Tritium Probe for Investigation of Humic Materials

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1. INTRODUCTION

The research of the properties of humic substances (HS) and their behaviour in different systems including biological systems is convenient by using radioactive label. It seems perspective to use tritium as the labeling agent of HS. Due to the specific organization of HS, to our mind, the most suitable method of introducing tritium into them is the tritium thermal activation method (1,2). Varying the conditions of label introduction it is possible to achieve the acceptable specific radioactivity of labeled compounds and to minimize secondary processes which modify the original compound. In this work the HS partition coefficients in octanol-water and toluene-water systems and their adsorption on liquid-liquid interface have been determined with the help of tritium labeled HS and different variants of liquid scintillation counting method.

2. MATERIALS AND METHODS

Humic (HA) and fulvic (FA) acids used in this study were extracted from peat, soil and coal according to standard technique using alkaline extraction with 0.1 M NaOH. Also a sample of brown coal humic acids (CHA) (commercially available preparation Powhumus (Humintech, Germany)) was used.

Tritium labeling was carried out on a special vacuum set as described in (1,2). After the atomic tritium bombardment the target was dissolved in phosphate buffer (0.028 M) under pH 6.8. The ^{3}H-HS samples obtained were purified from labile tritium by means of dialysis through membranes with pores MWCO 2 кDa (Merck, Germany) in phosphate buffer during a month until the radioactivity of buffer was stabilized. Size exclusion chromatography (SEC) analyses of the labelled HS samples were conducted according to (3) using ABIMED-HPLCsystem. The SEC column was packed with "Toyopearl" TSK HW-55S gel (Toso-Haas, Japan) with the fractionate range from 1 to 200 kDa on polydextranes. Phosphate buffer (0.028M) with pH 6.8 was used as eluent with the flow rate of 1 ml/min.

To determine the partition coefficients between water and toluene K_{tw} and the quantity of adsorption of ^{3}H-HS samples on the liquid-liquid interface, the scintillation phase method was used. The experiment procedures and techniques of separation of "volumetric" and "interfacial" contributions in counting of the samples are described in (4,5). Also partition coefficients of ^{3}H-HS in octanol-water system K_{ow} were determined by means of selection of samples and measurement of radioactivity by the scintillation method. Radioactivity was measured using the liquid scintillation counter RackBeta 1215 (LKB Wallac, Finland).

3. RESULTS AND DISCUSSION

For HS samples of different origin the influence of conditions of tritium label incorporation by means of tritium thermal activation method on yield of labeled products and their specific radioactivity was examined. The influence of gas pressure, target mass and reaction time in general coincides with the results found for label introduction into amino acids and carbohydrates (6-8). The best results were obtained by small reaction time and by use of gas pressure when the direct path of atoms from the wire to the target cooled with liquid nitrogen (77K) was provided. In this case the maximum radiochemical yield of labelled products was achieved. The influence of tungsten wire temperature resembled the results obtained for free amino acids in temperature range 1600-1800 K (8). However at higher wire temperature the reduction of the labeled product yield was not always observed. The cause of this difference is that HS include not only carbohydrate-peptide periphery but also the hydrophobic core with high content of aromatic and aliphatic hydrocarbon fragments. The specific radioactivity of ^{3}H-HS was 0.17-0.73 GBq/g.

The difference in behaviour of labeled and unlabeled HS was not found by means of used analyses procedures. The identity of labeled and initial HS samples was confirmed by size exclusion chromatography. In many cases the complete coincidence of elution profiles of initial and labeled HS was observed. Sometimes a small shift of SEC elution profile of labeled HS to the area of higher molecular weight occurred. The reason of this shift was in a partial loss of the low molecular weight fractions during the dialyses. However in this case the radioactivity and UV-absorption profiles of labeled samples were absolutely the same that proved the similar distribution of the label on the molecules forming the HS sample.

The partition coefficients for HS between water and toluene were determined and also value of adsorption on the interface was found using tritium labeled humic materials and the scintillation method. The partition coefficients between the phases did not depend on substance concentration in water phase and varied in range $0.23 \cdot 10^{-3} - 3.74 \cdot 10^{-3}$. K_{tw} increased in series: FA soil < HA soil < HA peat ≈ FA peat < HA coal. Good correlation between K_{tw} and K_{ow} was found. Thus by means of partition coefficients in toluene-water system the scale of HS hydrophobicity can formed.

Most of isotherms of HS sorption received by means of the scintillation method were the Langmure type. At the increase of HS samples concentration in water phase in the range from 0.1 to 5 mg/l a rapid growth of sorption of the labeled molecules was observed at the toluene-water interface. At the concentration in water phase from 20 to 60 mg/l the saturation of the adsorbed layer took place and at further increase of HS samples concentration in water phase the quantity of HS molecules on the interface practically did not change. The maximum adsorption value almost was corresponded to the monolayer of HS macromolecules on the toluene-water interface. The average values of HS adsorption in the area of HS concentration in water phase of 40-100 mg/l were from 0.08 to 2.8 mg/m^2. The row of the increase in maximum adsorption of the examined samples is as follows: FA soil < HA soil < HA coal < FA peat < HA peat.

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