Use of the p*K* Spectroscopy Method in the Study of Protolytic Properties of Humic Substances and Other Soil Polyelectrolytes

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Abstract: A common technique for the potentiometric analysis of complex protolytic systems is mathematical data processing. This approach allows for transforming the experimental titration curve into a function of the ionogenic group distribution of the titrated object by the values of the p*K* ($-\lg K$a) acid dissociation. This distribution function is called the p*K* spectrum, and the approach itself is called p*K* spectroscopy. In this case, solutions are found not in the form of discrete dissociation constants but as a distribution function of the concentrations of ionogenic groups over a continuous spectrum of dissociation constants. The purpose of this work is to test the possibility of using the p*K* spectroscopy method for assessing the acid–base properties of humic substances, water-soluble fraction, and soils. The results of testing the p*K* spectroscopy method showed that this method is expressive and more informative than the traditional method of continuous potentiometric titration. The use of the p*K* spectroscopy method for studying the protolytic properties of humic substances and other environmental objects is justified. The content and values of p*K* of ionogenic groups of fulvic acids, water-soluble fraction, and soil suspensions were determined. In the objects, the presence of three to five ionogenic groups with p*K* values from 3.2 to 9.6 was revealed.

Keywords: acid–base properties; titration; p*K* spectrum; soil; fulvic acids; water-soluble organic matter

1. Introduction

The acidity and acid–base buffering of soils are largely the products of soil formation and represent the fundamental characteristics that control the mobility of chemical elements, including many nutrients and pollutants in soils and landscapes. The reaction of the medium has a significant effect on the redox processes occurring in soils, the reactions of complex formation, precipitation, and dissolution [1,2].

The study of protolytic equilibria in complex natural systems, such as soils [3,4], their particle size fractions [5], humic substances [6,7], bottom sediments [8], wine [9,10], coals [11], and organic compounds mixtures [12–14] is performed by the potentiometric method. Potentiometric titration is widely used in analytical chemistry to determine the concentration of substances in solutions. Potentiometric titration of soil samples is performed in two ways, which are conventionally called continuous (CPT) and equilibrium potentiometric titration (EPT). There are no unified methods for determining the acid–base buffering of both options, but when using CPT, the pH is measured a few minutes after adding the titrant, and with EPT, after a longer period of time measured in hours and days [15].

The CPT method allows only fast-flowing buffer reactions to be recorded. These include homogeneous reactions in solutions, most of the cation exchange reactions, and the protonation–deprotonation of pH-dependent sorption centers on the surface of humus substances or soil minerals [16]. For many buffer systems present in the soil, the equilibrium in the soil-solution system does not have time to be established in the process of CPT. At the same time, the advantage of this method is the ability to supplement the titrant in small portions and, accordingly, to study the buffer reactions in sufficient detail.

In EPT, cation exchange reactions and slow dissolution reactions of some difficult-to-dissolve components, such as humic acids or silicates, are more fully recorded [17]. The limitations of the method are that it is impossible to obtain information about buffer reactions with such a degree of detail, as in the case of CPT, since the titrant is added to the system in large portions. In addition, with a longer interaction with the titrant, secondary reactions may occur in the soil-solution system, which complicate the interpretation of the titration results. For some of the most difficult-to-dissolve components, the equilibrium does not have time to be established even during EPT [18].

The main problem that arises when interpreting the results of both methods is that the soil is a polydisperse and multicomponent system. The most important component of the soil is organic matter, which is a complex combination of plant and animal residues that are in various stages of decomposition and specific soil organic substances called humus. Humic (HAs) and fulvic acids (FAs) contain various functional groups of both acidic and basic natures. At the same time, FAs have a relatively low molecular weight, can interact intensively with soil minerals, and easily migrate along the soil profile, participating in the leaching processes and podzol formation. Aside from this, the soil organic matter contains a wide range of low-molecular compounds: organic acids, carbohydrates, amino acids, phenols, alcohols, etc. [19]. The mineral soil matrix also consists of a large number of buffer components, usually having close dissociation constants. Therefore, the titration curve of soil samples is often a flat line without noticeable inflection points and plateau-like areas. Reliable identification of buffer reactions occurring in the process of CPT and EPT requires special model experiments, in which the changes in various soil-chemical characteristics are observed during titration.

However, it is almost impossible to identify acid–base groups that determine the chemical properties of the substrate in this way [20]. Mathematical methods are now becoming an important tool for understanding environmental objects [21–24].

Many computational methods have been proposed for processing the potentiometric titration curves. However, all of them are characterized either by low stability of solutions and, as a rule, are applicable only to the simplest (two-component) systems or require preliminary information about the values of the dissociation constants of the components.

Recently, for the potentiometric analysis of complex protolytic systems, it has been proposed to use data processing methods that allow for calculating the distribution function of ionogenic group concentrations of the titrated object by the pK ($-$lg$K$a) values of acid dissociation from the acid–base titration curve. This distribution function is called the pK spectra, and the approach itself is called pK spectroscopy. In this case, solutions are found not in the form of discrete dissociation constants but in the form of a function of the distribution of ionogenic group concentrations over a continuous spectrum of dissociation constants [21]. This distribution, in contrast to the classical method of potentiometric analysis, allows for the study of complex mixtures of acids and bases with sufficiently close values of dissociation constants and, simultaneously, the determination of both the concentrations of the components and their pK values.

A method based on such a distribution (the pK spectroscopy method) is promising in the study of complex acid–base systems. It has been successfully applied to study the protolytic properties of humus substances [6], γ-Al$_2$O$_3$ suspensions [20,25], and bottom sediments [8], namely systems for which there are no reliable data on the functional composition and for which classical methods of analysis are difficult to be applied and do not always provide adequate information about their structure.

The concentrations of ionogenic groups in the studied substrates c(pK_i) can be found by solving a system of equations describing the protolytic equilibrium at different pH values (during titration). However, it should be kept in mind that such a problem belongs to the class of incorrect (in the mathematical sense) problems, the solution of which is extremely sensitive to small errors in the initial experimental data, that is, errors in the titration function [20]. The stability of the solution to experimental errors increases if a priori information about the obtained results is used; in particular, it is obvious that the

values of $c(pK_i)$ must be positive. In [21], when solving this system of equations, the NNLS algorithm was used [26], in which this system of equations is solved by the method of least squares with a restriction on the non-negativity of solutions. A similar program ("PKSVD"), written in Turbo Pascal 7.0, was used.

The purpose of this work is to test the possibility of using the p*K* spectroscopy method for assessing the acid–base properties of fulvic acids, water-soluble fraction, and soils.

2. Materials and Methods

2.1. Isolation of Fulvic Acids

Preparations of FAs were extracted from organogenic horizons of Eutric Albic Stagnic Histic Retisol (Loamic), two Eutric Albic Retisols (Loamic)—virgin and arable according to the method [27]. Plots of virgin soils are laid on the catena: Eutric Albic Retisols (Loamic) and Eutric Albic Stagnic Histic Retisol (Loamic). The high position of the catena in the relief makes it possible to assess the effect of the moisture degree on the acid–base properties of humus substances. The Eutric Albic Retisol (Loamic) plot is located at a distance of 8 km west of the city of Syktyvkar (Komi Republic, Russia) on the top of a watershed hill at the back of a microdepression of 1.5 m low (61°39′44.6″ N, 50°41′10.4″ E; 160 m.a.s.l.). The vegetation is a bilberry-green-moss birch-spruce forest with many fallen trees. The Eutric Albic Stagnic Histic Retisol (Loamic) plot is located at a distance of 74 m from the previous one (61°39′42.4″ N, 50°41′8.4″ E; 155 m.a.s.l.). It is a microhollow between low and flat elevations. The vegetation is a long moss-sphagnum birch-spruce forest. Near the plot, there is sphagnum cover. The plot of Eutric Albic Retisols (Loamic) arable is located in the fields of the Syktyvkar state farm, which is 5 km southwest of Syktyvkar, on the watershed of the Sysola and Vazhel-yu rivers (61°38′46.1″ N, 50°44′20.7″ E; 145 m.a.s.l.). The terrain is flat, with small hills. The development period is about 40 years. The plot is drained by a covered drainage system. It is sowed with a pea-oat mixture.

The ground soil sample was dried to an air-dry state and extracted with a mixture of NaOH and $Na_4P_2O_7$ solutions; the molar concentrations of both components in the solutions were 0.1 mmol·dm^{-3}. A solution of Na_2SO_4 ($\omega = 20\%$) was added to the alkaline extract to coagulate colloidal particles and centrifuged for 2 h at a rotation speed of 5000 rpm. Furthermore, humic acids were precipitated with a solution of sulfuric acid.

FAs were purified on activated carbon AG-3 (JSC Sorbent, Perm, Russia) and desalted by passing through KU-2 cationite (Ural Chemical Company, Chelyabinsk, Russia) in H$^+$-form. The mass concentrations of FAs in the eluates were determined gravimetrically by evaporation of the solution aliquot.

2.2. Liquid Phase of Soils

To study the protolytic properties of the soil liquid phase, organogenic horizon samples of three Retisols were taken: Distric Albic Retisol (Loamic), Eutric Albic Retisol (Loamic), and Distric Gleyic Retisol (Loamic). Retisol plots are located at a distance of 1 km northwest of the Krutotyla village in the Priluzsky district (Komi Republic, Russia).

The Distric Albic Retisol (Loamic) plot is located on the top of a hill on the interfluves and a "ridge" of microhigh 1.5 m. (59°38′25.3″ N, 49°22′40.8″ E; 170 m.a.s.l.). The vegetation is an aspen-birch-spruce forest with bilberry, cowberry, and green mosses. The Eutric Albic Retisol (Loamic) plot is located at a distance of 50 m from the previous one (59°36′23.4″ N, 49°22′37.1″ E; 164 m.a.s.l.). The composition of the stand is the same. The soil cover is dominated by blueberry and green mosses. The plot of Distric Gleyic Retisol (Loamic) is located in a microhollow between a small flat microelevation, 60 m from the Eutric Albic Retisol plot (59°38′41.6″ N 49°22′52.4″ E; 152 m.a.s.l.). The vegetation is a birch-spruce-Sphagnum-Polytrichum forest. The cover of *Sphagnum* is prominent near the peat.

Water extracts from air-dry soil samples, porphyrized, and sifted through a sieve with $d = 0.1$ mm were prepared as follows: three soil samples of 2.00 g were filled with 50.0 cm^3 of DL water. The suspensions were shaken in a rotator for 15 min, left overnight, shaken

for other 15 min, and filtered through a paper filter "blue ribbon". In order to elucidate the nature of the water-soluble components that did not enter the water extract after a one-time eluant treatment, the filter with substrate was scoured with distilled water until the volume of 'scour waters' reached 20.0 cm^3.

2.3. Solid Phase of Soils

The protolytic properties of the solid phase were studied on the example of water suspensions of the mineral horizons of Eutric Albic Stagnic Retisol (Loamic), as well as one sample from the Eg horizon, calcined at a temperature of 500 °C for 5 h. The plot of Eutric Albic Stagnic Retisol (Loamic) is located at a distance of 3 km west of the Troitsko-Pechorsk settlement (62°41′21″ N, 56°08′59.1″ E; 140 m.a.s.l.). The soil under the green-moss spruce forests covers the periphery part of the top. The Eutric Albic Stagnic Histic Retisol (Loamic) develops under long moss and sphagnum-long moss spruce forests near the center of the hill. The vegetation is a bilberry-green-moss spruce forest. The floor vegetation is dominated by *Vaccinium myrtillus* L. and green mosses.

During calcination, almost all organic compounds decompose, which eliminates their influence of the soil mineral components on the acid–base properties. Water suspensions were prepared from air-dry soil samples, porphyrized, and sifted through a sieve with d = 0.1 mm in the mass ratio soil:water = 1:2.5. Before titration, the suspensions were shaken in a rotator for 15 min.

2.4. Potentiometric Titration

The potentiometric titration of the solutions was performed at a temperature of (25 ± 1) °C. The EMF of titrated solutions was measured on a pH-150 ionomer with an ESL-15-11 glass electrode (Plant of Measuring Equipment, Gomel, Belarus). An NaOH solution with a concentration of c = (0.0088 ± 0.0011) mmol·dm^{-3} was used as the titrant of the FA solutions. The aliquots of water extracts from soil samples and "scour waters" were titrated with HCl or NaOH solutions with molar concentrations (0.02000 ± 0.0026) mmol·dm^{-3} prepared from fixanals (MercK, Darmstadt, Germany). All solutions were prepared on bidistillate.

The pH values were recorded 2 min after the addition of the next titrant portion. Titration was carried out in the pH ranges from the initial titration point to 3.0, when titrated with an acid solution, and to 10.0, when titrated with a base solution. Titration of suspensions and extracts was carried out in a 3–5-fold repetition. The average values and limits of the confidence intervals were calculated; the significance of the differences was estimated at p = 0.95.

2.5. Calculation of pK Spectra

The computer program "PKSVD" for calculating the pK spectra of systems from the curves of potentiometric titration used in this work was developed by M. A. Ryazanov, Doctor of Chemical Sciences [28]. The construction of pK spectra included several stages. The first stage involved the calculation of the titration curve (function), the dependence of the ratio of the acid group number and the sample mass (n/m) on the system pH.

When working with natural polyelectrolytes, it is possible to use different approaches. (1) In the case where the substrate has a slightly acidic or slightly alkaline reaction, it is possible to perform titration with both an acid solution and an alkali solution. As a result, each experimental dependence $n/m = f$ (pH) included two fragments $n(\text{OH}^-)/m = f$ (pH) and $n(\text{H}^+)/m = f$ (pH) (Figure 1a). (2) The second option is acceptable in the study of substrates with a strongly acidic reaction. In this case, titration is performed only with an alkali solution.
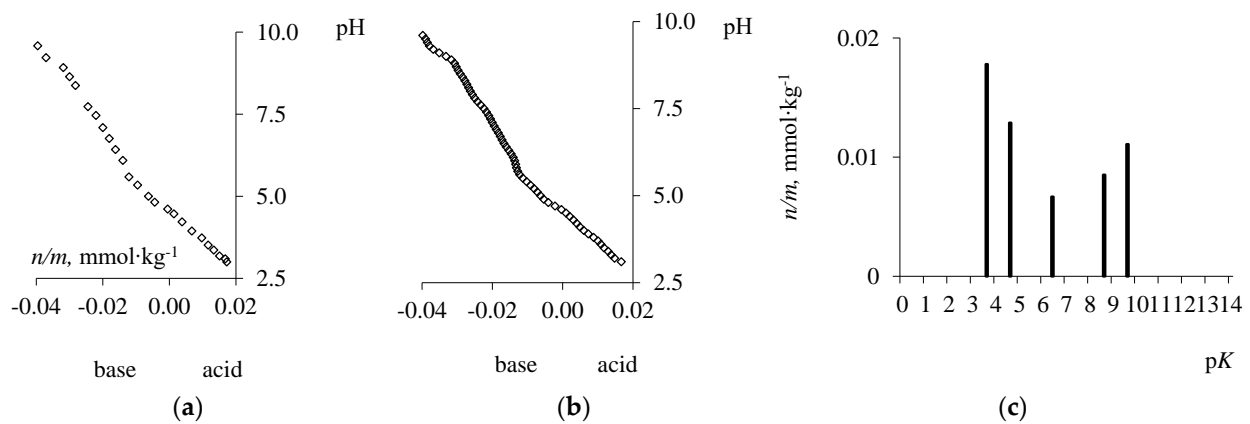
Figure 1. Example of the experimental (**a**) and smoothed (**b**) titration curves and the p*K* spectra (**c**). Negative values of n/m are associated with the introduction of "−" in the calculation formula.

To reduce the influence of errors on the calculation results (due to the sensitivity of this method of calculating the p*K* spectrum to errors), the program "SPLINE" [28,29] was used, which allowed smoothing out the experimental dependence $\alpha = f(\text{pH})$ and, using interpolation, reducing the value of Δp*K* to 0.1. In this procedure, three replicate titration curves obtained under repeated conditions are transformed (smoothed), and their coordinates are calculated at a specified interval ΔpH [30,31]. As a result, three smoothed titration curves were obtained from the three initial experimental titration curves, which made it possible to calculate the standard deviation of the titrant volume at each pH point of the titration curves (s_k^2), find the smoothed averaged titration curve (Figure 1b), and determine its standard deviation:

$$s = \sqrt{\frac{\sum_k^K s_k^2}{K}}, \tag{1}$$

where *K* is the number of points on the smoothed averaged titration curve.

The final stage was the use of the computer program "PKSVD" for the construction of p*K* spectra. The calculation of the p*K* values for the p*K* spectra was carried out according to the formula for finding the weighted average value:

$$\overline{\text{p}K} = \frac{\sum_{i=1}^{N} x_{\text{p}K_i} \cdot \text{p}K_i}{\sum_{i=1}^{N} x_{\text{p}K_i}}, \tag{2}$$

where $x_{\text{p}K_i} = \frac{c_{\text{p}K_i}}{\sum_{i=1}^{M} c_{\text{p}K_i}}$ are the nearby nonzero values of the mole fractions for the given p*K*, interpreted by us as a single peak; p*K*ᵢ are the corresponding values of p*K*; $M = (\text{p}K_{\max} - \text{p}K_{\min})/\Delta\text{p}K$ is the number of segments with a width of Δp*K* (the p*K* basis interval) in the given interval p*K*; and *N* is the number of nearby nonzero values of q_i.

The range of p*K* was limited from 0 to 14, and the change interval in p*K* was assumed to be 0.1–0.25. With a smaller interval, the peaks corresponding to the polybasic acid are split [20]. The p*K* spectra were represented as the distribution of the number of acid groups per unit mass (n_i/m) having similar p*K* values (Figure 1b), as well as the molar fractions $(x_i, \%)$ of acid groups according to the p*K* values:

$$x_i = \frac{n_i/m}{n/m} \cdot 100, \text{ where } n/m = \sum_{i=1}^{I} n_i/m \tag{3}$$

As an example, a fragment of the statistical processing results of three titration curves of water suspensions of the Eg horizon sample of Eutric Albic Stagnic Retisol (Loamic) is given (Table 1). Averaged over all points of the three titration curves, the error characteristic of the titrant volume required to establish a given pH value of the water suspension of the soil (s) was used in the construction of p*K* spectra.

Table 1. Experimental data on titration of water suspensions of the Eg horizon of Eutric Albic Stagnic Retisol (Loamic).

1st Repetition		2nd Repetition		3rd Repetition	
$n(\mathrm{OH}^-)/m$ [1], mmol·kg^{-1}	pH	$n(\mathrm{OH}^-)/m$, mmol·kg^{-1}	pH	$n(\mathrm{OH}^-)/m$, mmol·kg^{-1}	pH
−0.301 [2]	3.92	−0.274	3.96	−0.274	3.96
−2.782	4.16	−4.100	4.43	−3.561	4.22
−4.103	4.42	−5.463	4.64	−4.301	4.43
−5.463	4.64	−7.040	4.85	−5.852	4.73
−7.038	4.88	−8.024	5.08	−7.430	4.98
−8.826	5.06	–	–	−9.419	5.19

[1] Hereafter, n/m is the number of ionogenic groups. [2] The negative values of $n(\mathrm{OH}^-)_i/m$ are associated with the introduction of "−" in the calculation formula.

3. Results

3.1. Analysis of Fulvic Acids

FAs are natural polyelectrolytes of complex variable composition. They are characterized by a wide variety of functional groupings of different acidity, from strongly acidic (pK < 3) to very weakly acidic (pK > 10). From the point of view of the chemical nature, a large proportion of acid groups are carboxylic and phenolic [32]. In natural objects (soils, soil solutions, surface waters), the number of groups and their acidity constants determine the intensity of the reactions of exchange, addition, neutralization, adsorption of organic compounds, and the possibility of the formation of complex compounds with metal ions. In this regard, the study of protolytic properties allows for the evaluation of the contribution of humus substances in the formation of ion-conducting soil structures, the migration of elements in the earth's crust, etc. [33].

Based on the data of the potentiometric titration of FA solutions, the pK spectra are calculated. Figure 2 shows examples of the pK spectra of FAs isolated from one Eutric Albic Stagnic Histic Retisol (Loamic) and two Eutric Albic Retisols (Loamic), virgin and arable, respectively. They clearly show from three to four bands with a maximum in the pK region from 2.9 ± 0.3 to 10.5 ± 0.3, which indicates the presence of several groups of molecular fragments in the studied samples that differ in the values of pK and determine the acid–base properties of the FAs.
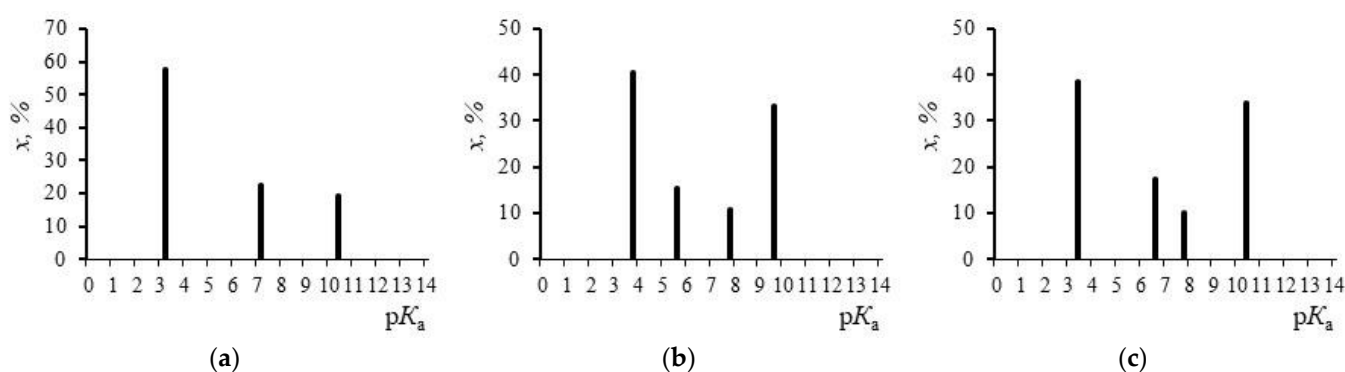

| (a) | (b) | (c) |

Figure 2. pK spectra of FAs isolated from the Oe horizons of Eutric Albic Stagnic Histic Retisol (Loamic) (**a**) and Eutric Albic Retisols (Loamic) virgin (**b**) and arable (**c**).

3.2. Analysis of the Soil Liquid Phase

To study the protolytic properties of the liquid phase of soils, samples of the organogenic horizons of three Retisols were used. As seen in Figure 3, the smoothed averaged titration curves of water extracts and scour waters differ significantly.

In the pK spectra, five bands with the same ranges of pK values are allocated: 3.5–3.8, 4.6–4.9, 6.4–7.1, 8.6–8.9, and 9.6–9.8 (Figure 4).

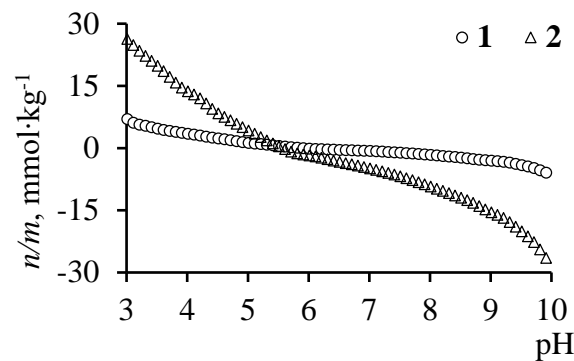**Figure 3.** Smoothed averaged titration curves of water extract (**1**) and scour waters (**2**); the O horizon of Distric Albic Retisol (Loamic).
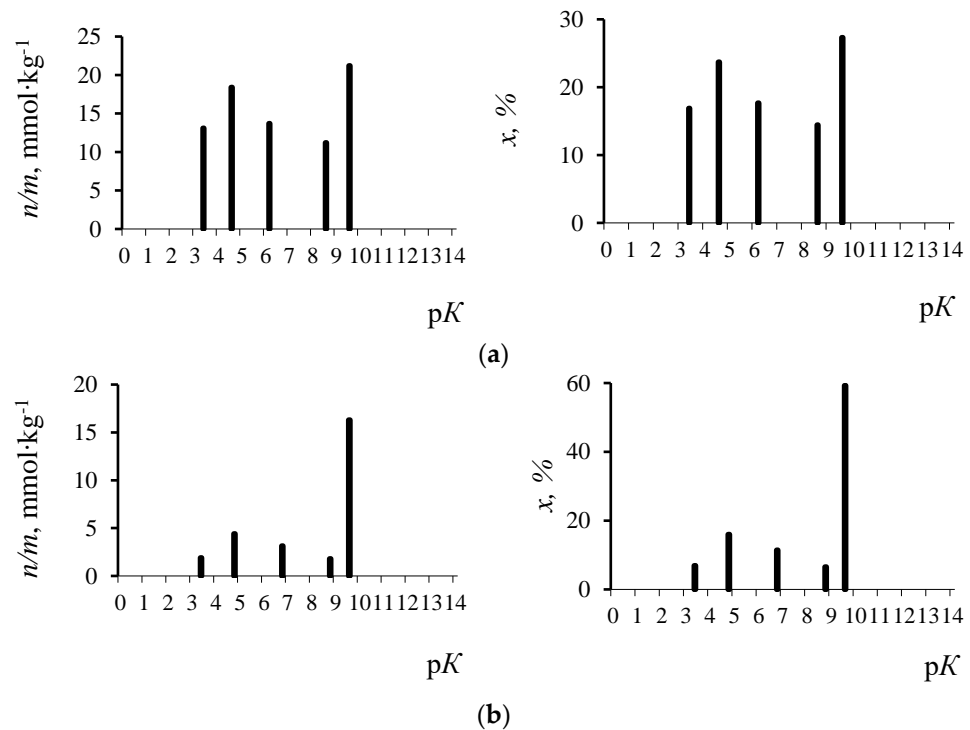

Figure 4. pK spectra of water extracts (**a**) and scour waters (**b**) of the O horizon of Distric Albic Retisol (Loamic): n/m–the number of acid groups (mmol·kg^{-1}), x–the molar fraction (%).

The functional group –COOH, which has a pK of 3.5–3.8, is a part of low-molecular formic, malic, glycolic, and lactic acids. Fulvic acids have carboxyl groups that are characterized by values of pK from 2.9 to 7.8 (Table 2) and, depending on the position in the molecule, can have both stronger and weaker acid properties [23]; this is also observed in hydroxalate and dihydrocitrate anions, butyric, fumaric, and other acids. Carbonic and maleic acids can form the third band in the pK spectrum. Amino groups of amino acids, as well as –OH-groups of phenols and phenolic acids, have a pK of more than 8.0.

Aluminum compounds represented by organometallic complex compounds can also participate in protolytic equilibria in a wide pH range. At low pH values, the stability of complex ions decreases, and when water extracts are titrated with an acid solution, the complex ion is protonated stepwise to form Al^{3+}. With an increase in pH values of more than 5, the basicity of organometallic complexes increases up to the formation and precipitation of $Al(OH)_3$. At pH > 7.5, the precipitate dissolves, and negatively charged aquahydroxo complexes of aluminum are formed. Similarly, the destruction of Fe- and Mn-organic complex compounds can occur (if any). The maximum total amount of acidic

groups of organic compounds that have passed into the water extract is contained in Eutric Albic Retisol (Loamic), while the minimum total is contained in Distric Gleyic Retisol (Loamic), the most moistened soil (Table 2).

Table 2. The number of acid groups of compounds extracted by distilled water.

Soil	Total Number Of Groupings, n/m, mmol·kg^{-1}			The Molar Fraction of the Groups That Passed into the Water Extract, x, %
	In Extract	In Scour Waters	Sum	
Distric Albic Retisol (Loamic)	78	28	105	74
Eutric Albic Retisol (Loamic)	110	45	155	71
Distric Gleyic Retisol (Loamic)	51	15	65	78
Error characteristic, $\pm\Delta n/m$, mmol·kg^{-1}	±6	±4	±5	–

3.3. Analysis of the Solid Phase of Soils

The smoothed averaged titration curves of the water suspensions of the Eg horizon of Eutric Albic Stagnic Retisol (Loamic) before and after calcination are shown in Figure 5.
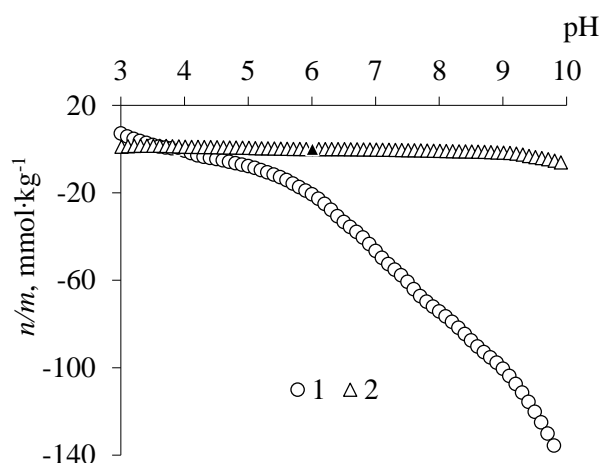

Figure 5. Smoothed averaged titration curves of water suspensions of the Eg horizon sample of Eutric Albic Stagnic Retisol (Loamic) before (**1**) and after (**2**) calcination.

The mineral horizons of Eutric Albic Stagnic Retisol (Loamic) have a peculiar combination of organic and mineral components containing acidic groups. In the Eg, Eh, and EB horizons, all acid centers are divided into five groups according to the values of pK, and in the B and C horizons, into four groups. After calcination, only three groups with p$K_{2,3,5} \approx 5, 7$ and, 10 were identified in the Eg sample.

4. Discussions

4.1. Fulvic Acids

Analysis of the pK spectra (Figure 2) allows us to make a conclusion about a similar set of carboxyl and phenolic groups in FAs located within the same soil. The main characteristics of the pK spectra of FAs are given in Table 3. The presence of such a wide range of groups of different acidity in the FA preparations indicates the presence of carboxyl and phenolic functional groups in their structure, occupying different positions in the carbon skeleton of the FAs.

Table 3. Characteristics of pK spectra of fulvic acids.

Horizon	Parameter	Band Number					
		1	2	3	4	5	6
Eutric Albic Stagnic Histic Retisol (Loamic)							
Oe	pK	3.2 ± 0.3	-	-	7.1 ± 0.4	-	10.4 ± 0.3
	x, % [1]	57.95	-	-	22.71	-	19.34
Eh	pK	3.4 ± 0.4	-	-	7.1 ± 0.3	9.5 ± 0.7	10.5 ± 0.3
	x, %	57.80	-	-	24.63	0.0550	12.07
B	pK	4.0 ± 0.3	-	-	7.3 ± 0.4	-	10.4 ± 0.3
	x, %	30.05	-	-	26.53	-	43.42
virgin Eutric Albic Retisol (Loamic)							
Oe	pK	3.8 ± 0.3	5.6 ± 0.4	-	7.8+0.7	9.5 ± 0.5	-
	x, %	40.65	15.30	-	0.1088	33.17	-
Eh	pK	2.9 ± 0.3	4.9 ± 0.4	6.2 ± 0.3	-	9.7 ± 0.3	-
	x, %	54.65	10.08	15.23	-	20.04	-
B	pK	-	5.0 ± 0.2	6.8 ± 0.7	-	9.4+0.8	10.5 ± 0.3
	x, %	-	30.67	16.44	-	3.64	49.25
arable Eutric Albic Retisol (Loamic)							
Oe	pK	3.4 ± 0.3	-	6.7 ± 0.4	7.8 ± 0.3	-	10.4 ± 0.4
	x, %	38.60	-	17.32	10.24	-	33.84

[1] x is the molar fraction of groups with given pK.

The lowest values of pK = 2.9 are the most acidic carboxyl groups, which are located in the ortho position with hydroxyl or other carboxyl groups at the benzene ring. Slightly less acidic (pK = 3.2–3.8) are the carboxyl groups that are in the meta- or paraposition with the above groups at the benzene ring or at the β-position in the aliphatic chain. Carboxyl groups of fatty acids have pK values = 4.9–7.8, as do carboxyl groups that have a nucleophilic group in the immediate vicinity but dissociate at the second or third step. The maximum values of pK = 9.4–10.5 have hydroxyl groups of phenolic fragments of FAs. The presence of carboxyl and phenolic groups in these positions in the FA's carbon skeleton has been confirmed by many researchers [32,34].

As seen in Table 3, the proportion of carboxyl groups (the sum of the concentrations of the groups having p$K \leq 8$) increases in the Eh horizons in both virgin Retisols and further decreases in the B horizons. The obtained data indicate that FAs of the podzolic horizon are most enriched in carboxyl groups and, consequently, are better dissolved in water and participate in complexation reactions with cations Ca^{2+}, Mg^{2+}, Fe^{3+}, Al^{3+}, etc. The relative decrease in acidic groups and the growth of phenolic fragments during the transition from organogenic horizons to mineral B can be explained by the microbiological destruction of FA molecules and the cleavage of carboxyl groups. Better aeration of the podzolic horizon E of the Eutric Albic Retisol (Loamic) compared to the Eutric Albic Stagnic Histic Retisol (Loamic) causes an increase in the number of oxygen-containing groups (hydroxyl and carboxyl) in the composition of humus substances, as well as their acidity (pK values become less). And this, in turn, leads to an intensification of the mineral leaching process, which is the essence of the podzol formation process [17].

Phenolic fragments are more resistant to decomposition and therefore accumulate in the FA structure. Scant data on the study of the acid–base characteristics of humus substances by pK spectroscopy also indicate the presence of three to four ionogenic groups with different values of pK in the FA structure [6,21].

As a result of the conducted studies, it was found that the cultivation and agricultural use of soils practically does not change the total number of acidic groups in the structure of FAs in comparison with the virgin analogues (Table 3). However, their qualitative composition significantly changes, namely the share of strongly acidic groups decreases in FAs of the Oe horizon from 55 to 39%, and peaks with pK values of 6.7 ± 0.4 and 7.8 ± 0.3,

corresponding to medium-acid carboxyl groups, appear. Thus, the data obtained indicate that FAs of the Oe horizon have weaker acidic properties and are less aggressive compared to FAs of the organogenic horizons of virgin soils.

Since the p*K* spectroscopy method allows us to determine the relative content of carboxyl and phenolic groups in the structure of humus substances, the results with the content of these groups obtained earlier by ^{13}C-NMR spectroscopy were compared [35,36] (Table 4).

Table 4. Relative content of acid–base groups in the composition of fulvic acids.

Horizon	Share of –COOH Groups, %		Discrepancy		Significance
	Results Obtained by ^{13}C-NMR Spectroscopy	Results Obtained by p*K* Spectroscopy	Actual, $\|X_1 - X_2\|$	Acceptable, $t_{0.95}\sqrt{\Delta_1^2 + \Delta_2^2}$	
	Eutric Albic Stagnic Histic Retisol (Loamic)				
Oe	0.72 ± 0.11	0.67 ± 0.05	0.05	0.52	insignificant
Eh	0.86 ± 0.13	0.80 ± 0.06	0.06	0.62	insignificant
	virgin Eutric Albic Retisol (Loamic)				
Oe	0.86 ± 0.13	0.81 ± 0.07	0.05	0.63	insignificant
Eh	0.89 ± 0.13	0.82 ± 0.07	0.07	0.63	insignificant
	arable Eutric Albic Retisol (Loamic)				
Oe	0.73 ± 0.11	0.66 ± 0.05	0.07	0.52	insignificant

From Table 4, it is visible that the discrepancy between the results of the content analysis of carboxyl groups in the composition of FAs obtained by different methods is insignificant. The calculated correlation coefficient between the content of –COOH groups obtained using the ^{13}C-NMR spectroscopy method and the p*K* spectroscopy method is 0.992 ($p = 0.95$; $n = 5$).

4.2. Soil Liquid Phase

With a single treatment of the soil with distilled water, only 70–80% of the groups of water-soluble organic compounds from their total amount passed into the extract. It should be noted that during this soil treatment, compounds containing groups with a p*K* of 9.6–9.8 are extracted only by 60% from samples of the Distric Albic Retisol (Loamic) and Eutric Albic Retisol (Loamic), and by 70% from the Distric Gleyic Retisol (Loamic). The compounds forming the fourth band (p*K* 8.6–8.9) pass into the solution to a greater extent (85–90%). The general regularities of the transition of the first three bands' compounds from different types of soils to the water extract have not been established.

A single soil treatment with distilled water is not sufficient to extract water-soluble organic compounds, since the composition of the compounds' mixture that have passed into the extract and been remained in the wet residue is different. Compounds containing groups with a p*K* of 9.6–9.8 are the most strongly retained by the soil during a single treatment with distilled water. The identification of the composition and properties of water-soluble compounds based on the results of the study of single water extracts only can lead to unsubstantiated conclusions (Table 5).

Quantitative estimates of the composition of the mixture of the soils' water-soluble organic compounds were carried out taking into account the results of the study of both extracts and scour waters. In all the studied soils, compounds containing groups of a p*K* of 9.6–9.8 accumulate, which is consistent with previously obtained results [19,37,38]. The shares of all types of acid groups in the Distric Albic Retisol (Loamic), Eutric Albic Retisol (Loamic), and Distric Gleyic Retisol (Loamic) are 40, 50, and 25%, respectively. Attention is drawn to the large value of the confidence error interval for the number of groups in the specified range of p*K* (Table 5). This is probably due to the most stable

retention of compounds containing these groups by the soil and their difficult transition to the liquid phase.

Table 5. Molar fractions (*x*) of acid groups in scour waters from their total amount contained in the extracts and scour water, according to the ranges of p*K*.

Band Number in the pK Spectra	Range pK	Distric Albic Retisol (Loamic)	Eutric Albic Retisol (Loamic)	Distric Gleyic Retisol (Loamic)
		x, %		
1	3.5–3.8	13	19	28
2	4.6–4.9	19	24	15
3	6.4–7.1	18	27	21
4	8.6–8.9	15	9	15
5	9.6–9.8	43	38	28

The distribution of acid groups over one to four bands of the spectrum in the samples of Eutric Albic Retisol (Loamic) and Distric Gleyic Retisol (Loamic) is the same ($r = 0.93$, $p = 0.95$). In the organogenic horizon of the automorphic soil, a low proportion of groups with a p*K* of 3.5–3.8 is noted. This conclusion is in accordance with the data obtained when studying the composition of low-molecular acids (Figure 6a,b).
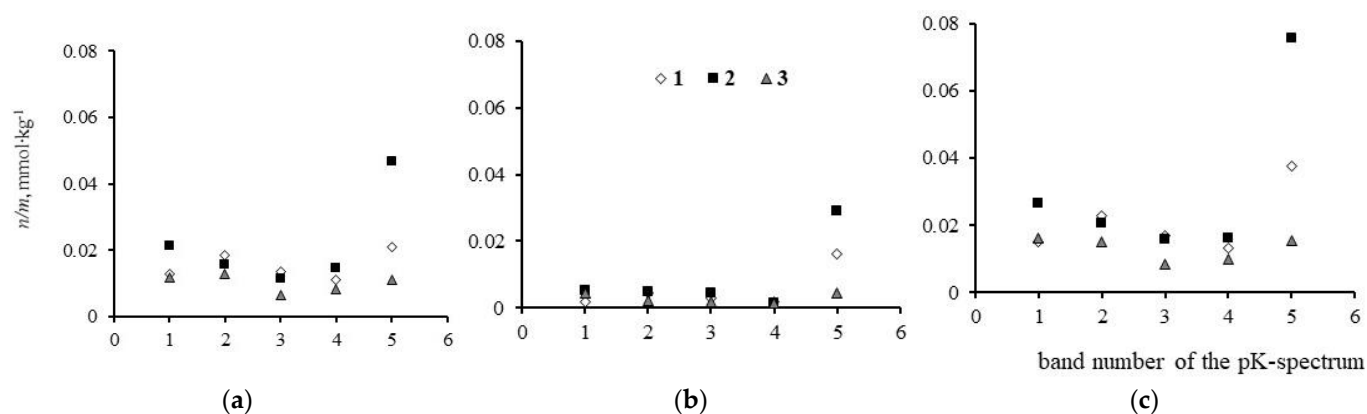

Figure 6. The number of acid groups (n/m, mmol·kg^{-1}) of water extracts (**a**), scour water (**b**), and their total amount (**c**) in the samples of organogenic horizons in the p*K* intervals: **1**: Distric Albic Retisol (Loamic); **2**: Eutric Albic Retisol (Loamic); and **3**: Distric Gleyic Retisol (Loamic).

The revealed patterns may be related to the mesorelief of the key area. The newly formed organic compounds are probably irreversibly displaced with the lateral runoff, accumulating in the Eutric Albic Retisol, which is facilitated by precipitation and snowmelt. In addition, the p*K* spectroscopy data are in accordance with the data obtained when studying the composition of low-molecular acids of soils by gas chromatography and chromatography–mass spectroscopy methods.

In particular, it is shown that a certain increase in soil moisture, which is characteristic of Eutric Albic Retisol (Figure 6c), in comparison with automorphic (Distric Albic Retisol) soil, contributes to the accumulation of low-molecular-weight acids. They contain phenolic compounds (p*K* > 9) and the strongest aliphatic oxyacids (p*K* < 4.0). This feature can be explained by the slowing down of the reactions of the dehydration of oxyacids to unsaturated and the oxidation to polybasic acids under conditions of high humidity [19,39]. Some researchers associate the accumulation of aromatic acids with a biological factor: the activity of microorganisms, the decomposition of vegetation residues, and root exudates [40,41].

Thus, when separating the water extract from the solid fraction, water-soluble organic compounds are retained by 20–30% by the moist soil and do not pass into the solution. The

ratio of the components in the extract and those remaining in the sediment is different, the compounds with a pK of 9.6–9.8 are mostly retained by the wet residue of the soil.

4.3. Solid Phase of Soils

The total molar fraction of the first two is 15% of their total amount (Table 6). It can be assumed that the remaining acid centers belong to aluminum, iron, and silicon oxides [42]. This corresponds to the data obtained during the study of the acid–base properties of γ-Al_2O_3 by pK spectroscopy [20]. The authors note the presence of four acid–base groups on the surface of aluminum oxide with a pK of 4.0–4.3, 5.3–6.1, 7.8–8.0, and 9.7–10.0.

Table 6. The values of the pK_i groups (above the line) of Eutric Albic Stagnic Retisol (Loamic) and the number of groupings (n_i/m, mmol·kg^{-1}) corresponding to them (below the line).

Horizon	Band Number in the pK Spectra									
	1		2		3		4		5	
	pK_1	n_1/m	pK_2	n_2/m	pK_3	n_3/m	pK_4	n_4/m	pK_5	n_5/m
Eg calcined	–		5.0 ± 0.3	1.3	6.8 ± 0.4	0.6	–		9.9 ± 0.2	11.2
Eg	3.9 ± 0.2	12.2	5.5 ± 0.1	11.6	6.7 ± 0.4	43.2	8.1 ± 0.4	30.0	9.5 ± 0.3	66.0
Eh	4.0 ± 0.4	27.0	5.8 ± 0.2	23.8	7.0 ± 0.3	24.6	8.4 ± 0.2	26.0	9.5 ± 0.4	76.0
EB	4.1 ± 0.2	19.3	6.0 ± 0.4	33.6	7.1 ± 0.3	22.1	8.4 ± 0.3	17.0	9.5 ± 0.4	42.0
B	4.2 ± 0.2	22.4	6.2 ± 0.5	21.3	–		8.5 ± 0.2	31.5	9.5 ± 0.5	35.0
C	4.5 ± 0.3	14.0	–		6.7 ± 0.6	15.0	8.6 ± 0.4	25.6	9.6 ± 0.3	25.8

The complete disappearance of the first, third, and partial second bands in the pK-spectrum of the Eg soil horizon after calcination is associated with the decomposition of organic compounds; the absence of the fourth band is due to the loss of the –OH-groups of amorphous Fe^{3+} and Al^{3+} hydroxides, as well as some of the most poorly crystallized clay minerals; and the absence of the fifth band is explained by the loss of phenolic groups of organic acids. In addition, prolonged calcination could lead to partial destruction of the kaolinite crystal lattices with a corresponding loss of hydroxo groups at the lateral cleavages.

The acidity of all the studied horizons of Eutric Albic Stagnic Retisol (Loamic) is explained by the presence of organic acids (pK from 3.9 to 7.0), as well as mineral components [43,44]. It is not possible to clearly divide pK of –COOH groups into the second and third groups (Figure 7), since when moving down the soil profile, the nature of the organic compounds forming these groups of acid centers changes: the –COOH of the second group of compounds in the Eg horizon have p$K \approx 5.5$, and in the deeper horizons, there is a gradual accumulation of weaker acid groups (for the C horizon, p$K \approx 6.7$). The appearance of the third group (p$K \approx 7.0$) only in the upper horizons (Eg, Eh, EB) is probably connected to the fact that the –COOH are part of the HAs, which do not penetrate into the lower horizons. Numerous data on the profile distribution of humus substances in the mineral horizons of soils indicate a significant decrease in their concentration with depth. However, against the background of the expected decline for individual acids and individual soil horizons, this trend may not manifest itself or reverse. This is explained by different ratios of the rate of acid formation, their migration, sorption, and biodegradation [18]. The sorption of acid anions in soils increases with a decrease in pH and with an increase in the composition of the solid phase of Fe and Al oxides/hydroxides and amorphous aluminosilicates [42].
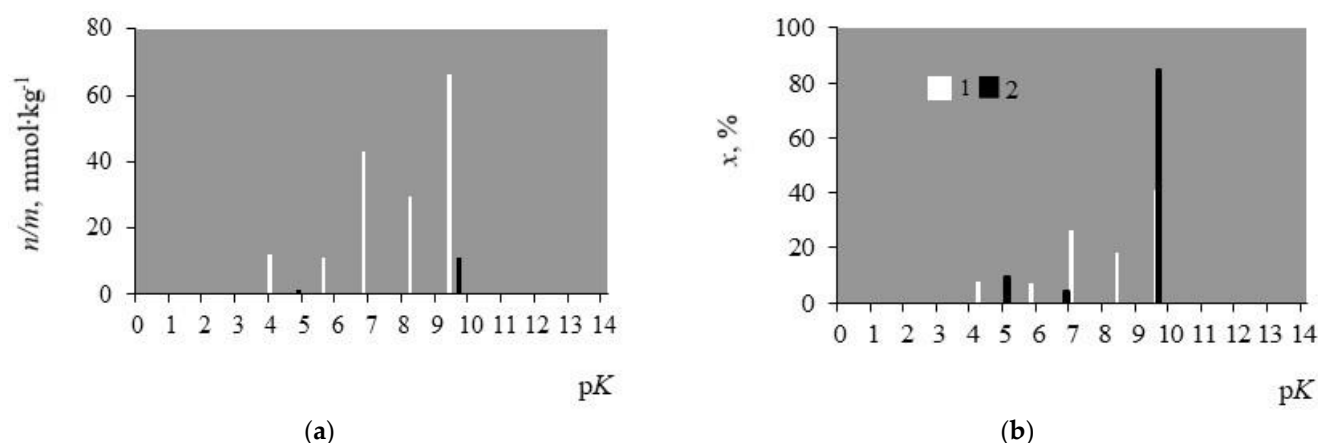
(**a**)

(**b**)

Figure 7. pK spectra of water suspensions of the Eg horizon sample of Eutric Albic Stagnic Retisol (Loamic) before (**1**) and after (**2**) calcination: n/m–the number of acid groups (**a**), x–their molar fraction (**b**).

To assess the relationship between the acidity indicators and the amounts of acid groups calculated according to the program, a correlation analysis was performed (Table 6).

The highest correlation coefficients are characterized by the series reflecting the relationship between:

- Exchange acidity and the number of acid centers with pK_{2-4}–n_{2-4}/m (r = 0.995);
- Hydrolytic acidity and n_{2-5}/m, with the exception of the groups with pK_2 = 5.8 of the Eh horizon (r = 0.989).

Thus, the heterogeneity of the composition of acidic components that cause different types of potential acidity of soil mineral horizons is established.

5. Conclusions

"PKSVD" software allows calculating the pK spectra with sufficiently high accuracy for complex natural polyelectrolytes, such as humic substances, water-soluble organic matter, soil suspensions, etc. The use of the "SPLINE" program for smoothing the experimental dependence allows improving the results of plotting the pK spectrum. The use of the pK spectroscopy method made it possible to determine the values of the acidity constants of groups of complex natural systems, calculate the number of acid groups, and obtain new results of both a fundamental and applied nature.

The pK spectroscopy method, which uses linear regression analysis with restrictions on the non-negativity of solutions, opens up wide possibilities for the potentiometric analysis of complex protolytic systems, including various environmental objects.

This method significantly expands the understanding of the physicochemical properties of humic substances and soil organic matter. It can be used for a more correct calculation of the doses of lime required for agricultural use of acidic soils and for increasing their productivity.

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