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Sizing of Colloidal Particles and Protein Molecules in a Hanging Fluid Drop

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ABSTRACT

We report non-invasive particle size measurements of polystyrene latex colloidal particles and bovine serum albumin (BSA) protein molecules suspended in tiny hanging fluid drops of 30 μL volume using a newly designed fiber optic probe. The probe is based upon the principles of the technique of dynamic light scattering (DLS). The motivation for this work comes from growing protein crystals in outer space. Protein crystals have been grown previously in hanging drops in microgravity experiments on-board the space shuttle orbiter. However, obtaining quantitative information on nucleation and growth of the protein crystals in real time has always been a desired goal, but hitherto not achieved. Several protein researchers have shown interest in using DLS to monitor crystal growth process in a droplet, but elaborate instrumentation and optical alignment problems have made in-situ applications difficult. We demonstrate that such an experiment is now possible. Our system offers fast (5 seconds) determination of particle size, utilize safe levels of very low laser power (≤0.2 mW), a small scattering volume (~2 x 10^{-5} mm^3) and high spatial coherence (ß) values. This is a major step forward when compared to currently available DLS systems.

1. INTRODUCTION

Fabrication of good quality and larger size protein crystals is required in elucidating detailed crystal structure[1]. This would be helpful to pharmaceutical industries in designing and developing novel drugs[2-3] and molecular biologists in identifying causes of certain life threatening diseases, e.g., cancer[4], hypertension, diabetes, arthritis, and AIDS[5]. Effects of sedimentation and convection under terrestrial conditions severely limit fabrication of larger size and good quality crystals[6]. This is specially true for crystals grown from solutions of protein monomers. Experiments conducted on-board the space shuttle orbiter has shown that relatively larger size and good quality crystals can be produced in space from protein solutions compared to terrestrial conditions. In recent years, however, the focus has been shifted to understand the protein crystallization process at a fundamental science level under reduced gravity conditions. This requires detailed studies of nucleation and growth phenomena. Since nucleation may depend upon temperature, monomer concentration, pH, solution ionic strength, and precipitating agent concentration, dynamical control of these parameters and a specific monitoring technique would be very useful. If one can dynamically control nucleation, then it will be much easier to produce good quality crystals reliably and routinely. This requires suitable quantitative and noninvasive monitoring techniques.

Several different techniques are available to grow protein crystals[7]. Experiments using a variety of crystal growth hardware and crystallization techniques have been conducted on twenty six U.S. space shuttle missions since April 1985[8]. We focus our experiments on the technique of vapor diffusion since this method is currently being used and it has been extensively used on-board the space shuttle orbiter in the past few years. The vapor diffusion apparatus (VDA) produces protein crystals inside a hanging drop. The drop is hanged by the tip of a syringe inside a sealed chamber of approximate volume 2 cm^3. The hanging drop can be visualized through observation windows located on sides of the sample assembly. The vapor diffusion hanging drop hardware and protein crystals grown on space shuttle flights (STS 51-D/61-C) are described in detail elsewhere[9].

The physical size, operator safety, power, stowage, and astronaut/cosmonaut involvement impose stringent requirements for experiments conducted on-board space vehicles (space shuttle system, space stations Mir and Alpha). This calls for payloads and experimental apparatus to be very small in size, consume very low electrical power, generate low levels of heat, minimize human interaction, and have no moving parts; thus avoiding a need for optical alignment.

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2. INSTRUMENTATION

We chose to exploit the technique of dynamic light scattering (DLS) to reliably characterize dynamical properties (diffusion coefficients or size) of the protein molecules in a hanging drop. Up until recently, such experiments were not possible, because of optical alignment problems, control of penetration depth inside the sample, physical size of the instrumentation, and power requirements for light source (laser) and photo detectors.

The technique of DLS is commonly used in the characterization of macromolecular solutions. The principles of DLS are well documented and can be found elsewhere[10-12]. In last few years, however, the DLS instrumentation has embraced emerging technologies associated with the advent of miniaturization in solid state lasers, photo detectors, optical components, and fiber optic communication devices. This include utilization of solid state lasers[13-15], avalanche photo diode (APD) detectors[16-18], charged coupled device (CCD) camera[19], and the development of fiber optic probes for macromolecular characterization[20-25].

2.1. Optical design

One of the difficulties in probing protein monomers in dilute solutions is their small size (~3-8 nm in diameter). Conventional DLS systems require laser powers of the order of few hundred milliwatts and data collection times of tens of minutes to ascertain good-quality time correlation functions (TCF). A good-quality TCF depends upon several factors. One of which is the precise control of the scattering volume or coherence area in the sample[26]. In conventional DLS systems the coherence area is controlled by making use of lenses and apertures as part of the launch and detection optics. Fiber optic systems reported more recently have made the use of two optical fibers; one for launch and the other for detection, mounted on one gradient index (GRIN) micro lens[25]. A lensless fiber optic system[20-21, 24-25] for doing DLS measurements has also been reported. The former (Figure 1/1A)[25] can only be used as an immersion probe while the lensless probe[20-21] can be used both inside and outside the sample cell. But both designs have certain limitations. In the former case, in our experience, reflections arising from the lens and fiber surfaces give rise to heterodyning effects; thus compromising the reliability and accuracy in obtaining correct particle sizes. The lensless fiber optic system on the other hand solves the problem of spurious reflection but, for outside use, it must be placed within a very close proximity ($\leq$ 2 mm) of the sample being interrogated. Further, its use is limited in interrogating very dilute samples and samples of larger colloidal particles.

In a previous study[21], a lensless fiber optic probe was used to characterize protein solutions of lysozyme and BSA. The experiments were performed using a gas laser in very good optical quality glass cuvettes. The data acquisition time (or experiment duration) for obtaining a TCF was of the order of several minutes (~20 minutes). The probe design employed a monomode and a multimode fiber for launching a laser beam and detecting the scattered light from the sample respectively. Special translation stages for optical alignment were used for launching the laser beam from a gas laser into the probe. Although, the particle size obtained in that study using a conventional DLS system and the lensless probe reasonably corroborated, and despite the fact that a gas laser was used, the ß values remained extremely low.

Our newly designed system alleviates all these problems. We attach two separate micro GRIN lenses (see paper #2632-18) on each optical fiber (launch and detection) in such a way that the scattering volume (~2 x 10^{-5} mm^3) and coherence area[26] are very tightly controlled resulting in high spatial coherence (ß>0.5). Several experiments were conducted with an older design (lensless) probe[20] and the new probe described in this paper. The lensless probe[20] used in this paper was developed under a NASA contract and purchased from Dr. H.S. Dhadwal of SUNY-Stony Brook. A detailed discussion on the comparison of two approaches will soon be presented elsewhere[27]. However, we give one example to illustrate the performance of two probes. We used both probes to characterize a 0.1 (wt.%) dispersion of 52 nm polystyrene latex spheres in a cuvette using a laser diode and a APD detector. The old approach (lensless probe) gave a ß value of 0.12 and a photon count of ~ $2x10^5$ /sec at a laser power of 0.5 mW. Our approach (new probe) gave a ß value of 0.75 and a photon count of ~ $5x10^5$/sec at a laser power of 0.02mW from the same sample. The new approach, as shown in Figure 1, increases the ß value by a factor of > 6 and since it reduces the laser power by a factor of 25 and enhances the count rate by a factor of 2.5, an overall gain in the performance of the new probe by a factor of >62 is achieved. This is a significant improvement over the previous approach.

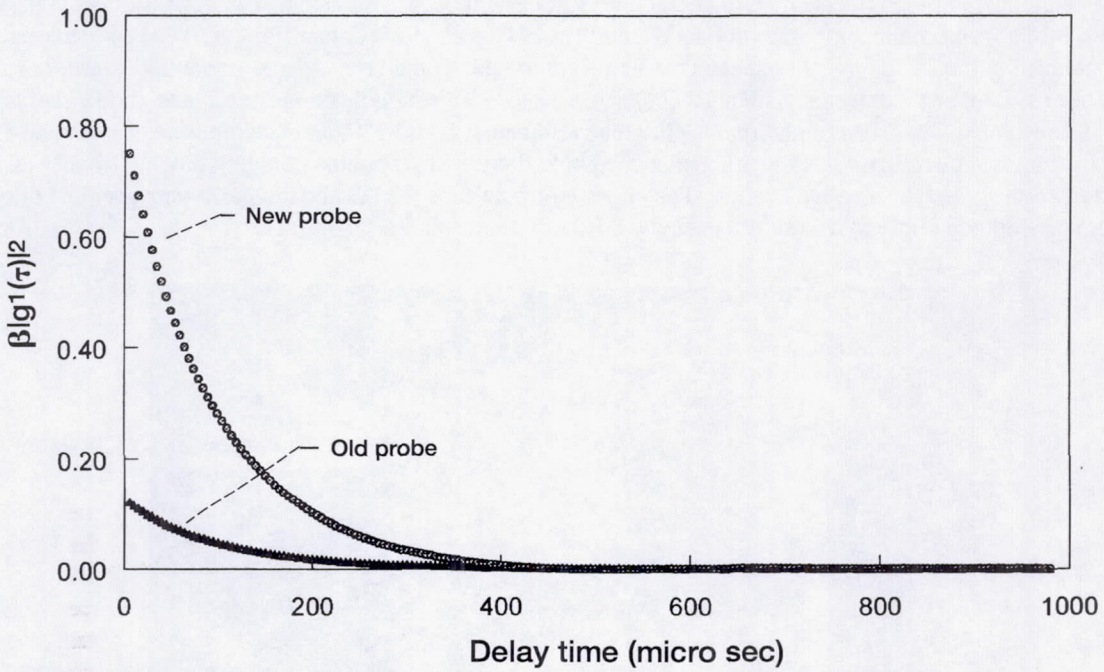

Figure 1.—Performance comparison of the older (▲ lensless probe)[20-21, 24-25] with the new (● two-lens) DLS probe described in this paper. The overall performance of the new probe is better by a factor of 62 (see text section 2.1).

2.2. Improvements

Our system is a step up from previously reported DLS systems as it provides major improvements. These improvements include accurate determination of particle sizes from very small (3 nm) to very large (~1000 nm) with very small data acquisition times (within seconds) and extremely small laser power levels (0.1-1.0mW) from a laser diode, and the ability to place the probe outside the sample container anywhere from 0-17 mm from the sample's surface. The probe can also be immersed in the solution. Another major improvement is its ability to probe solution dynamics in sample containers of varying size and shapes. We have performed accurate and reliable particle size measurements with this probe on dispersions contained in regular spectrographic cuvettes, capillary tubes, common laboratory utensils (beakers, graduated cylinders, conical flasks, glass/clear plastic container vessels), cells of 3 mm thickness made of acrylic material, concentric cells containing two different solutions, and hanging fluid drops. Extremely small sample quantities of protein solutions (~ 30 μL) were interrogated in the experiments reported in this paper.

2.3. Experimental set up

The experimental set up comprises four parts; a laser/detector module, a sample holder, a fiber optic probe, and a data acquisition system. An aluminum holder was fabricated to hold sample cuvettes. This holder was mounted on an optical rail using an optical post with adjustable vertical and horizontal positions. For hanging drop experiments we simply replaced the cuvette holder by a 0.22 μm filter (Millipore corporation, USA) carrying a suspended hanging drop at its tip. This is illustrated in Figure 2. The hanging drop was suspended in the ambient air throughout the experiment duration. The probe is mounted on the same optical rail using another optical post with a V-clamp to hold the probe in its place. The probe

comprises two monomode optical fibers and two GRIN lenses in a stainless steel housing. The optical fibers, 3 m in length and mated with FC/PC-type connectors, were obtained from Rifocs Corporation (Camarillo, CA). The connectorized fibers were directly coupled to the same type of connectors which were mounted on a laser/detector module. A laser/detector module housing a laser system and a detector system was built[28] and used. This module comprises a laser diode(Toshiba model TOLD 9215), a constant power driver, a photomultiplier tube (Hamamatsu HC-120) and an amplifier-discriminator system (Brookhaven Instruments Corporation, NY). An avalanche photo diode (APD) photon counting module (model PCS-2, EG&G Canada) was also used as a photodetector. The photomultiplier tube (PMT) and the APD were used to collect scattered light from concentrated polystyrene solutions and the BSA protein solutions respectively.

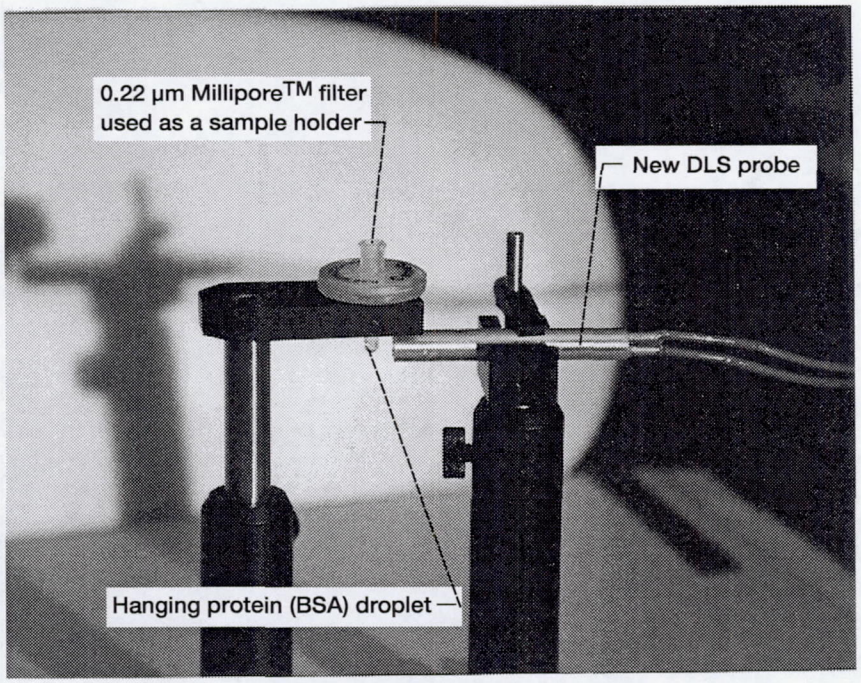

Figure 2.—Experimental setup: sizing of protein molecules in a hanging drop using a new (two-lens) fiber optic DLS probe.

2.4. Experimental procedure

Laser light (λ = 670 nm) from a laser diode at two power levels (0.2 mW and 1.0 mW) was launched into one of the monomode optical fibers. The light is focused inside the sample volume after passing through a micro GRIN lens (NSG SLW-1.8) mounted at the end of the fiber. Light scattered from the particles inside the scattering volume (~2 x 10^{-5}mm^3) is collected at an scattering angle of 154° by another GRIN lens mounted onto the second fiber and is guided back to the PMT or APD. The signal from the PMT system or the APD module is fed to a digital autocorrelator (Brookhaven Instruments BI9000). The digital autocorrelator card is housed in a docking station of a Pentium™ based notebook computer (7600 Series, EPS). For dilute dispersions of spherical particles the slope of the autocorrelation function provides a quick and accurate determination of the particle's translation diffusion coefficient, which can be related to its size via a Stokes-Einstein equation[26], provided the viscosity of the suspending fluid, its temperature, and its refractive index are known. From this information we determine particle size, spatial coherence (ß) values and a polydispersity factor (PDF) in a similar fashion as shown in paper # 2632-18 of this proceedings. ß is a measure of the efficiency of the detection system (signal-to-noise) and thus refers to the quality of the TCF and lies between 0-1. PDF is a measure of the polydispersity of the sample. Lower PDF values (<0.01) refer to narrow size distributions. The data analysis was carried out using Brookhaven's software package containing cumulants and CONTIN analysis programs. These are discussed in detail elsewhere[29-35].

2.5. Sample preparation

Samples of polystyrene standards (10 wt.%) were obtained from Bang's Laboratories (Indianapolis, Indiana). For lower concentration measurements, these samples were diluted using doubly distilled deionized water (~20 MΩ-cm). BSA protein samples ranging in concentration from 2% to 10% were obtained from Sigma Chemicals (St. Louis, Missouri) and were used as supplied. All the measurements were conducted under ambient conditions. Plastic cuvettes of 10 mm path length (Fisher Scientific Company, USA) were used for sample containment. A 0.22 μm filter (Millipore Corporation, Massachusetts) was used as a sample holder for the hanging drop measurements. Polystyrene and BSA solution drops were hanged by the exit tip of the filter. The volume of the hanging drop was measured to be ~30 μL.

3. RESULTS AND DISCUSSION

The newly designed fiber optic probe was thoroughly tested. Results on the characterization of polystyrene monodisperse standards ranging in size from 20 nm to 0.8 μm at several concentrations are presented in Table 1. Table 2 lists results for hanging drop measurements of BSA samples ranging in concentration from 2% to 10% and droplets of aqueous polystyrene solutions (PS) ranging in size from 20 nm to 0.8 μm. A comparison was made with the same samples stored in cuvettes. The results (Table 2) clearly demonstrate that the particle size values obtained in hanging drops are in full agreement with those performed in a plastic cuvette.

Table 1

Standard Size (nm)	Sample Concentration (wt %)	Spatial Coherence (β)	Diameter (nm)	Polydispersity PDF ($\mu_2/\langle\Gamma\rangle^2$)
20	10.0	0.54	19.8±0.2	0.205
20	1.0	0.66	19.4±0.1	0.086
20	0.05	0.58	22.0±0.3	0.123
43	10.0	0.49	37.8±0.9	0.116
43	0.5	0.53	40.0±0.7	0.037
43	0.01	0.64	42.1±1.0	0.076
85	1.0	0.44	82.1±1.2	0.107
85	0.05	0.52	83.3±1.4	0.062
85	0.002	0.66	85.0±1.5	0.064
165	0.2	0.32	161.1±4.4	0.052
165	0.05	0.51	175.4±2.1	0.014
165	0.0002	0.61	193.1±5.5	0.025
270	0.05	0.50	282.2±8.9	0.037
270	0.01	0.45	289.3±5.4	0.028
270	0.001	0.66	298.2±4.5	0.026
480	0.01	0.22	579.5±7.6	0.055
480	0.001	0.42	567.8±44.2	0.062
800	0.05	0.28	931.1±49.1	0.089
800	0.005	0.16	915.7±104	0.321

Table 2

Sample	Concentration	Cuvette			Hanging Drop		
	(wt %)	β	Size (nm)	PDF	β	Size (nm)	PDF
BSA	2.0	0.60	7.28±0.18	0.066	0.66	7.08±0.18	0.089
BSA	4.0	0.58	7.20±0.20	0.033	0.55	7.12±0.22	0.079
BSA	6.0	0.65	7.16±0.16	0.066	0.56	6.88±0.18	0.124
BSA	8.0	0.62	6.98±0.18	0.055	0.67	6.94±0.14	0.081
BSA	10.0	0.60	7.18±0.08	0.052	0.67	6.92±0.12	0.097
20 nm PS	10.0	0.62	17.1±0.2	0.477	0.61	22.4±0.5	0.451
43 nm PS	0.5	0.56	39.0±0.4	0.005	0.58	42.7±3.6	0.149
85 nm PS	0.05	0.58	80.8±2.4	0.088	0.62	80.0±3.3	0.068
165 nm PS	0.05	0.58	165.7±10.0	0.094	0.62	166.6±8.0	0.062
270 nm PS	0.05	0.60	282.9±12.7	0.074	0.57	286.3±17.7	0.066
480 nm PS	0.01	0.36	533.9±42.5	0.256	0.21	572.6±59.1	0.171
800 nm PS	0.05	0.28	951.2±179.5	0.170	0.30	1056±194.8	0.005

3.1. Effects of laser power and experiment duration

The nucleation and growth phenomena during temperature-induced protein aggregation or crystal growth can be very fast (~few seconds), thus fast and reliable non-invasive and quantitative techniques to study this phenomena will be very useful. For this reason we tested the effect of data acquisition time (experiment duration) and the incident laser power using

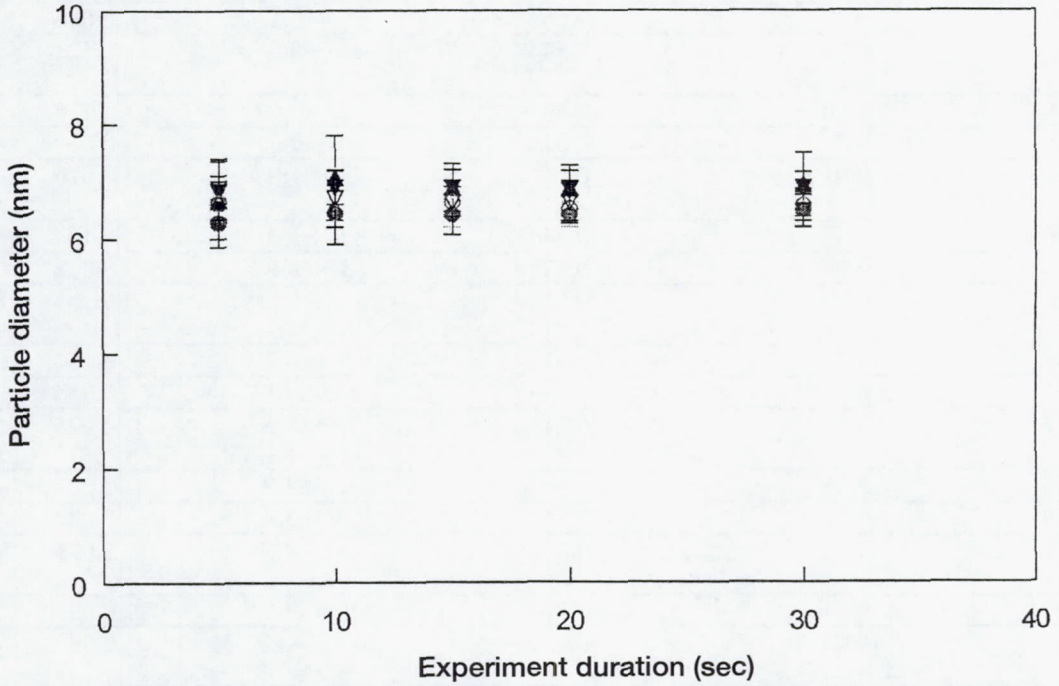

Figure 3.—Characterization of BSA proteins using method of cumulants. ▲2%, 0.2 mW; ▼6%, 0.2 mW; ●10%, 0.2 mW; △2%, 1.0 mW; ▽6%, 1.0 mW; ○10%, 1.0 mW;

our fiber optic probe and evaluated its reliability in yielding correct particle sizes. Figure 3 shows the sizes of BSA protein solutions of three different concentrations from 5 seconds to 30 seconds duration at two power levels of 0.2 mW and 1.0 mW. Particle sizes were obtained using cumulant analysis. These measurements clearly demonstrate consistent particle size values. The rms error (test[33]) values obtained from fitting the raw TCF data to the form (method of cumulant[29]) of a second order polynomial is plotted in Figure 4. As expected, the rms error values are slightly higher for low concentration samples at lower laser power and shorter data collection times. But in all cases they yield correct recovery of the particle sizes as shown in Figure 3. Figures 5-8 show TCF's obtained for two different concentrations of BSA samples at two power levels of 0.2 mW and 1.0 mW at 5 and 30 seconds duration. This data is analyzed using CONTIN[34] to obtain particle size distributions. The corresponding size distributions are shown as insets in Figures 5-8. In all cases the experimentally determined size distributions at both low laser power levels and extremely low data collection times, are identical.

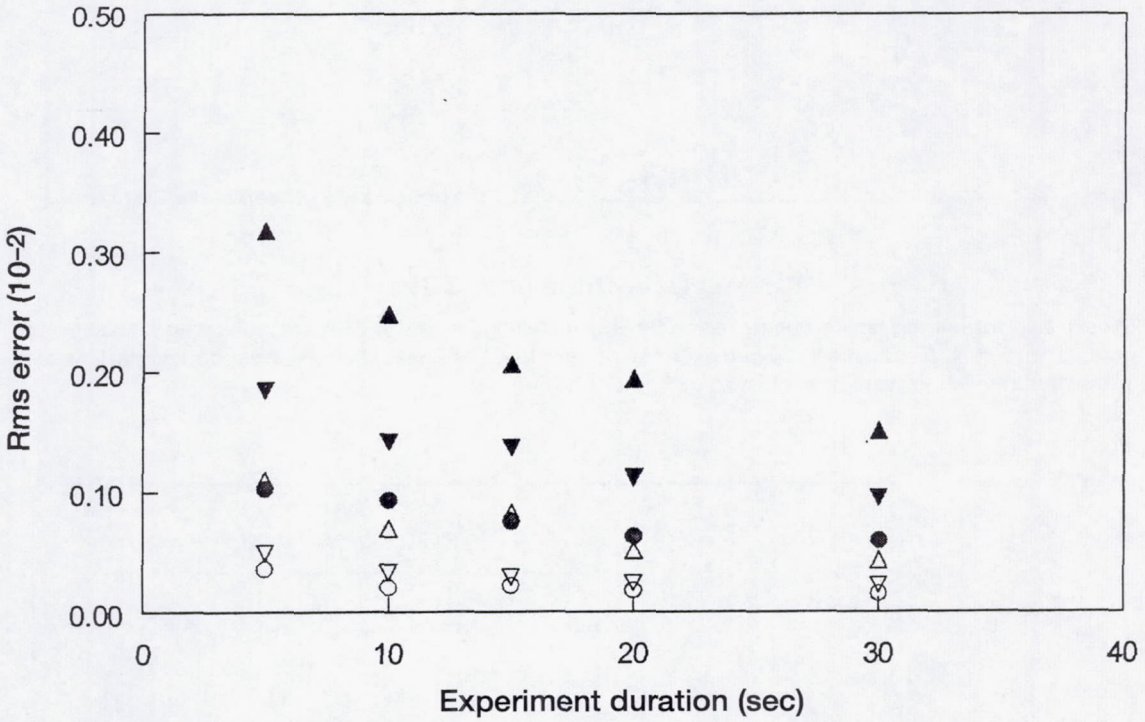

Figure 4.—Effect of experiment duration and laser power on the quality of DLS data. ▲2%, 0.2 mW; △2%, 1.0 mW; ▼6%, 0.2 mW; ▽6%, 1.0 mW; ●10%, 0.2 mW; ○10%, 1.0 mW;

3.2. Vibration Effects

DLS measurements normally require vibration isolation. Experiments reported in this paper did not use any optical tables or vibration isolation. The experiments were conducted on a regular laboratory bench top. We do not find any adverse effect on our droplet measurements due to any mechanical vibrations (e.g. vibrations from the floor, vents, etc.) or due to evaporation of liquid from the liquid-air interface. The ability to conduct reliable particle size measurements without vibration isolation makes these experiments very cost effective and opens new possibilities for conducting experiments involving containerless processing in space and in challenging industrial/field environments on earth.

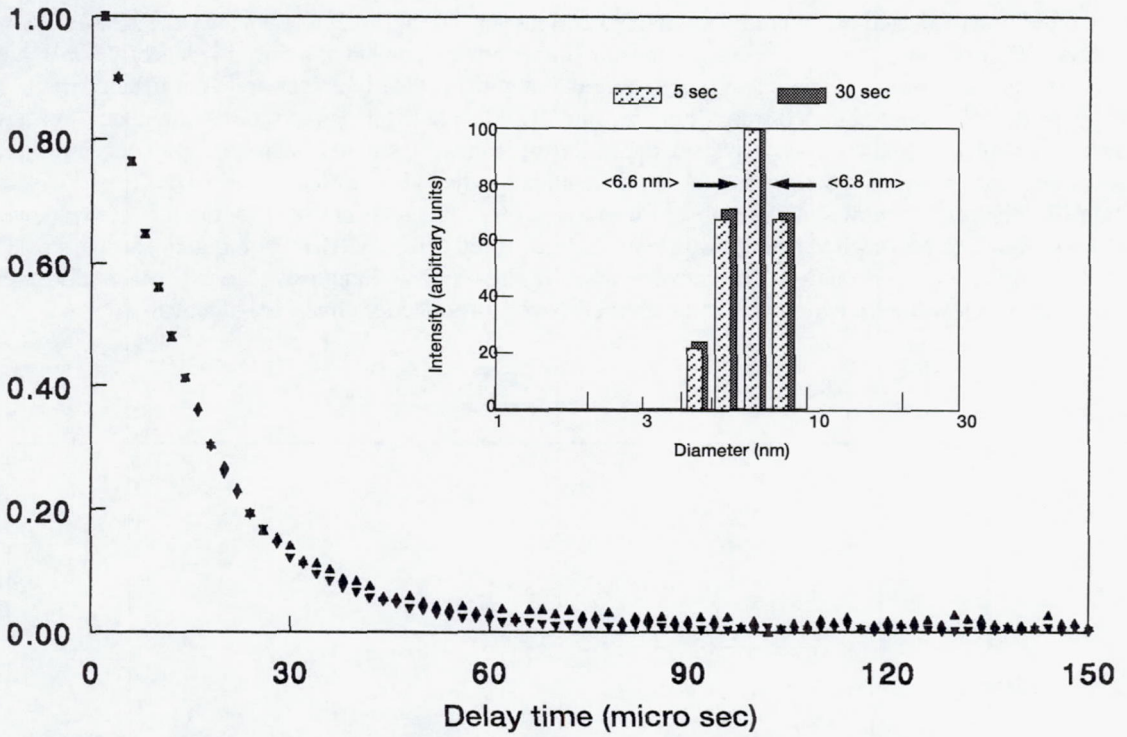

Figure 5.—Normalized time autocorrelation functions (TCF's) for a 10% BSA solution using a laser power of 0.2 mW at ▲ 5 sec and ▼ 30 sec experiment duration. The inset shows corresponding particle size distribution with <mean> particle size using CONTIN.

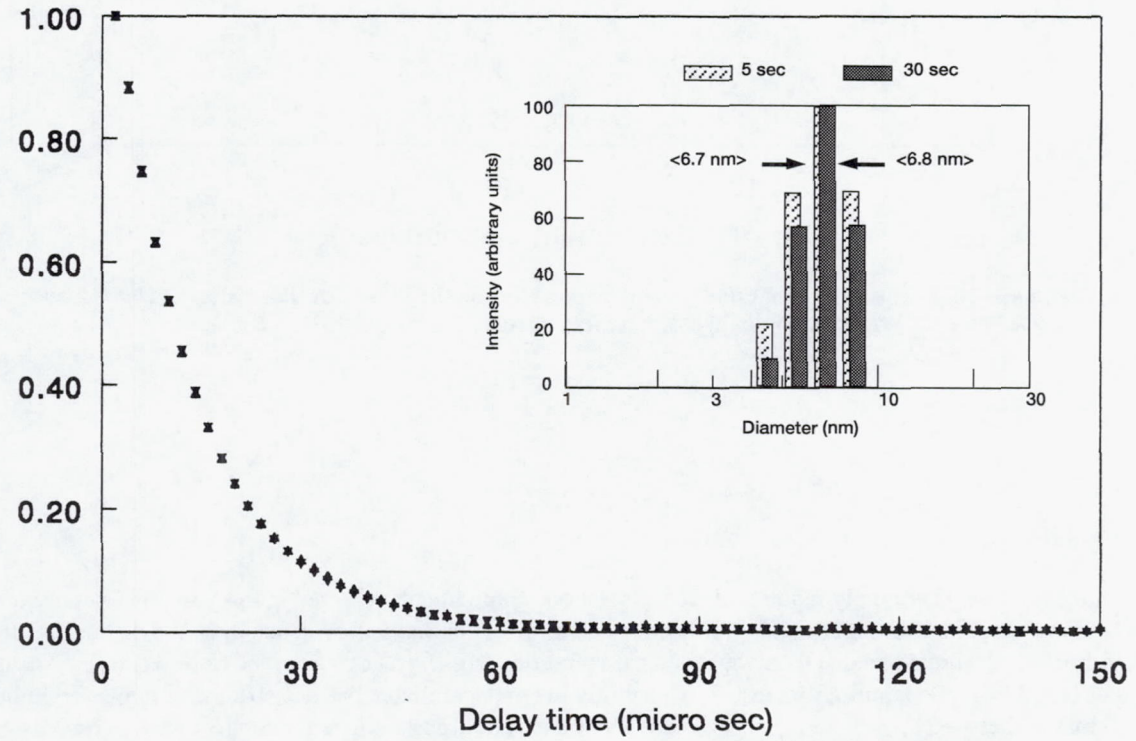

Figure 6.—Normalized TCF's for a 10% BSA solution using a laser power of 1.0 mW at ▲ 5 sec and ▼ 30 sec durations. The inset shows corresponding particle size distributions.

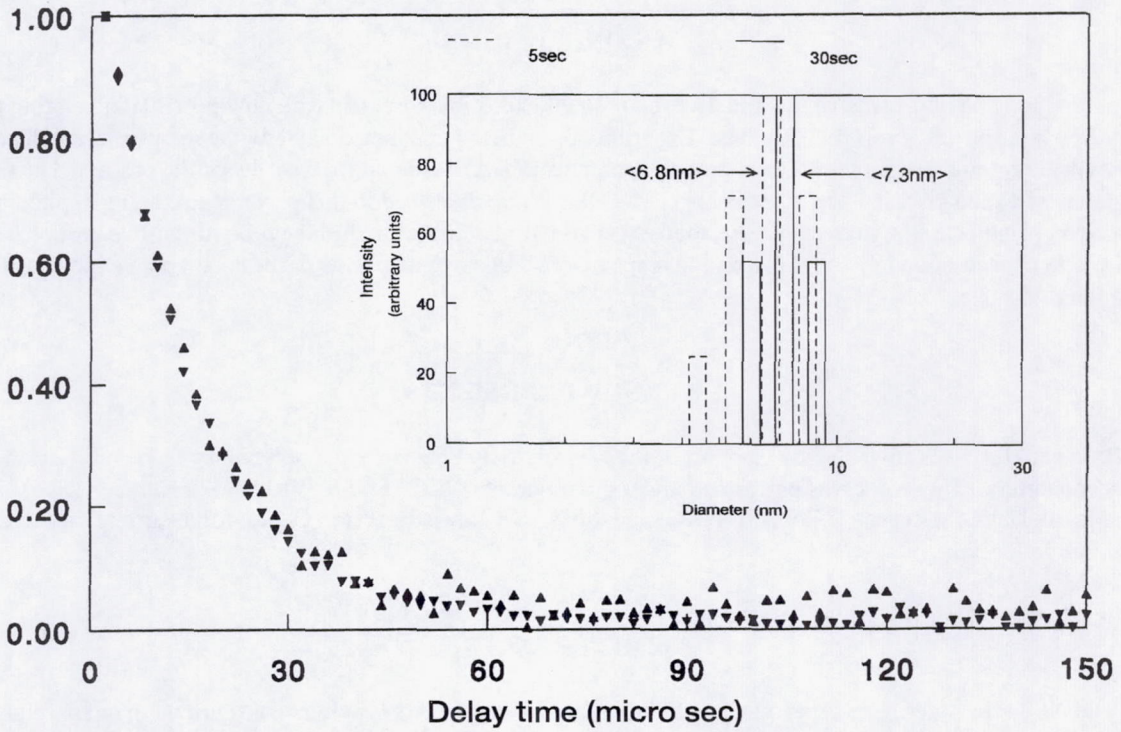

Figure 7.—Normalized time autocorrelation functions (TCF's) for a 2% BSA solution using a laser power of 0.2 mW at ▲5 sec and ▼30 sec experiment duration. The inset shows corresponding particle size distribution with <mean> particle size using CONTIN.

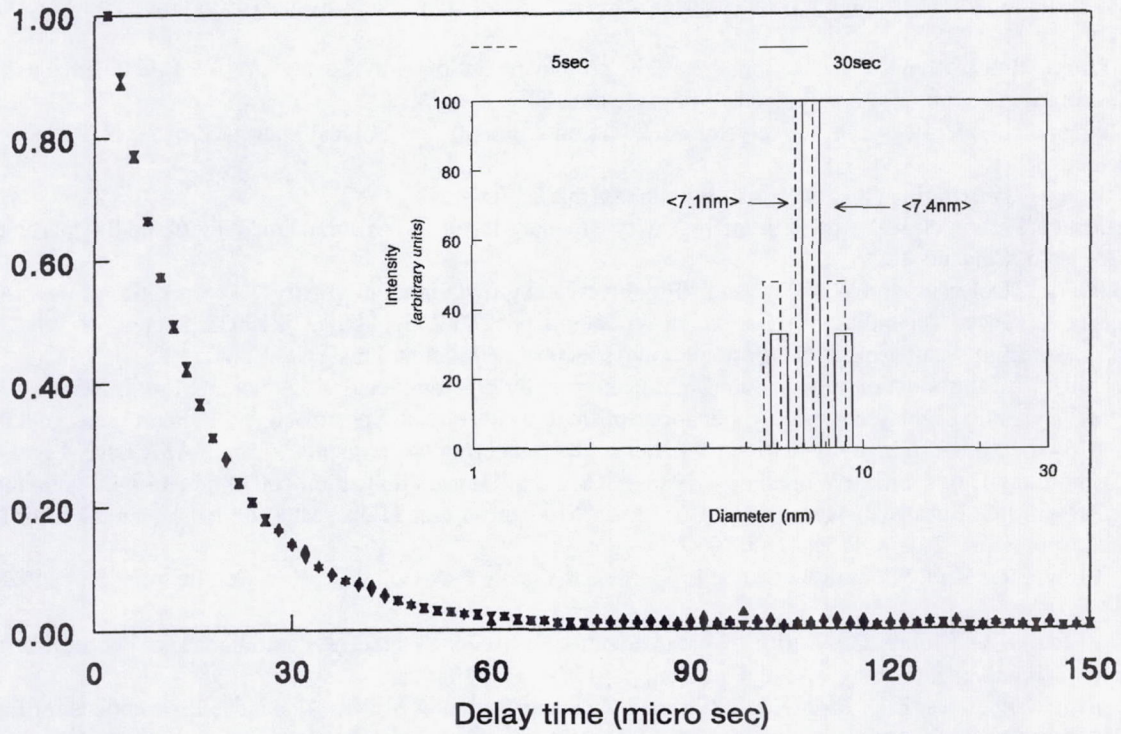

Figure 8.—Normalized TCF for a 2% BSA solution using a laser power of 1.0 mW at ▲5 sec and ▼30 sec experiment duration. The inset shows corresponding particle size distributions.

4. CONCLUSION

We conclude that non-invasive, quantitative, and reliable DLS measurements are now possible in hanging protein drops at very low laser power levels in 5 seconds. Experiments are being conducted to study the temperature induced aggregation in lysozyme proteins in a protein crystal growth apparatus (VDA) designed for a space shuttle orbiter. The results of this investigation will soon be discussed elsewhere[36]. The DLS probe described in this paper is also being applied to study aldolase proteins in small capillary cuvettes. We soon expect the implementation of this probe for on-line particle size measurements in another space-bound material dispersion apparatus (MDA)[37]. The results of these on going experiments will be reported at a later date.

5. ACKNOWLEDGMENTS

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13. ABSTRACT ***(Maximum 200 words)***

We report non-invasive particle size measurements of polystyrene latex colloidal particles and bovine serum albumin (BSA) protein molecules suspended in tiny hanging fluid drops of 30 μL volume using a newly designed fiber optic probe. The probe is based upon the principles of the technique of dynamic light scattering (DLS). The motivation for this work comes from growing protein crystals in outer space. Protein crystals have been grown previously in hanging drops in microgravity experiments on-board the space shuttle orbiter. However, obtaining quantitative information on nucleation and growth of the protein crystals in real time has always been a desired goal, but hitherto not achieved. Several protein researchers have shown interest in using DLS to monitor crystal growth process in a droplet, but elaborate instrumentation and optical alignment problems have made in-situ applications difficult. We demonstrate that such an experiment is now possible. Our system offers fast (5 seconds) determination of particle size, utilize safe levels of very low laser power (≤0.2 mW), a small scattering volume (~2 x 10^{-5} mm^3) and high spatial coherence (ß) values. This is a major step forward when compared to currently available DLS systems.

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