

DESTABILIZATION AND AGGREGATION OF AQUEOUS HUMIC ACIDS SOLUTION BY METAL IONS

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Abstract. *The aggregation of soil humic acid (HA) after addition of cations was investigated by nephelometric measurements of HA solution turbidity. Here we show that aggregation is promoted by the presence of positive ions in the solution and that the order of increasing effectiveness in turbidity follows Schulze-Hardy rule. Also, the obtained results indicate that the cation with the largest ionic radius within a common valence is the most effective coagulant. Influence of anions follows the sequence: chloride < nitrate < sulphate, but it is insignificant comparing the influence of cation. This confirms that HA molecules are of negative charge.*

Key words: *cation, humic acid, aggregation, turbidity*

1. INTRODUCTION

Humic substances (HS) comprise a general class of biogenic, refractory, yellow-black organic substances that are ubiquitous, occurring in all terrestrial and aquatic environments [1]. This heterogeneous mixture of compounds is composed of three operationally distinct fractions: 1) fulvic acid, which is soluble in both acidic and basic solutions; 2) humic acid (HA), which is soluble in basic solution but insoluble in acidic solutions; 3) humin, which is insoluble in both acidic and basic solutions. Although, the structure of HS is not fully understood, it is generally accepted that HS are complex phenolic polymers which possess both carboxyl and phenolic hydroxyl acidic functional groups [2].

Chemically, HS are heterogeneous and polydisperse macromolecules that behave like flexible, linear chains or as random coils that could form fairly compact spheres [3].

Their reactivity in the environment depends on their functional group chemistry and macromolecular structure, their shape and size. So, changes in HS macromolecular structure are important for their geochemical reactions: they play a vital role in the transport, bioavailability and deposition of many toxic heavy metal ions [4] and organic micropollutants as biocides and their degradation products [5].

In addition, they are classified as macroions because they have a polyelectrolytic character and therefore carry electrostatic charge in aqueous solution. Although, a variety of positive and negative sites can be identified in HA molecules, the predominant charge is negative (at pH 6, the most common value for water and soil environment), due to dissociation of carboxylic groups. According to literature data [2, 3], soil HA have 3 – 4 charged sites per 1000 Daltons, which is relatively high level of charge for a naturally occurring polyelectrolyte.

Recent investigations [6] have shown that aqueous solutions of soil humic acids respond to the addition of cations by forming intramolecular and intermolecular aggregates, compact structures with relatively hydrophobic interiors and hydrophilic surfaces.

Upon the addition of cations, HA macromolecules tend to shrink or contract; mutual repulsion among negatively charged carboxyl groups is minimized and they fold forming intramolecular and intermolecular aggregates. This is thought to be due to two mechanisms: charge neutralization and functional group bridging. Functional group bridging enhances this effect, especially with multivalent cations, by drawing together various groups on the humic acid chain.

Evidence for this model is confirmed, in the present investigation, by nephelometric measurements of aqueous HA solution turbidity, after cation addition. The intensity of scattering light, according to Rayleigh, is shown in the equation (1):

$$I = k \cdot a \cdot v^2 \quad (1)$$

Here I is the intensity of scattered light, a is particle number, v is particle volume and k is constant.

As the volume of particle increases, turbidity of solution increases, as well. Therefore, particle size and number can be monitored by measuring intensity change of scattered light, if concentration of colloid is constant.

Turbidity studies, performed in the present study, revealed influence of cation valence and radius on turbidity of HA solution. This is in agreement with the Schulze-Hardy rule.

2. EXPERIMENTAL

Chemicals. All reagents used were of analytical grade purity from Merck, Aldrich or Sigma. Solutions were prepared using double deionised and turbidity-free water. This water was obtained by passing distilled water through a membrane filter having 0.45 μm porosity.

Experiments were conducted on well-characterized soil HA is [7]. Soil samples were collected from a well-humified organic horizon of old beech-forest soil (10 cm depth). Samples were air dried for 5 days and sieved to pass a 2.0 mm sieve.

HA isolation and purification. HA isolation and purification was based on method as recommended by the International Humic Substances Society [8], except that in initial treatment, phosphoric acid was used rather than HCl, which is recommended by International Humic Substances Society, because H_3PO_4 would prevent the oxidation of organic substances by the Fe(III) ion.

Briefly, 1000g of soil was suspended in 10 l of 0.1M H_3PO_4 and pH was adjusted to 1-2 with 1M H_3PO_4 . The suspension was shaken for 4 hours and then left to settle over night. The supernatant was discarded and the pH of the solid phase was adjusted to 7.0

with 1M NaOH, followed by addition of 10 l of N₂-purged 0.1M NaOH, to minimize oxidation of HA. The solution was left under N₂ atmosphere for 24 h and then centrifuged.

The humic material in the liquid phase was acidified to pH 1.0 with HCl. The obtained humic acid precipitate was dissolved in a suitable volume of 0.1M KOH solution under N₂ atmosphere. KCl was added to concentration of 0.3M K⁺ and mineral phase was separated by centrifugation.

Supernatant was treated with 0.1M HCl – 0.3M HF mixture in order to remove mineral impurities (silicates). The ash-free HA was purified using ion exchange resin Dowex 50W - X8 (H⁺-form). The protonated HA was centrifuged, dried at 30°C and left over CaCl₂.

HA characterization. Purified HA was characterized by elemental analysis, E₄/E₆ ratio and total, carboxyl and phenolic acidity.

Absorbances of 0.04% humic acid solution in aqueous 0.05M NaHCO₃ solution was recorded in 1 cm cell on a Secomam Anthelie 306 spectrophotometer at 465 nm (E₄) and 665 nm wave lengths (E₆), in order to obtain E₄/E₆ ratio.

Total acidity (mmol g⁻¹) was determined by pH titrimetry with barium hydroxide and back titration with standard acid [2]. Carboxylic group determination (mmol g⁻¹) was carried out by addition of calcium acetate followed by pH titration of the liberated acetic acid with standard base [2]. Phenolic groups were obtained by the difference between total acidity and carboxylic acidity [2].

Turbidity experiments. The turbidity of HA aqueous solution was determined by turbidimeter HACH model 16800. The intensity of light scattered by the HA solution was compared with the intensity of light scattered by a standard reference suspension of formazin polymer, because it is easy to prepare and is reproducible.

Stock turbidity suspension (400 NTU-nephelometric turbidity units) was made with hydrazine sulphate and hexamethylenetetramine [9]. Dilute turbidity standards (40 NTU and 4.0 NTU) were prepared with turbidity-free water, daily.

Several turbidity ranges are used to obtain sufficient sensitivity for low turbidities. In each used instrument range at least two dilute turbidity standards were used.

Possible interference for this turbidity determination is color of solution due to HA which absorb light. Although, this causes measured turbidity to be low this is insignificant in this work, because we monitored relative ratio of measured turbidity values and not their absolute values.

Before measurements, every sample was vigorously shaken. After air bubbles were released, the turbidity was measured.

HA solution was made by dissolving 300 mg of HA in 1000 ml turbidity-free water. To ensure complete dispersion of the HA, the pH was first increased to pH 10 by NaOH addition and then lowered to the desired value by HNO₃ addition. HA was converted in H⁺-form by ion exchange resin Dowex 50W - X8.

The nephelometric measurements were performed by adding increments of solid metal salt to a HA solution (0.3 g/dm³) and adjusting the pH with NaOH and HCl to a pH of 7.0.

Turbidity of this HA solution, without presence of electrolyte, was 5.0 NTU.

After addition of increments of solid metal salt and mixing with a magnetic stirrer, turbidity of solution was monitored. Metal salts used were: NH₄Cl, NaCl, KCl, MgCl₂, CaCl₂ and Ce(NO₃)₃.

3. RESULTS AND DISCUSSION

Purified soil HA was analysed for C, H, N, S and O and the obtained elemental composition was (%): 44.86, 4.82, 2.66, 0 and 47.66, respectively. Oxygen percent was calculated by the difference: $100 - (\%C + \%H + \%N + \%S)$. These values are similar to the average composition of soil HA [2]. Ash content was 2.69%, which indicates good removal of mineral matter in the extraction and purification procedure.

Values for total acidity, COOH content and phenolic OH content are (mmol g^{-1}): 5.81, 3.4 and 2.4, respectively. It could be seen that RCO_2^- groups are predominant in isolated soil HA macromolecule and so, they are principal binding sites for Na^+ , NH_4^+ , K^+ , Mg^{2+} , Ca^{2+} and Ce^{3+} in our experiment.

The E_4/E_6 ratio of isolated HA is 2.71, which is in agreement with literature data (it is generally less than 5 for humic acid) [10].

Cation influence on HA solution aggregation. Nephelometric measurements were performed on HA solution before and after addition of increments of solid metal salts in order to estimate cation influence on HA aggregation.

Turbidity studies, as summarized in Table 1, show that HA aggregation is promoted by the presence of positive ions in the solution, which is expected since HA molecules have negative polyelectrolytic character.

Table 1. For monovalent ions: effectiveness cation radius (nm) and concentration (mM) on HA solution turbidity increase (from 5.0 to 10.0 NTU); for polyvalent ions: effectiveness of valence and radius (nm) on HA solution turbidity increase (electrolyte concentration is 1mM)

Cation	Radius of cation (nm)*	Electrolyte concentration (mM)
Monovalent		
Na^+	0.98	35
K^+	1.33	24
NH_4^+	1.43	33
Cation	Radius of cation (nm)*	Turbidity (NTU) of HA solution
Divalent		
Mg^{2+}	0.78	12.0
Ca^{2+}	1.06	17.0
Trivalent		
Ce^{3+}	1.18	43.0

*after Goldschmidt for six-fold coordination

Experiments were done at pH 7.0 (close to most naturally occurring pH values), when HA molecules are predominantly of negative charge, due to the dissociation of carboxylic acid groups. Mutual repulsion among these functional groups causes the humic acid polymers to adopt a stretched configuration, so the molecule expands [1].

Upon the addition of cations, this effect is minimized and HA polymers fold forming intramolecular and intermolecular aggregates, and the turbidity of solution increases (change in scattered light intensity is caused by change of particle size and number) (Fig. 1).

Also, the results reveal relatively small differences between effectiveness in turbidity increase of different cations of a common valence.

For the monovalent ions the order of increasing effectiveness in turbidity follows the sequence:



because 100% turbidity increase of HA solution (from 5.0 NTU to 10.0 NTU) was observed for following electrolyte concentration: 35 mM NaCl, 33 mM NH_4Cl and 24 mM KCl (Tab.1.). It is obvious that 100% turbidity increase cause the smallest concentration of the cation with the largest ionic radius (K^+). So, this is in agreement with the radius effect that the cation with the largest ionic radius within a common valence is the most effective coagulant.

Also, Table 1. shows that NH_4^+ ion does not follow this rule, possibly because NH_4^+ ion does not occur as simple cation specie in the solution.

Fig. 1. shows the change in HA solution turbidity upon the addition of sodium ion. Other investigated monovalent ions show similar turbidity change curve with difference only in its slope. It is obvious that higher ionic strength of the solution leads to greater turbidity increase, which must be ascribed to aggregation of humate molecules, so they tend to shrink or contract. The reason for this is that more counter ions are brought closer to negatively charged sites (mostly carboxylic), the electrostatic free energy of macro-molecule is decreased (Swift, 1989).

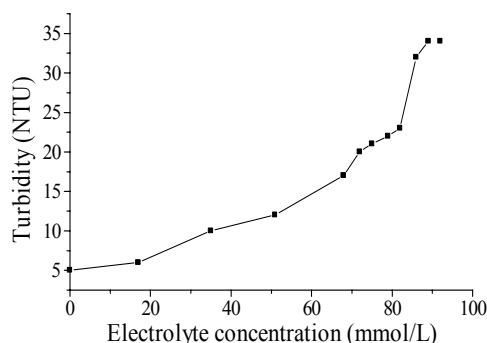


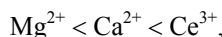
Fig.1. Variation of HA solution (0.3 g/dm^3 , pH 7.0) turbidity after addition of solid NaCl.

If, instead, the added cation was di- or trivalent, then described effect occurs at much lower concentrations of added electrolyte. Thus, concentration of only 1.0 mM divalent ion electrolytes will cause turbidity of HA solution as follows: 17.0 NTU for CaCl_2 and 12.0 NTU for MgCl_2 (Tab.1). Multivalent cations enhance this effect of molecule shrinking by drawing together various groups on the humic acid chain. The divalent ions are more effective in turbidity increase than monovalent ions are.

This data follow the same radius effect. Thus, mono- and divalent ions which have larger ionic radius, thus smaller hydrated radius, can approach closer to negatively charged sites of HA polymer. Those cations have the greatest tendency to form absorbed Stern layer and so, aggregation and coagulation occurs. It is obvious that the double-layer theory of colloid coagulation applies for HA macromolecules.

For trivalent ion, electrolyte concentration of only 1.0 mM $\text{Ce}(\text{NO}_3)_3$ will cause turbidity of 43.0 NTU (Tab. 1). This data follow the same valence rule as the divalent ions.

Addition of these di and trivalent ions even leads to precipitation of HS. So, for the polyvalent ions the order of increasing effectiveness in turbidity follows the sequence:



which is in agreement with Schulze-Hardy rule. Obtained results for divalent and trivalent ions are shown in Figure 2. Those higher valence cations also, form bridges between anionic groups of different humic macromolecules and thereby enhance their associations. This allows the macromolecules to approach one another more closely, so that intermolecular attractive forces predominate and coagulation or precipitation can occur [1].

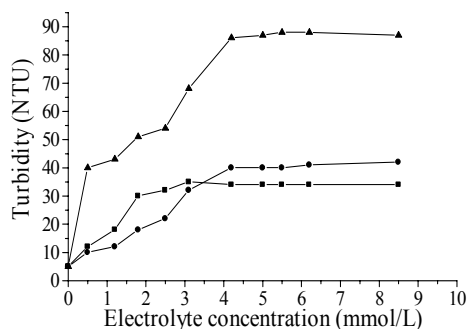
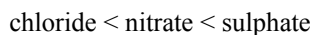


Fig. 2. Effects of di- and trivalent cations concentrations on turbidity increase of HA solution (0.3 g/dm³, pH 7.0). For: Mg²⁺ (●), Ca²⁺ (■), Ce³⁺ (▲).

Anion influence on HA solution aggregation. Besides these cation-enhanced aggregation experiments, the turbidity increase effect of different anions was also investigated. Nephelometric measurements were performed on HA solution before and after addition of increments of solid sodium salts. Concentrations of sodium salts that increased HA solution turbidity from 5.0 NTU to 6.0 NTU were: 14.5, 16.0 and 17.0 mM for Na₂SO₄, NaNO₃ and NaCl, respectively. So, the order of increasing effectiveness in turbidity increase was:



It is obvious that the influence of anions is insignificant comparing the influence of cation on HA solution turbidity increase. This confirms that humic acid macromolecules are predominantly of negative charge.

3. CONCLUSION

Nephelometric measurements of HA solution turbidity upon the addition of cations revealed that the sequence of increasing effectiveness in turbidity increase is: Na⁺ < NH₄⁺ < K⁺ < Mg²⁺ < Ca²⁺ < Ce³⁺. This is in agreement with Schulze-Hardy rule. Also, the results showed that the cation with the largest ionic radius within a common valence is the most effective coagulant. Influence of anions on HA solution turbidity increase follows the sequence: chloride < nitrate < sulphate, but it is insignificant comparing the influence of cation. This confirms that humic acid molecules are of negative charge.

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DESTABILIZACIJA I AGREGACIJA VODENOG RASTVORA HUMINSKIH KISELINA METALNIM JONIMA

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Agregacija terestrične huminske kiseline nakon dodatka katjona je ispitana nefelometrijskim merenjem mutnoće rastvora huminskih kiselina. Agregacija je pospešena prisustvom pozitivnih jona u rastvoru, pri čemu doprinos različitih metalnih jona porastu mutnoće, samim tim agregacije HA, sledi Šulc-Hardijevo pravilo. Takođe, dobijeni rezultati pokazuju da će od katjona iste valentnosti, katjon sa najvećim jonskim radijusom pokazivati najveću moć agregacije HA molekula. Redosled uticaja anjona na agregaciju je sledeći: hlorid < nitrat < sulfat, mada je on zanemarljiv u odnosu na uticaj katjona. Ovo potvrđuje da su HA molekuli negativnog naelektrisanja.