Radionuclide Diagnostics of Hydrophobicity and Surface Activity of Humic Substances

Gennadii A. Badun[a], Maria G. Chernysheva[a*], Andrey I. Konstantinov[a], Natalia A. Kulikova[a,b], Irina V. Perminova[a]

[a]Lomonosov Moscow State University, Leninskie Gory, 119992, Moscow, Russia;
[b]Bach Institute of Biochemistry of Russian Academy of Sciences, Leninskii 33, 119071, Moscow, Russia
E-mail: masha.chernysheva@gmail.com

1. Introduction

Humic substances (HS) is a widespread reactionary active component of soils. Dual structure and composition of HS provide their hydrophobicity and surface activity. Understanding fundamental physical properties of HS is important because of their direct relevance to the behavior of HS in the environment. Among analytical methods, radiochemical assay is the most suited and perspective for the experimental studies because of its sensitivity, selectivity and universality. In our previous work [1, 2] we have shown, that huge advantage of tritium thermal activation technique is the possibility of labeling HS from different origin. Specific radioactivity of labeled product was at least 2 times higher than one obtained by chemical reduction of fulvic acids by treated reagents [3] Furthermore, we have used tritium labeled HS for studying their interaction with bacteria and plants [4, 5] and also their hydrophobicity and surface activity by scintillation phase technique in aqueous/toluene system [6] In this study, we have shown that it is possible to achieve higher values of radioactivity of labeled HS and investigated sorption of HS at different hydrophobic surfaces by liquid scintillation spectrometry of tritium. This work has two component goals. To achieve the first of these goals, HS were labeled with tritium by means of tritium thermal activation method. The second critical goal was to investigate hydrophobicity and surface activity of [^{3}H]-HS by liquid scintillation spectrometry of tritium. We have study the behavior of HS in aqueous/oil system by liquid scintillation spectrometry in the application of scintillation phase technique. Not only toluene but alkanes and alcohols were used as an oil phase.

2. Materials and methods

Brown coal humic acids (CHA) (commercially available preparation Powhumus (Humintech, Germany) was used. Tritium labeling was carried out by means of thermal activation method [7]. One mL of 0.3 g/L solution of CHA in 0.05% NaOH was distributed on the walls of glass reactor, frozen with liquid nitrogen and lyophilized. Then the reactor was connected to

vacuum system. Air was pumped out under resent pressure 0.01 Pa and then filled with tritium gas. To increase specific radioactivity of the product the reaction was conducted at target temperature 293–298 K [8]. W-wire temperature, gas pressure and treated time were verified in the wide possible range. After the reaction gas was pumped out, the target was dissolved in 0.4% NaOH. To purify [^{3}H]-CHA from labile tritium and radioactive by-products 30 days dialysis through 2 kDa membrane against 0.028 M phosphate buffer (pH 6.8) was carried out. Final product was analyzed by size exclusion chromatography detecting both UV-absorbance and radioactivity [**¡Error! Marcador no definido.**].

The behavior of HS in aqueous/oil system was studied by means of scintillation phase method [**¡Error! Marcador no definido.**]. The experiments were carried out in 7 mL polyethylene vials. To 1 mL of [^{3}H]-CHA solution 3 mL of scintillation cocktail was added. Scintillation phases based on octane, toluene and octanol were used. The system was incubated during 5 days at 22 °C. Tritium counting rate of both whole system and the bit of organic phase was measured. The distribution coefficient and the value of adsorption of CHA were calculated according to the procedure described in Ref. [9].

3. Results and Discussion

In this work tritium thermal activation technique was applied for labeling of CHA at target temperature 295±3 K. It was found, that under this condition neither variation in W-wire temperature nor in gas pressure does not lead to significant changing in specific radioactivity of final product. Only the extension of the reaction time from 10 to 30 s led to specific radioactivity growth from 0.4 to 1.3 TBq/g. It has to be noted that at target temperature 77 K specific activity of labeled HS was decreased when exposition time was longer then 10 s. Unfortunately the formation of by-products of high molecular weight because of polymerization was also increased with the extension of exposition time. Comparing the results of the experiments allowed us to determine the conditions under which maximum specific radioactivity exist with minimum by-products, which can be separated by size exclusion chromatography. They are 10 s exposition at W-wire temperature 1880 K and 1.2 Pa of tritium gas pressure. Specific radioactivity of [^{3}H]-CHA was 3.5 times higher then one obtained at target temperature 77 K.

In order to provide sorption experiments at aqueous/oil interface [^{3}H]-CHA was used. We have compared the influence of the nature of organic liquid on the behavior of CHA. Liquid scintillation spectrometry of tritium in the application of scintillation phase technique allowed us to investigate both the adsorption of [^{3}H]-CHA at aqueous/oil interface and its distribution

in the bulk of the system of two immiscible liquids. In this study, we have measured the distribution coefficient of CHA as a ratio of the concentration of CHA in the organic phase to its concentration in aqueous phase. It is evident that distribution coefficient of the compound is a characteristic of its hydrophobicity. Furthermore, we have determined the adsorption isotherms of [^{3}H]-CHA at aqueous/octane, aqueous/toluene and aqueous/octanol interfaces. The investigation of adsorption kinetics has shown that the formation of the adsorption layer occurs in the first three hours. Adsorption isotherms were described by Langmuir equation. HS posses rather low surface activity at aqueous/oil interfaces. The parameters of adsorption isotherms and distribution coefficients for the investigated liquids were differing. The values of adsorption and distribution coefficient were highest for aqueous/octanol system. The reason for these differences related to the specific interaction between the components of HS with molecules of the organic phase.

4. Conclusions

Specific radioactivity of tritium labeled HSs can be increased if the reaction with atomic tritium is carrying out at target temperature 295 K. The behavior of HS in aqueous/oil systems is determined by the interaction of HS with molecules of the organic phase. Radionuclide diagnostics of humic materials can reveal their important and unique properties.

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