Dynamic Light Scattering

Marisol Chang
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Abstract

A dynamic light scattering apparatus was built with a HeNe laser; the beam incident on a sample of particles(microspheres of one or multiple diameters or casein undigested and digested) suspended in water scatters into a random pattern product of the coherent superposition of the outgoing waves scattered from the spheres. Because the spheres are in constant Brownian motion, the pattern randomly changes in time. A phomultiplier tube was placed in the pattern and the random fluctuations in the light intensity were measured and analyzed. The signal's autocorrelation functions were computed to verify the spheres' diameter. The results show that the apparatus is able to measure the diameter of single sized particles but not on a mixture with two different size microspheres. Further analysis needs to be done in order to determine the size of protein digested fragments.

Introduction

When monochromatic, coherent beam of light is incident on a dilute solution of macromolecules such as proteins or suspension of colloidal particles such as polystyrene or latex microspheres and the solvent refractive index is different from that of the solute (proteins or microspheres) the incident light is scattered by each illuminated particle in all directions **[1]**. The scattered light waves from different macromolecules or particles mutually interfere or combine, at a distant fast photomultiplier tube (PMT) and produce a scattered intensity I(t) that is not uniform on the scattering or detection plane. If all the particles are stationary, the scattered light intensity at each direction would be a constant (independent of time). However in reality all the scatters in solution are undergoing constant Brownian motions **[2]**, and this fact leads to fluctuations of the scattered intensity pattern on the detection plane and the fluctuations in I(t)) if the detection area is sufficiently small. Dynamic or quasi-elastic light scattering measures the intensity fluctuations instead of the average light intensity as in static light scattering. When incident light is scattered by a moving macromolecule or particle, the detected frequency of the scattered light will be slightly higher or lower than that of the original incident light owing to Doppler Effect, depending on whether the particle moves towards or away from the detector **[1]**. Thus, the frequency distribution of the scattered light is slightly broader than that of the incident light. This is why dynamic light scattering is called quasi elastic. This frequency broadening is so small (~10^5-10^7 Hz) in comparison to the light frequency (~10^{15} Hz) that it is very difficult to detect in the frequency domain, but it can be recorded in the time domain via a time autocorrelation function (eq 1). At time t, the scattered light intensity is I(t) and at a very small time later (t+τ) the diffusing particles will have new positions and the intensity at the PMT will have a value I(t+t) which correlates with I(t), the closer the measurement is to time zero, the more similar I(t+t) is to I(t) since the particles have not had much time to move. As time goes on there is no more similarity between the starting state and the current state; the measured intensities do no correlate anymore to the beginning one. At time there is a 100% autocorrelation; as time progresses, the autocorrelation diminishes reaching zero as there is no more similarity between starting and ending states. The decay of the autocorrelation is described by an exponential decay function (eq 2) which relates the autocorrelation to the diffusion coefficient D and the measurement vector K, where n is the refraction index of the solvent and λ is the beam frequency (eq 3), then using stokes equation the hydrodynamic diameter can be calculated, where K_B is Boltzman's constant, T is temperature, η is viscosity of the solvent and d is the hydrodynamic radius (eq 4).

$$r_\tau = \frac{\sum_{i=1}^{N-k}(Y_i-\bar{Y})(Y_{i+k}-\bar{Y})}{\sum_{i=1}^{N}(Y_i-\bar{Y})} \quad (1)$$

$$G(\tau) \propto e^{-2DK^2\tau} \quad (2)$$

$$K = \frac{4\pi n}{\lambda}\sin\frac{\theta}{2} \quad (3)$$

$$D = \frac{K_B T}{3\pi\eta d} \quad (4)$$

The objective of this study is to use dynamic light scattering as a mean to estimate the diameter in a mixture containing a population of particles with different diameters. For this purpose a dynamic light scattering apparatus will be built and its accuracy will be determined by individually measuring the diameter of different particles in a solution containing one type of microspheres at the time. Subsequently a mixture containing two or more types of microspheres will be used to determine the diameter of the particles in the solution. Finally a solution containing, casein the main protein in milk, will be use to test the accuracy of the apparatus to estimate the diameter of complex macromolecules such as proteins. Furthermore, casein will be subjected to trypsin digestion to measure the ability of the apparatus to measure protein fractions.

Methods

Experimental Set Up

The dynamic light scattering set up is shown in figure 1, the schematic of the set up is shown figure 2. A HeNe 632.8nm laser (1144P, JDS Uniphase Corporation, San Jose, CA) with 0.7mm beam diameter was used as the light source. The laser beam was focused to a sharp, horizontal line parallel to the wall of the scattering cell through which the scattered light was observed. The laser beam was decreased by the use of a Keplerian telescope consisting of a 150mm objective lens and a 25.4mm eye piece lens. The telescope was used to focus the laser beam to about 100μm (1:6) to produce a higher power density for the incident beam, which leads to a smaller scattering volume and a higher coherence factor. The laser was aligned making sure the beam was hitting the middle of a crosshair set up at the wall, before adding the lenses and aligning them one by one by first focusing on the crosshair. The augmented laser beam then passed through a square disposable cuvette cell (Kendall Elkay, Boston, MA, ultra-vu 10mm sides), where a 2mm diameter iris was used to define the scattering area (approximately 0.2mm^2). A 150mm lens was used to focus the scattered light to a second 2mm iris. A photomultiplier (PMT, H742240, Hamamatsu Photonics Ltd, 5mm effective area) positioned at 90^o from the sample cell was placed at a position that will cover most of the PMT effective area; a small mirror was used to make sure the beam hit the middle of the PMT. The photomultiplier had a bandpass filter with a center wavelength of 632.9 nm and a bandwidth of 2.9 nm (10LF03-633, Newport) attenuating the high frequency components of the PMT. An amplifier with a 1x10^6 gain was connected to the PMT and to a NI-DAQ board (AT-MIO-16E-1, National Instruments). Matlab was used to acquire and process the data.

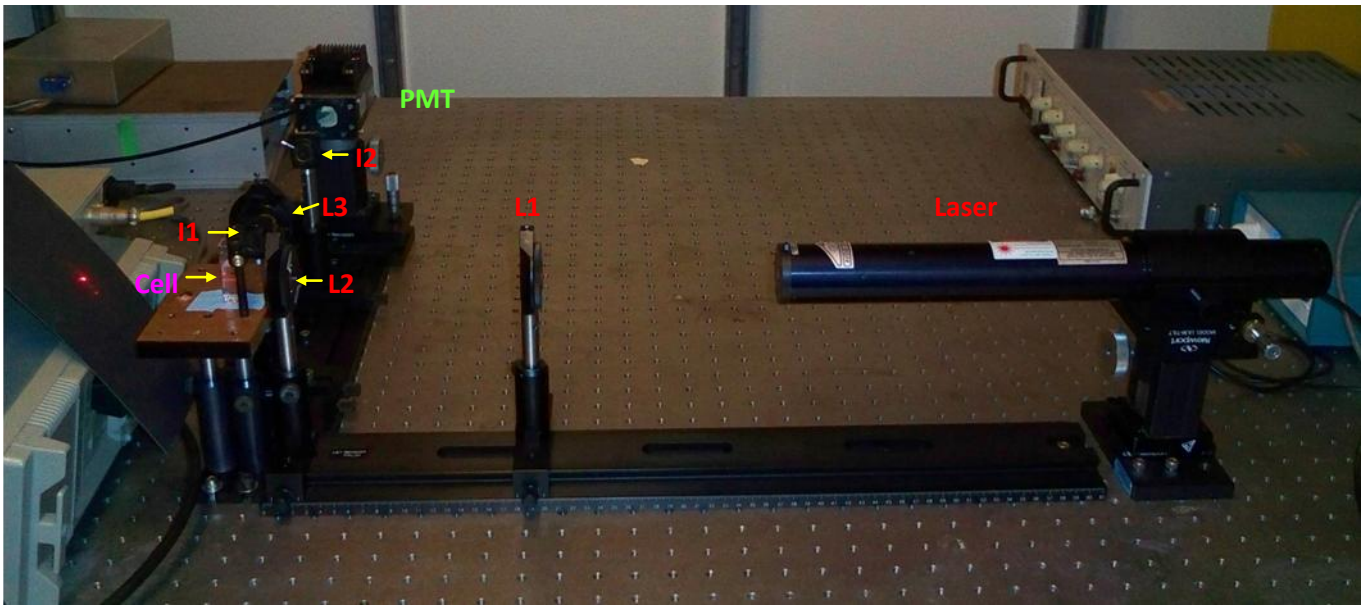

Figure 1. Actual Dynamic Light Scattering Experimental Set up. L1= 150mm lens, L2=25.4mm lens, L3=150mm lens, I1=2mm iris, I2=2mm iris, PMT=photomultiplier tube.

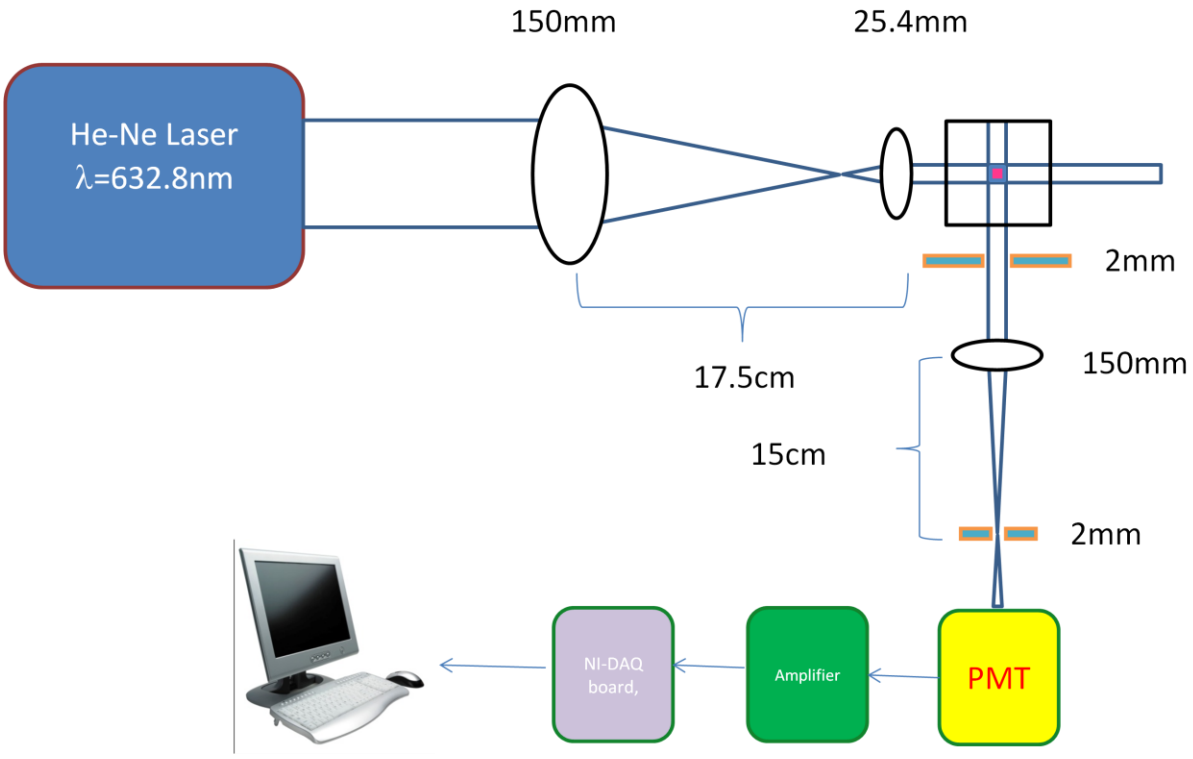

Figure 2, Schematic Representation of Dynamic Ligth scattering Set Up.

Sample Preparation

Beads of different diameters were used for dynamic light scattering experiments (Table 1) and diluted in distilled water at the concentrations shown in table 1. Casein, 2mg/ml (Sigma) was diluted in water in filtered in 5 μm pore size filter (Millipore). Casein digestion was performed with trypsin, 1mg/ml (Sigma) for 10 min.

Diameter (μm)	True diameter (μm)	Standard Deviation (μm)	Material	Concentration (μl/ml)	Company
6.0	5.883	0.283	Polystyrene	1	Polysciences, Inc
4.5	4.452	0.127	Polystyrene	1	Polysciences, Inc
2.9	2.88	0.144	Latex	1	Interfacial Dyanmics Corp
2.0	2.06	0.024	Polystyrene	1	Polysciences, Inc
1.0	1.025	0.01	Polystyrene	1	Polysciences, Inc
0.2	0.214	0.007	Latex	1	Interfacial Dyanmics Corp

Table 1, Beads used for dynamic light scattering.

Data Analysis

Raw data (fig. 3) was analyzed using the Matlab autocorrelation function part of the GARCH toolbox. Data was collected at a frequency of 100 or 200 kHz for 120 or 60 minutes respectively and it was repeated 3-6 times. The autocorrelation function was performed for every second of data collected and the resulting autocorrelation functions were averaged in order to remove the effects of random noise. The autocorrelation function was fitted to a decaying exponential function by first finding the roll-off (fig.4) point and substituting the corresponding values to equation__ in order to find the diffusion coefficient. The diameter of the spheres or protein was calculated by substituting the diffusion coefficient into equation__ .

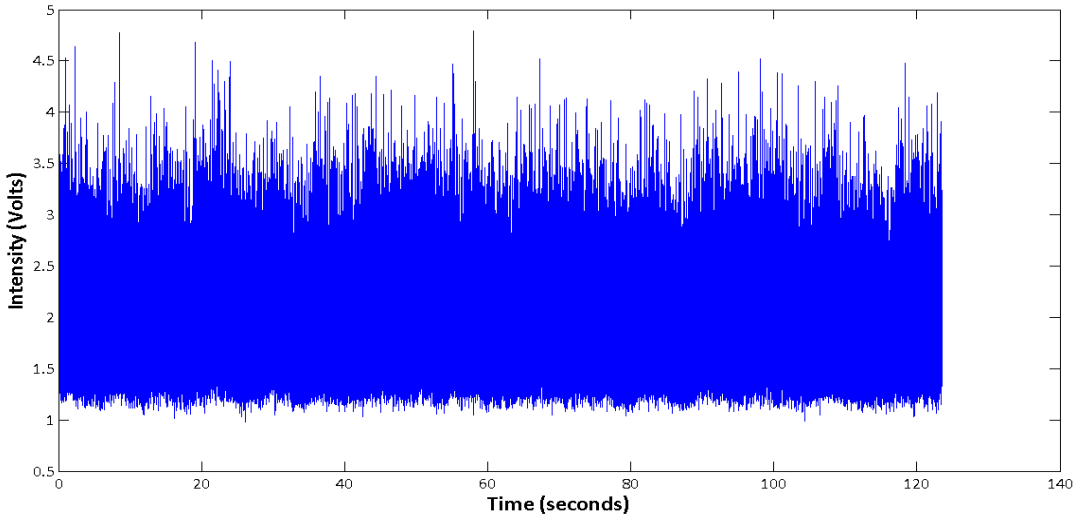

Figure 3, Time series of a typical dynamic light scattering measurement.

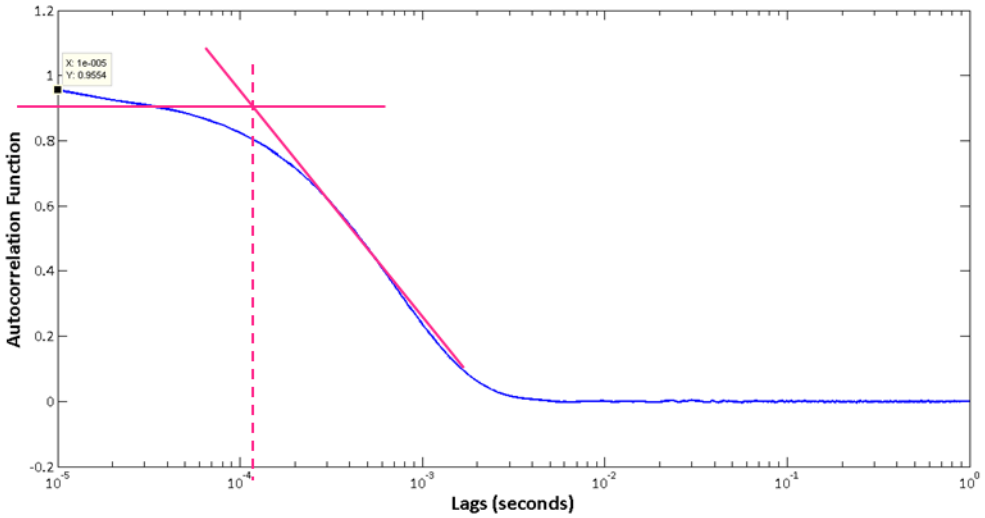

Figure 4. Curve fitting process. Roll-off point is used to calculate the diffusion constant.

Results

First, in order to determine that the system was working properly measurements were made in which the data was recorded without having the laser on to account for the shot noise. Data was also recorded with just water, the solvent used to dilute the microspheres, and then data was recorded with microspheres. Figure 1 shows the non-normalized autocorrelation function for these three conditions. From the figure it can be seen that the contribution of the noise and just the solvent is very small as compared to the solution with the beads.

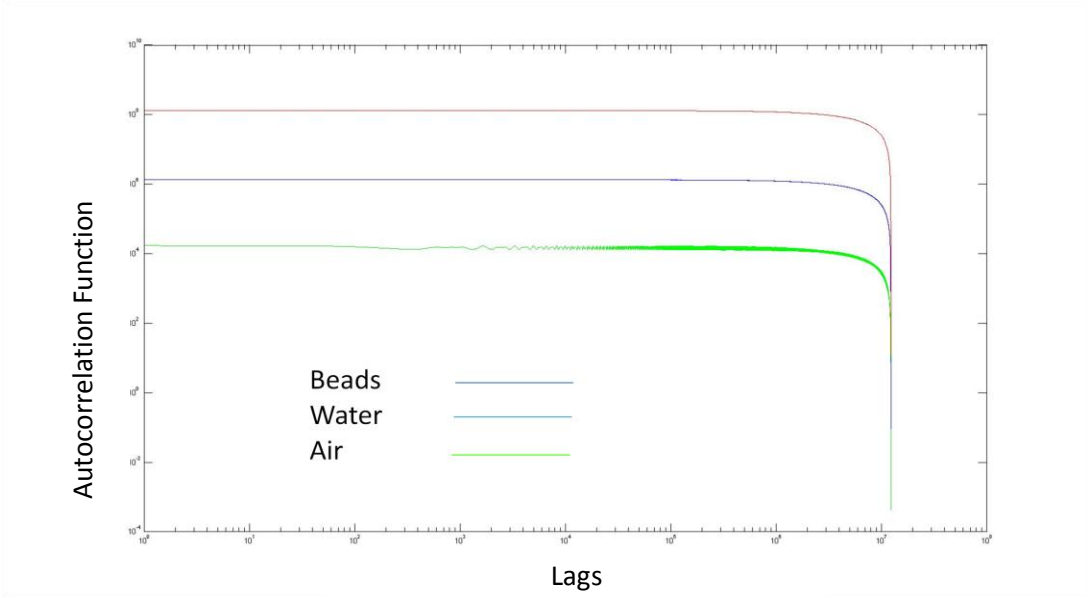

Figure 5, Non-normalized autocorrelation function. Red line is for beads, blue line is for water and green line is for noise.

The time constant of the PMT was determined to be about $2x10^{-5}$ (50kHz) by placing a piece of paper inside an empty cuvette, the autocorrelation function is shown in figure 6. The time constant of shot noise was found to be about $1x10^{-3}$ (1kHz).

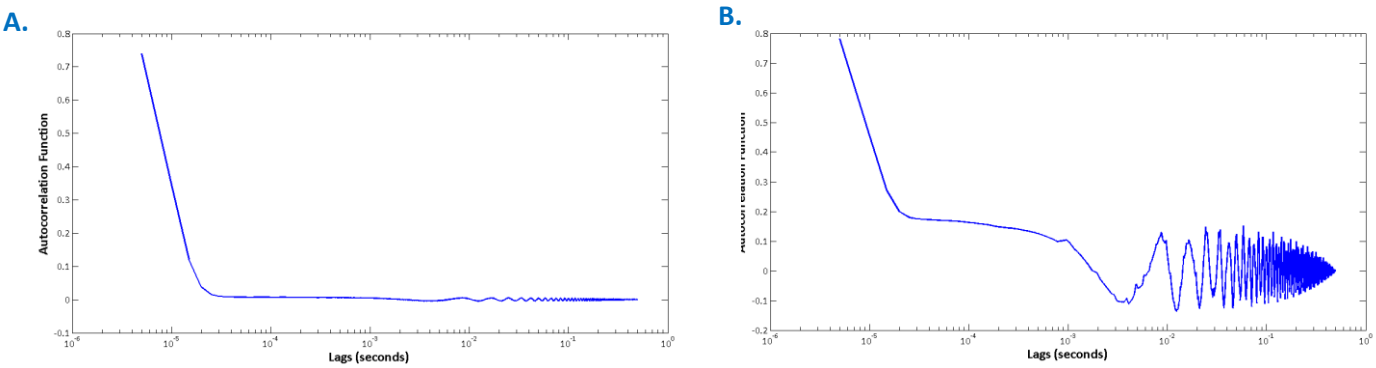

Figure 6, Time constants, A. Autocorrelation function of light passing through paper. B, autocorrelation of shot noise.

Microspheres

Table 2 shows the calculated diameter of different microspheres and figure 7 shows the averaged autocorrelation function of the intensity of the scattered light for each microsphere with the corresponding fitted curve. As it can be observed it was possible to measure the diameter of one type of microspheres using the dynamic light scattering with a percent error of 0.3-6.9%. In order to determine if it is possible to determine different diameters in a solution containing a mixture of microspheres a solution containing polystyrene 6.0µm beads and 0.2µm latex beads was used at a concentration of 0.5µl/ml each, and sampled at a frequency of 200kHz for 60 seconds with six repetitions. The averaged autocorrelation function was performed for a period of 1 second (fig.8), it can be seen that the autocorrelation function has 2 roll-off points that possibly corresponds to the distribution of the two different size microspheres. Fitting of the two curves reveals a calculated diameter of 0.4501µm and 51.12µm, with diffusion coefficients of $9.35x10^{-13}$ and $8.232x10^{-15}$ respectively and percent error of 125% and 752% respectively.

Protein

Figure 9 shows the averaged autocorrelation function of casein sampled at 200kHz for 60 minutes with 6 repetitions. By fitting the autocorrelation function, the calculated hydrodynamic diameter was found to be 195 nm with a diffusion coefficient of $2.155x10^{-12}$. The calculated diameter has a 57.3% error as compared to the value reported by Rui et al of 124nm at 20°C **[3].** When trypsin digestion of casein was performed for 10 minutes followed by dynamic light scattering for 60 seconds at 200kHz; the average autocorrelation function (fig. 10) revealed a hydrodynamic radius of 179nm and the diffusion coefficient $2.354x10^{-12}$. No fragments were detected after enzymatic digestion of casein.

Diameter (μm)	Diffusion coefficient	Calculated diameter (μm)	% Error	Sampling frequency (kHz)	Time (sec)
6.0	$6.975x10^{-14}$	6.033	0.6	100	120
4.5	$X10^{-13}$	4.601	1.6	100	120
2.9	$1.351x10^{-13}$	3.115	6.9	100	120
2.0	$2.068x10^{-13}$	2.035	1.7	100	120
1.0	$4.428x10^{-13}$	0.997	0.3	100	120
0.2	$2.097x10^{-12}$	0.201	0.4	100	120

Table 2 Calculated diameter of different microspheres

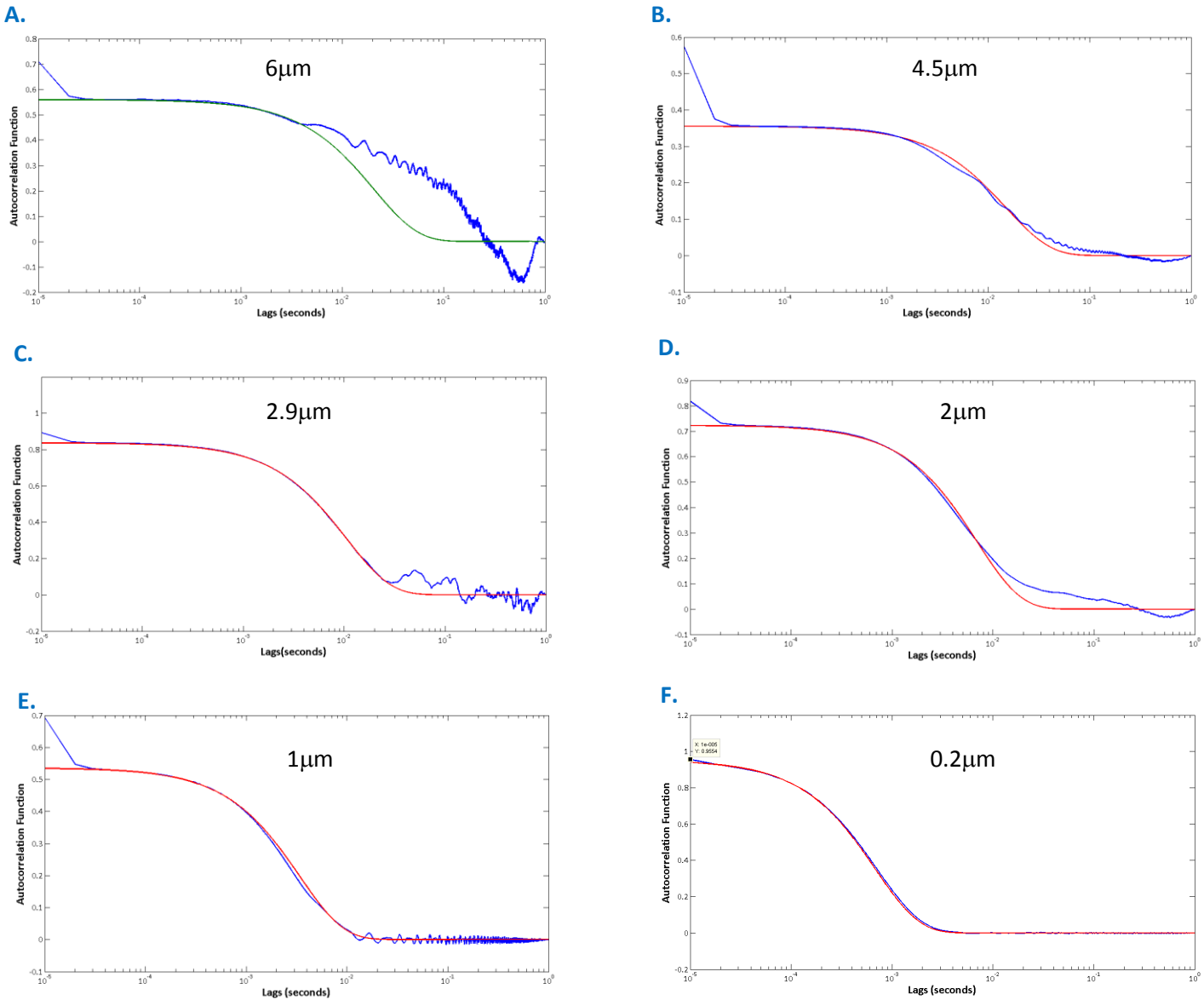

Figure 7, Autocorelation function of microspheres of different diameter. Blue line is the autocorrelation function and red line is the fitted curve.

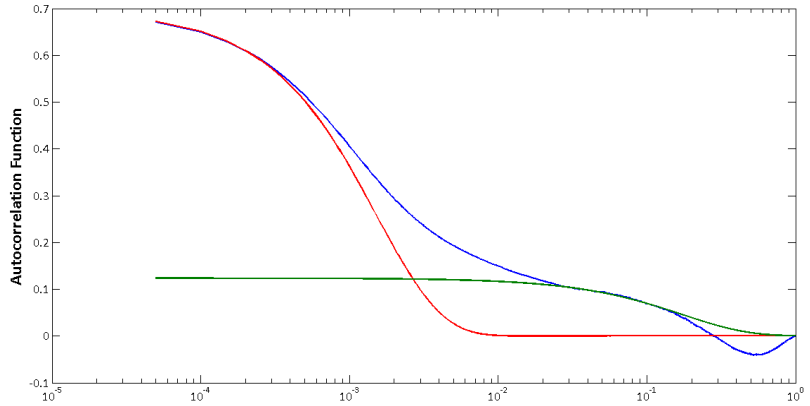

Figure 8. Solution with 6µm and 0.2µm microspheres. **Blue line corresponds to autocorrelation function; red and green lines corresponds to fitted curves.**

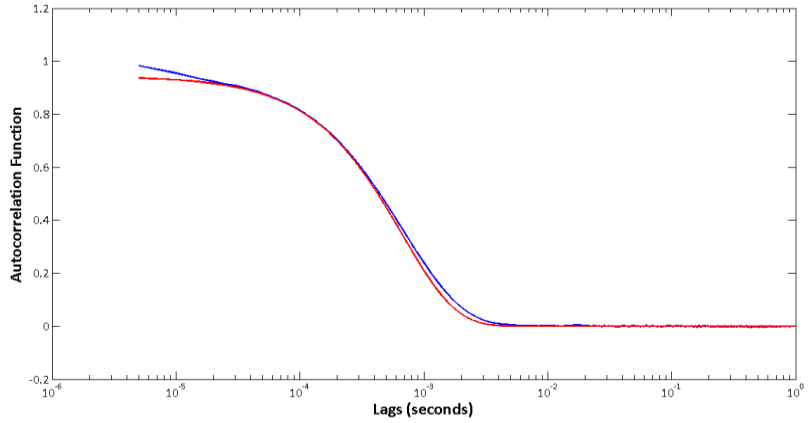

Figure 9, Casein Autocorrelation Function. **Blue line corresponds to autocorrelation function; red line corresponds to fitted curve**

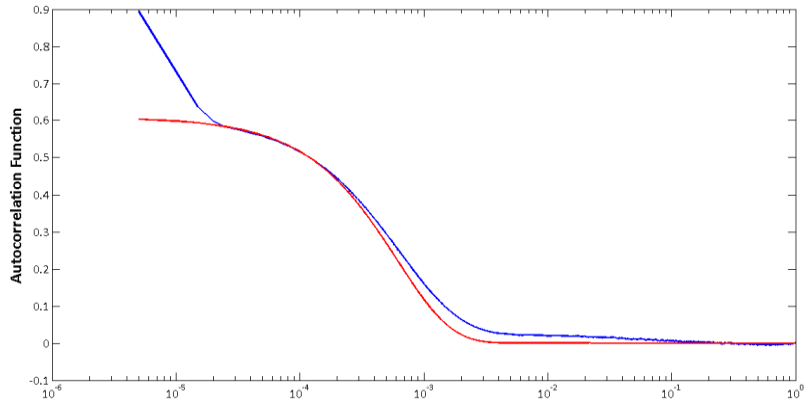

Figure 10, Casein after 10min digestion with trypsin. **Blue line corresponds to autocorrelation function; red line corresponds to fitted curve**

Discussion

In this study the diameter of a solution containing microspheres of the same diameter was able to be determined very accurately however when the solution contained microspheres of two different diameters the calculation was far from being accurate. This result may have been due to the fact that the microsphere were of different materials polystyrene and latex causing aggregation therefore erroneous estimation of the diameter of the microspheres.

Calculation of the diameter of casein was different than the value reported in the literature; possible causes for major difference could be fact that Rui et al used PBS as a solvent instead of water as used in this study. The ions present in PBS could cause slight changes in the protein quaternary structure leading to changes in the hydrodynamic radius.

Digestion of casein did not produce any visible fragments however Farrell et al reported that trypsin digestion of casein produces 8nm fragments **[4]**. Although not visible fragments were detected in the autocorrelation function graph the diameter of the digested casein decreased about 8.2% as compared to casein without digestion. This might indicate that digestion of peptides took place but either the fragments were too small or too few to be detected. Another reason why fragments were not observed could be due too short time for digestion; Rui et al, also used a 10 minute digestion time and no fragments were detected either, the only difference is that Rui et al found an increase in diameter instead of a decrease as it was found in this study.

More studies are needed in order to determine if dynamic light scattering can be used to detect the diameter of particles in a heterogeneous solution. Additional studies with beads of the same material and different sizes need to be performed. Similarly, different concentrations of the digested protein need to be studied in order to be able to detect the core protein and its peptide fragments

References

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2. Clark, N., J. Lunacek, and G. Benedek, *A study of Brownian motion using light scattering.* American Journal of Physics, 1970. 38: p. 575.
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4. Farrell, H., et al., *Characterization of the particles of purified -casein: trypsin as a probe of surface-accessible residues.* Journal of Protein Chemistry, 1999. 18(6): p. 637-652.