Optical microrheology measurements for determination of sol-gel transitions of chitosan hydrogels

P Domagalski, M Orczykowska, P Owczarz and M Dziubinski
Faculty of Process and Environmental Engineering, Lodz Technical University, ul. Wolczanska 213, 93-005 Lodz, Poland

Abstract. The combination of medicinal substance and an appropriate polymer matrix with specially adjusted permeability has become a basic form of medicine in the controlled drug delivery. One of main solutions used recently in pharmacology is the application of polymer matrices which form colloidal systems. The sol-gel phase transition is inhibited by a change of pH or temperature. Thermosensitive systems are produced from polymers whose sol-gel transition in water solutions depends on temperature. Thermosensitive hydrogel systems are currently obtained mainly from poly(N-isopropylacrylamide) (PNIPA), copolymer (polyethylene oxide – polypropylene oxide – polyethylene oxide), polyethylene glycol, lactic acid copolymer with glycolic acid and polyethylene glycol, and polysaccharides (xyloglucane, ethylcellulose, hydroxyethylcellulose and chitosan). At the temperature below 25°C these systems occur in the form of sols which at human body temperature are transformed into gels. Such systems belong to the so called intelligent ones and are a subject of special interest, particularly in view of their possible use as drug carriers and also as material for cell culture in the past.

Keywords: passive optical microrheology, chitosan, sol-gel transition

1. Introduction
At present, when ecology is given much attention in industrial production, of special interest is the use of natural polymers in the formation of pH- and thermally-sensitive colloidal systems. Such polymers include chitin derivative, N-acetyl-D-glucosamine, called chitosan. Chitosan is a polymer insoluble in water and organic solvents. However, in the form of salt it shows high solubility. The most frequently used solvents are diluted organic acids, and the main solvent is acetic acid. Hydrochloric acid is also quite often applied as a solvent for chitosan.

Acid solutions of chitosan salts show the ability of sol-gel transition under environment (temperature or pH) change. Such ability allows for using the chitosan and its thermogels as polymer matrix carrier of active bio-compounds like medicines. However, for use of such carrier in vivo, the precise knowledge on lower critical solution temperature (LCST) is needed.

Position of the critical solution temperature results from an unstable equilibrium between hydrophilic and hydrophobic interaction of a polymer with water and depends on the content of hydrophilic and hydrophobic elements in a polymer molecule, their distribution, topology as well as molecular mass and presence of low-molecular substances in the solution. Below the phase transition temperature, a macromolecule is retained in the solution owing to the salvation zone. It is formed as a result of formation of hydrogen bonds between hydrophilic fragments of polymer chain and water molecules. A temperature increase weakens the hydrogen polymer-water bonds and promotes hydrophobic polymer-polymer interactions (dipole, hydrogen, van der Waals forces, etc.). Precipitation of the polymer from the water solution at increased temperature is accompanied by an increase of the system entropy. As a consequence, the chain structure changes into a ball, phase separation occurs, and in the case of a 3D polymer network a volumetric phase transition is observed.
Determination of the lowest critical solution temperature (LCST) proceeds by way of rheological measurements, i.e. determination of the point of intersection of $G'$ and $G''$ as the function of oscillation frequency $\omega$ or by determining a dramatic increase in viscosity of the system investigated during its heating.

Rheological measurements are dependent on volume of sample. In classical rheometry with coaxial cylinders or cone and plate measurement systems volume of sample is equal from several to several dozen millilitres. In pharmacological practice such a volume of samples is practically unattainable and simultaneously cost of their production is very high. During rheological measurement in rotational rheometer structure of investigated media is partially deformed (destroyed) according to acting on sample mechanical forces.

In addition, the dynamic nature of measurement, where the structure of investigated medium is deformed due to shear inducted by measurement’s system rotor results in both temperature and mechanical effects responsible for destroying the building up structure.

The above measurement problems can be solved by use of passive optical microrheology.

1.1. Aim of work

Due to huge amount of possible hydrogels and their derivatives they represent vast spectrum of properties. Rheology can quickly provide information on gel structure changes like sol-gel transition, therefore can act as primary, very effective characterization techniques. The main aim of presented study was determination of lower critical solution temperature LCST and transition kinetics of chitosan gel by high precision classical rotational rheometry and passive optical microrheology and comparison of results obtained by those two different rheological techniques.

2. Experimental

As an experimental media Shrimp chitosan -based matrix (average molecular weight Mw 862 kDa and degree of deacetylation 83.4 DD) was used. A method to form chitosan thermogels based on solutions of chitosan salts in 0.1 M hydrochloric acid using sodium $\beta$-glycerophosphate as a neutralizing agent has been developed [1-5].

Rheological properties of chitosan gel were determined by Physica MCR 301 Anton Paar rotational rheometer using cone and plate system. The angle of 50 mm cone diameter was equal 1 deg. Investigations were carried out in four temperatures 25, 30, 35 and 40°C and range of oscillation frequency $\omega$ from 0.01 to 500 s$^{-1}$ with constant amplitude $\gamma$ equal 1%.

The measurements were performed by placing the chitosan salt solution sample in measurement gap at temperature of 5°C and increasing the temperature to desired lever of 25, 30, 35 or 40 °C with speed of 10°C/s.

The passive microrheology techniques relies in general on tracking the Brownian motion of fluorescent tracer particles suspended in investigated medium. The rheological parameters are calculated based on mean square displacement MSD of tracer evolution with time and generalized Stokes-Einstein equation [6].

$$\tilde{G}(s) = \frac{k_BT}{\pi \alpha (\Delta r^2(s))}$$ (1)

where $k_B$ is Boltzmann constant, T is temperature, $\alpha$ is the radius of the tracer particle, $\tilde{G}(s)$is the Laplace transformed complex shear modulus, s is the Laplace frequency and $\Delta r^2(s)$ is Laplace transformed mean square displacement (MSD) defined as:

$$\langle MSD \rangle = \langle \Delta r^2(\tau) \rangle = \langle (r(t + \tau) - r(t))^2 \rangle$$ (2)

where $r$ is particle position and $\tau$ is the lag time.

The passive microrheology measurements were carried out in temperature range between 39 and 45 °C on Leica DMI3000B inverted microscope equipped with HCX PL FLUOTAR 100x/1.30 oil immersion objective. The light was provided by Thorlabs Mounted High Power LED (470 nm, 830 mW) and filtered/splitted on Leica I3 (BP 450–490/510/LP 515) filtercube. The images were recorded by Lumenera Infinity 1 camera (1.3 MPix, ½”CMOS). Polystyrene, fluorescent yellow-green tracers
of 100 nm diameter were used (Sigma Aldrich, Poland). The temperature was controlled by Anton Paar Viscotherm V2 & PZO Poland temperature control system. Data processing was done in MATLAB (MathSoft, USA) using PolyParticleTracker script [7].

3. Results

Experimental values of storage $G'$ and loss modulus $G''$ for chitosan gels obtained in different temperatures in rheometrical measurements (rotational rheometer Anton Paar) are presented on fig.1.

![SPECTRE OSCILLATING at 25 °C](image1)

![SPECTRE OSCILLATING at 35 °C](image2)

![SPECTRE OSCILLATING at 30 °C](image3)

![SPECTRE OSCILLATING at 40 °C](image4)

Fig. 1. The frequency-dependent storage $G'(\omega)$ and Loss Modulus $G''(\omega)$ for investigated hydrogel in different temperatures.

Presented on Fig. 1. graphs show that in 25-35°C the investigated chitosan salts solutions present non-Newtonian character with dominating viscous effects. Measurements performed at 35°C show however the sample viscoelastic properties, indicating the polymer network build up due to temperature rise.

The obtained values of complex viscosity and initial results of optical microrheology measurements are presented on fig. 2.
Fig. 2. Comparison of complex viscosity (Anton Paar) with optical micro rheology results.

Visible at fig. 2 abrupt change in the complex viscosity in case of classical rotational rheometric measurements and mean squared displacement <MSD> in case of initial results of optical rheology measurements reflects the material mechanical parameters changes due to structural sol-gel transition.

4. Conclusions
As we show the passive optical microrheology can provide efficient way for lower critical solution temperature determination of sol-gel transition as the sample volume (order of μl) of expensive chitosan gel can be reduced by order of magnitude compared with classical rheological techniques, therefore the heat inertia and temperature control issues can be minimized. Another advantage of passive optical microrheology is easiness to maintaining sample sterility. This, together with low equipment cost, time of analysis of order of seconds and the potential of automated sample handling proves that optical microrheology is a very versatile and powerful rheological technique.

Acknowledgements
Work was supported by The National Science Centre (project N N209 764640)

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