INTRODUCTION

Fractionation of dissolved aquatic organic matter on the resin Amberlite XAD designed by J.A. Leenheer [12, 15, 23] is the most common method for the study of organic compounds in natural waters. Most studies [8, 11, 13] devoted to the study of dissolved organic matter and research of carbon behavior in soils and ecosystems are preceded by fractionation of DOM by this method. This is a kind of primary analysis which is followed by more detailed studies of other properties of natural organic substances. The advantages of XAD resins [12, 26] include their relative inexpensiveness, as well as ease and the rate of separation of DOM, compared to other methods [3], the possibility of obtaining fractions directly from the aqueous sample, the possibility of preparative isolation and accumulation of fractions (including under field conditions, for example, on cartridges), the use of inorganic eluents that facilitates purification of DOM fractions for subsequent analysis.

In the Russian literature, the data on the use of XAD are limited by the isolation of humic acids from waters [2], although the use of the method by Leenheer for studying soil is no less important, as the hydrophilic–hydrophobic properties of DOM define their role and functional behavior (sorption, migration, accumulation, resistance, etc.) in the soil [1]. The discussed technique also allows one to separate each of the fractions of DOM into another three according to the acid–base properties that is of interest for the prediction of their participation in the exchange reactions. The current evidence confirms the specifics of different factions: the differences in the qualitative composition, molecular weight, spectral properties, biochemical and chemical resistance, complexing, and migration abilities [1, 14]. Implementation of the method for DOM fractionation is important for domestic practices of soil science, although like any other technique, along with advantages, it has several drawbacks. Among them is the possible influence of eluents (with high and low pH values) used for removing the targeted components on their structure. Some conventions are associated with the principle itself of separation of substances and should be considered when interpreting the results.

Fractionation on XAD resins refers to the frontal adsorption chromatography. Resins (XAD-2, -4, -7, -8, -16, etc.) are manufactured by Rohm & Haas (from 2009 year—Dow Chemical Company [6, 7]) and are hydrophobic polymeric macroporous crosslinked sorbents with a high surface area (>380 m²/g). Particle size, porosity, dipole moment, and their other characteristics vary [5, 6], which allows using resins in combination to extract substances of different polarity from solutions. The most common material for the fractionation of organic substances from natural waters is the resin XAD-8 (now it is replaced by analogs XAD-7 and XAD-7H). Resins DAX-8, Diaion HP2MG (Supelco, Sigma-Aldrich), etc., are also applied [24].

Fractionation is based on the affinity principle. Substances of different polarities differ in their affinities for the sorbent surface allowing their selective removal from the solution. In this case, the hydrophobic resin adsorbs hydrophobic substances from water. The components of DOM, which are products of the destruction of lignin and humic substances, exhibit hydrophobicity (relative nonpolarity). Their mole-
molecules include fragments of both aromatic and aliphatic structure and can contain hydroxyl, methoxyl, carboxyl, and carbonyl groups. Hydrophilicity (relative polarity) of the substances is determined by the presence of a substantial amount of oxygen and nitrogen containing groups in their structure—acids, proteins, amino sugars, as well as carbohydrates and their derivatives. Transition from hydrophilic and to hydrophobic compounds is not clear; i.e., DOM is amphiphilic, as it contains both hydrophilic and hydrophobic fragments. Therefore, fractionation by hydrophobicity is somewhat conventional. The results of fractionation also depend on the specific conditions of extraction: pH, type of resin, and elution rate, as well as the volume ratio of the sorbent and the solution defining the sorption capacity value of column (resin) under the conditions of an experiment.

Despite the fact that the separation of DOM on XAD resins has been used for more than 30 years and is the most common method of research of natural waters, a single fractionation protocol does not exist, and the technique has many modifications [5, 10, 13, 17, 18, 20, 25]. However, it is clear that for the correct interpretation of the results it is necessary to use the same fractionation conditions. This is important in both assessing the ratio of fractions and for their preparative separation.

The most important factor influencing the separation of DOM is the capacity of a column (k'): the higher the k', the greater is the volume of the solution that can be passed through it (elution rate, the amount of resin in the column, etc.).

According to Leenheer’s definition [12], under the conditions of a certain experiment, the substances that are adsorbed on the resin by 50% are taken as hydrophobic and those that are 50% or more pass through the resin without being adsorbed are taken to be hydrophilic. Based on the breakthrough curve parameters describing the process of separating substances in frontal adsorption chromatography, the author introduces the concept of the volume of semiretention (V0.5r) of a hypothetical substance 50% of which is retained on the column and 50% passes through it. This volume equals V0.5r = 2V0(1 + k0.5r), in which V0 is the dead volume (void volume) of the resin; k0.5r is the coefficient of the distribution of the substance in the column, which is equal to the ratio of its mass in the resin and in the solution and reflects the extent of its affinity to the sorbents. Thus, the dead volume can be calculated: V0 = VbP, in which Vb is the resin bed volume in the column, taking into account porosity P (the void volume is given in the specification to the sorbent). The equation can be used to calculate the quantity of resin V0 required for separating the hydrophobic and the hydrophilic fractions of the solution with a volume of V0.5r or conversely, for determining the volume of the solution that can pass through a given volume of the resin. For the calculation it is necessary to select the k' value. It is also possible to find the k' value, which is created under certain volume ratios of the resin and the solution. The question is how to choose the k' value.

According to the assumptions by Leenheer [12], the process of dynamic sorption during chromatographic separation (a) is in equilibrium; (b) it is described by the Freundlich equation, \( Q = K \cdot C^{1/n} \), in which \( Q \) is the absorption capacity, \( K \) and \( n \) are constants, and \( C \) is the equilibrium concentration of the sorbate; (c) where the absorption capacity is not dependent upon the concentration of DOM in the solution (due to the high affinity of the sorbate to the sorbent, the \( 1/n \) value is negligible and \( Q = K \)). Thus, the value of k' of the equation (p. 00) corresponds to the meaning of distribution coefficient \( K \) of the Freundlich equation and simultaneously \( k' = Q \), being a measure of the ratio of the resin quantity to the quantity of the passed solution [19, 22, 26].

The required value of k', as was stated, is selected by the researcher. Depending on it, the ratio of the volumes of the resin—solution will change, which ultimately will affect the number of yielded fractions. For example, if k' = 15 is accepted, then 208 ml of the solution can be passed through the resin volume of 10 mL (P = 0.65) and at k' = 5 only 78 mL can be passed through. In the latter case, a problem may occur to form the desired amount of the substance for analysis, particularly at its low concentrations in water. Even more important is another case: the higher k' the larger the passed volume and the amount of substance (load on the resin), which creates competition among the components of the solution for the exchange places. Since k' is a measure of the affinity of the substance to the resin, by limiting the exchange capacity of the column (the experimental conditions), researchers deliberately limit the sorption of certain components on it. For example, taking a value of k' = 10, we create the conditions when only substance with k' ≥ 10 are adsorbed from the solution, and substances with lower affinity pass through without adsorbing. As a result, the composition of the fractions obtained at various values of k' will be different. Since k' reflects the measure of affinity (in this case, the degree of hydrophobicity) of the sorbate to the sorbent, by increasing k', we create the conditions for adsorption of components with more pronounced hydrophobic properties on the resin. The weakly hydrophobic will slip into the effluent flowing from the column being mixed with hydrophilic substances. It was shown [19, 22] that an increase in k' leads to a marked reduction in the amount of the extracted hydrophobic fraction and increase in the degree of hydrophobicity of its constituent substances (with increasing k' from 2 to 30 the content of the hydrophobic fraction contracted by 22–31%, the values of specific extinction \( E_{254} \) increased by 19–43%).

Thus, both the ratio of the hydrophilic and hydrophobic fractions and their qualitative composition