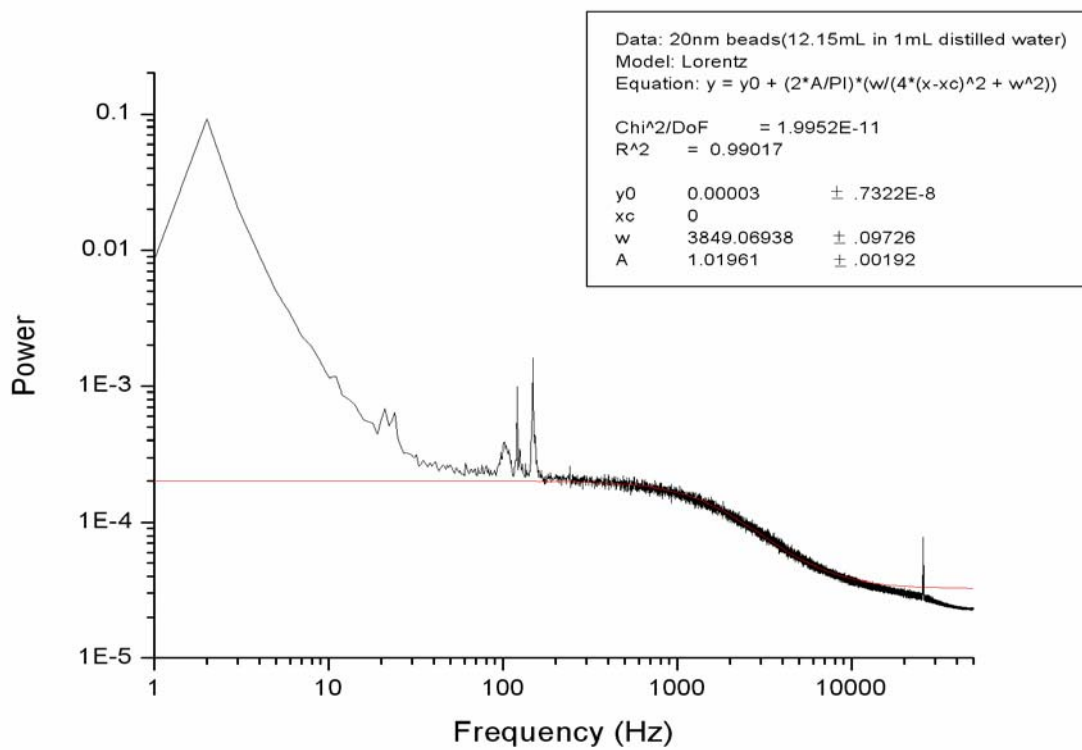
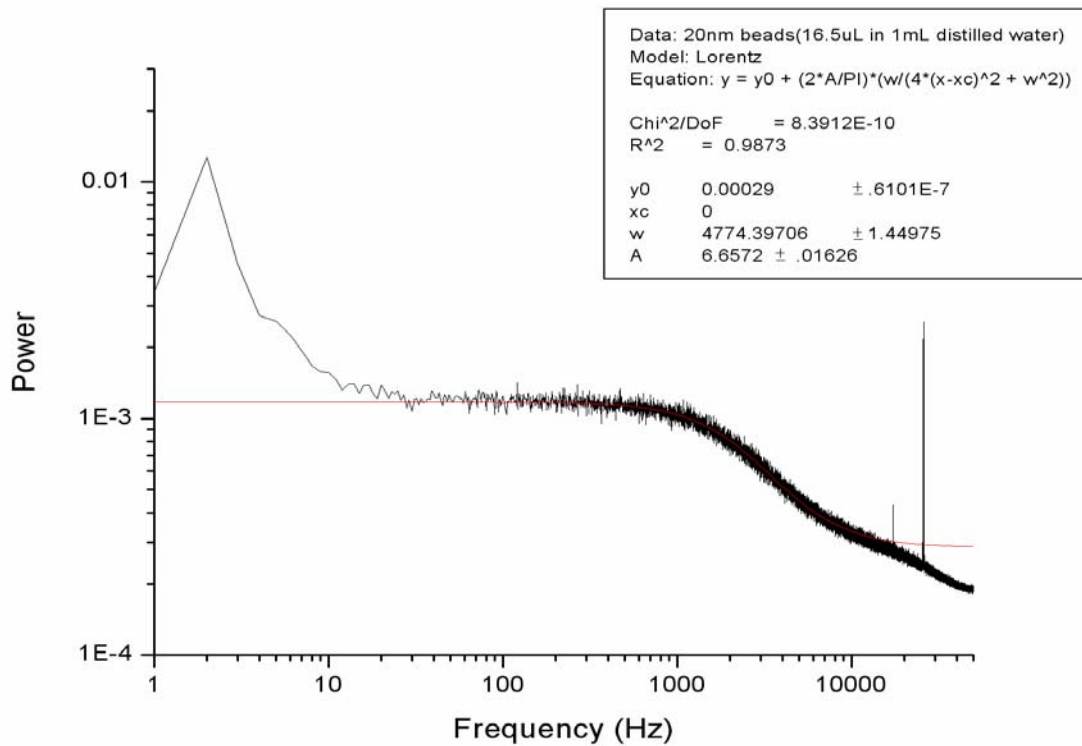
Conc ($\mu\text{l}/\text{ml}$)	Mm3 Beads	%vol beads	Roll-off (Hz)	Diameter (nm)	% difference
16.5	1.65	0.16	2387	20.1	0.5
16.5	1.65	0.16	2430.5	19.8	-1
50	5	0.48	2413	19.9	-0.5
50	5	0.48	2495	19.3	-3.5
150	15	1.32	2385	20.2	1
150	15	1.32	2367	20.3	1.5
150	15	1.32	2389	20.1	0.5
450	45	3.2	2349	20.5	2.5
450	45	3.2	2382	20.2	1
1350	135	6.09	2321	20.7	3.5
1350	135	6.09	2096	22.9	14.5
4050	405	8.72	2031.5	23.7	18.5
4050	405	8.72	1920	25	25
12150	1215	10.18	1843	26.1	30.5
12150	1215	10.18	1924.5	25	25

The standard error (given by the interpolation of the data) for the 20nm beads is of 115.99 Hz.

⁴ the same result is given comparing the roll-off versus the percent of volume of beads in the solution

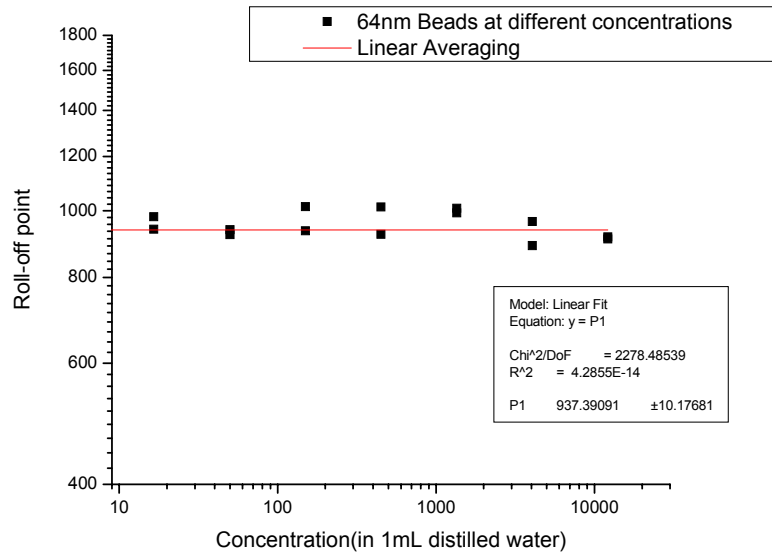


The graphs show a deviation from the predicted value of both the diameter and the roll-off point for high concentration. A possible explanation to that is that for such a high value of mm^3 of beads in water is not possible to consider the material a solution, since the solid part influence the condition of working. In this situation the formula we used is not more valid and it has to be replaced by a new one. The interpolation of the roll-off (f) versus the concentration (C) gives that the expected value for the roll-off frequency is given by $f = -0.0453 C + 2359.4$.

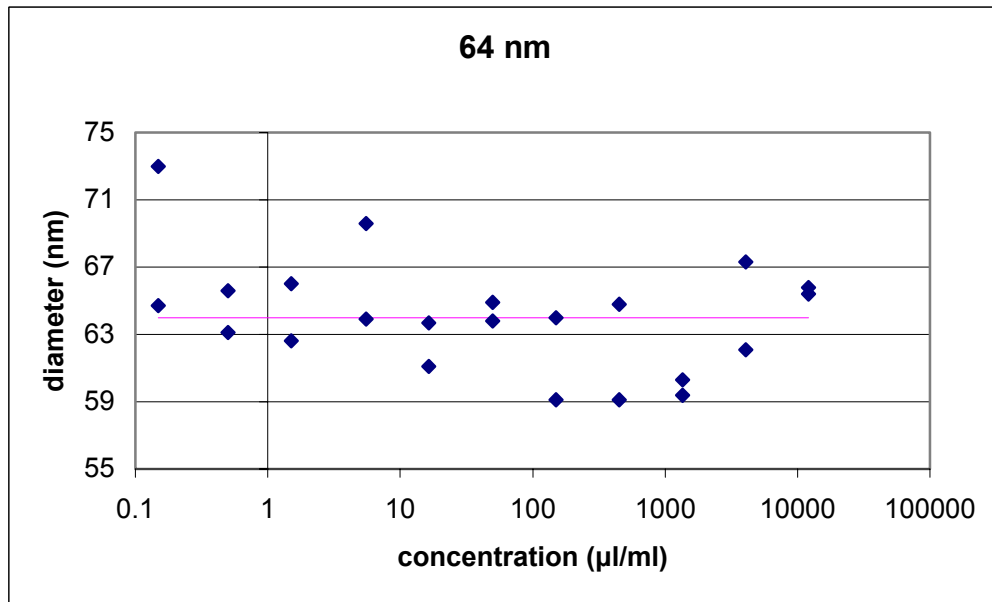


64nm

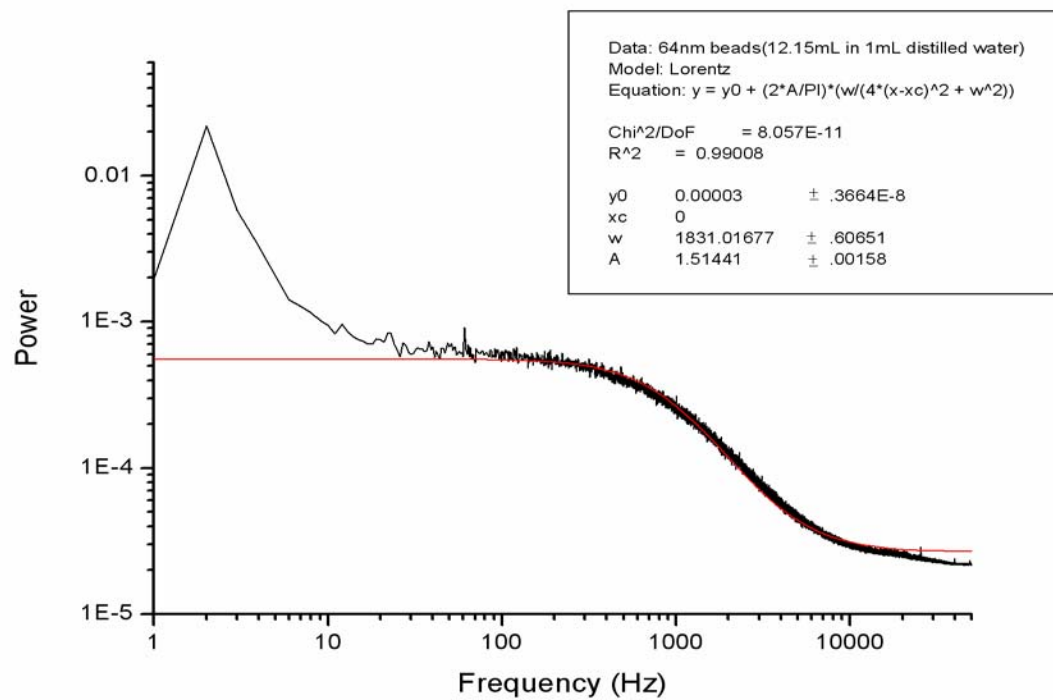
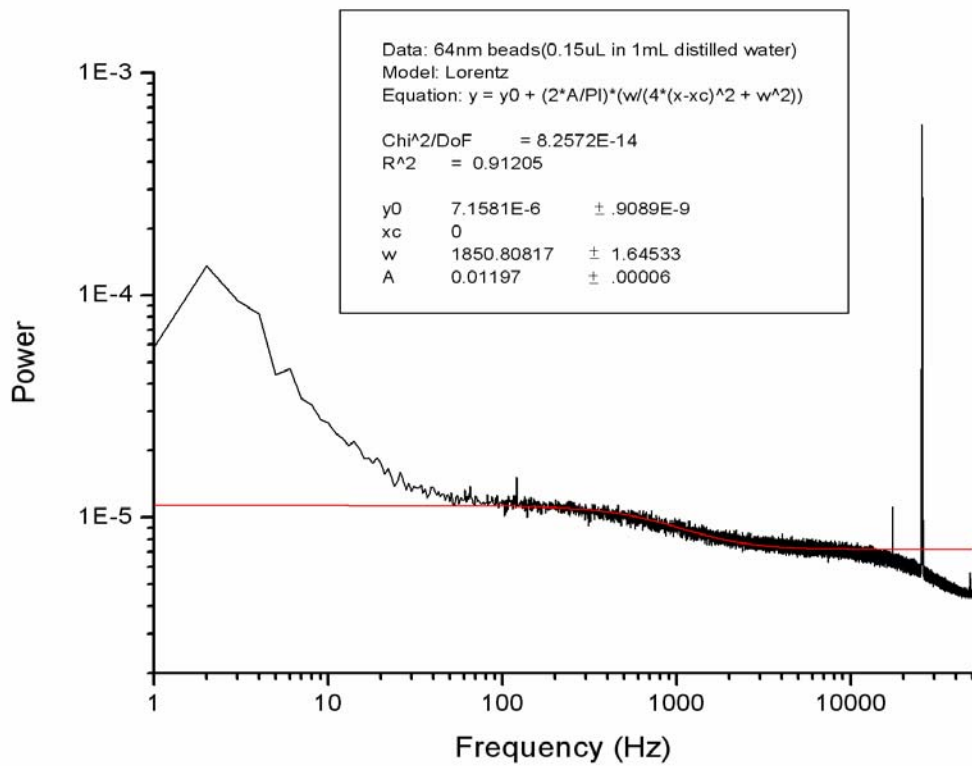
Conc ($\mu\text{l}/\text{ml}$)	mm ³ Beads	%vol beads	Roll-off (Hz)	Diameter (nm)	% difference
0.15	0.004	0.00039	820.4	73	14.06
0.15	0.004	0.00039	925.4	64.7	1.09
0.5	0.01	0.0013	949.25	63.1	-1.4
0.5	0.01	0.0013	913.35	65.6	2.5
1.5	0.04	0.0039	907.35	66	3.12
1.5	0.04	0.0039	957.3	62.6	-2.19
5.5	0.14	0.014	860.9	69.6	8.75
5.5	0.14	0.014	937.05	63.9	-0.16
16.5	0.42	0.042	940.3	63.7	-0.47
16.5	0.42	0.042	980.7	61.1	-4.53
50	1.29	0.12	938.9	63.8	-0.31
50	1.29	0.12	923.2	64.9	1.41
150	3.87	0.34	1013.55	59.1	-7.66
150	3.87	0.34	935.5	64	0
450	11.61	0.81	924.05	64.8	1.25
450	11.61	0.81	1012.8	59.1	-7.66
1350	34.83	1.5	993.4	60.3	-5.78
1350	34.83	1.5	1008.5	59.4	-7.19
4050	104.49	2.11	964.7	62.1	-2.97
4050	104.49	2.11	890	67.3	5.16
12150	313.47	2.44	916	65.4	2.19
12150	313.47	2.44	910	65.8	2.81



The standard error (given by the linear regression) for the 64nm beads is 48.51 Hz.



For the 64 nm beads there is not the deviation seen with the 20nm beads. The fact that the deviation effect is so evident for the smaller beads can be referred to the fact that for them the percent of beads into the volume of water was much higher, at the same concentration, than the one for the bigger beads.



It is possible to observe in almost all the power spectra a peak around 25 kHz, this noise is most likely produced by the alimentation of the laser.

Conclusions:

The experiment gave good results, above all for the 64nm beads. The water as background did not interfere drastically in all the experiment, in fact its roll-off point was so high respect the data from the beads, that we did not even subtract it. It is possible to notice the presence of this background noise observing a second roll-off in the power spectra of the beads, anyway the interpolated data are out from this zone.

Unfortunately this method seems to depend critically from the characteristic of the material used. In fact for the 64 nm beads the concentration's range is broader than the one for the 20nm beads.

Future work:

On the future several improvements might be implemented done to the light scattering apparatus.

A first step will be reducing all the noise at low and high frequencies. That way it will be possible to take into consideration also beads with higher (or lower) diameter.

Once that the noise problem is solved, the instrument can be calibrated using beads having not only different sizes, but also different index of refraction. It will be interesting to find out whether is there any fixed formula that gives the radius considering that there is a limit for the volume of beads into the solution (it will be probably around the 3%).

Another interesting test might be done on the sensitivity of the system. Analyzing several beads of near value will allow knowing how small the sensitivity of the system is. In other words, it will be possible to know how

small the variation in diameter could be to consent the instrument to notice the change in size.

Once that the instrument is calibrated, it will be possible to take a sample with some round-shaped virus (tomato viruses might be fine) to measure the diameter of it.

More applications:

Dynamic light scattering is not only used to determine the size of round particle, but it has a broad range of applications. This method is used in medicine to detect molecular changing in the cornea⁵, in biology to measure the rate of diffusion on proteins⁶, and in material science to studying the orientational fluctuation in the liquid crystals⁷.

⁵ <http://clinicaltrials.gov/ct/show/NCT00050466?order=11>

⁶ <http://www.protein-solutions.com/vol2no2.htm>

⁷ <http://www-rim.sci.kun.nl/lcr/publication/1996/mclc96.pdf>

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