DLS MICRORHEOLOGY OF BIOPOLYMER SOLUTION, PROBE SURFACE EFFECT

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Abstract

Structures of biopolymers play important role for its industrial applications and affect their potential usability. The more information about the micro and macrostructure of the sample the better is evaluation of potential behavior of the sample and suitability of use. For microstructure determination we used new approach – passive microrheology method by utilizing dynamic light scattering device and fluorescence correlation spectroscopy (FCS) with microrheology probes to characterize properties of the sample. Three different particles with unlike particle surfaces were chosen to determine the most suitable one for biopolymer solutions measurements. For estimating possible probe – matrix interactions, which are undesirable for microrheological measurements, zeta potential of particles in the solutions was studied. Data from DLS microrheology were compared with FCS nanorheology measurements.

Our results show importance of appropriate particle choice, with an emphasis on the particle surface chemistry. The most appropriate particles for biopolymer solutions are polystyrene particles without any surface modification or also polystyrene particles with –COOH groups that avoid particle – biopolymer interaction. DLS and FCS microrheology data exhibit good agreement and provide wide frequency range data.

Key words:

Diffusing light spectroscopy, microrheology, microstructure, zeta potential, fluorescence correlation spectroscopy

1. INTRODUCTION

Biopolymers are sustainable, renewable and some of them biodegradable materials. For these unique properties, biopolymers are used and studied worldwide. Knowing structure of the sample can lead for better understanding its behavior and potential usage. Macrostructure of the sample is usually measured via classical rheometer. Nowadays new group of techniques were developed to measure microstructure of the sample, which can be diametrically different from the sample macrorheology. These methods are called microrheology. Their principle lies in the small particles embedded into the material and from their movement one can calculate viscoelastic properties of material and characterize its microstructure based on the particle size. Microrheology can be divided into two groups based on driving force that moves with the particle [1,2,3,4]. Passive microrheology group utilizes thermal motion of probes based only on the particle size, temperature and viscosity of the surrounding microenvironment. On the other hand, active microrheology needs external force like electric or magnetic field or optical tweezers to manipulate with particles. Our experiments were performed by DLS [5] and FCS [6] microrheology methods, which belong to passive microrheology group.

Generally, passive techniques provide advantages for measuring weakly structured materials which have low values of predominantly viscous moduli, whereas active techniques better facilitate measurements on materials which have significant elastic properties.

A key requirement of microrheology experiment is to minimize particle-biopolymer interaction, since the existence of physical or chemical interactions of the embedded probe with matrix can alter the local material environment and affect diffusivity in a measureable way. Ensuring an appropriate choice of particle surface

chemistry is an essential element of method development for DLS Microrheology measurements for a particular complex fluid.

1.1 Dynamic Light Scattering (DLS) microrheology

Dynamic Light Scattering is method also called Photon correlation spectroscopy (PCS) or Quasi-Elastic light scattering (QUELS) and it is a common method for particle size measurements in sub-micron scale [1].

DLS microrheology is able to offer significant advantages of weakly-structured, low viscosity complex fluids since it offers wider frequency range compared to classical rheology method. Next advantage lies in small sample volume (microliter scale) required for one measurement that enables rheological characterization of biological, expensive or hard to reach materials such as proteins. On the other hand, this method is not suitable for heterogeneous and opaque samples, because it requires single scattering of the incident beam.

For particles moving due to thermal energy in a Newtonian fluid, mean squared displacement (MSD) as a function of time increases linearly, and diffusion coefficient can be determine from the slope:

$$(\Delta x^2(t)) = 2dD\tau^{\alpha},$$

where *d* is dimension, *τ* is time and *α* is time exponent. According to the Stokes-Einstein equation one can calculate viscosity of the material if the size of the particle and temperature is known [3].

$$D = \frac{k_B T}{6\pi\eta a},$$

In case of non-Newtonian fluid, function MSD(t) is not linear and sample properties has to be characterized using viscous and elastic moduli as a function of frequency, which can be determine from MSD generalized Stokes-Einstein equation.

In DLS microrheology, the autocorrelation function (ACF) of the light scattered by particles undergoing thermally driven motion within a material under study is measured. ACF can be written as a function of the MSD, and can be extracted from this function:

$$g_1(\tau) = g_1(0)exp[-q^2\langle\Delta r^2(\tau)\rangle/6]$$

where $g_1(0)$ is the value of the autocorrelation function at zero time and *q* is the scattering vector.

2. MATERIALS AND METHODS

Hyaluronic acid HA (750 kDa) was chosen as a biopolymer. Two different concentrations of HA (1 g/l and 5 g/l) were prepared. Three different microrheology probes were used for DLS measurements – polystyrene particles (PS), 1 μm, polystyrene particles –COOH coated,1 μm, SiO_2-NH_2 coated particles, 1.16μm.

DLS and FCS microrheology techniques were used to perform microrheological experiments. Its scheme is visible in figure 1 and figure 2. The main difference of these methods is sample volume needed for measurements. For DLS around 500 μl and for FSC it is much less (usualy one droplet – 10 μl) is used.

Whilst FCS probes the local rheological properties of materials on length scales of the focus dimension of the confocal microscope (around 1 fl), length scales measured by DLS are higher (comparable with microrheology probe size).

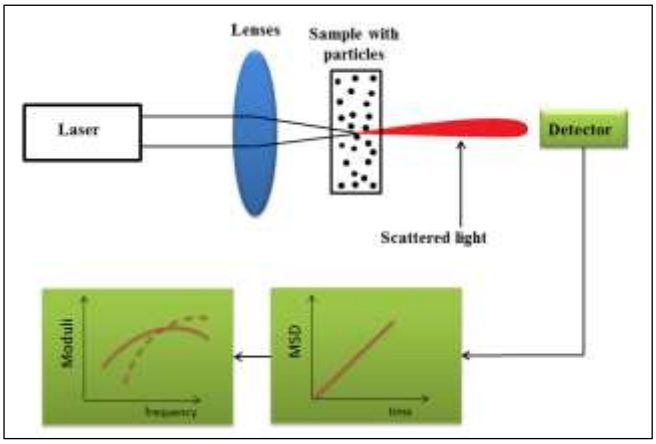

Figure 1: Scheme of DLS [7]

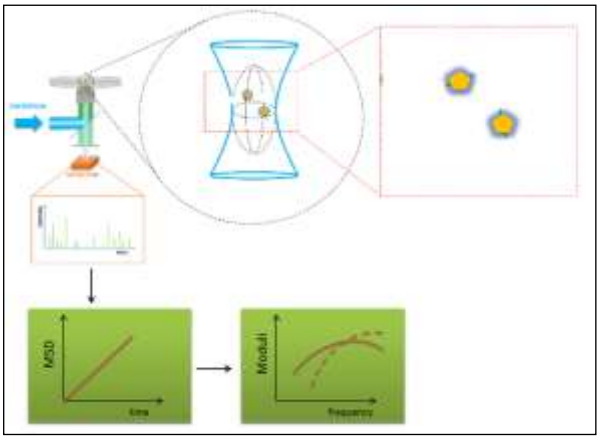

Figure 2: Scheme of FCS [5,8]

3. RESULTS AND DISCUSSION

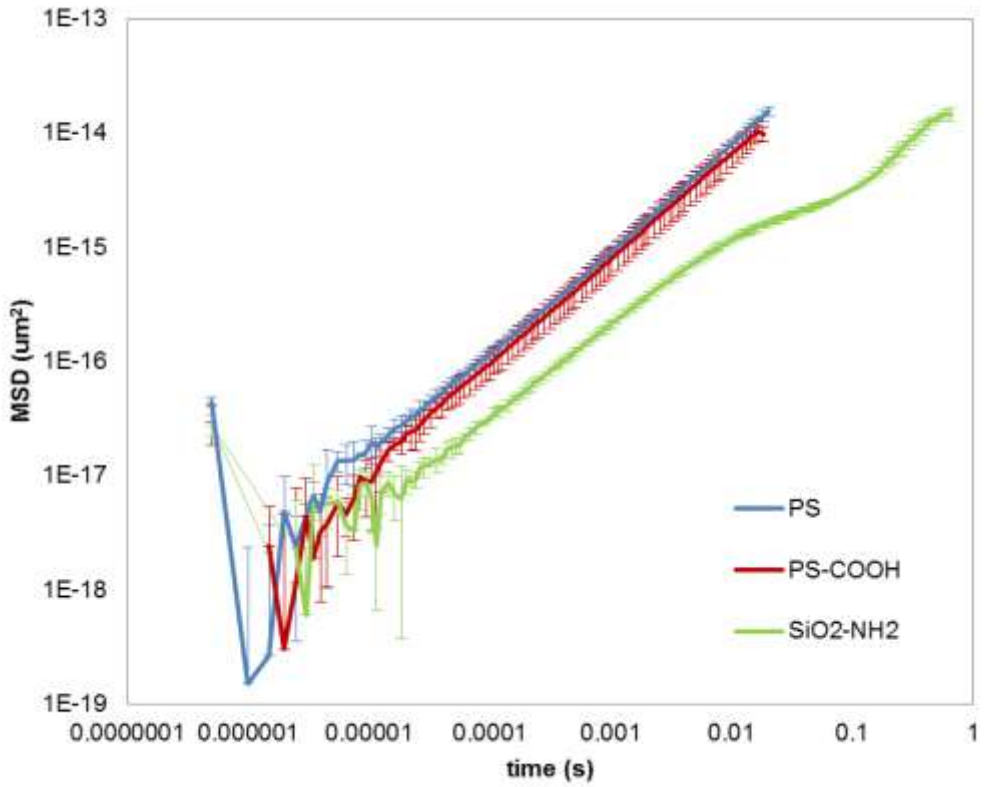

Figure 3: MSD of HYA solution 1 g/l for 3 different particle surfaces

In figure 3 and 4, substantial changes in MSD(t) are observable for different particle surfaces in case of 1 g/l and 5 g/l hyaluronic acid solution. Efect of hyaluronic acid – particle interaction is most noticeable for SiO_2-NH_2 probes. Consequently, these particles are not good choice for biopolymer solutions measurements. This phenomena is supported by zeta potential measurements showing biggest change for SiO_2-NH_2 particles in

water and in hyaluronic acid solutions (Table 1.). The lowest change in zeta potential was observed for polystyrene particles, which are supposed to be inert as well as negatively charged particles repelling negative hyaluronic acid groups. Whereas positively charged SiO_2-NH_2 particles exhibit considerable changes in zeta potential, confirming adverse interaction [9,10].

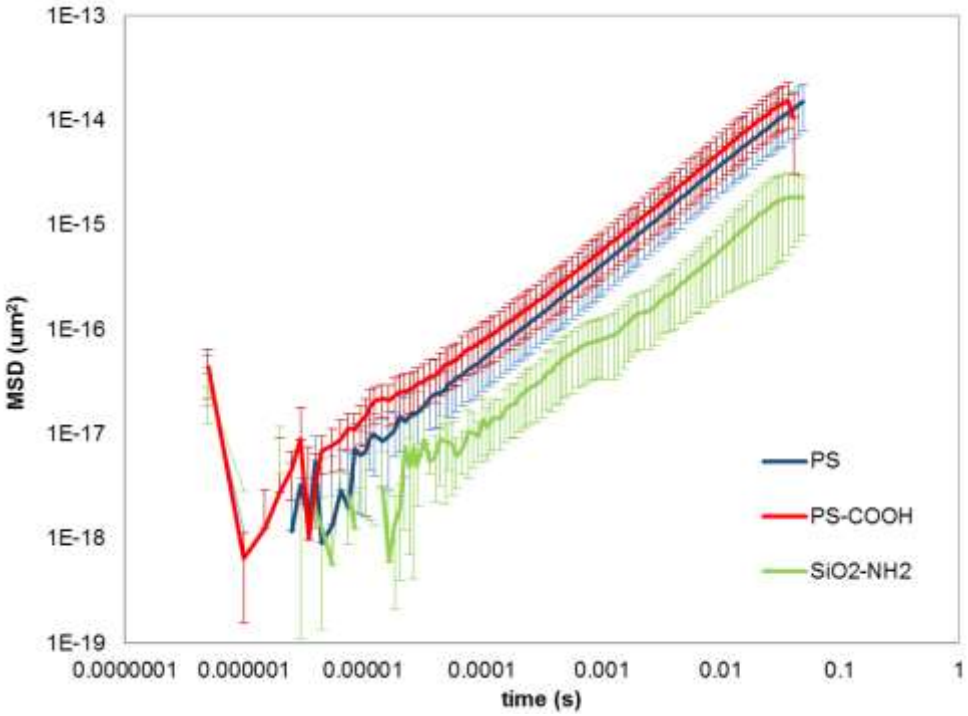

Figure 4.: MSD of HYA solution 5 g/l for 3 different particle surfaces

Larger error bars are drawn in figure 4 for 5 g/l hyaluronic acid solution. This effect is probably caused by the hyaluronic acid solution microenvironment, which is more heterogeneous in case of 5 g/l than 1 g/l.

Table 1: Zeta potentials for HA solution 1 g/l, Mw = 750 kDa

Particles	**Zeta potential of particles in water**			**Zeta potential in HA**		
PS	-16.8	-18.4	-18.4	-21.8	-24.7	-23.2
SiO_2-NH_2	39.4	31.6	30.4	-41.7	-41.8	-42.4
PS-COOH	-19.1	-18.8	-21.6	-52.5	-54.5	-54.7

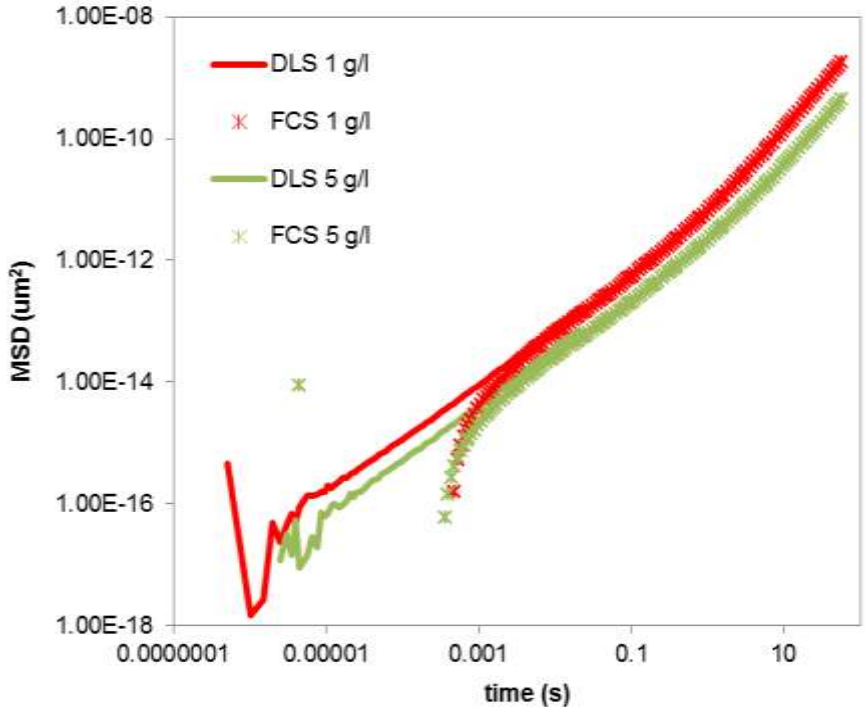

Figure 5: MSD of HYA solution 5 g/l and 1g/l measured by DLS and FCS microrheology using PS particles (100 nm)

Comparison of DLS and FCS microrheology is shown in figure 5. Frequency scale of DLS microrheology is located in lower values (1E-5; 1E-1). FCS data follow values of DLS measurement in higher frequency range (1E-2; 1E2). Combination of these two microrheology method can lead to obtain sample information in a very wide frequency range.

4. CONCLUSION

Possible utilization of different microrheology probe material and surface chemistry to study viscoelastic properties of viscoelastic and low viscosity materials using FCS and DLS passive microrheology methods has been demonstrated. It has been shown that positive particle surface is not suitable for biopolymers solution measurements due to strong particle – biopolymer interaction. This claim was supported by zeta potential measurements and this phenomenon was observable for both concentrations of hyaluronic acid solution. Combination of DLS and FCS microrheology measurement can lead to obtain desirable wide frequency scale data.

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