AGGREGATION AND DEGRADATION PROCESSES OF BIOPOLYMERS AND BIOCOLLOIDS STUDIED BY LIGHT SCATTERING TECHNIQUES

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Abstrakt

The stability of natural substances is very important parameter, which nowadays still lacks for deeper research. The main aim of this work was to shed a new light in the area of natural substances ongoing aggregation and degradation processes especially in longer time scale. Any deviation of these natural substances, which are often used in industrial productions, as final products for distribution to customers or just in long-term research, can cause serious problems such as different yield of product, bad purity or unfavourable property of obtained product, or in the case of research wrong result. All these effects are of course highly undesirable. This contribution presents light scattering techniques as easy and universal methods for studying of aggregation/degradation processes and time stability of selected group of biopolymers (bovine serum albumin, carboxymethyl cellulose, glucose, hyaluronic acid, sucrose) and biocollods (humic acids). The experimental work and obtained results indicated suitability of all presented light scattering methods for purposes of discussed effects. The results also showed good correlation between individual light scattering techniques. Combination of different light scattering techniques caused the results became more reliable, which was of course desirable.

Klíčová slova:

biopolymers, biocolloids, light scattering, molecular weight, particle size, zeta potential

1. INTRODUCTION

Biopolymers are natural macromolecules, which are composed of large number of repetitive monomeric units linked together by covalent bonds. The supramolecular structure, which is essential for proper functions of these substances in nature, is mainly stabilized by non-covalent interactions. On the other side biocolloids can be described as dispersions of natural substances with particle sizes from the colloidal scale (1 nm – 1 μm). Contrary to biopolymers, biocolloids does not contain in their molecules regularly repeating unit – so called "mer". Similarly to biopolymers, supramolecular structure of biocolloids can be formed using non-covalent interactions [1,2,3].

Structure and conformation of biopolymers as a specific group of naturally formed polymers can be characterized by large variety of analytical methods. Typical and often used and discussed example is nuclear magnetic resonance, infrared spectroscopy or liquid chromatography [4]. There are only few methods that find utilization exclusively in the field of biopolymer chemistry, such as light scattering, dilute solution viscosimetry, size exclusion chromatography and flow field fractionation. The most fundamental parameters obtained by these methods are molecular weight (and/or molecular weight distribution) and particle size (and/or particle size distribution) [5]. These parameters supplemented by zeta potential and mobility in the case of proteins and peptides are the main parameters used for characterization of natural substances such as proteins, polysaccharides, oligonucleotides and antibodies. Biological macromolecules are often synthetized in precisely defined molecular weight and narrow particle size distribution, allowing almost perfect monodisperse solutions. These systems are perfect for characterization using dynamic light scattering (DLS). DLS can be essential for studying of the size and conformation of

proteins, nucleic acids, polysaccharides, lipids and their supramolecular assemblies [6,7]. Electrophoretic light scattering can support the results obtained from particle size distribution analysis with the information about the electrostatic stability of studied system, which is often desirable. Application of light scattering techniques (dynamic, static and electrophoretic light scattering) for studying of the time stability and ongoing aggregation/degradation processes on selected group of biopolymers (bovine serum albumin, carboxymethyl cellulose, hyaluronic acid, glucose, sodium alginate, sucrose) was one of the main goal of this contribution. The main advantage of application of light scattering techniques is the fact, that these methods are non-destructive to the sample and the analysis is relatively fast and low cost.

The second part of this work was dealing with colloidal dispersions, which are nowadays well-known and indispensable part of our lives. These systems are widespread in many areas such as medicine, food and beverage industry, chemistry of paints and inks, fertilizers etc. As an example of natural colloidal system studied in this work humic acids were chosen. Humic acids are remarkable natural compounds, which play significant roles in natural processes [1,8]. This fact hand by hand with their colloidal size, rich natural availability and relatively low-cost extraction techniques create from them extremely important material for practical applications. Similarly to biopolymers particle size and shape as well as molecular weight can be listed as main parameters that must be taken into account in the case of investigation of the structure, reactivity and also in the possible future application of this natural material. Application of light scattering techniques nowadays widely used for characterization of biopolymers can shed a new light also in the area of humic acids behaviour in aqueous solutions, ongoing aggregation processes and their stability in time. This area of research is still encountered by few experimental difficulties mainly caused by the heterogenic and polydisperse character of humic material.

The literature provides publications dealing with utilization of different methods and also acting of different parameters and modifications on protein and polysaccharides stability [4,5,9]. But almost nothing can be found in the case of studying of the time stability, ongoing aggregation/degradation processes and aging of the biopolymers itself. Practically the same can be told about humic acids. Many articles are dealing with characterization, reactivity or modifications of humic acids [10,11,12,13], but nothing can be found about time development of the stability of humic acids. All these mentioned substances are often used in many industrial application and synthesis or just as studied compounds in many researches. Degradation or aggregation processes running in time can lead research to incorrect results or in the case of synthesis can lead to bad purity or even to another product etc…

Lack of the stability information of biopolymers and biocolloids lead us to thing about this phenomenon deeply. The main objective of this study was the utilization of light scattering techniques as simple controlling methods for studying of degradation and aggregation processes in "bio" substances.

2. MATERIALS

Experimental part of this work was studying aggregation/degradation processes of chosen biopolymers and biocolloids systems. Utilized biopolymers from the group of polysaccharides were carboxymethyl cellulose (CMC), glucose, hyaluronic acid, sodium alginate and sucrose. As an example of the group of proteins, bovine serum albumin (BSA) was used. Studied biomaterials were dissolved in millipore distilled water. Concentration of biopolymers solutions was 0.1, 1, 2 and 5 $g \cdot dm^{-3}$. After overnight stirring the samples were filtered using 0.45 μm syringe filters. All studied samples were used in p.a. purity grade.

As an example of biocolloids, humic acids (HA) were chosen. HA studied in this work originated from South-Moravian lignite. Details on the process of extraction and purification of HA can be found elsewhere [10,11]. For preparation of humic sols three different methods were used. First method was according to International Humic Substances Society (IHSS) – solid HA were dissolved in 0.1 M NaOH [14]. In second method (sol B), solid HA were dissolved in millipore distilled water and the pH of the sol was adjusted to 12 [15]. For preparation of sol C, sol A was neutralized with 0.1 M HCl. Utilized final concentration of humic acids in all

studied sols were 0.1, 1, 2 and 5 g·dm⁻³. Samples were stirred overnight and filtered through 5 μm syringe filters.

3.   METHODS

The main measuring techniques utilized in experimental part of the work were light scattering techniques. This group of techniques can be divided on three main methods: static light scattering, dynamic and electrophoretic light scattering. Generally light scattering is very sensitive to any changes in the structure, degradation or aggregation of molecules. These processes will reflect in average molecular weight of studied compound. For purposes of characterization of biopolymer samples size exclusion chromatography (SEC) coupled with multiangle static light scattering (MALS) and differential refractive index (dRI) detection (SEC chromatographic system from Agilent Technologies with detectors from Wyatt Technology) was used. Utilized size exclusion column was PL aquagel–OH MIXED–H 8 μm with the flow rate 0.6 ml·min⁻¹. Used mobile phase during the measuring was 0.1 M $NaNO_3$ and injected volumes of analysed biopolymers were in the range between 50 and 100 μl. All SEC–MALS–dRI measurements were performed at 25 °C. As a supplementary method for studying of aging and aggregation/degradation processes of biopolymers, electrophoretic light scattering (Zetasizer Nano ZS instrument) was also utilized. Determined parameter – zeta potential – was used for characterization of stability of the sample against aggregation. If the zeta potential is between –30 and 30 mV, the sample is non-stable. Time development of obtained zeta potential values in combination with obtained values of molecular weight can provide the information about ongoing processes in the samples.

Degradation or aggregation processes of biocolloids (humic acids) were investigated by means of dynamic and electrophoretic light scattering on Zetasizer Nano ZS (Malvern Instruments). This method was used to detect any changes in size distributions of HA particles in all studied sols. Again the time development of both methods can provide valuable parameter describing running processes in the samples.

4.   RESULTS AND DISCUSSION

The main aim of the contribution was deeper study of the aging of selected biopolymers and biocolloids. First part of this work is dealing with the study of time stability of biopolymers. The time development of zeta potential of selected biopolymers can be found in Fig 1. Obtained stability curves display negative zeta potential for all studied biopolymers. With increasing observation time all the stability curves are increasing from low negative values approaching more or less depending on the sample the value of zero zeta potential.
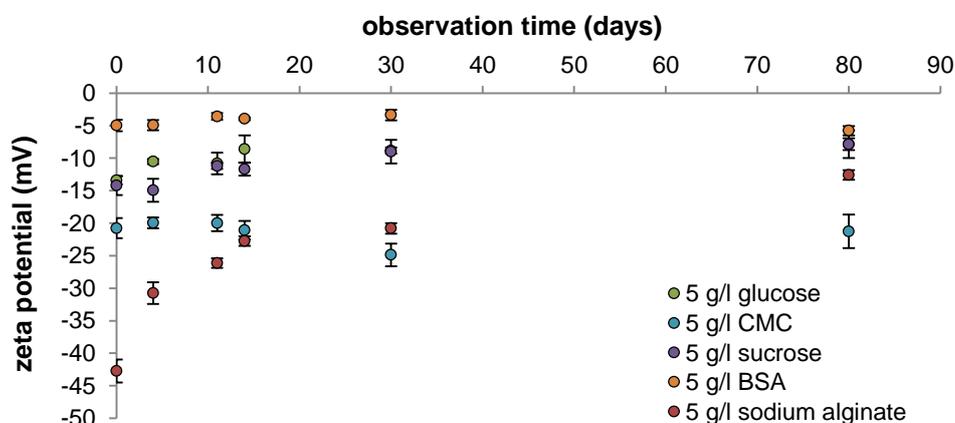

Fig. 1: Stability curves of selected biopolymers

Generally sample is electrostatically stable against aggregation if the absolute value of zeta potential is higher than 30 mV. According to this condition all samples except of the sodium alginate early after

its preparation were non–stable. Highest stability was measure in the case of sodium alginate and carboxymethyl cellulose, on the other side the lowest stability during the whole observation time of experiment was measured for BSA. This also confirms the fact that BSA had started to aggregate and settle at the bottom of the flask with sample early after preparation. Fig. 1 also indicates gradual destabilization of all samples with time. The rate of destabilization is decreasing and each of the samples after certain time met the dynamic equilibrium. This state was indicated by constant value of zeta potential in time. All these equilibrium values of zeta potential were in the region of electrostatic instability, indicating ongoing aggregation and degradation processes in these natural systems with increasing observation time. Generally using the shape of the dependences of stability curves we can predict the behaviour of studied compound in time.

Results from the electrophoretic light scattering measuring were also expanded with data from time development of molecular weight distribution of studied biopolymers. Molecular weight of selected biopolymers was determined using size exclusion chromatography (SEC) with multiangle static light scattering (MALS) and differential refractive index detection (dRI). Calculated molecular weights obtained from SEC–MALS–dRI in comparison with values certified by producers are summarized for selected biopolymers in Table 1.

Table 1: Molecular weights (MW) of selected biopolymers determined using SEC–MALS and their comparison with values certified by producers (listed only data for fresh solutions and solutions after 80 days from their preparation)

sample name	certified MW (kDa)	measured MW (kDa)
BSA (fresh)	82.0	82.3±1.1
BSA (80 days old)	–	104.2±3.2
CMC (fresh)	90.0	63.2±0.4
CMC (80 days old)	–	55.9±0.4
Hyaluronic acid (fresh)	200.0–300.0	175.8±0,9
Hyaluronic acid (80 days old)	–	112.9±1,2
Sodium alginate (fresh)	250.0	247.7±1.8
Sodium alginate (80 days old)	–	30.4±0.2

Results show good agreement of measured molecular weight of BSA and sodium alginate with producers certified values. On the other side value determined for CMC and hyaluronic acid were significantly lower than producer´s certified values. Observed difference was probably caused by degradation of biopolymers in powders. Comparison of molecular weights of fresh biopolymers and samples measured after 80 days indicates very low degradation rates in the case of CMC. On the other side, molecular weight of hyaluronic acids decreased by 35 % and especially sodium alginate reduced its molecular weight almost 10times. In opposite to these findings BSA was showing increase of molecular weight probably caused by ongoing aggregation processes running in time. These results perfectly correlate with stability curves obtained from electrophoretic light scattering measurement (Fig. 1). Higher decrease of stability observed by electrophoretic light scattering was reflected in higher degradation of sample during molecular weight analysis in time. Especially molecular weight of CMC was almost constant in time and same can be told about the zeta potential value. On the other side molecular weight of sodium alginate changed in time significantly and the same trend was observed during zeta potential measuring by significant decrease of stability.

The second studied group of natural substances – biocolloids were in this work represented by humic acids. HA aggregation processes were in experimental works studied in three different colloid sols. For preparation of sol A, solid humic acids were dissolved in 0.1 M NaOH. The second sol B was utilizing water as a solvent for humic acids followed by adjusting of the pH of the sol on 12 and finally the third sol C was prepared by neutralization of sol A with 0.1 M HCl. Basic characterization of all three prepared humic sol is summarized in Table 2. Table 2 indicates the difference in main parameters such as pH and conductivity. Measured pH value of the sol was mainly influenced by basicity of used solvent for solid humic acids. On the other side

measured value of conductivity was connected to the amount of low molecular ions in the sample. With increasing amount of low molecular ions in the sol probably affected the hydratation of chains of humic acids. With increasing conductivity higher value of density were measured. This effect can be attributed to the formation of more compact and dense structure in the presence of higher amount of low molecular ions. Higher content of low molecular ions caused also shielding of negative charge of functional groups of humic acids, which is often assumed as the stabilization element of humic structure. This destabilization was observed by decreasing zeta potential and increasing average particle size.

Table 2: Basic characterization of humic sols (concentration of humic acids 1 g·dm^{-3})

Sample	pH (–)	conductivity (S·m^{-1})	density (g·cm^{-3})	average particle size (nm)	zeta potential (mV)
SOL A	12.75±0.03	15.19±0.03	1.003	376.70±5,.65	-35.34±0.77
SOL B	11.90±0.13	4.16±0.14	1.001	262.60±3.92	-38.74±1.43
SOL C	3.70±0.02	5.16±0.01	0.999	351.20±8.64	−32.64±0.48

Deeper exploration of aggregation processes in studied humic sols was done by measuring their particle size distributions. Obtained volume particle distributions are presented on Fig. 2a. Ongoing aggregation processes in different humic sols were also studied in longer time scale. Measured time development of particle size and zeta potential of all three studied humic sols can be found in Fig. 2b.
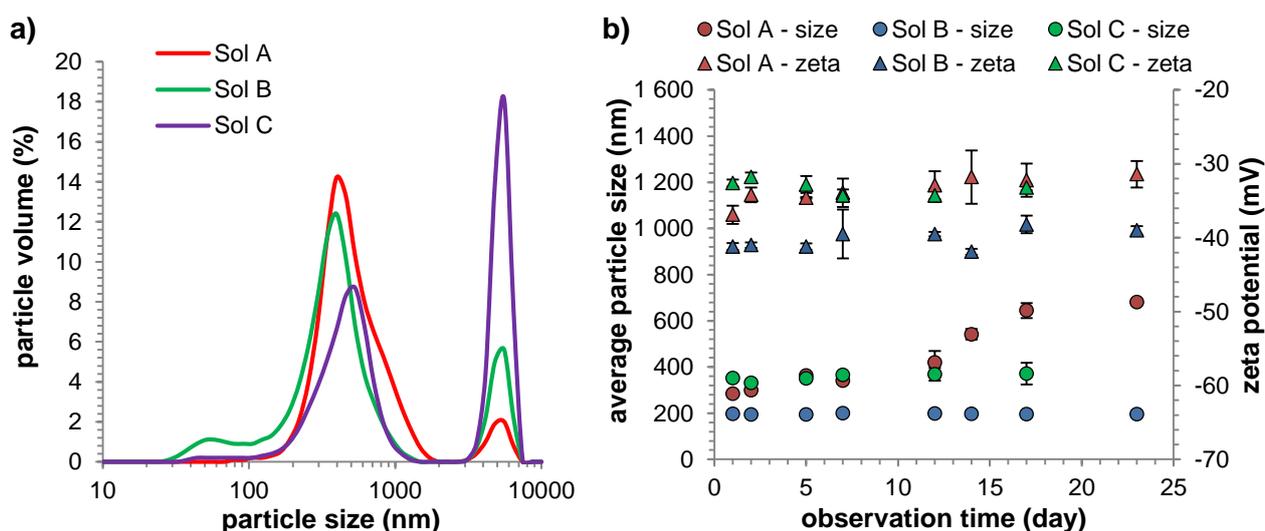

Fig. 2: a) Comparison of particle size distribution of studied humic sols; b) time development of average particle sizes and zeta potential of humic sols.

Results displayed in Fig. 2a for studied humic sols are showing different particle distributions of matter in the samples. Humic sol A and B proved almost similar particle distribution for fresh solution. But in longer time scale (Fig. 2b) is obvious, that stability of humic sol A was lower in comparison with other two samples. The main reason of this lower stability and observed increase of average particle size was caused by the higher content of low molecular ions in this sample. Low molecular ions have shielded the negative charge of humic acid. This effect was followed by destabilization of humic structure in time (decreasing value of zeta potential) and by aggregation of humic molecules. On the other side Sol B was containing lowest amount of low molecular ions which was reflected in almost constant value of zeta potential in time and almost constant average particle size during the observation time.

5.   CONCLUSIONS

The time stability of natural substances is nowadays very important parameter. This contribution is discussing the application of simple light scattering techniques for the study of ongoing aggregation and degradation processes especially in longer time scale. These processes running in natural substances,

which are used in industrial productions, as final products for distribution to customers or just in long-term research, can cause serious difficulties such as different yield of product, bad purity or unfavourable property of obtained product, or in the case of research wrong result. Results of this work showed to be valuable in this area of research. The main benefit of light scattering techniques is the fact, that the measuring is simple, non-destructive to the sample and relatively low cost. Obtained results indicated suitability of all light scattering methods for purposes of simple overview about ongoing biopolymers and biocolloids aggregation or degradation processes but also for deeper study of these processes. The results also showed good correlation between individual light scattering techniques, which became results more reliable.

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