In this work, the dissociation of humic acids is investigated from the point of view of their mean activity coefficients. They are determined on the basis of two different concepts: sparingly soluble substance and multistep mechanism. It was found that the mean activity coefficients are generally higher, if the traditional concept is applied to the data, excepting the HA-A sample. Both the used concepts provide the mean activity coefficients dependent on the ionic strength, the amount of dissolved humic acids, and the types of electrolyte added in the studied suspensions. Their values based on the concept of a multistep mechanism and determined for humic acids in NaCl and NaI form a continuous curve and the individual character of their ions did not assert. It means that activity coefficients were affected only by the ionic strength and valence factor of the electrolytes irrespective of their chemical composition. The mean activity coefficients obtained for humic acids in HCl are lower in comparison with NaCl and NaI due to the common ion H⁺. Comparing the results obtained for individual humic acids, we can state that the results obtained for the HA-E sample are very different from those of other samples. It seems that its solubility is very high, but the majority of the dissolved particles remained in the molecular form and only a small amount of ions is formed. 

1. INTRODUCTION
The dissociation of humic acids and their behavior in an aqueous environment should be of our interest if we want to understand their functioning in nature.¹⁻³ Solid humic acid preparations are usually considered to be insoluble or sparingly soluble in water. Isolated preparation is a mixture of plenty of substances, which differ in their solubility in water environments, undoubtedly.⁴,⁵ Their behavior in water and aqueous solutions can be suggestive of sparingly soluble solid substances. The solubility of sparingly soluble substances is well understood, including the effect of foreign ions or their own ions, and described by solubility products. These substances are dissolved by dissociation of their acidic functional groups (carboxylic, enolic, and phenolic) and the solution in contact with the solid phase is quickly saturated and equilibrated. Ion concentrations in saturated solutions are very low.⁶ Unlike sparingly soluble solid substances, the behavior of humic acids in water environments is more complex due to their mixture character.⁷⁻¹² Sharp difference in the solubility of fresh humic acid precipitate and dried solid preparation indicates the great influence of hydration of humic molecules and aggregates on their properties.¹³ The dissolved fraction of humic acids is thus considered as the most active one playing the principal role of organic matter in nature¹⁴⁻¹⁹ and their molecular organization in aqueous solutions can influence their activity and function in natural systems.²⁰⁻²⁵

The equilibria between organic matter and water are important mainly in water-saturated soils, in which they can affect the immobilization of pollutants as well as the transport of nutrients.¹¹⁻¹⁳ Active coefficients of the humic substances and solute can strongly influence these processes and has been the subject of several studies.¹¹,²⁶⁻³² but the determination and analysis of activity coefficients of humic acids are really scarce. Collazo-Lopez et al.¹¹ determined the activity coefficients of nonpolar organic solutes in sedimentary organic matter and its different extractable fractions by means of gas chromatography. The highest values were obtained for solutes in the presence of humic acids, which are considered as the fraction with very little affinity for the probes. Spurlocl and Biggar²⁶ dealt with the thermodynamics of organic chemical partition in soils. They determined the activity coefficients of different organic compounds dissolved in water and sorbed in the humic phase. Zhu et al.²⁹ studied the π⁻π interactions between aromatic compounds and soil organic matter. They focused on the pH effect on sorption including the solute activity coefficients. Shoba and Sen Kov⁳⁰ determined the activity coefficients and the chemical potentials of ions and minerals in different soils on the
basis of the physicochemical simulations and the data on the composition of the water extracts and soil solutions. Shoba and Chudnenko31,32 studied the ion-exchange properties of humic acids. They developed the quantitative concepts of the composition and ion exchange between soil humic acids and cations of different valences. On the basis of these, the activity coefficients of humic acids considered as monoionic forms were determined.

Some authors, e.g., Fukushima et al.33,34 Sutheimer et al.35 Santos et al.36 and Porasso et al.37 included the activity coefficients of protons eliminated in the titrations of humic acids in the models of their acidity and dissociation behavior. In contrast, Salma and Lang38 neglected the deviation of the activity coefficients from the unity in their model of the dissociation of humic substances.

As we can see, the significance of activity coefficients in the functioning of humic substances is known but their effect is often neglected or discussed as a possible factor influencing some processes as dissociation or sorption. Determinations of activity coefficients of humic substances are really sparse, and their values and concentration dependencies are usually unknown. Some authors31,32 approximated the missing values by means of the physicochemical simulations.

2. MATHEMATICAL MODELS

The method, used in this study, is different. The determination of the activity coefficients of humic acids is based on their solubility in water and aqueous solutions. Two different approaches are used. The first one is the traditional concept of a sparingly soluble substance,6,38,39 the second one is the concept of a multistep mechanism of their dissolution and dissociation derived in our previous works.5,13 The dissociation behavior of humic acids as sparingly soluble substances can be described by following simple scheme

\[ \text{HA}^{(s)} \leftrightarrow \text{H}^{+}^{(aq)} + \text{A}^{-}^{(aq)} \]  

where \( \text{HA}^{(s)} \) is the sparingly soluble substance (model monobasic humic molecule in this case), \( \text{H}^{+}^{(aq)} \) is the eliminated hydrogen ion, and \( \text{A}^{-}^{(aq)} \) is the dissociated humic anion. If the sparingly soluble substance is in equilibrium with their saturated solution, its chemical potential \( \mu_{\text{HA}} \) as well as the mean chemical potential of its ions \( \mu_{\pm} \) is (at given temperature and pressure) constant (\( \nu \) is the number of ions formed by the dissociation)

\[ \mu_{\text{HA}} = \nu \mu_{\pm} = \text{const} \]  

and can be expressed as

\[ \mu_{\text{HA}} = \mu_{\text{HA}}^{(0)} + \nu RT \ln c_{\pm} + \nu RT \ln \gamma_{\pm} = \text{const} \]  

where \( \mu_{\pm}^{(0)} \) is the standard chemical potential, \( R \) is the universal gas constant, \( T \) is the temperature, \( c_{\pm} \) is the mean concentration of ions, and \( \gamma_{\pm} \) is the mean activity coefficient. Equations 2 and 3 are valid regardless of the presence of other ions and can be simplified as

\[ c_{\pm} \gamma_{\pm} = S \equiv \text{const} \]  

where \( S \) is the solubility product defined by means of activities of ions \( a_{+} \) and \( a_{-} \)

\[ a_{+} a_{-} = S \]  

Equation 4 can be used as a basis for the calculation of the mean activity coefficients. According to this relation, the mean activity coefficient of a sparingly soluble substance is inversely proportional to the mean concentration of its ions. If we know the solubility of the substance for different ionic strengths (\( I \)), we can extrapolate it to infinite dilution (\( c_{\pm} \rightarrow 0 \)), where \( \gamma_{\pm} = 1 \), and to obtain the value of \( \sqrt{S} \). The extrapolation can be realized by means of the following relation

\[ \lim_{I \rightarrow 0} \left( \frac{1}{c_{\pm}} \right) = \frac{1}{\sqrt{S}} \]  

In contrast, our previous results5,13 showed that the dissociation of humic acids is more complex and can be described by a multistep mechanism

\[ \text{H}^{+}^{(aq)} + \text{A}^{-}^{(s)} \leftrightarrow \text{HA}^{(s)} \leftrightarrow \text{HA}^{(aq)} \leftrightarrow \text{H}^{+}^{(aq)} + \text{A}^{-}^{(aq)} \]  

where humic acids behave like weak electrolytes and can exist as dissolved molecules, which are able to dissociate up to the equilibrium. Therefore, dissolved humic acids can exist in a molecular form and as dissociated ions (right side of eq 6). Simultaneously, the surface functional groups of insoluble residual humic acids can dissociate and contribute to the acidity of the system (left side of eq 6), although this contribution is usually low.5,13

We can define three different equilibrium constants in the result from eq 1. The constant \( K_{1} \) is for the dissolving of solid humic acids \( \text{HA}^{(s)} \) (it means its transition into a dissolved form of humic acids \( \text{HA}^{(aq)} \)), the constant \( K_{2} \) for the dissociation of the dissolved humic acids \( \text{HA}^{(aq)} \) into ions, and the constant \( K_{3} \) for the dissociation of surface functional groups of solid humic residue. If we assume that the contribution of surface groups is small and can be neglected, we can use the constant of dissociation \( K_{2} \) for the determination of activity coefficients or the product \( K_{1} K_{2} \) as the summary constant of dissociation of humic acids. In view of the fact that the same ions \( 
H^{+}^{(aq)} \) and \( \text{A}^{-}^{(aq)} \) are included in the constant \( K_{2} \) and the summary constant \( K_{1} K_{2} \), both approaches should provide the same values of activity coefficients \( \gamma_{\pm} \).

The constant \( K_{1} \) is defined as the ratio of the activities of dissolved and residual undisolved humic acids

\[ K_{1} = \frac{a_{\text{HA}^{(aq)}}}{a_{\text{HA}^{(s)}}} \]  

The constant \( K_{2} \) can be expressed as

\[ K_{2} = \frac{a_{\text{H}^{+}^{(aq)} a_{\text{A}^{-}^{(aq)}}}}{a_{\text{HA}^{(aq)}}} = \frac{c_{\text{HA}^{(aq)}} c_{\text{A}^{-}^{(aq)}}}{c_{\text{HA}^{(s)}}} = K'_{2} \gamma_{\pm}^{2} \]  

where \( K'_{2} \) is the concentration dissociation constant. According to the Debye–Hückel theory, the following equation is valid for the mean activity coefficient \( \gamma_{\pm} \)

\[ \log \gamma_{\pm} = -A z_{\pm} z_{\pm} \sqrt{I} \]  

where \( A \) is a constant valid for a given solvent and temperature; \( z_{+} \) and \( z_{-} \) are the charges of dissociated ions; and \( I \) is the ionic strength. Since \( \gamma_{\pm} \rightarrow 1 \) in this case, we can combine eqs 9 and 10 to form the following relation

\[ \log K'_{2} = \log K_{2} + 2 \log \gamma_{\pm} = \log K_{2} + 2A \sqrt{I} \]  

3. RESULTS AND DISCUSSION

In Figure 1, the example of the measured pH values is shown. As can be seen, they decrease with the increasing content of humic acids in a suspension as a result of the dissociation of acidic
functional groups and the production of H⁺ ions. The data obtained for HA-E differ from those of other samples, and the pH values are relatively high. It resulted in different solubility products and activity coefficients as described in the following text. The values were obtained for HA-D, which is the standard of the International Humic Substances Society (IHSS). The results measured for lignitic humic acids of technical quality (HA-A) are between those of both purified samples (HA-B and HA-C).

As discussed in the Experimental Section, the ionic strength was adjusted by means of three different substances: HCl, NaCl, and NaI. The HCl has one common ion with humic acids and therefore its influence should be different from the influence of NaCl and NaI, which have only foreign ions. The effects of NaCl and NaI should be the same or very similar because the individual character of foreign ions does not assert in the system and activity coefficients are determined only by the ionic strength and the valence factor of the electrolyte if the solutions are very diluted.38,39

3.1. Concept of Sparingly Soluble Substance. In Figure 2, the experimental data obtained for humic acids suspended in deionized water and processed according to eq 6 are shown. Ionic strength was adjusted only by humic acids, and no foreign ions were added.

We can see that only the data obtained for the HA-E sample can be fitted by a line and the data from other humic acids show a continuous curve, which can be approximated by the power-law function. In our previous study,5,6 dilute solutions with activities equal to the concentrations were supposed. It was found that the pH values increased with the number of humic acids added to the constant volume of water. Consequently, the concept of a sparingly soluble substance was rejected.5 In contrast, the unit activity coefficients were not supposed in this study and the hypothesis of a sparingly soluble substance was tested again. The obtained results showed that differences between samples of humic acids (excluding HA-E) cannot be distinguished and this hypothesis cannot be used for the humic acids suspended in water without the addition of other ions. If an electrolyte is added to the suspension of humic acids, the results are much better. In Figure 3, we can see the comparison of the experimental data obtained for the HA-C sample in three different electrolytes. We can see that the experimental data as well as the calculated activity coefficients differ for individual electrolytes, which is more noticeable in linear dependencies in Figure 3. The results obtained for humic acids in HCl are much different in comparison with those in NaCl and NaI. The reason is the common ion (H⁺) of the electrolyte HCl and humic acids. If we compare the activity coefficients for the same c1, we obtain the order HCl, NaI, and NaCl. The calculated values of the solubility product S in individual electrolytes are listed in Table 1. The solubility product S is expressed as dimensionless because the concentrations used were relative (related to the standard state concentration cm = 1 mol dm⁻³). We can see that values of S in salts (NaCl and NaI) are usually higher in magnitude in comparison with those in HCl, which has the common ion H⁺ with humic acids.

In Figure 4, a comparison of the mean activity coefficients of the studied humic acids is shown. Values obtained for HA-B and HA-C, which are purified samples isolated from the same matrix, are very similar and form a continuous line. Comparing the results obtained for individual humic acids, we can see that the HA-D and HA-E samples are different from lignitic samples. It seems that the HA-E sample has the lowest solubility, but the color of the aqueous solution above its solid sample was very dark and practically nontransparent. In contrast, the HA-D sample seems to be the most soluble.

3.2. Concept of Multistep Mechanism. In our previous works,4,13 the concept of a multistep mechanism of the dissociation of humic acids was developed and verified. In this study, the same samples are studied and data are analyzed from the point of view of activity coefficients. As mentioned above, a contribution of the dissociation of functional groups on the surface of solid undissolved particles is usually small and can be neglected for the purpose of the determination of activity coefficients. There can be thus calculated on the basis of eq 11, in which case, the dissociation constant K₁ or the product of K₁ and the equilibrium constant between solid undissolved humic acids and dissolved one K₂ can be used. The same resulting activity coefficients for these two ways of their determination are assumed. In Figure 5, the activity coefficients obtained on the basis of the K₁ dissociation constant and the K₁·K₂ product are

Figure 1. Measured pH values of humic acids in water: HA-A (green), HA-B (violet), HA-C (blue), HA-D (black), and HA-E (red).

Figure 2. Experimental data processed according to the concept of a sparingly soluble substance (eq 6): HA-A (green), HA-B (violet), HA-C (blue), HA-D (black), and HA-E (red).
compared. We can see that the obtained results are in agreement with the presumption that the determined activity coefficients based on the $K_2$ constant and $K_1K_2$ product have to be the same. The amounts of the dissolved humic acids were calculated on the basis of the results obtained in our previous works. The values of the activity coefficients were calculated by means of the mathematical apparatus described in eqs 8–11. Extrapolation to the infinite dilution was carried out using eq 11.

Table 1. Solubility Products of Humic Acids Determined in Three Different Electrolytes on the Basis of the Concept of Sparingly Soluble Substance (Equation 6)

| sample | $S_{\text{HCl}}$ (−)          | $S_{\text{NaCl}}$ (−)          | $S_{\text{NaI}}$ (−)          |
|--------|-------------------------------|-------------------------------|-------------------------------|
| HA-A   | $(5.83 \pm 0.10) \times 10^{-8}$ | $(3.74 \pm 0.05) \times 10^{-7}$ | $(7.07 \pm 0.09) \times 10^{-7}$ |
| HA-B   | $(9.13 \pm 0.48) \times 10^{-8}$ | $(1.54 \pm 0.07) \times 10^{-7}$ | nd                            |
| HA-C   | $(2.11 \pm 0.03) \times 10^{-8}$ | $(1.17 \pm 0.02) \times 10^{-7}$ | $(9.72 \pm 0.08) \times 10^{-8}$ |
| HA-D   | $(5.85 \pm 0.08) \times 10^{-7}$ | $(2.95 \pm 0.03) \times 10^{-6}$ | $(2.77 \pm 0.05) \times 10^{-6}$ |
| HA-E   | $(1.26 \pm 0.01) \times 10^{-12}$ | $(1.18 \pm 0.01) \times 10^{-11}$ | nd                            |

Mean activity coefficients obtained for the HA-A sample in four different media are compared in Figure 6. It was confirmed that their values in NaCl and NaI form a continuous curve and the individual character of their ions does not assert in the systems. The activity coefficients are affected only by the ionic strength and the valence factor of the electrolytes. Similar results were obtained also for other humic acids. Similarly, as in the case of the concept of a sparingly soluble substance, the mean activity coefficients obtained for humic acids in HCl are
It is not easy to explain this result. HA-E is a commercial product in the molecular form and only a small amount of ions is formed. The HA-D sample was relatively easily soluble, but their molecular form is preferred and the basic characterization of the studied samples is shown in Figure 2. Other samples were isolated from lignite mined in the Czech Republic. They differed in the methods of their isolation and purification. Two different concepts were applied to the obtained experimental data: sparingly soluble substance and multistep mechanism. It was found that the simple traditional concept of sparingly soluble substance is less suitable for the characterization of the behavior of humic acids in water or aqueous solutions than the concept of a multistep mechanism. In contrast, the concept of a multistep mechanism provided results corresponding with some principles of physical chemistry, e.g., the assumption of activity coefficients is affected only by the ionic strength and the valence factor of the electrolytes (without the influence of the type of electrolyte). The order of humic samples according to their calculated mean activity coefficients was $\text{HA-E} < \text{HA-B} < \text{HA-C} < \text{HA-A} < \text{HA-D}$. The behavior of the HA-E sample was very different in the comparison with other humic acids. It seems that this sample is relatively easily soluble, but their molecular form is preferred and only a small amount of H$^+$ ions is formed. The HA-D sample was also well soluble, but its degree of dissociation was much higher as well as their mean activity coefficients for given concentrations of dissociated ions were the highest. In conclusion, this study showed that the principles and processes of physical chemistry can be applied to the behavior of humic acid in aqueous solutions and their activity coefficients can be calculated on the basis of their rules.

4. CONCLUSIONS

The mean activity coefficients of humic acids in different electrolytes were determined and analyzed. Five different samples of humic acids were studied, including the standard of the International Humic Substances Society and a commercial sample purchased from Fluka. Other samples were isolated from lignite mined in the Czech Republic. They differed in the methods of their isolation and purification. Two different concepts were applied to the obtained experimental data: sparingly soluble substance and multistep mechanism. It was found that the simple traditional concept of sparingly soluble substance is less suitable for the characterization of the behavior of humic acids in water or aqueous solutions than the concept of a multistep mechanism. In contrast, the concept of a multistep mechanism provided results corresponding with some principles of physical chemistry, e.g., the assumption of activity coefficients is affected only by the ionic strength and the valence factor of the electrolytes (without the influence of the type of electrolyte).

The order of humic samples according to their calculated mean activity coefficients was $\text{HA-E} < \text{HA-B} < \text{HA-C} < \text{HA-A} < \text{HA-D}$. The behavior of the HA-E sample was very different in the comparison with other humic acids. It seems that this sample is relatively easily soluble, but their molecular form is preferred and only a small amount of H$^+$ ions is formed. The HA-D sample was also well soluble, but its degree of dissociation was much higher as well as their mean activity coefficients for given concentrations of dissociated ions were the highest. In conclusion, this study showed that the principles and processes of physical chemistry can be applied to the behavior of humic acid in aqueous solutions and their activity coefficients can be calculated on the basis of their rules.

5. EXPERIMENTAL SECTION

5.1. Humic Acids. Five different humic acids were used in this work. Three samples were extracted from lignite mined in the Czech Republic (Mikulcice in South Moravia). Other samples were purchased from the International Humic Substances Society (IHSS) and from Fluka. All samples were studied in our previous works from the point of view of the multistep mechanism of dissociation.

Sample HA-A was extracted from lignite by means of alkaline extraction and characterized previously. Samples HA-B and HA-C were extracted from lignite and purified by the mixture of HCl and HF (HA-B) and dialyzation (HA-C). Extraction and purification procedures are described in detail in previous works.

Sample HA-D was a leonardite standard purchased from the International Humic Substances Society. Sample HA-E was purchased from Fluka. Both samples were studied and characterized in previous work.
5.2. Determination of Activity Coefficients. The dissociation behavior of humic acids was investigated after suspending them in water (4–40 g dm\(^{-3}\)). The dried sample was mixed with deionized water and stirred. The pH value was measured after 24 h, when the suspension was equilibrated (pH 3 to 1). The symbols are presented.

| sample  | C (atom %) | H (atom %) | N (atom %) | S (atom %) | O (atom %) | t.a. (mmol g\(^{-1}\)) | ref |
|---------|------------|------------|------------|------------|------------|-----------------------|-----|
| HA-A    | 40.3       | 41.0       | 1.5        | 0.4        | 16.8       | 5.77                  | 5   |
| HA-B    | 44.3       | 39.6       | 1.2        | 0.3        | 14.6       | 6.23                  | 13  |
| HA-C    | 47.7       | 33.9       | 1.0        | 0.7        | 16.7       | 6.19                  | 13  |
| HA-D    | 48.1       | 33.2       | 0.8        | 0.2        | 17.7       | 6.81                  | 13  |
| HA-E    | 40.3       | 38.2       | 1.1        | 0.6        | 19.8       | 4.39                  | 13  |

Table 2. Elemental Composition and Total Acidity (t.a.) of Humic Acids (Normalized on Dry Ash-Free Sample)

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c03511

Notes
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References
(1) Atalay, Y. B.; Carbonaro, R. F.; Di Toro, D. M. Distribution of proton dissociation constants for model humic and fulvic acid molecules. Environ. Sci. Technol. 2009, 43, 3626–3631.
(2) Badr, M. H.; El-Halafawi, M. H.; Zeid, E. R. A. E. Comparison between the effect of ionic strength on acidity and dissociation constants of humic acids extracted from sewage sludge and Nile water hyacinth composts. Global J. Environ. Res. 2012, 6, 36–43.
(3) Klucáková, M.; Kolajová, R. Dissociation ability of humic acids: Spectroscopic determination of pKa and comparison with multi-step mechanism. React. Funct. Polym. 2014, 78, 1–6.
(4) Kipton, H.; Powell, J.; Town, R. M. Solubility and fractionation of humic acid – effect of pH and ionic medium. Anal. Chim. Acta 1992, 267, 47–54.
(5) Klucáková, M.; Pekař, M. Solubility and dissociation of lignitic humic acids in water suspension. Colloids Surf., A 2005, 252, 157–164.
(6) Stewart, W. J. Physical Chemistry; Longman Publishing Group: London, 1998.
(7) Conte, P.; Spaccini, R.; Šmejkalová, D.; Nebbioso, A.; Piccolo, A. Spectroscopic and conformational properties of size-fractions separated from a lignite humic acid. Chemosphere 2007, 69, 1032–1039.
(8) Piccolo, A.; Conte, P.; Cozzolino, A. Chromatographic and spectrophotometric properties of dissolved humic substances compared with macromolecular polymers. Soil Sci. 2001, 166, 174–185.
(9) Klucáková, M.; Kalina, M. Composition, particle size, charge and colloidal stability of pH-fractionated humic acids. J. Soils Sediments 2015, 15, 1900–1908.
(10) Klucáková, M. Characterization of pH-fractionated humic acids with respect to their dissociation behaviour. Environ. Sci. Pollut. Res. 2016, 23, 7722–7731.
(11) Collazo-Lopez, H.; Yates, R. R.; Cooper, W. T. Applications of inverse chromatography in organic geochemistry. II. Measurement of solute activity coefficients in organic geopolymers by gas chromatography. Org. Geochem. 1989, 14, 165–170.
(12) Ames, T. T.; Gruulke, E. A. Group contribution method for predicting equilibria of nonionic organic compounds between soil organic matter and water. Environ. Sci. Technol. 1995, 29, 2273–2279.
(13) Klucáková, M.; Pekař, M. Behaviour of partially soluble humic acids in aqueous suspension. Colloids Surf., A 2008, 318, 106–110.
(14) Boguta, P.; Sokolowska, Z. Interactions of Zn(II) Ions with humic acids isolated from various type of soils. Effect of pH, Zn concentrations and humic acids chemical properties. PLoS One 2016, 11, e0153626.
(15) Bonn, B. A.; Fish, W. Variability in the measurement of humic carboxyl content. Environ. Sci. Technol. 1991, 25, 232–240.
(16) Orsetti, S.; Andrade, E. M.; Molina, F. V. Modeling ion binding to humic substances: Elastic polyelectrolyte network model. Langmuir 2010, 26, 3134–3144.
(17) Leenheer, J. A.; Wershaw, R. L.; Brown, G. K.; Reddy, M. M. Characterization and diagenesis of strong-acid fulvic groups in humic substances. Appl. Geochem. 2003, 18, 471–482.
(18) Klucáková, M. Dissociation properties and behavior of active humic fractions dissolved in aqueous systems. React. Funct. Polym. 2016, 109, 9–14.
(19) Klucáková, M. Conductometric study of the dissociation behavior of humic and fulvic acids. React. Funct. Polym. 2018, 128, 24–28.
(20) Jung, A. Y.; Frochot, C.; Villieras, F.; Lartiges, B. S.; Parant, S.; Viriot, M. L.; Bersillon, J. L. Interaction of pyrene fluorophore with natural and synthetic humic substances: Examining the local molecular organization from photophysical and interfacial processes. Chemosphere 2010, 80, 228–234.
(21) Shaffer, L.; Von Wandruszka, R. The effects of conformational changes on the native fluorescence of aqueous humic materials. Chem. Sci. Int. J. 2014, 4, 326–336.
(22) Aquino, A. J. A.; Tunega, D.; Pasalic, H.; Schaumann, G. E.; Haberbauer, G.; Gerzabek, M. H.; Lischka, H. Molecular dynamics simulations of water molecule-bridges in polar domains of humic acids. Environ. Sci. Technol. 2011, 45, 8411–8419.
(23) Salma, I.; Lang, G. G. How many carboxyl groups does an average molecule of humic-like substances contain? Atmos. Chem. Phys. 2008, 8, 5997–6006.
(24) Klucáková, M.; Věžníková, K. The role of concentration and solvent character in the molecular organization of humic acids. Molecules 2016, 21, No. 1410.
(25) Klucáková, M.; Věžníková, K. Micro-organization of humic acids in aqueous solutions. J. Mol. Struct. 2017, 1144, 33–40.
(26) Spurlocl, F. C.; Biggar, J. W. Thermodynamics of organic chemical partition in soils. 1. Development of a general partition model and application to linear isotherms. Environ. Sci. Technol. 1994, 28, 989–995.
(27) Spurlocl, F. C.; Biggar, J. W. Thermodynamics of organic chemical partition in soils. 2. Nonlinear partition of substituted phenylureas from aqueous solution. Environ. Sci. Technol. 1994, 28, 996–1002.
(28) Spurlocl, F. C.; Biggar, J. W. Thermodynamics of organic chemical partition in soils. 3. Nonlinear partition from water-miscible cosolvent solutions. Environ. Sci. Technol. 1994, 28, 1003–1009.
(29) Zhu, D.; Hyun, S.; Pignatello, J. J.; Lee, L. S. Evidence for π–π electron donor-acceptor interactions between π-donor aromatic
compounds and π-acceptor sites in soil organic matter through pH effects on sorption. *Environ. Sci. Technol.* 2004, 38, 4361–4368.

(30) Shoba, V. N.; Sen’kov, A. A. Equilibrium composition and properties of soil solutions. *Eurasian Soil Sci.* 2011, 44, 1068–1076.

(31) Shoba, V. N.; Chudnenko, K. V. Physicochemical simulation of the ion exchange between humus acids and cations of different valencies. *Eurasian Soil Sci.* 2012, 45, 1138–1146.

(32) Shoba, V. N.; Chudnenko, K. V. Ion exchange properties of humus acids. *Eurasian Soil Sci.* 2014, 47, 761–771.

(33) Fukushima, M.; Tanaka, S.; Hasebe, K.; Taga, M.; Nakamura, H. Interpretation of the acid-base-equilibrium of humic-acid by a continuous pK distribution and electrostatic model. *Anal. Chim. Acta* 1995, 302, 365–373.

(34) Fukushima, M.; Tanaka, S.; Nakamura, H.; Ito, S. Acid-base characterization of molecular weight fractionated humic acid. *Talanta* 1996, 43, 383–390.

(35) Sutheimer, S. H.; Ferraco, M. J.; Cabaniss, S. E. Molecular size effects on carboxyl acidity: Implications for humic substances. *Anal. Chim. Acta* 1995, 304, 187–194.

(36) Santos, E. B. H.; Esteves, V. I.; Rodrigues, J. P. C.; Duarte, A. C. Humic substances’ proton-binding equilibria: Assessment of errors and limitations of potentiometric data. *Anal. Chim. Acta* 1999, 392, 333–341.

(37) Porasso, R. D.; Benegas, J. C.; van den Hoop, M. A. G. T.; Paolo, S. Analysis of potentiometric titrations of heterogeneous natural polyelectrolytes in terms of counterion condensation theory: Application to humic acid. *Biophys. Chem.* 2000, 86, 59–69.

(38) Atkins, P.; de Paula, J. *Physical Chemistry*, Oxford University Press: Oxford, 2010.

(39) Monk, P. *Physical Chemistry. Understanding Our Chemical World*; John Wiley & Sons, Ltd.: Chichester, 2004.