

BINDING OF CADMIUM TO SOIL HUMIC ACID AS A FUNCTION OF CARBOXYL GROUP CONTENT

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The binding of Cd(II) to soil humic acid (HA) at pH 6.5 and in 0.1 mol/L KNO₃ ionic medium, was studied by potentiometric titration with a cadmium ion selective electrode. The influence of carboxyl groups in cation-humic interactions was investigated by selective blocking of humic acid carboxyl groups with thionyl chloride and methanol. Infrared spectroscopic analysis confirmed that esterification took place. Differences between underivatized and derivatized HA complexation properties are ascribed to carboxyl groups. The Scatchard plots and incremental formation constants were used to obtain values for Cd-binding constants, for both HAs. The derivatization decreased the number of HA complexing sites by approximately 60 %, which correlates with acid-base properties of both HAs, studied by barium hydroxide and calcium acetate exchange methods. The stability constants for binding at the strongest sites ($\log K_{INT}$) was larger for underivatized HA (5.40) than for derivatized HA (4.92), indicating greater stability in the case when carboxyl groups are involved in complexation reaction.

Key words: cadmium; humic acid; carboxyl group content; Scatchard plot; ion-selective electrode

ВЛИЈАНИЕ НА СОДРЖИНАТА НА КАРБОКСИЛНИТЕ ГРУПИ ВРЗ ВРЗУВАЊЕТО НА Cd(II) ЗА СОЛИ НА ХУМИНСКАТА КИСЕЛИНА ОД ПОЧВАТА

Врзувањето на Cd(II) за хуминската киселина од почвата при pH 6,5 во 0,1 mol/L јонски медиум на KNO₃ беше изучувано со потенциометриска титрација со примена на јон селективна електрода за кадмиум. За да се испита влијанието на карбоксилните групи во катјон-хумински интеракции, карбоксилните групи беа селективно блокирани по пат на естерификација со тионил-хлорид и метанол. Естерификацијата на карбоксилните групи беше потврдена со примена на инфрацрвена анализа. Разликите помеѓу особините на комплексирање на дериватизираните и недериватизираните хумински киселини се должат на присуството на карбонилни групи. Графичкиот приказ и константите на формирање на комплекси се искористени за да се добијат вредностите на константите на сврзување за Cd со двете хумински киселини.

Клучни зборови: кадмиум; хуминска киселина; содржина на карбоксилни групи; Скачардов графикон; јон-селективна електрода

1. INTRODUCTION

Nowadays, the interest in humic substances (HS) research, is obvious from the role they play in a range of environmental issues, such as soil and water acidification, nutrient control, weathering, soil formation, soil structure, mobility and distribution of heavy metals, radioactive waste disposal, pesticides, xenobiotics, ecosystem buffering, etc. [1]. In almost all of those issues, cation binding is recognised to be an important factor. Even though there are many papers dealing with cation-humic interactions [2–5], there is still some uncertainty about how humic functional group heterogeneity affects proton and metal binding [6].

It is well known that HS are too heterogeneous in nature. They are considered as poly-disperse mixtures of natural organic polyelectrolytes, containing a large number of different functional groups [7]. The ability of humic acids to form stable complexes with polyvalent cations is attributed to their high content of oxygen-containing functional groups including carboxyl, phenol, hydroxyl, enol and carbonyl structures of various types [8]. The most important cation-humic interactions involve both phenol and carboxyl groups, while reactions of less importance involve only carboxyl groups [9, 10]. Bearing in mind the fact that cation-humic interactions depend on the presence of reactive acidic functional groups, such as carboxylic and phenolic groups [11], their characterization and quantification can provide a guide to modeling metal-humate interactions.

There are various analytical methods for determination of HA oxygen-containing functional groups, such as direct and indirect potentiometric titrations [12], radiometric determination [13], infrared spectroscopy [14], carbon-13 nuclear magnetic resonance spectroscopy (^{13}C -NMR) [15], ^{29}Si -NMR [16]. Beside those methods, some authors studied the differentiation between various hydroxyl groups of HA by ^{13}C -NMR spectroscopy using methylated HS [17]. This modification process that resulted in HA

with selectively blocked functional groups, can be used for metal ion-humic binding studies.

Even though, it is known that HS carboxyl groups play a prominent role in the complexing of cadmium ions [18, 19], no reports have appeared on derivatization of those groups in order to estimate their effect on cadmium binding. This can be achieved by selective blocking of HA carboxyl groups which would prevent their contribution to the complexation reaction. Obtained derivatized HA, with blocked carboxyl groups, could be used in comparison to underivatized HA, for investigation of the influence of carboxyl functional groups on the HA complexation properties.

In this context, selective blocking of carboxyl groups could be achieved by their esterification. Among many derivatization methods described in the literature [20], probably one of the most attractive and efficient is the methanol/thionyl chloride procedure [20–23], which was modified and is thus described later in detail. The applied methanol/thionyl chloride procedure is efficient route for synthesis of methyl esters of substituted aromatic carboxylic acids without affection of phenolic hydroxy groups [23]. Due to its high selectivity, the method was suitable for functionally very heterogeneous humic macromolecule.

Ion-selective electrode (ISE) for cadmium was used to measure Cd complexation. The ISE experimental technique has been shown to be a reliable for measuring Cd concentrations in the range 10^{-7} to 10^{-2} mol/L Cd in a complex organic matrix [24–27]. A number of modeling approaches have been developed to describe binding of metals by humic material [28, 29]. One of them which is basically graphical technique, the Scatchard approach [30, 31] is used in this study. Discrete site ligand model of humate-metal binding used in the study (the “Scatchard model”) merely provide a convenient mean to mimic the observed metal binding capabilities of humic material. Even though, the ligands in the discrete ligand model may not represent actual binding sites, this model can successfully

predict metal ion binding by humic material in the absence of competing ions (protons excluded) and within the range of the conditions of the calibrating titrations. The model should not be used to extrapolate outside the range of calibrating titrations because the postulated ligands are justified only by their ability to provide an empirical fit of available data.

Heterogeneity of humic substances and possibility that cation binding to humic substances may also occur as a result of counter-ion accumulation to the negative charge of the humic, suggests that a large number of site types are involved in metal binding and that these site types are best characterized by a continuous distribution models (Gaussian normal distribution model [32], The Stockholm Humic Model [33], NICA-Donnan model [34]).

The choice of whether discrete or continuous model to employ in the description of metal-humate binding depends on the objective of the data analysis. The discrete ligand approach is easily adapted to experimental data using graphical methods and fitting parameters that are varied until the experimental data are appropriately described. In cases when one deals with unknown nature of humic substances, an "average" approach may be employed if it provides an adequate representation of the results. Considering the fact that the Scatchard approach shows some limitations, the Incremental Stability Constants model [35] was applied, as well. In this model, instead of calculating an average value of stability constant from a slope of the Scatchard plot, incremental stability constants from successive slope values were obtained for each point in the Scatchard plot. The intrinsic constant, K_{INT} , was obtained by extrapolation. This constant represents the metal binding at the lowest Cd concentration, thus describing the strongest binding site [3]. This approach is more promising than the Scatchard approach, since it expresses the HS heterogeneity.

The objectives of the study presented in this paper are: evaluation of differences in carboxyl group content between underivatized

and derivatized HA, quantification of the complexation of Cd and estimation of the strength of Cd binding due to the number of available carboxylic complexation sites.

2. EXPERIMENTAL

2.1. Extraction and purification of humic acid

HA was obtained following the procedure suggested by the International Humic Substances Society (IHSS) [36] from a well-humified organic horizon of old beech-forest soil (10 cm depth), in autumn 2003, using standard grinding equipment. The soil was air-dried and sieved to pass a 2.0 mm sieve. Briefly, HA was extracted from the soil with 0.1 mol/L NaOH at 1:10 solid to solution (mass/volume) ratio under N_2 for 4 hours. The suspension was then centrifuged at 4500 rpm for 30 min, the supernatant was acidified with 6 mol/L HCl to pH 1.0 and suspension was allowed to stand overnight. Precipitated HA was separated from fulvic acid by centrifugation at 4500 rpm for 30 min. Obtained HA precipitate was redissolved in 0.1 mol/L KOH, under N_2 . Solid KCl was added to attain 0.3 mol/L (K^+) and then suspension was centrifuged at high speed to remove suspended solids. The HA was reprecipitated with 6 mol/L HCl to pH 1.0. The HA precipitate was suspended in a solution of 0.1 mol/L HCl/0.3 mol/L HF. In order to minimize the ash content, this procedure was repeated three times. The acid was then dialyzed against distilled water until Cl^- was eliminated. Bound metal ions were removed by putting the HA solution (at pH 8 adjusted with 0.1 mol/L NaOH) into a 300×10 mm column with ion-exchange resin Dowex 50W-X8 (H^+ -form) at a flow rate of 0.5 mL min^{-1} . The protonated HA was diluted in a volumetric flask and stored at 4°C. The concentration of the stock was determined as 1 g/L, by dry weight of measured volume of well homogenized HA suspension and stored in the dark, at 4 °C.

2.2. Complexometric titrations

Complexometric titrations were performed with two Hach sension 3 pH/ion-meters; one of them was used for pH measurements and the other was used with an ion selective electrode (ISE-Metrohm, 6.0502.110) for Cd(II) against an double junction Ag/AgCl reference electrode (Metrohm, 6.0726.100) filled with 3 mol/L KCl in the inner and the outer compartment. For pH monitoring a Hach gel-filled combination glass electrode (51935-00) was used. ISE electrode was conditioned before use by polishing, followed by immersion in 0.1 mol/L Cd(NO₃)₂ solution, overnight, while reference electrode was conditioned by soaking in 0.1 mol/L solution of the ionic strength adjuster.

Both electrodes were calibrated before use. Calibration of the Cd electrode was performed with Cd(NO₃)₂ at a concentration of 10⁻⁷ to 10⁻³ mol/L Cd and the measured electrode voltage was linear to Cd concentrations with a Nernstian slope (29 mV). The calibration was performed immediately before humic acid titration. Each experiment was run three times to ensure the reproducibility.

Throughout the titrations a N₂ gas was initially bubbled through the solution and then a constant atmosphere was maintained above the solution which was thermostated in a water bath at 25.0 ± 0.1 °C. The pH of the solution was adjusted to 6.50 ± 0.05, by addition of minute amounts of diluted KOH or HNO₃ after each addition of Cd²⁺ solution, as needed. Titrations were performed with 40.00 mL of humic acid suspension with concentration 980 mg/L, titrated with 5.19 · 10⁻⁴ mol/L Cd²⁺ in ionic medium of 0.1 mol/L KNO₃. The potential was read only when its variation was smaller than 0.555 mV/min, which was monitored by the drift control of the ion-meter.

The same set of experimental conditions was applied for complexometric titration of esterified humic acid.

2.3. Esterification of HA

Selective blocking of carboxyl functional groups was achieved by esterification, by isolation of esterified HA derivative (EHA). Esterification was performed by the methanol/thionyl chloride procedure suggested by Schnitzer and Skinner [21] and Hosangadi and Dave [23]. The synthesis of derivatized humic acid is shown in the reaction scheme (Figure 1). To a stirred solution of 500 mg of HA in 20 mL of methanol, under ice-cooling (approximately -5 °C), thionyl chloride (5 mL) was added dropwise from a dropping funnel, over 2 hours. The reaction mixture was then heated on the water bath for 5 minutes to decompose excess thionyl chloride. The suspension obtained was centrifuged at 2000 rpm and the separated ester was washed with distilled water until free of chlorides. The ester was dried in a rotary evaporator and finally in a vacuum desiccator over P₂O₅. The esterification procedure was repeated and the obtained twice esterified product was marked as EHA. The obtained ester in the second esterification procedure was diluted with distilled water and the concentration of the stock EHA suspension was determined to be 1 g/L.

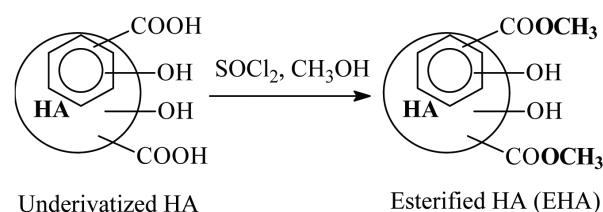


Fig. 1. Reaction scheme for the synthesis of derivatized humic acid

2.4. Spectrophotometric characterization of derivatized and underivatized HA

IR-spectra were recorded on a Bomem Hartman & Braun MB-Series FT-IR spectrometer from 4000 to 1000 cm⁻¹, using KBr pellet (1 mg of each sample + 200 mg of dry KBr).

2.5. Determination of HA functional groups

Total acidity [$\text{Ba}(\text{OH})_2$ method] and carboxylic acidity [$\text{Ca}(\text{OAc})_2$ method] were determined according to Schnitzer and Gupta [37]. Phenolic acidity was determined by difference between total and carboxylic acidity.

2.6. Potentiometric data evaluation

Interactions between metal ions and macromolecules are well described by the discrete site model proposed by Scatchard [30]. This model assumes that macromolecule poses a small number of different complexing sites with no interaction among them. The charge at the macromolecule should be constant and stoichiometry of the complex is 1:1. Organic ligand–humic macromolecule (HA) is considered as the central group which reacts with metal (Cd^{2+}) giving complexes CdA , Cd_2A , ..., Cd_nA . Successive formation constants can be described as:

$$K_1 = \frac{c(\text{CdA})}{c(\text{HA}) \cdot c(\text{Cd}^{2+})}; K_2 = \frac{c(\text{Cd}_2\text{A})}{c(\text{CdA}) \cdot c(\text{Cd}^{2+})} \quad (1)$$

and overall:

$$K_n = \frac{c(\text{Cd}_n\text{A})}{c(\text{Cd}_{n-1}\text{A}) \cdot c(\text{Cd}^{2+})} \quad (2)$$

For binding at identical and independent sites, the Scatchard plot, $c(\text{CdA})/c(\text{Cd}^{2+})$ vs. $c(\text{CdA})$ is obtained by plotting the equation:

$$\frac{c(\text{CdA})}{c(\text{Cd}^{2+})} = C_c \cdot K - c(\text{CdA}) \cdot K \quad (3)$$

where $c(\text{CdA})$ is the concentration of the complexed metal in mol/L, obtained from the mass balance: $c(\text{CdA}) = c(\text{Cd}^{2+})_T - c(\text{Cd}^{2+})$, $c(\text{Cd}^{2+})$ is the concentration of free metal in mol/L and $c(\text{Cd}^{2+})_T$ is the concentration of total Cd equivalent to the amount of Cd added to the vessel in mol/L, C_c is complexing capacity (total concentration of binding sites) and K is

the average equilibrium constant. This equation yields average stability constant K as the slope and C_c as y-axis intercept divided by the slope.

The Scatchard plot of HA is curvilinear, due to heterogeneity of its binding sites [38–40]. Incremental stability constants, $\log K_i$, were obtained as successive slopes calculated from neighboring data points in the Scatchard plot. Those constants are related to data points $[\text{CdA}]_{\text{SR}}$, which are equidistant between the original data points of the Scatchard plot. A plot of $\text{p}K_i$ ($\log K_i$) vs. $[\text{CdA}]_{\text{SR}}$ shows the range of strengths of binding sites. Intrinsic constants, K_{INT} , were obtained by third-degree polynomial fitting of data from $\text{p}K_i$ vs. $[\text{CdA}]_{\text{SR}}$ plot and by extrapolation toward $[\text{CdA}]_{\text{SR}} = 0$ using Microcal Origin® software.

3. RESULTS AND DISCUSSION

Evidence that esterification of the carboxyl groups had occurred was gained by differences in the IR absorption before and after derivatization. There are two important IR regions related to this study: (1) 3200–3600 cm^{-1} region, characteristic for absorption due to O–H stretching vibrations in carboxyl, phenol and hydroxyl groups and (2) 1100–1450 cm^{-1} region, characteristic for absorption due to C–O stretching vibrations. The spectrum of HA showed a broad and strong peak at about 3430 cm^{-1} (Figure 2). In the spectra of esterified HA, there was reduction in O–H stretching vibrations, since less hydroxyl groups were present. Also, the C–O absorption (region 1100 cm^{-1}) increased in esterified humic acid due to the formation of methyl ester (Figure 2). Higher OCH_3 content of esterified derivative was indicated by strong bands for C–H stretching at 2920 and 2850 cm^{-1} . Esterification also increased the intensity of the 1722 cm^{-1} band and reduced absorption at 1622 cm^{-1} . Intensification of the 1722 cm^{-1} band confirmed the conversion of carboxyl group to ester because carbonyl groups of esters absorb within a narrower range than carboxylic acids, thereby giving a more pronounced maximum [41]. The 1600 cm^{-1} band in HA is at least in

part due to the antisymmetrical stretching mode of carboxylate groups. Following esterification absorption of these groups, which are converted to esters, shifts from 1600 to 1700 cm^{-1} [21]. The assumption was made that during esterification procedure only carboxyl groups and not phenolic groups were modified. This is based on the literature findings [23] that the

esterification method is highly selective, thus do not affect phenolic hydroxy groups, and that infrared spectroscopic analysis confirmed that esterification of the humic acid did occur. Thus, the functional group content of both HAs, as well as, their complexation properties were compared.

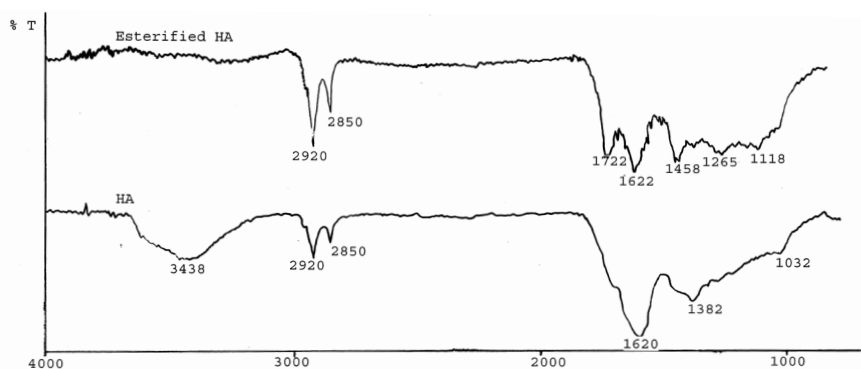


Fig. 2. FT-IR spectra of the humic acid (down) and esterified humic acid (up) in KBr pellet humic acid

The C_c values for each humic acid, obtained by the Scatchard plot are shown in Table 1. The capacity of compost and sediment HA for Cd binding, as reported by Kaschl *et al.* [39] and Abate and Masini [27] were in the same range as obtained results for the soil HA.

The Scatchard plots for underivatized and derivatized HAs (Figures 3 and 4) were curvilinear, as it was expected due to a range of binding sites with different binding energies and variety of electrostatic and conformational changes on the humic macromolecule [3, 27, 35, 42, 43].

Obtained average values for conditional stability constant ($\log K$) from the Scatchard plots are shown in Table 1. Stability constants values were compared with the literature data [25, 27, 39] and obtained values were found to be in the range of published results. Underivatized acid formed more stable complex, due to availability of carboxyl groups in complexation and chelation reactions.

The total concentration of binding sites was 0.980 mmol/g for HA and 0.425 mmol/g for EHA, which indicates that 59.8 % of humic acid

metal binding sites can be attributed to carboxyl groups. This result obtained by the Scatchard plot is confirmed by the barium hydroxide and calcium acetate methods for total and carboxylic acidity determination. According to the obtained values of total acidity for HA and EHA (Table 1), 58.1 % of humic acid proton binding sites can also be attributed to the carboxyl groups. Considering that very similar percentage (59.8 and 58.1 %) of COOH groups is involved in cadmium and in proton binding, our findings suggest that there is correlation between complexing capacities and carboxyl acidity in humic acid. Due to this correlation one can predict Cd-complexation capacity of HA on the base of COOH group content. The estimated value of COOH groups content (58.1 %) is in accordance with the value obtained by COOH content determination with calcium acetate method applied on underivatized HA, that is 2.80 mmol/g or 60 % of total acidity. Obtained values are consistent with view that roughly 50 % of humic acid acidity is due to carboxyl moieties [10].

In derivatized HA only 10 % of ionizable groups correspond to carboxyls. This result confirms high selectivity of the methanol/thionyl chloride derivatization procedure (93 % of carboxyl groups in HA were blocked by the method).

The values of the total concentration of binding sites and conditional stability constants were directly related to availability of carboxylic groups for interaction with ions, indicating their importance in complexation.

Incremental stability constants (pK_i) for Cd binding by underivatized and derivatized HA are shown in Figures 5 and 6. Third-degree polynomial fit was satisfactory, thus the strongest binding sites for Cd (pK_{INT}) at low ratios of Cd to ligand were obtained (Table 1). The stronger binding for Cd was found on underivatized humic acid ($pK_{INT} = 5.40$) than on esterified humic acid ($pK_{INT} = 4.92$), indicating greater stability in the case when carboxyl groups are involved in complexation reaction. Graphs relating $\log K_i$ against $[Cd]_{SR}$ (Figures 5 and 6) showed decrease of incremental stability constants, due to accumulation of charge on macromolecule and thus decreasing tendency of functional groups to complex further metal ions [38].

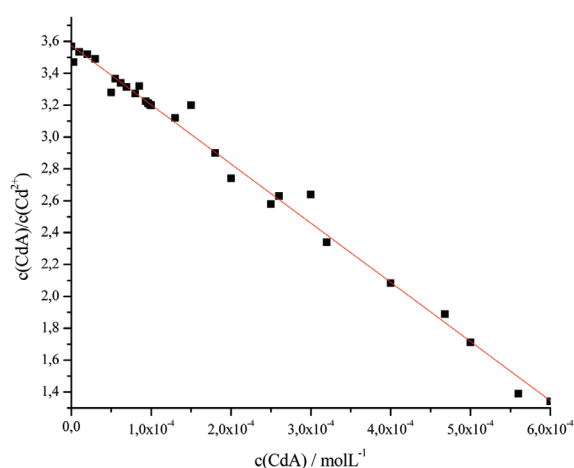


Fig. 3. Scatchard plot obtained for underivatized humic acid (40.00 mL of 980 mg/L HA suspension, $pH = 6.50 \pm 0.05$, 0.1 mol/L KNO_3 titrated with $5.19 \cdot 10^{-4}$ mol/L Cd^{2+})

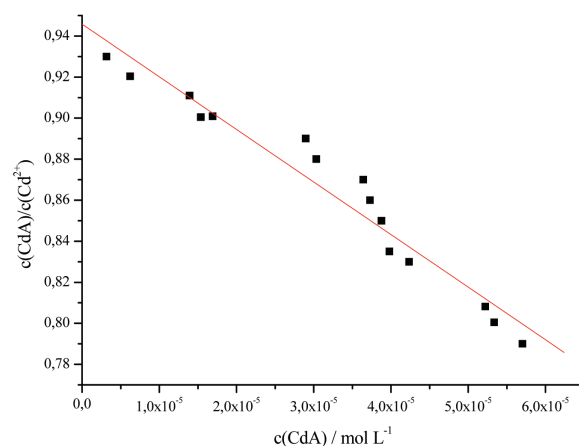


Fig. 4. Scatchard plot obtained for esterified humic acid (40.00 mL of 980 mg/LEHA suspension, $pH = 6.50 \pm 0.05$, 0.1 mol/L KNO_3 titrated with $5.19 \cdot 10^{-4}$ mol/L Cd^{2+})

Table 1

Conditional stability constants (K) and complexing capacities (C_c) for the underivatized and derivatized humic acids at 25 ± 0.1 °C, $pH = 6.50 \pm 0.05$ and ionic medium of 0.1 mol/L KNO_3 obtained according to Scatchard plots (obtained results from triplicate experiments)

	HA	Derivatized HA
log K	3.57 ± 0.1	3.35 ± 0.1
C_c (mmol/g)	0.980 ± 0.1	0.425 ± 0.05
Phenolic OH (mmol/g)	1.88	1.76
COOH (mmol/g)	2.80	0.2
Total acidity (mmol/g)	4.68	1.96
pK_{INT} (R^2)	5.40 (0.70)	4.92 (0.72)

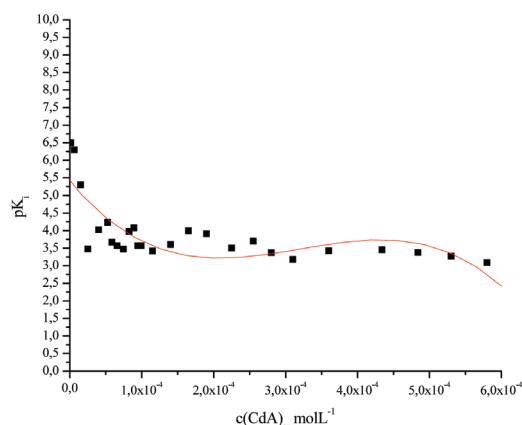


Fig. 5. Plot relating $\log K_i$ of Cd(II)-humic acid

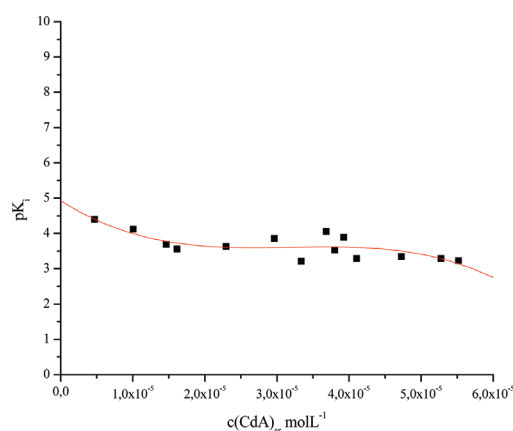


Fig. 6. Plot relating $\log K_i$ of Cd(II)-esterified humic acid

4. CONCLUSION

This study revealed that chemically modified HA by methanol/thionyl chloride esterification procedure can be used in comparison to unmodified HA for investigation of the influence of carboxyl functional groups in HA on the humic proton/metal interactions. IR spectroscopic analysis confirmed that carboxyl groups had been esterified. Selectivity of the derivatization method was confirmed by carboxyl group content determination by calcium acetate method since it was indicated that 93 % of carboxyl groups in humic acid were blocked by the method.

Cadmium used in this study, showed a tendency to preferably associate with unmodified HA, due to higher content of carboxyl groups available for cadmium interaction. The

values of the total concentration of binding sites and conditional stability constants were directly related to availability of carboxylic groups for interaction with ions, indicating their importance in complexation. Unmodified HA showed higher capacity for binding and existence of ionizable groups for stronger Cd-binding. Thus, approximately 60 % of humic acid metal and proton binding sites can be attributed to carboxyl groups. There is direct correlation between the amount of carboxyl groups and Cd-complexing capacity, that can be used in HA complexometric properties prediction.

The binding constant was larger for un-derivatized HA (5.40) than for derivatized HA (4.92), indicating greater stability in the case when carboxyl groups are involved in complexation reaction.

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