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Radical Stabilization in Dissolved Humates

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Abstract

Quinoid entities, in which quinone and hydroquinone groups equilibrate via a semiquinone radical intermediate, are a common structural feature in humic materials. The electron paramagnetic resonance (EPR) signals of these radicals are significantly enhanced in the presence of diamagnetic divalent metal ions such as Mg^{2+}, while monovalent ions do not show the effect. The addition of trivalent ions leads to rapid precipitation, leaving little room for observation. It was noted that the metal ions producing EPR signal enhancement were also underwent effective bridging interactions with humic subunits, forming pseudomicellar structures. Particle growth determined through dynamic light scattering measurements coincided with the onset of EPR signal enhancement, and surface tension measurements further corroborated the coincidence of aggregation. The addition of a chaotrope (urea), which broke up the humic structures, eliminated the EPR signal increases. These observations strongly suggested that bridging interactions by divalent metal ions, and the intramolecular aggregation that accompanied it, led to significant stabilization of semiquinone radicals within the humic structure.

Keywords: Humic Acid, Semiquinone, EPR Enhancement, Bridging Interactions, Aggregation, Radical Stabilization

1. Introduction

The transformation of natural organic matter leads to the formation of humic substances. Under aerobic conditions of composting or humification in terrestrial ecosystems the main components of these materials are fulvic acid (FA) and humic acid (HA). They are a complex, operationally defined suite of substances built around a highly aromatic backbone and containing an abundance of oxygenated functionalities, including carboxylic, phenolic, alcoholic and carbonyl groups [1].

Structural changes during ageing of HA lead to an increase in the number of polyphenolic and quinoid units. The latter generally exist as equilibrated quinone/ Hydroquinone structures encompassing a radical semiquinone intermediate that can be investigated by electron paramagnetic resonance spectroscopy (EPR) [2].

The quinone-semiquinone-hydroquinone equilibria are sensitive to various chemical and physical factors such as redox conditions, radiation, pH, and metal concentration [2,3]. Jerzykiewicz *et al.* [4] noted that the carboxylic, phenolic and quinoid groups of HA are involved in metal complexation, and interactions of humic acids with metal ions can play a significant role in the shifting the reaction equilibria. The effect of metal binding can be observed as a change in the free radical structure and/or spin concentration [4-6].

The metal ions that affect the quinoid equilibria in HAs also play a significant role in their aggregation behavior [7]. Under the influence of the metals, the materials aggregate to form pseudomicellar structures similar to surfactant micelle, but constrained by the intramolecular nature of the arrangement and the polydispersity of the material [8,9]. Upon the addition of cations, especially polyvalent ones, HA folds and shortens, forming compact structures with relatively hydrophobic interiors and hydrophilic surfaces. This it thought to be due to a combination of charge neutralization and functional group bridging [10].

The present study was prompted by the observation that the quinoid EPR resonance of HA is strongly enhanced by the presence of metal ions, especially Mg^{2+} [11], which also has been shown to have a major influence on its aggregation behavior. The possible correlation between these two phenomena has been investigated.

2. Materials and Methods

2.1. Chemicals

The source of HA in this study was a South Moravian lignite collected from the Mir mine in the area of Mikulcice, near Hodonin, Czech Republic. HA were extracted by standard alkaline method with 0.5 M NaOH and 0.1 M $Na_4P_2O_7$. After separation from parental lignite, humic acids were precipitated with 4 M HCl, demineralised with 0.5% HF, dialyzed against distilled water until chloride and fluoride free, and freeze-dried.

Sodium chloride (Fisher Scientific), lithium chloride (Allied Chemical, NY), magnesium chloride hexahydrate (Baker), barium chloride (Fisher Scientific), calcium chloride (EM Science), zinc chloride (Fisher Scientific), aluminium chloride hexahydrates (Fisher Scientific), samarium chloride (Aldrich), cerium chloride (Fisher Scientific), ethylene-bis(trimethylammonium) iodide (Aldrich), potassium bromide (Fisher Scientific), sodium hydroxide (Fisher Scientific), hydrochloric acid (EMD Chemicals), 3-carbamoyl-2,2,5,5,-tetramethyl-3-pyrrolin-1-yloxy free radical 97% (Acros), and urea (Baker) were used without further purification.

2.2. Procedures

For EPR analysis HA solutions (500 mg/L) were prepared by dissolution in water with minimal addition of NaOH. Various metals were added as their chlorides in the concentration ranges 5.0×10^{-5} - 1.0×10^{-3} M. The cations in question were Na^+, Li^+, Mg^{2+}, Ba^{2+}, Ca^{2+}, Zn^{2+}, Al^{3+}, Sm^{3+} and Ce^{3+}; one non-metallic cation, the ethylene-bis(trimethylammonium) ion $[(CH_3)_3N\text{-}(CH_2)_2\text{-}N(CH_3)_3]^{2+}$ ("N2") at a concentration of 1.0×10^{-3} M, was also included.

EPR spectra were recorded on a Bruker EMX 6/1 Spectrometer equipped with an AquaX Aqueous Sample Cell (30 L/cm volume). Spectra were obtained at a resonant frequency 9.862 GHz, a microwave attenuation of 40 db, a modulation frequency 100 kHz, a conversion time of 40.9 ms, a time constant of 1.280, and an average of 4 scans. Infrared spectra were obtained with a Nicolet FT-IR spectrometer using KBr pellets containing approximately 5% (w/w) HA. For HA samples containing Mg^{2+}, 8 g/L aqueous HA solutions with 1.0×10^{-2} M $MgCl_2$ were prepared and freeze dried. The dry material was used in the KBr pellets. Standard 3-carbamoyl-2,2,5,5,-tetramethyl-3-pyrrolin-1-yloxy free radical was used for spin count calibration.

The acid content of HA samples was determined by conductometric replacement titrations as described in earlier reports [12-14]. Measurements were made with a platinum conductivity probe manufactured by Vernier (Beaverton, OR) and recorded with a Vernier LabQuest module. HA solutions of 500 mg/L were dissolved in a slight excess (ca. 5 µeq) of NaOH and titrated with 2 M HCl.

Surface tension studies were performed on 500 mg/L aqueous HA solutions, including various cations, using a Fisher Surface Tensiomat Model no. 21, fitted with a 19-mm-diameter platinum-iridium ring. Solutions were placed in a shallow glass dish of 50 mm diameter, and the ring was inserted in the middle of the container to avoid edge effects. The ring was raised through manual operation of the torsion mechanism, and the tension reading at the instant of surface detachment was noted. All measurements were taken in triplicate at a temperature of 22˚C.

Dynamic light scattering experiments were performed with a Coulter N4 Plus Submicron Particle Sizer equipped with a 10-mW helium-neon laser (λ = 632.8 nm). The concentration of aqueous humic acid (ca. 500 mg/L) was adjusted to give scattering intensities in range 5×10^4 - 1×10^6 counts per second. All measurements were taken at a 62.6 detection angle and all reported sizes are averages from 10 sequential runs of 300 s each.

3. Results and Discussion

Before The EPR spectra of HA obtained in this study displayed a narrow line at an average value of g = 2.00361 (**Figure 1**), which is consistent with the characteristic sharp resonance produced by the semiquinone free radical [3,5,15].

Spectra obtained with HA metal complexes showed similar peaks with slight variations in position and line width, but notable differences in signal intensities (**Figure 1, Table 1**).

The data in Table 1 show that the addition of cations generally increased the g-values of the resonances in the respective EPR spectra. Jezierski and coworkers ascribed the increased g-values in the presence of metal ions to the formation of complexes with oxygen-rich groups in HA [5]. Conductometric titration [12] revealed that the humic material use in these studies had a carboxylic acid content of ca. 5 meq/g, providing ample opportunity for such interactions. They were confirmed by changes in the carboxylate IR bands (not shown) upon the addition of Mg^{2+}: the 1710 cm^{-1} – C = O stretch and 1620 cm^{-1} asymmetric stretch coalesced into an unresolved band at 1629 cm^{-1}, while the 1398 cm^{-1} HA band shifted to 1426 cm^{-1}.

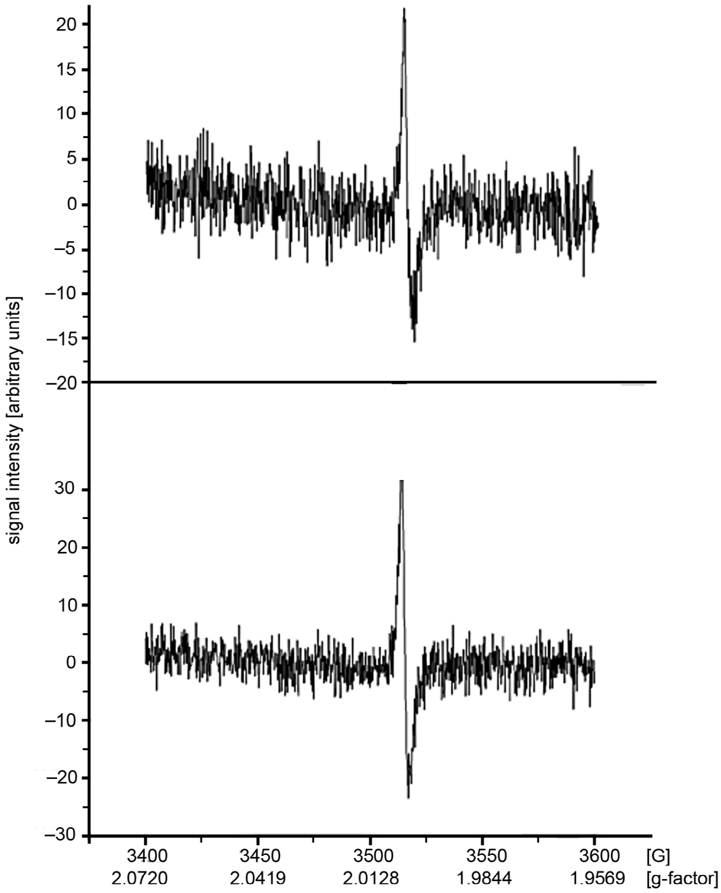

Figure 1. EPR spectra of 500 mg/L HA (top) and 500 mg/L HA + 1.0 × 10^{-3} M Mg^{2+} (bottom).

Table 1. EPR parameters for humic acids with addition of several cations.

Sample	g value	Line width (G)	Free radical concentration (spins/g × 10^{-17})
HA 500 ppm	2.00361	5.38	2.32
HA + 1 E-3 M Na	2.00418	3.68	3.00
HA + 1 E-3 M Li	2.00435	4.25	2.80
HA + 1 E-3 M Mg	2.00401	4.53	8.45
HA + 7 E-4 M Mg	2.00387	3.12	4.68
HA + 5 E-4 M Mg	2.00386	3.40	4.35
HA + 1 E-4 M Mg	2.00386	4.53	4.11
HA + 5 E-5 M Mg	2.00417	3.97	3.56
HA + 1 E-3 M Zn	2.00368	5.39	8.68
HA + 1 E-3 M Ba	2.00400	3.69	5.75
HA + 1 E-3 M Ca	2.00390	4.53	4.22
HA + 1 E-4 M Al	2.00415	4.53	3.13
HA + 1 E-4 M Sm	2.00447	3.97	2.64
HA + 1 E-4 M Ce	2.00433	3.69	3.01
HA + 1 E-3 M N2	2.00400	2.55	0.10

An interesting observation, also made by Golonka et al. [11], was that the addition of 1.0 × 10^{-3} M Mg^{2+} to the HA solution produced a more than 3-fold enhancement of the EPR signal of the humates, indicating a stabilization of the semiquinoid radical in the humic structure. In view of the fact that Mg^{2+} and other divalent metal ions are known to undergo bridging interactions with carboxyl groups on humates [10], this observation suggests that such bridged structures were involved in the stabilization of humic semiquinones.

Other divalent metal ions, Mg^{2+}, Ba^{2+}, Ca^{2+} and Zn^{2+}, were also found to give EPR resonance enhancements, albeit to different degrees (see spin counts in **Table 1**). In contrast, monovalent metal ions such as Na^+ and Li^+ produced little or no enhancement. The addition of a large organic divalent cation, $[(CH_3)_3N\text{-}(CH_2)_2\text{-}N(CH_3)_3]^{2+}$, did not enhance the humic EPR signal, but actually decreased it.

If the formation of bridged humates was the cause of the observed EPR enhancements, then it should be expected that trivalent species such as Al^{3+}, Sm^{3+}, and Ce^{3+} would have similar effects. In fact, relatively small signal increases (< 30%) were observed with these ions, but their propensity to cause HA to precipitate at ionic concentrations greater than ca. 1.0×10^{-4} limited the observable effects to very dilute solutions. The data in Table 1 show that low Mg^{2+} concentrations gave accordingly small EPR signal enhancements, so it can be cautiously inferred that the humic structures produced by interaction with trivalent metal ions can also increase humic EPR signals.

Bridging interactions between metal ions and (primarily) carboxyl groups in humates can be both intra- and inter-molecular. The latter case, especially, can lead to precipitation at higher ionic strengths. It has been shown [7] that this precipitation proceeds through a gradual process of aggregation in which humic particles grow from micellar to macroscopic size. The effect can also be caused by 1:1 salts containing non-bridging (monovalent) cations, which, when the ionic strength is high enough, can shield the negative charges on humic polyanions sufficiently to overcome mutual repulsion.

The question arises whether the apparent stabilization of semiquinoid entities in humates in the presence of metal ions depends entirely on bridging interactions, or whether nonspecific aggregation effects also play a role.

Since the particle size of dissolved humates provides one of the most direct measures of aggregation in HA solutions, dynamic light scattering (DLS) measurements were carried out on humic solutions with various additives. The results shown in **Figure 2** pertain to solutions in which no precipitation had taken place. It can be seen that the addition of a monovalent metal ion did not produce aggregate size changes [7], consistent with the view that these ions do not bind humic polyanions together.

Figure 2 also shows that low concentrations of Mg^{2+} caused a small reduction in particle size, which has previously been ascribed to intramolecular contraction [8]. At a Mg^{2+} concentration around 5.0×10^{-4} M the average particle size began to increase and continued to do so until humic flocs appeared. In this progression of events, EPR signal enhancement due to Mg^{2+} became noticeable at a concentration of 4.0×10^{-4} M, *i.e.* slightly before the HA particles began to grow significantly. This suggests that radical stabilization was primarily predicated on metal mediated intramolecular bridging.In the case of the addition of Ce^{3+} the effect was greatly compressed (**Figure 2**) and proceeded to intermolecular aggregation at low cation concentrations, quickly followed by precipitation. As noted above, this left room for the observation of only minor EPR signal enhancement.

Surface tension measurements, shown in **Figure 3**, further illustrate the relation between the state of cation induced humic aggregation and EPR signal enhancement. The data for Mg^{2+} show a gradual decrease in surface tension with cation concentration, which has previously been ascribedto the formation of humic amphiphiles [11] as the cations bind with and neutralize part of the anionic (carboxyl) groups in the humate. This regimen coincides with the conditions under which EPR enhancement was observed. At higher Mg^{2+} concentrations, pseudomicellar aggregation led to the removal of humate from the air-water interface and a commensurate increase in surface tension. EPR signal enhancement persisted in this region, as it did with the larger humic particles shown in **Figure 2.** The surface tension data obtained with the addition of

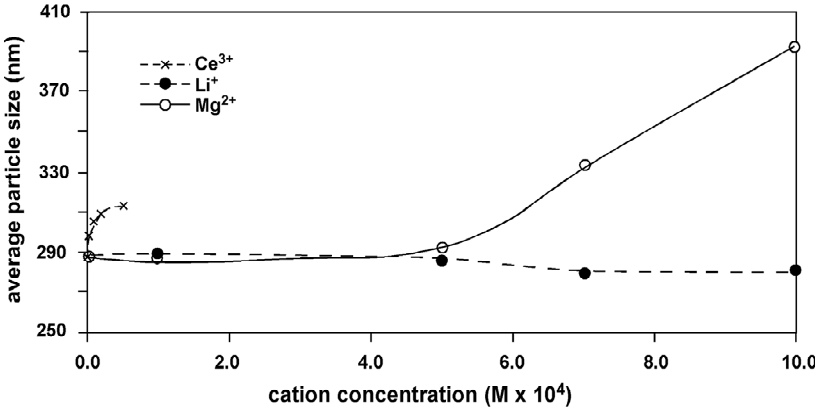

Figure 2. Average particle size of HA in concentration 500 mg/L with addition of Li^+, Mg^{2+} and Ce^{3+}.

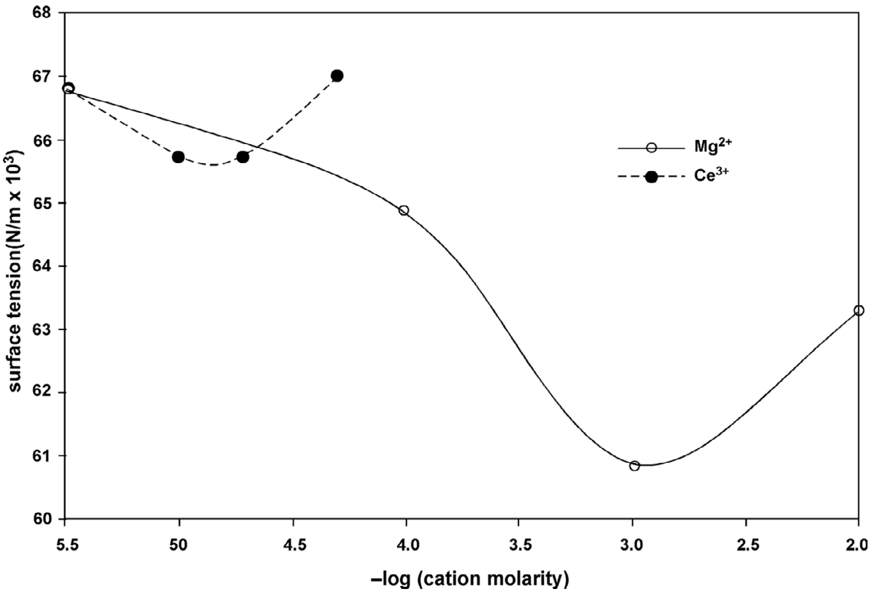

Figure 3. Variation of the surface tension of a 500 mg/L HA solution with addition of Mg^{2+} and Ce^{3+}.

Table 2. EPR characteristic of HA in water and urea.

Sample	g value	Line width (G)	Free radical concentration (spins/g × 10^{-17})
HA	2.00361	3.35	2.32
HA + $Mg^{2+\dagger}$	2.00401	4.53	8.45
HA + urea*	2.00409	5.38	2.60
HA + urea + Mg^{2+}	2.00375	4.54	2.23
$^{\dagger}1.0 \times 10^{-3}$ M			
*8 M			

Ce^{3+} again showed the aggregation range to be truncated by precipitation at low cation concentrations.

Urea is a well known chaotrope which, in high concentrations, disrupts the hydrogen-bonded network of water, allowing macromolecules more structural freedom and promoting protein extension and denaturation [16,17]. It has been shown that the ability of urea to destabilize hydrophobic aggregates extends to the break-up of HA structures in aqueous solution [18]. The data in Table 2 demonstrate that the disruption of these structures had a profound effect on the EPR signal. In solutions containing only HA, the addition of urea made little difference to the spin concentration, but the signal enhancement observed upon addition of Mg^{2+} disappeared when urea was added. This adds further credence to the contention that cation-bridged structures—which were disrupted by urea—are essential to radical stabilization in HA.

4. Conclusions

In conclusion it can be noted that diamagnetic species such as alkaline earth metal ions and zinc ions exert considerable influence on free radical concentrations in humates by stabilizing semiquinone structures within the humic framework. Disruption of the aggregates formed by metal bridging interactions lead to a loss of radical content and EPR enhancement.

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