MOLECULAR ORGANIZATION AND CONFORMATIONAL CHANGES OF HUMIC ACIDS IN DEPENDENCE ON THEIR CONCENTRATION IN VARIOUS AQUEOUS SOLUTIONS

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Abstract

Conformational changes of humic acids in different aqueous solutions were studied by means of two novelty methods: high resolution ultrasound spectrometry (HRUS) and micro-rheology (μR). Obtained results were compared with those of more conventional method of dynamic light scattering (DLS).

The HRUS method is based on the measurement of parameters of ultrasonic waves propagating through the sample. The attenuation describes the decay of the amplitude of the ultrasonic wave with the distance travelled. The velocity is the speed of this wave and is related to the wavelength and the frequency of oscillation of the deformation. It is determined by the density and elasticity of the sample, which is strongly influenced by the molecular arrangement. The μR technics (in contrast to conventional rheology) relies on tracking the motions of the micro-sized tracer particles embedded in the sample. It can give information about a local inhomogeneity especially in the ultra-small scale materials.

Our results showed that the organization of particles in diluted and concentrated solutions of humic acids is different. The conformational arrangement was strongly influence both the concentration and the character of surrounding medium. The decrease in compressibility indicated the formation of a more rigid structure, which could lead to the decrease in the binding ability of humic acids. Changes in sizes of particles detected using DLS resulted in their different mobility and obtained micro-rheological parameters. It was confirmed that both novelty methods employed were very sensitive and could be utilized as indicators of conformational changes in humic systems.

Keywords: Humic substances, molecular organization, conformation, ultrasound spectrometry, micro-rheology

1. INTRODUCTION

Humic substances are a mixture of different compounds with unique properties. They occur in all types of soils, waters and sediments of ecosphere. Due to the heterogeneous and polydisperse character of humic substances, their secondary structure has not been clarify yet nor their conformational arrangement. The conformation of humic substances in aqueous solutions are mainly stabilized by weak disperse forces such as Van der Waals, π-π, CH-π interactions and hydrogen bonds. Humic substances in aqueous solutions tend to form aggregates that are held together by intermolecular hydrophobic interactions. Therefore their conformation and molecular organization as well as their stability is strongly dependent on character of medium and by change of conditions in the solution is lead to changes in the arrangement and size of humic particles in studied systems [1]-[3].

The characterization of molecular organization of humic substances represents a major problem, due to their heterogeneity and tendency to associate when present in solutions with their increasing concentration. Variations in ionic strength and pH were frequently observed as affecting the apparent molecular size of humic particles [3]-[5]. Some authors stated that dissolved humic acids might be micelle-like, supra-molecular assemblies of small entities. The apparent conformation of humic acids at higher concentrations (>1 g.dm^{-3}) might differ strongly from the conformation in more dilute systems [3], [6], [7]. A critical examination of published data resulted in a new view evolving on the molecular structure of humic substances. In this view, humic substances are collections of diverse, relatively low molecular mass components forming dynamic associations stabilized by hydrophobic interactions and hydrogen bonds. These associations are capable of organizing into micellar structure in suitable aqueous environments [3], [6]-[10].

In this work, high resolution ultrasound spectrometry (HRUS) and micro-rheology (□R) supplemented by dynamic light scattering (DLS) were used in order to study the conformational changes in humic acids in dependence on their concentration in three different environments. HRUS is a novel technique for direct and non-destructive analysis of the intrinsic properties of materials. The method is based on measurements of the ultrasonic velocity and attenuation. Ultrasonic velocity is determined by the density and elastic response of the sample to the oscillating pressure (stress) in the ultrasonic wave and can be expressed in terms of compressibility or storage modulus (longitudinal). The elastic response is generally dominant. The attenuation, which is a measure of the ultrasonic transparency of the medium analyzed, is determined by the energy losses in compressions and decompressions in ultrasonic waves and so can be expressed in terms of the viscosity of the medium or its longitudinal loss modulus [3], [11], [12]. This parameter is extremely sensitive to the molecular organization and intermolecular interactions in the analyzed medium, which can influence strongly the reactivity and structural arrangement of humic substances and their complexes [13]-[16]. The ultrasonic analysis is based on the measurement of characteristics of ultrasonic waves propagating through a sample. Sound can be absorbed by matter which either attenuates the signal or it changes the velocity of the signal. Ultrasonic attenuation (*N*) describes the decay of the amplitude of the ultrasonic wave with distance travelled. Ultrasonic velocity (*U*) is the speed of this wave and is related to the wavelength and the frequency of oscillation of the deformation. It is determined by the density and compressibility of the sample [17]

$$U = \frac{1}{\sqrt{\beta\rho}} \tag{1}$$

where U is the ultrasound velocity of the sample in cell 1, β is the compressibility, and ρ is the density of the sample. The computed compressibility is usually normalized to the compressibility of water (β_w)

$$\beta_r = \frac{\beta}{\beta_w} \tag{2}$$

The compressibility is determined by the elastic response of the sample to the oscillating pressure (stress) in the ultrasonic wave. This parameter is extremely sensitive to the molecular organization and intermolecular interactions in the analyzed medium [3], [8]. Obtained ultrasound parameters characterize indirectly rheological properties of studied systems, which are studied in this work by other novel method - micro-rheology. The passive μR method is based on the thermal motion of particles inserted into the investigated sample. Small well defined particles are embedded into the investigated material. Size of the particles allows subjecting of the Brownian motion, which depends on the viscosity of surrounding material. Mean squared displacement (Δx^2) of particles with dimension *r* is used for the determination of their diffusion coefficient *D*

$$\Delta x^2 = 2Dr\tau^{\alpha} \tag{3}$$

where τ is time and α is time exponent. The determined value of diffusion coefficient is used for the calculation of viscosity η

$$D = \frac{k_B T}{6\pi\eta\, r} \tag{4}$$

where k_B is the Boltzmann constant and *T* is temperature. Viscosity obtained using μR is strongly influenced by character, size and charge of dissolved humic particles. The combination of HRUS with methods of μR and DLS can provide a complex view on the molecular organization of humic particles in studied systems.

2. MATERIALS AND METHODS

Humic acids were extracted from South-Moravia lignite using a mixture of NaOH and $Na_4P_2O_7$ by the procedure described and characterized previously [2], [3], [18]-[21]. Three different types of solutions were used in this work. The first type was the colloidal solution prepared by the dissolution of humic acids in 0.1 M NaOH and the addition of the same volume of 0.1M HCl. This preparation resulted in humic acids dissolved in the solution of NaCl with neutral pH-value. The second type was the solution prepared by dilution of stock solution of previous type ($10 g.dm^{-3}$) by means of 0.1M NaCl. Solutions of both these types have the same chemical character but they are prepared by different procedure which could influence the final organization of humic particles. Third type was the alkaline solution in 0.1M NaOH. The concentration range of humic acids in their solutions was 0.01 - 10 g dm^{-3}.

An ultrasonic spectrometer with high resolution HR-US 102 (Ultrasonic Scientific), was utilized for measurement of basic ultrasonic parameters at three frequencies (5110 kHz, 8220 kHz, and 12 200 kHz, respectively). Six values of ultrasonic parameters were measured and averaged at each frequency. The device consisted of two independent cells heated to 25 °C. Cell 1 was used for a humic sol measured, cell 2 for a blank solution. Ultrasonic velocity (U) and attenuation (N) in both cells was monitored; the resulting values ΔU and ΔN were calculated as the differences between sample and reference cells. The solvents were used as blank solutions for determination of the ultrasonic parameters of humic solutions. The density of humic sols was determined by DMA 4500 (Anton Paar).

Polystyrene well defined particles were used for the particle tracking μR. Solutions with particles were placed into the “glass pocket” to avoid its drying or flowing. The particle tracking was monitored using microscope (Nicon Eclipse e200) and CCD camera (Canon EOS 550D) and analyzed using SW VirtualDub and ImageJ.

The particle size distribution and zeta potential were determined using Zetasizer Nano ZS (Malvern Instrument).

3. RESULTS AND DISCUSSION

In **Fig. 1**, parameters determined on the basis of ultrasound spectrometry are shown.

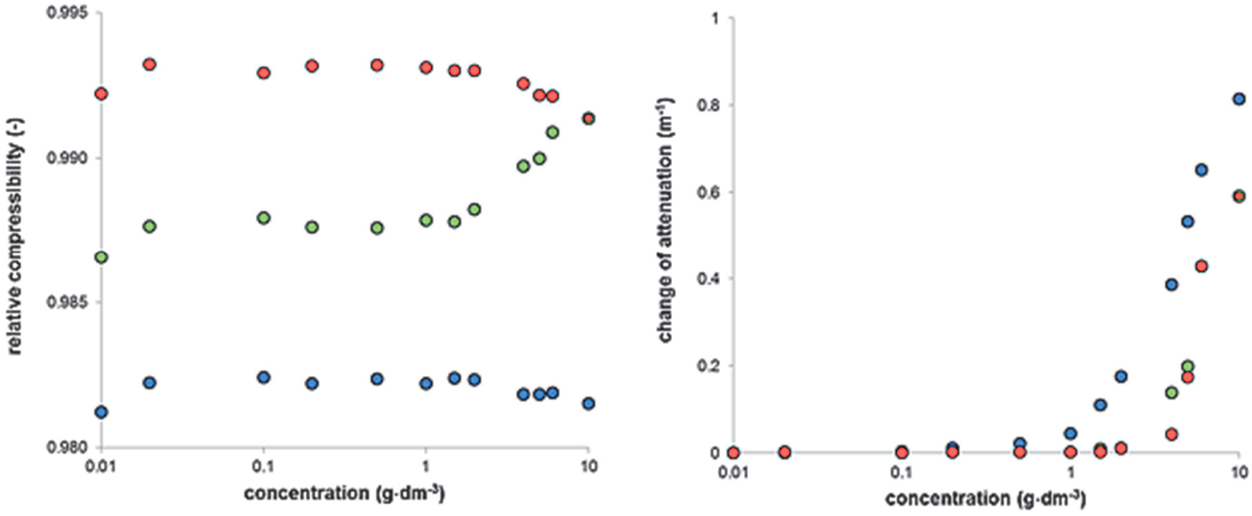

Fig. 1 Relative compressibility and change in attenuation of humic acids in NaOH (blue), NaOH / HCl (red) and NaCl (green)

The conformational changes and different stability affected both the ultrasonic parameters measured: velocity and attenuation. While the attenuation is determined by the energy losses in compressions and decompressions in ultrasonic waves, the ultrasonic velocity is determined by the elasticity and density of the sample measured. Since the dependence of density on concentrations of humic systems is strongly linear (not shown), the values of compressibility presented in **Fig. 1** must be a result of the elasticity, which is usually dominant. We can see that compressibility of humic systems with alkaline pH is much lower in comparison with neutral ones and depends on the concentration only weakly. Similarly, the concentration dependence of compressibility of humic acids in NaCl is not strong. On the other hand, the compressibility of humic systems prepared by neutralization of alkaline solution by HCl increases sharply for highly concentrated solutions. The way of preparation influences significantly behavior of studied systems. Humic acids dissolved in NaOH can spread out their structure which can change gradually during neutralization. Systems in NaCl are prepared by simple dilution and possibilities for changes are limited. Results obtained on the basis of measurement of attenuation are different. All three systems have low attenuation for low concentrations and their values increase for samples with concentration higher than 1 g·dm^{-3}. This "switch-over point" was detected by more methods which confirmed qualitative changes in molecular organization of studied systems with concentration.

High values of attenuation can indicate aggregation or presence of large particles which can sediment. Data obtained by means of DLS (**Fig. 2**) confirmed the increase of particle sizes only for humic acids in NaOH. The aggregation of neutral systems is much lower. The polydispersity of alkaline systems is practically constant and increases slightly for very low concentrations. Similar trend was observed for humic acids in NaCl but the increase is stronger. Results obtained for systems in NaOH neutralized HCl are opposite. The polydispersity of lowly concentrated solution is low and increases with concentration. Values obtained for medium and highly concentrated systems are practically constant.

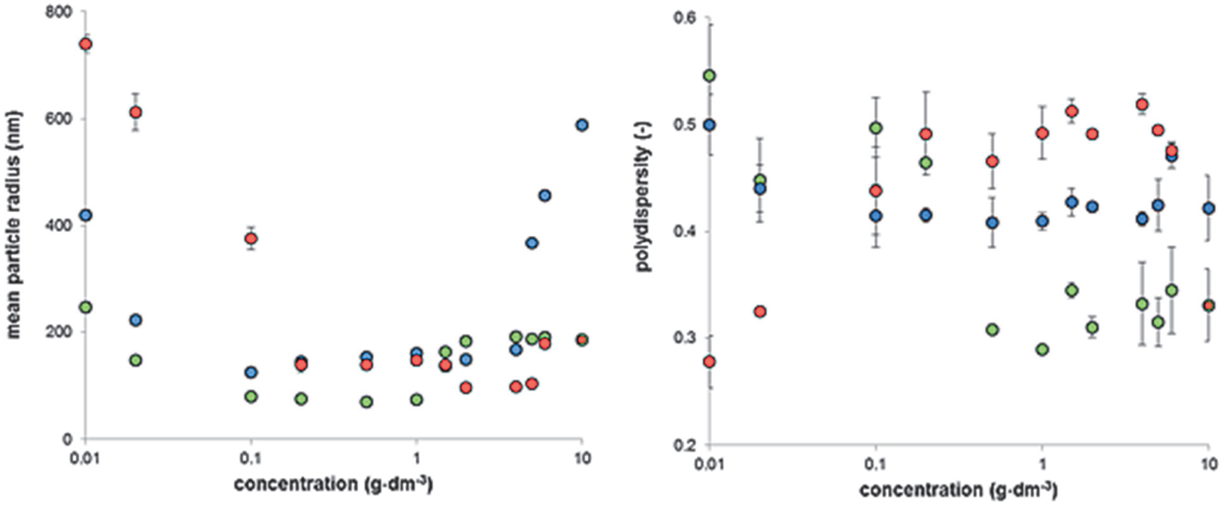

Fig. 2 Particle size and polydispersity of humic acids in NaOH (blue), NaOH / HCl (red) and NaCl (green)

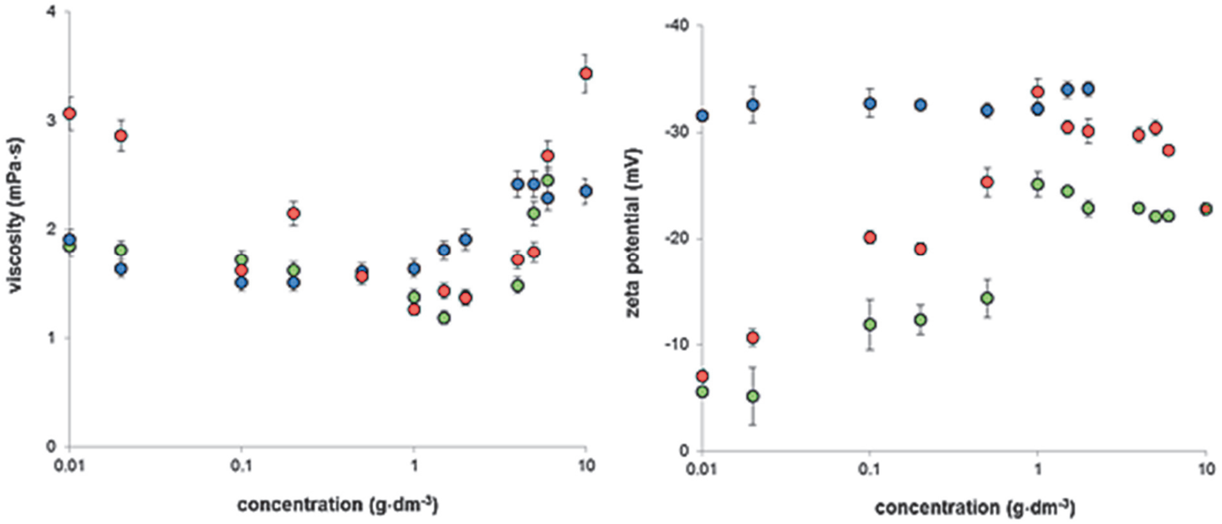

Fig. 3 Viscosity and zeta potential of humic acids in NaOH (blue), NaOH / HCl (red) and NaCl (green)

Charge of humic particles in studied system is influenced by the character of surrounding medium similarly as in previous cases. The decrease of zeta potential with increasing concentration indicates the increase of stability of humic solutions. Data obtained for humic acids in NaOH / HCl correspond with their polydispersity including a little decrease for very concentrated systems. The increase of stability is connected with the increase of polydispersity. On the other hand, the stability of humic acids in NaCl increases with decreasing polydispersity, which confirms the strong influence of preparation process on final properties of humic systems. Values of viscosity determined by the μR method indicated higher flow resistance for highly concentrated systems. Results are related to changes in the hydration shell of humic particles detected also by the ultrasound spectrometry. A minimum of viscosity was observed for all studied systems, which indicated the above mentioned “switch-over point”. Differences in behavior of lowly and highly concentrated systems were detected by all used methods. It shows that the molecular organization changes with the content of humic particles as well as with the character of solvent. The conformational arrangement of humic particles in different systems is the result of many factors as hydrophobic interactions, dissociation of functional groups, charge repulsions and others.

4. CONCLUSION

The novelty methods used in this work provided comprehensive illustration of molecular organization in complex humic systems. Presented approach to study on conformational changes and aggregation behavior is useful tool for characterization of systems containing particles sensitive on surrounding medium and conditions, especially biomaterials.

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