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A new humic acid remedy with addition of silver nanoparticles

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ABSTRACT: Previously known biogenic stimulator humic acid (HA) was the subject of this current study and HA based new remediation was developed by addition of silver (Ag) nanoparticles in its macromolecule. Extracted HA from a healing mud was characterized and used as reducing agent for Ag ion as well as a stabilizer for the formed Ag nanoparticles. The properties of the obtained hybrid composite were examined by XRD, UV and FTIR spectroscopic techniques. The diameter of the nanoparticles in the HA polymer was up to 8.6 nm and they were identified to be metallic Ag.

Keywords: Composite; Healing mud; Humic acid; Silver nanoparticles

INTRODUCTION

Aromatic nucleus and functional groups in the HA molecule react with cell walls of organisms and with receptors in a derma; furthermore, it shows a therapeutic effect by penetrating through tissues [1]. HA is a major proportion of organic matters in Mongolian healing mud [2] and it tends to form an organomineral particles. A hypothesis, therefore, was developed that HA may possess a stabilizing character for metal nanoparticles as other natural polymers do [3-4] and such behaviour has been recently investigated [5]. Humic substances from the healing mud differ from coal, turf and soil derived counterparts by their low molecular weight and high content of reactive functional groups [6]. Silver shows an antibacterial characteristic in addition to its chemical stability, good thermal and electrical conductivity and catalytic properties. Therefore organic and inorganic hybrid composites bearing Ag nanoparticles were synthesized and studied for a different purposes; for instance, as medical preparations, antimicrobial coatings for

biomaterials, as well as film castings for electronic and optoelectronic devices. The formation of Ag nanoparticles in a variety of polymers (e.g., natural and synthetic) has been examined as size and dispersion pattern of metal particles were unique for each case. Also natural polymers are biocompatible making them particularly interesting for researchers.

For obtaining metal nanoparticles, the most important controlling parameters include reducing agent type, stabilizer and the matrix in which nanoparticles are formed. Dendrimers usually take on a spherical three dimensional structure, which is very different from linear polymers. Additionally, it is predicted that HA may provide more active sites for stabilization of metal nanoparticles.

Akaihe et al examined the formation of Ag nanoparticle in the presence of HA (i.e, from the fresh water and marine sediment) under environmentally relevant conditions (e.g., concentration, pH and temperature), and discussed the possible formation by the natural processes and their stability depending on HA

characterization as well as environmental mobility [5].

Lipid, humic substances and water extracts from healing mud often used for therapy, among them HA based products are commercially well known. In this study, we aimed to develop a method to obtain a new prospective HA remedy based on nanotechnology approaches. It makes possible to overcome the disadvantages of conventional HA remedies by promoting biological activity, extended duration of effectiveness, elimination of toxicity and side effects, as well as increasing the selectiveness of treatments. In addition, it is one of a few studies on HA polymer as a stabilizer for metal nanoparticles.

EXPERIMENTAL

Materials and reagents. HA fraction was extracted from a sapropel peloid from Lake Gurvan nuur (geographical coordinates: 42°01' N; 111°40' E), in Khentii province, Mongolia. 0.2 N sodium hydroxide (NaOH) extraction was performed after HCl pretreatment and benzene/chloroform extraction as the peloid sample contains a significant amount of non HA organic matter [2]. The alkaline extract was decanted, centrifuged then passed through a nuclear filter with a pore size of 1 μ m (prepared at the Flerov Laboratory of Nuclear Reactions, Joint Institute for Nuclear Research, Dubna, Russia) to ensure the HA extract was free of clay particles. HA precipitated from the NaOH solution when acidified (pH~2) by using 0.1 N HCl solutions and washed by deionized water. Then dried and ground for further analyses and procedures. Silver nitrate (AgNO₃) and ethanol were all AR grade and were used as received from a supplier. Ultrapure water from an Arium®basic system (Sartorius Co., LTD) was used throughout this work.

Synthesis of HA/Ag hybrid composite. Synthesis of HA/Ag organic and inorganic hybrid composite with antibacterial capacity was performed based on a method [7]. For each set of experimental trials, 400 mg of peloid HA was dissolved in 6 mL of 1 N NaOH solution and heated for 15 minutes. AgNO₃ solutions with Ag concentrations ranging from 36-360 mg (Table 1) were added into the individual trials under constant stirring. Then the reaction

mixture was filtered and ethanol added into it in order to precipitate the reaction product, which was then isolated by centrifugation. This product was washed with deionized water and ethanol as well. Here, ethanol was used as an agent to facilitate [Ag⁺] reduction to Ag⁰ [8] and to precipitate the resultant product from the solutions. Ag concentrations of the composites for each trial are given in Table 1 along with the amount of Ag initially taken for synthesis.

Analytical techniques. The peloid derived HA and synthetic composites were studied by ultimate analysis and their structures were investigated by using an FTIR spectroscopy, UV spectrometer and an X-ray diffractometer (XRD). The XRD patterns were recorded at a scanning rate of 4°min⁻¹ in the 2 θ range of 20-90° using a Bruker D8 Advance diffractometer with Cu K α radiation (λ = 1.54178 E). UV-Vis spectra were measured on a Specord UV-Vis spectrophotometer, while FTIR was measured using an RAM II Bruker Vertex70 spectrophotometer. In order to get quantitative results from the FTIR spectra, the intensities of the bands due to the oxygen containing functional groups were compared to those for aromatic (1619 cm⁻¹) and aliphatic units (2920 cm⁻¹) (Table 2). The Ag concentration was measured using an atomic adsorption spectrophotometer (AAS) Perkin-Elmer Analyst 200.

RESULTS AND DISCUSSION

In terms of molecular structure, HA is an aromatic non organized polymer containing carboxylic, phenolic OH, quinon, hydroxyl, methoxyl, aldehyde, keton and enolic group as well as nitrogen and sulfur (often found in thiol group) atoms. Its main structural skeleton is alkyl and polyfunctional groups attached to condensed aromatic rings, with polysaccharides and polypeptides associated into it. We found efficient HA purification from clay particles using the nuclear filter and its ash content was negligible. It consisted of 48.9% C, 5.4% H, 5.0% N, 1.3 % Cl (percentages based on weight), and the molar ratio of H/C was 1.325, which indicates higher aliphaticity than typical value for other HAs from coal and soil specimens as reported by the International Humic Substances Society. Ag content in the hybrid composites fluctuated from 5.5 g/100 g⁻¹ to 24.8 g/100 g⁻¹, where the initial ratio of Ag⁺:HA ranged between 1:1 to 1:11 (Table 1).

Table 1. Silver concentrations in HA matrix

Trials	Added silver amounts, mg	The ratio of Ag + HA	Silver concentration s (g/100 g composite)
Trial 5	36	1:11.1	5.5
Trial 3	72	1:5.6	9.5
Trial 7	108	1:3.7	12.0
Trial 6	144	1:2.8	15.6
Trial 8	360	1:1.1	24.8

In FTIR spectrum (Fig.1), the peloid HA showed an intense band at 3425 cm^{-1} that was due to hydroxyl groups, and bands at 2920 cm^{-1} and 2850 cm^{-1} which attributed to the C-H of methyl and methylene groups. Bands at $1619\text{--}1591$, and 1379 cm^{-1} were due to benzene ring with C=O units. The CO in ether groups appeared at 1228 cm^{-1} , and in hydrocarbons registered between 1076 and 1041 cm^{-1} [8]. The FTIR spectrum of the parent HA and the obtained hybrid composites (not shown here) were similar, indicating that the HA molecular structure remained virtually unchanged after reaction. The parametrical calculation of HA structure from FTIR spectra revealed that the dominant oxygen bearing moieties were hydroxyl groups of phenol and carbohydrate, and carbonyls (C-O) of ether and carbohydrate (Table 2).

Table 2. Comparison of band intensities in FTIR spectra of the peloid-derived HA

Functional groups and its corresponding wavelength (X_1/X_2)	Band intensity		Ratio of I_{X1}/I_{X2}
	(I_{X1})	(I_{X2})	
$\text{OH}_{3425}/\text{C}=\text{C}_{1619}$	44	52	0.84
$\text{Calk}_{2920}/\text{C}=\text{C}_{1619}$	52	52	1.00
$\text{CO}_{1228}/\text{C}=\text{C}_{1619}$	57	52	1.10
$\text{OH}_{3425}/\text{Calk}_{2920}$	44	52	0.85
$\text{CO}_{1228}/\text{Calk}_{2920}$	57	52	1.10

plasmon band appeared at around 400 nm. Similarly, study on Ag nanoparticle formation in the presence of various origins of HAs (i.e., the fresh water and marine sediment HAs) showed the surface plasma resonance peak at around 400-420 nm [5]. These authors concluded that the aliphatic predominant HAs (e.g., sedimentary) more readily reduced Ag+ than did aromatic dominant HAs (i.e., from soils) and formed Ag nanoparticles were stable. The variation in peak wavelength may result from particle size and agglomeration state of

Therefore, the ratio of hydrophilic and hydrophobic moieties ($\text{OH}_{3425}/\text{Calk}_{2920}$) of HA was 0.85, while the ratio of aliphatic and aromatic ($\text{Calk}_{2920}/\text{C}=\text{C}_{1619}$) units was 1.

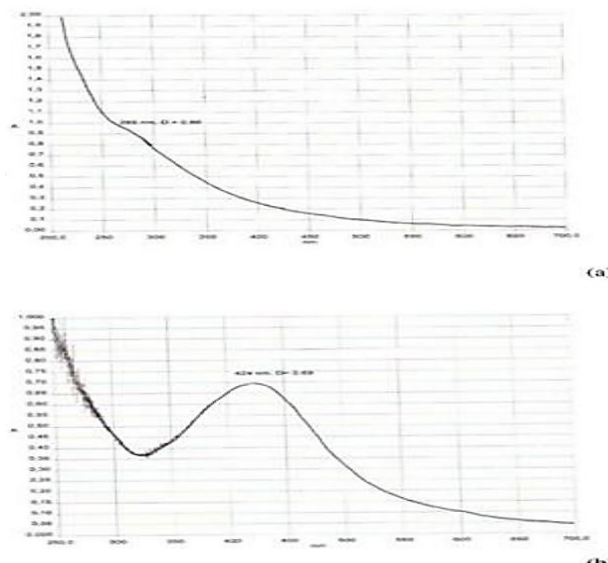


Figure 2. UV-Vis spectra of (a). HA in sodium hydroxide solution and (b). HA matrix contains Ag nanopartic

In the UV-Vis spectrum of peloid derived HA, an absorption maximum at 285 nm with high optic density was observed and that was assigned to -OH groups in phenolic moiety (Fig.2.a). But in the HA/Ag spectrum (for trial 8), an absorption peak was observed at 424 nm (Fig.2.b) which is associated with the surface plasmon resonance of nanoparticles of metal Ag. This absorption maximum in the hybrid composite resulted from electron transmission of excited metals. It is believed that Ag ion was reduced in the presence of HA by its reducing sites [9]. An earlier work on HA/Ag composite was studied this composite as a colorimetric sensor of herbicide [10]; the average size of nanoparticles found to be 5 nm and the surface nanoparticles were calculated by the 111 line; and a parameters of unit cell ranged $4.059(1)\text{--}4.077(1)\text{ \AA}$; and the average coherent scattering region of Ag nanoparticles was 8.6 nm in the HA/Ag composite. Aggregate resistant of colloidal system is defined by several parameters including small particles solvation and repulsion of electrical statistic force. It is certain that HA polyanions in aqueous solution react with positively charged Ag. This interaction could result in coagulation of the system. However, the colloid system was stable

formed nanoparticles. The surface plasmon bands for nano-sized Ag formed in natural rubber matrix were ranged between 425-484 nm [4], while it was at about 418 nm for Ag/polyvinyl alcohol [11] composite, where particles sizes were 4-10 nm and <10 nm, respectively.

in the given ratios of HA:Ag. This showed that HA functional groups occupy all possible coordination positions on the surface of metal nanoparticles. HA associates to the nanoparticle surface and increases the hydrophilic character of products; this will sustain its stability in

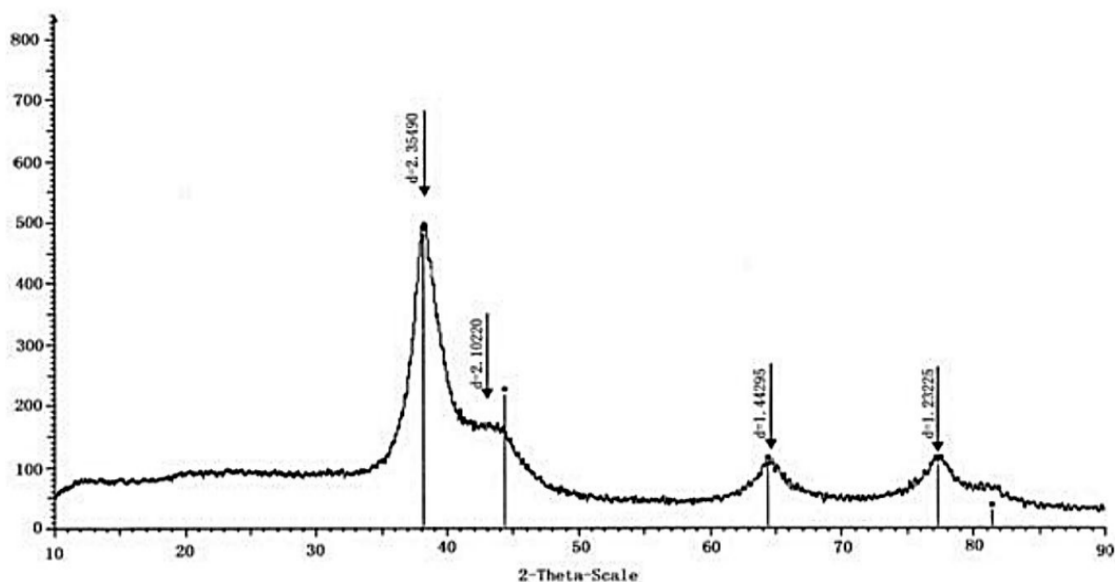


Figure.3. XRD pattern of Ag nanoparticles stabilized within peloid HA

The XRD spectrum, in Fig.3 (trial 8), was consistent with published data on nano-sized metal Ag [7] and demonstrated the existence of Ag^0 in the product. All the prominent peaks at 2θ values of about 38, 44.2, 64.4, 77.6 and 81.6 $^\circ$ represent the 111, 200, 220, 311 and 222 Bragg's reflections of Ag crystal. The diffraction line for the first precursor (i.e., Ag^+) was not observed, showing that the reduction process was completed. Also, diffraction line intensity and distance between planes were in good agreement with the standard lines of Ag. Both UV-Vis spectra and XRD analysis confirmed that the synthesized hybrid product contains Ag^0 . Here, reduction of Ag^+ could be induced by oxidation of phenolic hydroxyl and carbonyl groups in HA composition. The average unit cell parameters of Ag

aqueous solution. The resultant HA/Ag hybrid composite could be essential to improve the properties of HA preparations because of Ag's antibacterial properties and the enhanced solubility of the product.

CONCLUSIONS

We sought to develop a new HA remedy containing Ag nanoparticles. In this work, ethanol was used to precipitate the resultant product due to the stabilizer's (i.e., HA) analytical character and also to facilitate the reduction of Ag^+ to Ag^0 . The Ag nanoparticles formation within HA polymer was determined by analytical techniques and the average size of metal particles were determined to be 8.6 nm. This advanced HA/Ag hybrid composite, will contribute to overcome some problems observed in traditional HA based remedies.

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