Separation of photosensitive substances in humic acids using molecular imprinting method based on electrostatic interactions and hydrogen bond

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Abstract. The goal of this research was to provide an improved understanding of the existence of photosensitive structure in humic acids (HAs) that are extracted from Changbai Mountain soils. Molecular imprinting technique was used to separate phthalocyanine-like substances from HAs with the mechanisms of electrostatic interactions and intermolecular hydrogen bond. Copper phthalocyanine (CuPc) was used as template molecule and the fraction bound by CuPc-imprinted polymers (MIP) named F (bind) presented higher spectroscopic activity than that of effluent fraction named F (eff). The fluorescence intensity of F(bind) at emission wavelength of 462 nm was 5.5 times as high as that of F(eff) at 458 nm, and the UV-vis absorbance at 254 nm of F(bind) had been increased to 2.5 times as compared with F(eff). The results of this work show the key role of humic substances with special structures in the light or photo involved process.

1. Introduction
Humic acids (HAs) are one of the important components in natural environment. It has generally been accepted that the photodegradation of organic pollutants were affected significantly by the presence of HAs since these processes such as absorbing light by HAs were always accompanied by the formation and consumption of reactive oxygen species (ROS, e.g. •OH, ¹O₂, and ROO•).

However, previous studies have shown that HAs often control many photochemical reactions of pollutants in two ways. In brief, one theory assumed the presence of HAs could facilitate the photochemical degradation of organic compounds, which can be attributed to the excited triplet states (³HA*) and ROS produced through irradiation of HAs [1, 2]. The second theory assumed HAs could inhibit the photolysis of organic pollutants due to the competitive absorption for available photons and the hydrophobic binding interaction between pollutants and humic materials [3, 4]. These results reveal that different compositions of HAs display different photochemical behavior and photochemistry of HAs is closely related to their structures [5]. Therefore, to evaluate the contribution of different structures in HAs to the sensitized photodegradation of pollutants, recognition and separation of the substances with specific structure from HAs that are related to certain photochemical behavior become crucial [5, 6].

Molecular imprinting technique, widely applied in separation, synthesis, and catalysis, is usually created by imprinting template molecules in a rigid porous polymers matrix through the non-
covalent/covalent interactions between template molecule and functional monomer [7, 8]. Molecular imprinting technique is beneficial for the separation of molecules with the photosensitive structure from HAs. Chlorophyll, the key component during photosynthesis of plants, might absorb the sunlight and result in a series of electron transfer due to the presence of photosensitive structure, and would remain in humic substances through humification after plants’ life cycle finishes [9]. In this paper, copper phthalocyanine (CuPc) is selected as the analogue of chlorophyll due to their similar photochemical property and the chemical labilization of chlorophyll, and then CuPc is served as the template molecule during molecular imprinting.

The study aimed to contribute to a better understanding of photosensitive structure in humic substances by separating HAs components using the molecular imprinting technique. The spectroscopic techniques was used to characterize the different components that constitute the major fraction of HAs extracted from Changbai Mountain soils, making it possible to elucidate the relationship between photochemical properties and the structure of humic substances.

2. Materials and methods

2.1. Soil samples and humic acids analysis
HAs were extracted from broadleaf forest soil samples by the International Humic Substances Society (IHSS) methodology [10]. The sample sites were situated in Changbai Mountain, Jilin province, China. The HAs powder was dissolved in Milli-Q water that was adjusted by NaOH to pH 7.5–8.0, and the HAs concentrations were measured by a total organic carbon analyzer (TOC-VCPH, Shimadzu, Japan).

2.2. Standards and reagents
All chemicals were analytical reagent grade. α-Methacrylic acid (MAA) and 2,2’-azobis (isobutyronitrile) (AIBN) were purchased from Fuchen Chemistry Reagent Co. (Tianjin, China). Ethylene glycol dimethacrylate (EGDMA) was supplied by Alfa Aesar, A Johnson Matthey Co. (U.S.). Copper phthalocyanine (CuPc, purity ≥ 98%) was obtained from Tokyo Chemical Industry Co., Ltd. (Japan). Oxalic acid, citric acid, glucose, and sucrose were obtained from Kemiou Chemistry Reagent Co. (Tianjin, China). Milli-Q water (resistivity ≥ 18 MΩ·cm) was used in all experiments.

2.3. Characterization
UV-vis spectra were measured using a spectrophotometer (Jasco UV-550, Japan). The fluorescence was measured using a fluorescence spectrophotometer (RF5301-PC, Shimadzu, Japan) with the excitation wavelength at 350 nm and the emission wavelength ranged from 360 nm to 600 nm (10 nm bandwidth). The morphology of samples was observed using scanning electron microscope (SEM, Hitachi S-4800). FTIR spectra were recorded on an FTIR spectrometer (Prestige-21, Shimadzu, Japan).

2.4. Preparation of MIP
CuPc was used as the template molecule to prepare molecularly imprinted polymers (MIP). The functional monomer, cross-linking agent, and initiator are MAA, EGDMA, and AIBN, respectively. The preparation process of MIP was described in reference 11. Simply, 1 mmol CuPc was mixed with 4 mmol MAA, 20 mmol EGDMA, and 40 mg AIBN in a glass tube containing 12 mL chloroform. The mixture solution was sonicated for 5 min, flushed with nitrogen gas for 15 min, and then polymerized by heating at 60 °C in thermostatic water bath for 24 h. The obtained MIP was removed the template molecules by washing with methanol/acetic acid (9:1, v/v) solution. Nonimprinted polymers (NIP) were prepared in the same manner only without CuPc template. MIP and NIP after elution were dried and used for further experiments.

2.5. MIP-SPE cartridges preparation and fractionating procedure of HAs by MIP
CuPc-MIP (0.25g) was placed into empty polytetrafluoroethylene (PTFE) solid phase extraction (SPE) cartridges (6 mL) to prepare MIP-SPE cartridges. The fractionating procedure is that HAs aqueous
solution (20 mL, 5 mg L\(^{-1}\)) passed through CuPc-MIP cartridges at a flow rate of 0.5 mL min\(^{-1}\), effluent fractions were collected and named F\(_{\text{eff}}\), and the bound fractions were washed using 60 mL of fresh Milli-Q water, and then collected and named F\(_{\text{bind}}\). All fractions were concentrated by evaporation at 60 °C in a thermostatic water bath, and used for further experiments.

3. Results and Discussion

![Figure 1. FTIR spectra of MIP and NIP before elution.](image)

3.1. FTIR characterization of polymers

FTIR spectra of polymers (MIP and NIP) were recorded before template molecule CuPc removal to investigate intermolecular interactions between template molecule CuPc and functional monomer MAA. Figure 1 gave the FTIR spectra of MIP and NIP before elution. The changes of MIP compared with NIP were as follows: (i) decreasing in the intensity of \(\nu(–OH)\) at 3400–3600 cm\(^{-1}\); (ii) decreasing the bands assigned to the asymmetric and symmetric –COOH stretching at 1636 cm\(^{-1}\), 1614 cm\(^{-1}\) and 1400 cm\(^{-1}\) [6]. Furthermore, the band at 1728 cm\(^{-1}\) assigned to C=O stretching of carbonyl and carboxyl had the same intensity for MIP and NIP, which may be due to the rich carbonyl in EGDMA. These observations indicated that the interaction between CuPc and MAA was possible contributed by –OH···N hydrogen bonds and electro static attraction resulted from the interaction between protonated nitrogen and ionized carboxyl [7, 11].

![Figure 2. UV-vis spectra of MIP eluted by CH\(_3\)OH:CH\(_3\)COOH (9:1).](image)

![Figure 3. SEM image of MIP (a) before and (b) after elution.](image)
3.2. Elution of MIP and SEM characterization of polymers
The obtained MIP was removed the CuPc molecule by washing with methanol/acetic acid (9:1, v/v) solution until no template molecules were detected in the effluents with the UV-vis measurement. It can be seen from figure 2 that the mixture of methanol and acetic acid (9:1) was effective to remove the template molecule from MIP. The surface topography of MIP before and after elution was observed by SEM, which was shown in figure 3a and b. It is clear that both MIP before and after elution are obviously network materials with microporous structures. The surfaces of the MIP before elution appear to be smooth and porous, whereas the SEM micrographs of MIP after removal of template produced rough surfaces and more pores of different sizes. The result provided additional evidence for the efficient removal of template molecules with organic solvent.

3.2. Selectivity of MIP
To investigate the selective binding of Pc-like substances from HAs for the eluted MIP, some elementary structures in HAs, such as oxalic acid, citric acid, glucose, and sucrose, were used for binding tests onto MIP (NIP)-SPE. The chemical structures of these substances may be form hydrogen bonds and have electrostatic interactions with MIP because of the presence of carboxyl or hydroxyl groups in these substances. The concentrations of original solutions and effluents from MIP (NIP)-SPE cartridges were determined by UV-vis spectrophotometer. Figure 4 showed that differences of absorbance from 200 nm to 400 nm between original solutions and effluents for four organic matters were negligible, indicating that these substances did not interact with the eluted MIP or NIP according to hydrogen bonds or electrostatic interactions. Thus the four substances did not effectively bind onto MIP and the prepared MIP possessed high selectivity.

3.3. Fluorescence and UV-vis spectra of HAs fractions
Figure 5 showed the synchronous fluorescence spectra of HAs fractions recorded upon excitation at 350 nm. Compared with F(eff), the spectrum of F(bind) presented some differences as follows: (i) fluorescence intensities of F(bind) were obviously more intense than that of F(eff) and maximum intensity of F(bind) was 5.5 times as high as that of F(eff); (ii) the emission bands of F(bind) was
broad and that of F(bind) was narrow; (iii) the band with maximum intensity shifted from 458 nm for F(eff) to 462 nm for F(bind). These phenomena showed that the structures of F(eff) and F(bind) were indeed different and the fraction with similar structure of Pc such as extended κ-conjugated ring structures had more fluorophores. This result is of interest for better understanding the relationship between photochemical activities and the structure of HAs in respect that the substances with more fluorophores could display better photoinductive activity [12].

The UV-vis absorption spectra of HAs fractions were presented in figure 6. The spectra of F(eff) and F(bind) exhibited a featureless increase in absorbance during the wavelength decreasing and were dominated by broad humps at 254 nm. The shoulder at 254 nm is attributed to aromaticity structures or π–π* electron transitions of unsaturated bonds [13]. The UV-vis absorbance at 254 nm of F(bind) had been increased to 2.5 times as compared with F(eff), indicating that F(bind) possessed the better electron transfer capacity. It was clear that humic substances with special structures (e.g. Pc) would present the key role in the light or photo involved process.

![Figure 5. Fluorescence emission spectra of HAs fractions (5 mg L⁻¹).](image)

![Figure 6. UV-vis absorption spectra of HAs fractions (5 mg L⁻¹).](image)

4. Conclusion
The separation of HAs extracted from Changbai Mountain soils can be achieved well with molecular imprinting method. The functional monomer MAA can interact with N atoms of template molecules CuPc according to hydrogen bonds and electrostatic interactions. Based on the spectroscopic characterization of different HAs fractions by synchronous fluorescence and UV–vis, the fluorescence intensity and UV-vis absorbance of HAs fractions binding on CuPc-MIP were both much higher as compared with effluent fractions when HAs solutions passed through CuPc-MIP SPE cartridges. The results provide an improved understanding of the existence of photosensitive structure with better spectroscopic properties in humic material.

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References
[1] Canonica S, Jans U, Stemmler K and Hoigné J 1995 Environ. Sci. Technol 29 1822
[2] Gerecke A C, Canonica S, Muller S R, Scharer M and Schwarzenbach R P 2001 Environ. Sci. Technol 35 3915
[3] Torrents A, Anderson B G, Bilboulian S, Johnson W E and Hapeman C J 1997 Environ. Sci. Technol 31 1476
[4] Bachman J and Patterson H H 1999 Environ. Sci. Technol 33 874
[5] Yu C, Zhang Y, Quan X, Chen S, Han J, Ou X and Zhao J 2010 Environ. Sci. Technol 44 5812
[6] Ou X, Chen S, Quan X and Zhao H 2008 *Chemosphere* **72** 925
[7] Gao D M, Zhang Z P, Wu M H, Xie C G and Guan G J 2007 *J. Am. Chem. Soc* **129** 7859
[8] Alexander C, Davidson L and Hayes W 2003 *Tetrahedron* **59** 2025
[9] Chen J, Gu B H, LeBoeuf E J, Pan H J and Dai S 2002 *Chemosphere* **48** 59
[10] Swift R S 1996 Organic matter characterization *Methods of Soil Analysis* (Madison: Soil Science Society of America) pp 1018–1020
[11] Yu C, Chen S, Quan X, Ou X and Zhang Y 2009 *J. Agr. Food Chem* **57** 6927
[12] Richard C, Trubetskaya O and Trubetskaj O 2004 *Environ. Sci. Technol* **38** 2052
[13] Zhang H, Peng Q and Li Y 2005 *Modern Spectral Analysis of Organic Matters* (Beijing: Chemical Industry Press) pp 228