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INVESTIGATION OF THE COMPLEX FORMATION PROCESS OF LEAD (II) WITH NATURAL MACROMOLECULAR ORGANIC SUBSTANCES (FULVIC ACIDS) BY THE SOLUBILITY AND GEL CHROMATOGRAPHIC METHODS

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Abstract. Fulvic acids are one of the most important ligands, governing the geochemical cycling of metals in the environment. The objective of the work was to investigate the complex formation process between fulvic acids and *Pb*(*II*). The complex formation process was studied by the solubility and gel chromatographic methods at pH 5.0 and 8.0.

Keywords: fulvic acids, lead fulvate, average stability constant.

1. Introduction

Natural macromolecular humic substances are ubiquitous in natural materials: soils, waters, sediments and lignites.[1-7] These complex superstructures are derived from the decomposition of dead plant and animal matter. Humic substances can play an important role in determining the mobility and bioavailability of both organic and inorganic trace components in the environment. *pH* and free metal ion activity are the most important factors, affecting the binding of metal ions to humic substances. Humic substances have been classified according to their solubility in diluted acids and bases: humins, humic acids, and fulvic acids. Humins are insoluble at any value of *pH*. Humic acids represent the fraction that is insoluble at $pH < 2.0$. Fulvic acids, the third major group of humic substances averagely dissolve in acidic and alkaline regions.[1] Originally, the term fulvic acids was used for all natural organic substances which were solved in the acid and remained after the acidification of soil alkaline extracts. At present time, this term means substances which are solved in the acid, and their isolation from other organic substances can be performed during the fractionating by using activated charcoal or other sorbents.

Fulvic acids (FA) are one of the most important ligands, governing the geochemical cycling of metals in the environment. They are major organic substances of natural waters. FA concentrations are ranging from less than 1 mg/L to more than 100 mg/L.[3-9]

The chemical structure of FA has not been finalized yet. Elemental composition(%) of FA isolated from natural waters changes: *C* – from 46.7% to 59.7%; *H* – from 4.1% to 5.5%; *O* – from 31.8% to 49.7%; *N* – from 0.74% to 3.3% and *S* – from 0.4% to 1.7%.[4,6-9] $pK_{H,COOH}$ changes from 3.1 to 4.9 and $pK_{H,Ph-OH}$ changes from 8.3 to 10.6.[4,7,10,11]

Carboxyl and phenol groups of FA take an active part in complex formation and sorption processes, proceeding in natural waters, bottom sediments and soils and stipulate migration forms of heavy metals in natural objects.[3,7,12-23] Complex compounds of heavy metals with macromolecular organic substances are characterized with less bioavailability and accordingly with less toxicity.[24,25]

Lead is a typical toxic heavy metal which is ubiquitous in natural environment. Lead contamination of natural water systems has adverse behavioural, physiological, and biochemical effects on humans. The most toxic forms of lead are Pb^{2+}, $[PbOH^+]$, $[Pb(OH_2^0)]$ and different inorganic complexes. They react with the biomolecules of the body to form extremely stable biotoxic compounds.[26,27] FA acids may affect the transport, accumulation, bioavailability and toxicity of heavy metals in the environment.[28,29]

In spite of researches, experimental data on stability constants (β) of complex compounds of FA with lead are heterogeneous and they differ in several lines from each other.[7,19,30-39] This condition is mainly stipulated by

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ignoring the average molecular weight (Mw) of the associates of FA, which value in its turn depends on the value of pH and finally causes the wrong results. Therefore, it is difficult to investigate complex formation processes, taking place in natural waters, identify migration forms of $Pb(II)$ and evaluate and assess chemical-ecological condition of natural waters.

Objective of the work was to investigate complex formation process between the pure samples of FA, isolated from natural waters and $Pb(II)$ and to calculate average stability constant of lead fulvate complex. Complex formation process of fulvate complexes of lead was studied by the solubility method at $pH = 8.00$ and gel chromatographic method at $pH = 5.0$. Average molecular weight of associate and oligomer of FA was used for determination the composition and average stability constant of lead fulvate complex.

2. Experimental

2.1. Isolation and Obtaining Pure Samples of Fulvic Acids

For obtaining pure samples of FA, after filtration through membrane filters (0.45 μm pore size), water of Paravani Lake was concentrated by the frozen method. The concentrated water samples were acidified with 6M HCl to $pH = 2$ and were put for 2 hours on water bath at 333 K for coagulation of humin acids. Then the solution was centrifuged for 10 min at 8000 rpm (Centrifuge T-23). For isolation of FA from centrifugate the adsorption-chromatographic method was used, charcoal was used as a sorbent. The centrifugate was passed through the column of activated charcoal ($V = 1$ mL/min, $d = 2$ cm, $l = 60$ cm) until the colored solution was achieved. Sorption of amino acids and carbon acids practically did not occur at that time. But for the purposes of insurance, to remove possible fraction of amino acids, 0.1M HCl was passed through the activated charcoal. The column of activated charcoal was washed by bidistilled water, with silver nitrate to a negative reaction to chlorine. For desorption of polyphenols 90% acetone water solution was used, until the colourless solution was achieved. For the removing of acetone, the column of activated charcoal was washed by the bidistilled water until the smell of acetone disappeared in eluate. Elution process of FA fraction was performed with 0.1N $NaOH$ solution[7,15]. Obtained alkalic solution of FA, for the purification was passed through a cation-exchanger. For determination the concentration of FA in obtained solutions, the gravimetric method was used, the part of the solution was dried under vacuum, until the constant weight was obtained. Elemental composition of standard samples of FA, isolated from natural waters and average meaning of dissociation constants respectively equals to C – 53.75%; H – 4.29%; O – 40.56%; N – 0.89%; S – 0.51%; $pK_{H,COOH} = 4.37$; $pK_{H,Ph-OH} = 10.4$; ashing – 0.35%.

2.2. Solubility Method

0.1 mL suspension of lead hydroxide and increasing quantity of standard solution of FA were placed in fluoroplastic cylinders. The concentration of FA in model solutions changes from $1.35 \cdot 10^{-5}$ to $5.40 \cdot 10^{-5}$ mol/L (Mw(FA) = 6260 at pH=8.0). The initial concentration of $Pb(II)$ before adding ligand, at $pH = 8.0$ was $3.58 \cdot 10^{-6}$ mol/L, ionic strength $\mu = 0.01(KNO_3)$, $V = 10$ mL. Then, it was stirred in a mechanical mixer for 100 hours, until the balance was achieved and then the suspension was filtered through the membrane filters (0.45 μm pore size). In filtrates, the concentration of lead was measured by an atomic absorption spectrophotometer (Agilent 280Z AA).

2.3. Gel Chromatographic Method

Parameters of sephadex G-25: mass of dry gel - 17g, height of swelled layer of gel – 42 cm, inner diameter of column – 1.6 cm, the limits of fractionating 100–5000 dalton. For the calibration of sephadex G-25 blue dextran, polyethylene glycols with molecular weights 300, 600, 1000 dalton and glucose were used. Titer of standard substances – 1 mg/mL, transmission speed – 3 mL/min, apply volume of solution – 2 mL.

Increasing quantity of standard solutions of FA (concentration of FA in model solutions changes from $3.82 \cdot 10^{-5}$ to $15.28 \cdot 10^{-5}$ mol/L, Mw(FA) = 2210 at pH = 5.0) and lead (II) solutions with one and the same concentration ($(Pb(II))_{total} = 14.89 \cdot 10^{-5}$ mol/L), which were prepared from the standard solutions, intended for atomic absorption method, and were placed in fluoroplastic cylinders. Constant ionic strength was achieved by adding 0.1M 1 mL of potassium nitrate. The final volume of solutions was 10 mL. In model solutions at $pH = 5.0$, the concentration of hydrogen ions was regulated by 0.01M potassium hydroxide and 0.01M nitric acid.

2 mL of model solutions were placed at the top part of the column. The elution process was done by 0.01M potassium nitrate, that had the same pH as the aliquots of solution. The quantity of metals connected with FA was determined in fractions, which releasing volume fits substances with the molecular weight of $300 \leq Mw > 5000$. The lead concentration was measured by atomic absorption spectrophotometer (Agilent 280Z AA).

3. Results and Discussion

3.1. Investigation of Complex Formation Process by the Solubility Method

As it is shown from the results (Table 1), along with the increasing of FA concentration in the solution, the concentration of $Pb(II)$ increases as well due to the formation of fulvate complex. The fact that FA form associates in water solutions was taking into consideration during the calculation of molar concentrations of FA. While investigation of water solutions of FA by the gel chromatographic method was established, that in the interval from $pH = 4.0$ to $pH = 11.0$ there is a line dependence between the average molecular weight (Mw) of FA and the value of pH, which is expressed in the following way: Mw=1350pH–4540.[7] At pH = 8.0, $Mw(FA)$=6260.

In diluted water solutions, at $pH = 8.0$, the lead dihydroxy complex $Pb(OH)_2^0$ is the dominant form.[40] The reaction between the lead dihydroxy complex and FA may be written in the following way:

$$Pb(OH)_2^0 + mFA \rightleftarrows Pb(OH)_2(FA)_m \quad (1)$$

$$\beta = \left[Pb(OH)_2 FA_m\right] \Big/ \left\{\left[Pb(OH)_2^0\right]\left[FA\right]^m\right\} \quad (2)$$

In the solution, the concentration of lead dihydroxy fulvate complex equals to the difference between final $[(Pb(II)_{total}]$ and initial $[(Pb(II)_{free}]$ concentrations of lead received after formation of complex:

$$\left[Pb(OH)_2 FA_m\right] = \left[Pb(II)_{total} - Pb(II)_{free}\right]$$

and $$\left[Pb(OH)_2^0\right] = \left[Pb(II)_{free}\right] \quad (3)$$

Put these values in (2) equation

$$\beta = \left[Pb(II)_{total} - Pb(II)_{free}\right] \Big/ \left\{\left[Pb(II)_{free}\right]\left[FA\right]^m\right\} \quad (4)$$

From (4) equation

$$\beta\left[Pb(II)_{free}\right] = \left[Pb(II)_{total} - Pb(II)_{free}\right] \Big/ \left[FA\right]^m \quad (5)$$

At the fixed pH, the left part of the equation (5) is a permanent value and was marked as K'

$$K' = \left[Pb(II)_{total} - Pb(II)_{free}\right] \Big/ \left[FA\right]^m \quad (6)$$

Logarithm of this equation (6) is:

$$\lg K' = \lg\left[Pb(II)total - Pb(II)_{free}\right] - m\lg\left[FA\right] \quad (7)$$

The numeral value (m) of the stoichiometric coefficient or the number of ligands in an inner coordination sphere of complex equals to the slope of straight line built in coordinates:

$$\lg\left[Pb(II)_{total} - Pb(II)_{free}\right] — \lg\left[FA_{total}\right]$$

To calculate the exact value of slope the least square method was used

$$m = tg\alpha = \left(n\Sigma x_i y_i - \Sigma x_i \Sigma y_i\right) \Big/ \left(n\Sigma x_i^2 - \left(\Sigma x_i\right)^2\right) \quad (8)$$

$$x_i = \lg\left[FA_{total}\right] \quad (9)$$

$$y_i = \lg\left[Pb(II)_{total} - Pb(II)_{free}\right] \quad (10)$$

After the calculation, the numeral value of m ($Mw(FA)$=6260) was obtained which equals to 1.04. The complex, obtained at $pH = 8.0$ in a heterogeneous system

$$Pb(OH)_2(solid) — Pb(II)(solution) — FA — H_2O$$

is the lead dihydroxy fulvate, with the 1:1 ratio. So, the complex formation reaction ($pH = 8.0$) may be written in such a way:

$$Pb(OH)_2^0 + FA = Pb(OH)_2 FA \quad (11)$$

Table 1. The dependence of the solubility of lead hydroxide on the FA concentration and data for determination of the lead dihydroxy fulvate complex composition, pH=8.0; Mw(FA) = 6260; $[(Pb(II)_{free}]=3.58\cdot10^{-6}$ mol/L

mol/L			$[Pb_{total}]:[FA_{total}]$	$Log[FA_{total}]$	$LogPbFA_m$
$[FA_{total}]$	$[Pb_{total}]$	$[Pb(OH)_2FA_m]$			
$1.35\cdot10^{-5}$	$4.08\cdot10^{-5}$	$3.72\cdot10^{-5}$	1:0.33	–4.8697	–4.4294
$2.02\cdot10^{-5}$	$5.73\cdot10^{-5}$	$5.37\cdot10^{-5}$	1:0.35	–4.6946	–4.2700
$2.70\cdot10^{-5}$	$7.58\cdot10^{-5}$	$7.22\cdot10^{-5}$	1:0.36	–4.5686	–4.1415
$3.37\cdot10^{-5}$	$9.86\cdot10^{-5}$	$9.50\cdot10^{-5}$	1:0.34	–4.4724	–4.0223
$4.05\cdot10^{-5}$	$11.84\cdot10^{-5}$	$11.48\cdot10^{-5}$	1:0.34	–4.3925	–3.9400
$4.72\cdot10^{-5}$	$13.85\cdot10^{-5}$	$13.49\cdot10^{-5}$	1:0.34	–4.3260	–3.8699
$5.40\cdot10^{-5}$	$15.13\cdot10^{-5}$	$14.77\cdot10^{-5}$	1:0.36	–4.2676	–3.8306

Table 2. Data for calculation of average stability constant of lead fulvate complex by the Leden's method, pH=8.0; Mw(FA) = 2128; $[(Pb(II)_{free}]=3.58\cdot10^{-6}$ mol/L

mol/L				$F(FA)$
$[FA_{total}]$	$[Pb(II)_{total}]$	$[Pb(OH)_2FA]$	$[FA_{free}]$	
$3.97\cdot10^{-5}$	$4.08\cdot10^{-5}$	$3.72\cdot10^{-5}$	$0.25\cdot10^{-5}$	$4.17\cdot10^{6}$
$5.95\cdot10^{-5}$	$5.73\cdot10^{-5}$	$5.37\cdot10^{-5}$	$0.58\cdot10^{-5}$	$2.57\cdot10^{6}$
$7.94\cdot10^{-5}$	$7.58\cdot10^{-5}$	$7.22\cdot10^{-5}$	$0.72\cdot10^{-5}$	$2.78\cdot10^{6}$
$9.92\cdot10^{-5}$	$9.86\cdot10^{-5}$	$9.50\cdot10^{-5}$	$0.42\cdot10^{-5}$	$6.33\cdot10^{6}$
$11.91\cdot10^{-5}$	$11.84\cdot10^{-5}$	$11.48\cdot10^{-5}$	$0.43\cdot10^{-5}$	$9.65\cdot10^{6}$
$13.89\cdot10^{-5}$	$13.85\cdot10^{-5}$	$13.49\cdot10^{-5}$	$0.40\cdot10^{-5}$	$9.63\cdot10^{6}$
$15.88\cdot10^{-5}$	$15.13\cdot10^{-5}$	$14.77\cdot10^{-5}$	$0.75\cdot10^{-5}$	$5.47\cdot10^{6}$

Therefore, the formula for the average stability constant can be expressed in the following way

$$\beta = \frac{\left[Pb(OH)_2 FA\right]}{\left\{\left[Pb(II)_{free}\right][FA]\right\}} = \frac{\left[Pb(II)_{total} - Pb(II)_{free}\right]}{\left\{\left[Pb(II)_{free}\right][FA]\right\}} \quad (12)$$

In balanced solutions, on the average the correlation $[Pb(II)_{total}]:[FA_{total}]$ is equal to 1:0.34 (Table 1). This means, that during complex formation process, the associate of FA, which Mw at pH = 8.0 equals to 6260, divides and every 0.34 part of this associate inculcates into lead's inner coordination sphere, as an integral ligand. So it may assume, that Mw of the associate of FA which takes part in a complex formation process equals to 2128. This part of the associate of FA was conventionally called an "active associate".[41] The meaning of Mw of the „active associate" of FA was used for determination the concentration of free ligand and average stability constant. It should be noted, that in case of using the average molecular weight of the associate (6260), it will be impossible to calculate the concentration of free ligand. Without it, it's impossible to calculate the average stability constant of lead fulvate complex. It should be mentioned that changing of molecular weight does not cause the changing of the numeral value of stoichiometric coefficient.

For calculation of average stability constant of lead dihydroxy fulvate at pH=8.0 Leden's function $F(L)$ was used.[42]

$$F(L) = F(FA) = \frac{\left[Pb(OH)_2 FA\right]}{\left\{\left[Pb(II)_{free}\right]\left[FA_{free}\right]\right\}} = \frac{\left[Pb(II)_{total}\right] - \left[Pb(II)_{free}\right]}{\left(\left[Pb(II)free\right]\left[FA_{free}\right]\right)} = \beta_1 + \beta_2\left[FA_{free}\right] \quad (13)$$

$\left[Pb(II)_{free}\right]$ – initial concentration of lead in solution, before adding ligand

$$\left[FA_{free}\right] = \left[FA_{total}\right] - \left[Pb(OH)_2 FA\right] \quad (14)$$

When $[FA_{free}]$ aspires to zero, β could be found by the graphical method. The section which is cut on the ordinate by the straight line, is built in coordinates $F(FA)$–$[FA_{free}]$ and equal to the average stability constant. The value of β was calculated by the least square method:

$$\beta = \left(\sum y_i - a\sum x_i\right)/n \quad (15)$$

$$a = \left(n\sum x_i y_i - \sum x_i \sum y_i\right)\Big/\left(n\sum x_i^2 - \left(\sum x_i\right)^2\right) \quad (16)$$

$$x_i = \left[FA_{free}\right] \quad (17)$$

$$y_i = F(FA) \quad (18)$$

The data, necessary for calculation of average stability constant of lead dihydroxy fulvate complex, using the average molecular weight of "active associate" are given in Table 2 .

It was obtained that $\beta\left(Pb(OH)_2 FA\right) = 9.63x10^6$ (19);

$$\lg\beta = 6.98 \quad (20)$$

3.2. Investigation of Complex Formation Process by the Gel Chromatographic Method

As it is shown from the results (Table 3), the containing of lead in high weight molecular fraction $(300 \le Mw > 5000)$ increases with the increasing the concentration of FA, which could only be explained by the formation of fulvate complexes. At pH = 5.0, for calculation of molar concentrations of FA, the average molecular weight of the associate of FA was taken into consideration, $Mw(FA) = 2210$.

In diluted water solutions, at $pH = 5.0$, the dominant form of lead is Pb^{2+}.[40] If charges of ions are not taken into account, the reaction of formation of lead fulvate complexes, could be written in the following way:

$$Pb(II)_{free} + mFA_{total} = PbFA_m \quad (21)$$

$$\beta = [PbFA_m] / ([Pb(II)_{free}][FA_{total}]^m) \quad (22)$$

In homogeneous systems, where $[Pb(II)_{free}]$ is not a constant value, the numeral value of stoichiometric coefficient(m) equals to slope of straight line built in coordinates: $\lg([PbFA_m]) / [Pb(II)_{free}])$ and $\lg[FA]$. During the investigation by the gel chromatographic method, $[PbFA_m]$ equals to the concentration of metal in high weight molecular fraction $(300 \le Mw > 5000)$ and

$$[Pb(II)_{free}] = [Pb(II)_{total}] - [PbFA_m] \quad (23)$$

For the purpose of calculation of exact value of tangent ofslope of straight line the least square method was used, according to (8) equation, where

$$x_i = \lg[FA_{total}] \quad (24)$$

and $$y_i = \lg([PbFA_m] / [Pb(II)_{free}]) \quad (25)$$

It was obtained that the numeral value of m $(Mw(FA) = 2210)$ equals to 0.98. So, in homogeneous system $Pb(II)(soluton)—FA—H_2O$ at $pH = 5.0$, the lead fulvate complex with 1:1 ratio dominates.

Therefore, the complex formation reaction may be written in such way:

$$Pb(II) + FA = PbFA \quad (26)$$

For the calculation of average stability constant of lead fulvate at $pH = 5.0$ the Leden's function $F(L)$ was used.[42] Unlike $pH = 8.0$, at $pH = 5.0$ when the complex formation is proceeding more weakly, the calculation of free ligand, using the average molecular weight of the associates of fulvic acids is not a problem. The calculation of characteristics of the Leden's function

$$F(L) = F(FA) = = [PbFA] / ([Pb(II)_{free}][FA_{free}]) = -\beta_1 + \beta_2[FA_{free}] \quad (27)$$

is easy during the investigation of fulvate complexes by the gel chromatographic method. Concentration of $[PbFA]$ quantitatively equals to the quantity of metals (mol/L) determined in high weight molecular fraction $(300 \le Mw > 5000)$.

Table 3. Data for identification of the composition of lead fulvate complex, pH=5.0; $[Pb(II)_{total}]$=14.89·10^{-5} mol/L; $Mw(FA)$=2210; 300 ≤ Mw(PbFA)> 5000

mol/L			$PbFA_m$:$[Pb(II)_{free}]$	lgFA_{total}	Lg($PbFA_m$:$[Pb(II)_{free}]$)
[$FAtotal$]	$PbFA_m$	$[Pb(II)_{free}]$			
3.82·10^{-5}	2.47·10^{-5}	12.42·10^{-5}	0.20	–4.4179	–0.6990
5.73·10^{-5}	3.54·10^{-5}	11.35·10^{-5}	0.31	–4.2418	–0.5086
7.64·10^{-5}	4.67·10^{-5}	10.22·10^{-5}	0.46	–4.1169	–0.3372
9.55·10^{-5}	4.90·10^{-5}	9.99·10^{-5}	0.49	–4.0199	–0.3098
11.46·10^{-5}	5.86·10^{-5}	9.03·10^{-5}	0.65	–3.9408	–0.1871
13.37·10^{-5}	6.11·10^{-5}	8.78·10^{-5}	0.69	–3.8738	–0.1611
13.28·10^{-5}	6.55·10^{-5}	8.34·10^{-5}	0.78	–3.8159	–0.1079

Table 4. Data for calculation of average stability constant of lead fulvate complex by the Leden's method, pH=5.0; $[Pb(II)_{total}]$=14.89·10^{-5} mol/L; $Mw(FA)$=2210; 300 ≤ Mw(PbFA)> 5000

mol/L				$F(FA)$
$[FA_{total}]$	$[PbFA]$	$[Pb(II)_{free}]$	$[FA_{free}]$	
3.82·10^{-5}	2.47·10^{-5}	12.42·10^{-5}	1.35·10^{-5}	1.47·10^{4}
5.73·10^{-5}	3.54·10^{-5}	11.35·10^{-5}	2.19·10^{-5}	1.42·10^{4}
7.64·10^{-5}	4.67·10^{-5}	10.22·10^{-5}	2.97·10^{-5}	1.54·10^{4}
9.55·10^{-5}	4.90·10^{-5}	9.99·10^{-5}	4.65·10^{-5}	1.05·10^{4}
11.46·10^{-5}	5.86·10^{-5}	9.03·10^{-5}	5.60·10^{-5}	1.16·10^{4}
13.37·10^{-5}	6.11·10^{-5}	8.78·10^{-5}	7.26·10^{-5}	0.96·10^{4}
15.28·10^{-5}	6.55·10^{-5}	8.34·10^{-5}	8.73·10^{-5}	0.90·10^{4}

Table 5. Data for calculation of average stability constant of lead fulvate complex by the Leden's method. pH=5.0; $[Pb(II)_{total}]$=14.89·10^{-5} mol/L; $Mw(FA)$=1350; 300 ≤ Mw(PbFA)> 5000

mol/L				$F(FA)$
$[FA_{total}]$	$[PbFA]$	$[Pb(II)_{free}]$	$[FA_{free}]$	
6.25·10^{-5}	2.47·10^{-5}	12.42·10^{-5}	3.78·10^{-5}	5.26·10^{3}
9.37·10^{-5}	3.54·10^{-5}	11.35·10^{-5}	5.83·10^{-5}	5.35·10^{3}
12.50·10^{-5}	4.67·10^{-5}	10.22·10^{-5}	7.83·10^{-5}	5.84·10^{3}
15.62·10^{-5}	4.90·10^{-5}	9.99·10^{-5}	10.72·10^{-5}	4.57·10^{3}
18.75·10^{-5}	5.86·10^{-5}	9.03·10^{-5}	12.89·10^{-5}	5.03·10^{3}
21.87·10^{-5}	6.11·10^{-5}	8.78·10^{-5}	15.76·10^{-5}	4.41·10^{3}
25.00·10^{-5}	6.55·10^{-5}	8.34·10^{-5}	18.45·10^{-5}	4.26·10^{3}

Table 6. Data for calculation of average stability constant of lead fulvate complex by the Leden's method. pH=8.0; $Mw(FA)$=1350; $[Pb(II)_{free}]$=3.58·10^{-6} mol/L

mol/L				$F(FA)$
$[FA_{total}]$	$[Pb(II)_{total}]$	$[Pb(OH)_2FA]$	$[FA_{free}]$	
6.25·10^{-5}	4.08·10^{-5}	3.72·10^{-5}	2.53·10^{-5}	4.13·10^{-5}
9.37·10^{-5}	5.73·10^{-5}	5.37·10^{-5}	4.00·10^{-5}	3.75·10^{-5}
12.50·10^{-5}	7.58·10^{-5}	7.22·10^{-5}	5.28·10^{-5}	3.82·10^{-5}
15.62·10^{-5}	9.86·10^{-5}	9.50·10^{-5}	5.76·10^{-5}	4.60·10^{-5}
18.75·10^{-5}	11.84·10^{-5}	11.48·10^{-5}	6.91·10^{-5}	4.64·10^{-5}
21.87·10^{-5}	13.85·10^{-5}	13.49·10^{-5}	8.38·10^{-5}	4.50·10^{-5}
25.00·10^{-5}	15.13·10^{-5}	14.77·10^{-5}	10.23·10^{-5}	4.03·10^{-5}

$$\left[Pb\left(II\right)_{free}\right]=\left[Pb\left(II\right)_{total}\right]-\left[PbFA\right] \quad (28)$$

$\left[Pb\left(II\right)_{total}\right]$ is the total amount (mol/L) of metal in the sample, which is a constant value according to the condition of the experiment.

The necessary data, for calculation average stability constant of lead fulvate complex are given in Table 4. The value of β was calculated by the least square method according to (15) equation. After the calculations, the value of $\beta$was obtained:

$$\beta\left(PbFA\right)=1.63x10^4 \quad (29)$$

$$\lg\beta=4.21\left(Mw=2210\right) \quad (30)$$

The complexation of fulvic acids to metal ions cannot be described in strict frames because of the ill-defined nature of FA in contrast with the complexation of single ligands. So, to succeed the calculation of average stability constants of fulvate complexes it is necessary to make some assumptions. At high values of pH, the calculation of average stability constants of fulvate complexes of heavy metals according to associates, during the using of average molecular weight, as it is in the case of lead at pH = 8.0 is not always possible. Because it is not possible to calculate concentration of free ligand.[15,20,41] That's why it was necessary to introduce the term- "active associate".[41] But the value of average molecular weight of "active associate" in the case of different metal and pH is different.[15,20,41] For this reason the correct comparing and using in practice the values of stability constants is impossible. Therefore, for the correct comparison of constants we calculated average stability constants at pH = 5.0 and pH = 8.0 using the average molecular weight of oligomer of fulvic acids, which is a constant value and does not depend on the solution pH. The necessary data, for calculation average stability constants of lead fulvate complex according to oligomer Mw = 1350 are given in Tables 5 and 6. In the case of oligomer at pH = 5.0 and pH = 8.0 the values of stability constants of lead fulvate complex and lead dihydroxy fulvate complex were obtained: $\beta(PbFA)$ = 5.88·10^3 (31) lgβ= 3.77 (32) and $\beta(Pb(OH)_2FA)$ = 4.14·10^5 (33) lgβ = 5.62 (34).

4. Conclusions

It was established, that in a heterogeneous system $Pb\left(OH\right)_2\left(solid\right)$—$Pb\left(II\right)\left(solution\right)$—$FA$—$H_2O$ at pH = 8.0 lead dihydroxy fulvate complex $[Pb(OH)_2FA]$ with the 1:1 ratio dominates. Its average stability constants according to "active associate" (Mw = 2128) and oligomer(Mw = 1350) of fulvic acids are equal to β = 9.63·10^6 (lgβ = 6.98) and β = 4.14·10^5, respectively.

In the homogeneous system

$$Pb\left(II\right)\left(soluton\right)—FA—H_2O$$

at $pH = 5.0$ the lead fulvate complex $[PbFA]$ with the ratio 1:1 dominates. In the case of the associate ($Mw = 2210$) and oligomer ($Mw = 1350$), the values of stability constants of lead fulvate complexes correspondently equals to $\beta = 1.63{\cdot}10^4$ ($\lg\beta = 4.21$) and $\beta = 5.88{\cdot}10^3$ ($\lg\beta = 3.77$) Through the obtained results, (especially the results obtained by using the molecular weight of oligomer), it will be possible to calculate the migration forms of lead in natural waters, to determine toxicity and bioavailability and to evaluate the ecological condition of water reservoirs.

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ДОСЛІДЖЕННЯ ПРОЦЕСУ КОМПЛЕКСОУТВОРЕННЯ ПЛЮМБУМУ (II) З ПРИРОДНИМИ МАКРОМОЛЕКУЛЯРНИМИ ОРГАНІЧНИМИ РЕЧОВИНАМИ (ФУЛЬВОКИСЛОТАМИ) МЕТОДАМИ РОЗЧИННОСТІ ТА ГЕЛЬ-ХРОМАТОГРАФІЇ

Анотація. *Фульвокислоти є одними з найважливіших лігандів, що регулюють геохімічний кругообіг металів у навколишньому середовищі. Метою цієї роботи було дослідити процес комплексоутворення між фульвокислотами та Pb(II). Процес комплексоутворення вивчали методами розчинності та гель-хроматографії за pH 5,0 і 8,0.*

Ключові слова: *фульвокислоти, фульват плюмбуму, середня константа стійкості.*