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Preparation of Tritium-labeled Humic Substances Using Thermal Activation Method

Introduction

Humic substances (HS) represent one of the most abundant classes of non-living organic matter (Humic Substances, 1989). The HS from different sources have similar molecular design determined by a presence of highly oxidized aromatic core and bound to it saccharidic-peptidic periphery. The dualism of the structure results in surface activity of HS and their ability to participate in hydrophobic interactions. As a result, HS can cause a direct impact on the cells and living organisms (Visser, 1986). Despite the numerous findings on the biological effects of HS, until present the particular molecular features that govern the biological activity of HS are not known. To solve the problem, the labeled HS preparations are in need that would possess the same molecular properties as their unlabeled analogues. In this context, tritium label deserve particular consideration, while (1) substitution of ^{1}H with ^{3}H causes minimal distortions in the structure and properties of the labeled humic material; (2) half-life of ^{3}H is 12.3 year that allows long-term studies with the labeled material. A use of thermal activation method allows to introduce ^{3}H-label in any structural fragments of humic macromolecule regardless of their nearest surrounding. The objective of this study was (1) to prepare ^{3}H-HS using thermal activation method, and (2) to characterize the properties of the labeled humic material.

Material and Methods

Humic acids isolated from peat (PHA) and coal (CHA) according to standard technique were used in this study.

Preparation of the labeled ^{3}H-HS. The central part of the reactor used was a cylindrical glass vessel fitted with an axial tungsten wire heated by an electric current. The target material is deposited as a thin film on the side wall of the vessel, which is cooled in liquid nitrogen. The HS target was prepared by liophilisation of 0.7 ml of HS solution (1400 mg l^{-1}). The reactor was evacuated, and then gaseous tritium was let into the system. When the temperature of filament has reached 1500-2500 K, the tritium atomized on its surface, and bombarded the walls of the reactor. Low pressure of molecular tritium (0.5 Pa) and short time (10 s) of its atomization on W-wire was used to avoid the secondary reactions. For every HS sample, two targets were bombarded by atomic tritium. The ^{3}H-HS samples obtained were purified from the labile (exchangeable) label using dialysis. The latter was conducted during a month. This allowed to eliminate labile tritium of OH-, COOH-, and NH_2 –groups.

Size exclusion chromatography (SEC) analyses of the labelled HS-preparations were conducted according to (Perminova et al., 1998) using ABIMED-HPLC-system. The SEC column was 25x400 mm packed with Toyopearl HW-55S resin. Phosphate buffer (0.028M) was used as an eluent at a flow rate of 1 ml min^{-1}. The UV-absorbance of the eluted buffer was registered at a wavelength of 254 nm. Radioactive SEC profiles of the ^{3}H-HS were obtained off-line, using fractionation of the eluent into 2.5 ml aliquots with follow up measurements of their radioactivity. The latter was measured using the liquid scintillation counter RackBeta 1215 (LKB Wallac) and scintillation cocktail Ultima-Flo M (Packard Instrument Co.).

Results and Discussion

Tritium thermal activation method is a convenient way of producing atomic tritium under laboratory conditions (Badun et al., 2000). Thermal catalytic dissociation of tritium molecules occurs on tungsten catalyst at high temperature. The follow

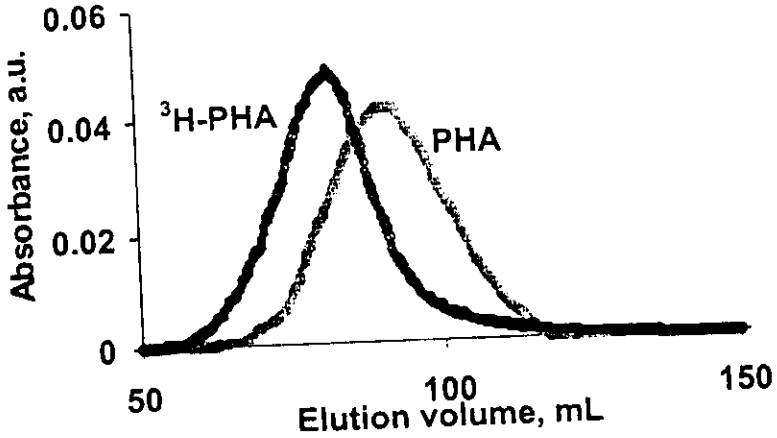

Fig. 1. SEC-profiles of the labelled and unlabelled samples of peat HA

reactions occur during interaction of tritium oms with the target molecules:

$RH + {}^3H \rightarrow R^* + {}^3HH$ (1)

$R^* + {}^3H \rightarrow R^3H$ (2)

The activation energy of reaction (1) for hot ^3H oms varies in the range of 20-27 kJ/mol, that of e reaction (2) equals zero, and occurs for all the H atoms present.

^{3}H-CHA and 3H-PHA samples prepared in this study, had initial radioactivity of 0.13 GBq (per 2 mg) and 0.24 GBq, respectively. The activity of the samples decreased up to 2-20 MBq after the labile tritium was washed out. The UV- and radioactive elution profiles of the labelled HS-samples had very similar appearance characterized by a broad monomodal distribution (Fig.1). However, the peak elution volumes of the labelled HS samples were shifted to the lower values indicating an increase in the molecular weights of the labelled preparations compared to the initial ones. The most probable reason of the observed phenomenon is depletion of the labelled samples with low molecular weight fractions during the purification stage due to a use of dialysis membrane with rather large pores.

To compare hydrophobicity of the labeled and initial humic materials, their distribution between aquatic and organic phase (toluene) was determined using the method of isotopic dilution. The obtained results showed the identity of the labeled and unlabeled preparations used in this study.

The presented research allows a conclusion on the method of thermal activation as a promising tool for preparing the radioactively labeled HS preparations identical to the initial humic materials suitable for multiple biological and chemical studies.

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