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# Self-aggregation of soil humic acids with respect to their structural characteristics

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*Abstract*: The main goal of this work was to estimate the influence of carboxyl and phenolic groups, as well as aromatic, aliphatic and polysaccharide components, on the soil humic acids (HA) self-aggregation process. Soil HAs (leptosol and regosol) were separated using base resin getting fractions with different functional group contents. Blocking of carboxyl groups was performed using the esterification procedure to estimate the participation of each functional group in the HA aggregation. The presence of HA structural components was evaluated by potentiometric titration and ATR-FTIR. The aggregation was monitored at pH 3 using dynamic light scattering. Results indicated that the higher group content, the HA aggregation is less pronounced. A significant positive correlation of aliphatic C and aggregate size revealed their dominant influence in the HA self-aggregation. A lower abundance of aliphatic C in HA fractions could be considered as not sufficient to start the process. An increase of aromatic C in esters likely pointed out to its participation in hydrophobic bonding and, consequently, more pronounced aggregation. The relation of HA self-aggregate size with carboxyl and phenolic group, as well as aliphatic C, at low pH, could be considered universal regardless of the structural characteristics of the original or modified HA forms.

*Keywords*: fractionation; esterification; carboxyl group; phenolic group; aliphatic C; aromatic C.

## INTRODUCTION

Humic substances (HS), having significant environmental functions, are the most important organic components present in water, soil, and sediments.[1] HS affect the soil and water properties through their participation in the dynamic processes where their constituent molecules interact with other molecules or ions

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(complexation/decomplexation), solid surfaces (adsorption/desorption) and among themselves (aggregation/deaggregation).[2]

The concept of HS molecular organization is based on the main principles of supramolecular chemistry, namely HS represents ensembles of relatively small organic molecules.[3] Humic acids (HA), as one of the HS fractions, behave as molecular aggregates or supramolecular structures, formed from small individual moieties.[4] The HA aggregation process depends on various environmental conditions such as suspension pH, ionic strength, HA concentration, residence time, type and concentration of organic and inorganic ions and presence of solid particles, *etc*., as well as on the HA structural properties (size, shape, conformation, functional groups).[4–6]

Humic acids have a large number of reactive functional groups influencing their environmental behavior. That is why the HA functional groups recently became the subject of great research interest. Most of the studies are related to their interaction with various organic and inorganic compounds in soil and water, predominantly pollutants. Some articles are dealing with the role of reactive functional groups in the HA aggregation process in the presence of ionic or molecular species.[7–9] Mecozzi and Pietrantonio[10] which suggested that, besides functional groups, other HA structural components such as carbohydrates, proteins and lipids also participate in the HA self-aggregation process. Chilom *et al.*[11] and Hoffman *et al.*[12] emphasized that lipid HA components play a facilitative role in the forming of HA aggregates. Hakima and Kobayash[13] concluded that a higher aromatic content induces more pronounced HA self-aggregation.

As the complex nature of HA makes it difficult to get precise information on their chemical structure and properties, their heterogeneity can be reduced by separating them into various fractions.[14] Different types of HA fractionations were performed in order to obtain fractions with various structural properties. Size-exclusion chromatography and electrophoresis, as well as their combination, are widely used for HA fractionation.[15] In addition to the above, other fractionation methods were used such as such as reversed-phase high-performance liquid chromatography, aqueous alkaline and organic solvent extractions, subsequent dissolution in buffers adjusted to different pH and sequential dissolution in buffers with increasing pH values, separation by secondary amine weak base resin *etc*.[11,14,16,17]

To the best of our knowledge, the relation between HA self-aggregation and their functional group content, although very important, was not comprehensively studied. Additionally, despite the participation of other structural components in HA self-aggregation has been previously studied, no unambiguous conclusions were drawn. Hence, the main goal of this work is to estimate the influence of carboxyl and phenolic groups as well as aromatic, aliphatic and polysaccharide components on the soil HA self-aggregation process. In order to achi-

eve this, two soil humic acids of different origin were separated using base resin thus getting fractions which have different functional group contents. To estimate the participation of each particular functional group in HA aggregation, blocking of carboxyl groups was performed using the esterification procedure. The presence of structural components in the original and modified HAs was evaluated by potentiometric titration and/or ATR-FTIR spectroscopy. The aggregation process was monitored using dynamic light scattering (DLS) measurements.

## EXPERIMENTAL

### *Humic acid extraction*

Rendzic Calcaric Leptosol humic acid (RCLHA) was isolated from soil classified as Rendzic Calcaric Leptosol (Loamic),[18] originating from Negotin, Serbia. The soil has developed on indurated limestone, at 199 m above sea level (MASL), area under forest. The soil sample taken from the A horizon (0–25 cm depth) had the following characteristics: light clay texture, 4.2 % $CaCO_3$, pH 7.71, 6.25 % total organic C. Leptic Calcaric Regosol humic acid (LCRHA) was isolated from soil classified as Leptic Calcaric Regosol (Loamic, Aric),[18] originating from Stari Slankamen, Serbia, developed on sandy marl, at 187 MASL, area under grassland. The soil sample taken from the A horizon (0–20 cm depth) had the characteristics as follows: sandy loam texture, 14.78 % $CaCO_3$, pH 7.69, 4.31 % total organic C. Soil texture, pH (soil/water = 1/2.5), carbonate and total organic C content were determined by common methods.[19]

Humic acid (HA) samples were isolated using a modified International Humic Substance Society (IHSS) method (HA gel was dried at 35 °C, powdered, and sieved using a 0.05 mm sieve).[20]

HA elemental composition was determined to be as follows: C 51.82 %, H 4.80 %, O 39.98 %, N 3.40 %, for RCLHA and C 52.04 %, H 5.18 %, O 37.96 %, N 4.82 % for LCRHA. Elemental composition was determined by using the elemental analyzer (CHNS 628, LECO Corporation, St. Joseph, MI, USA) after drying the samples over $P_2O_5$ under vacuum. Their percentage was calculated on an ash-free basis.

The IHSS standard humic acid (ESHA) was isolated from Elliott Soil.[20] Elliott Soil is silt loam, silty clay loam or loam, moderately acid to neutral. In this study, ESHA was used only in its unmodified form.

### *Humic acid fractionation*

HA fractionation was performed according to Lin *et al.*[16] The secondary amine weak base resin (Amberlyst® A21 free base, Aldrich Chemistry, Germany) was soaked with 10 % NaCl (Kemika, Croatia) solution, left for two hours and used as a column (1.95 cm diameter, 27 cm height) package (approx. resin volume 80 $cm^3$). The resin was washed using deionized water.

HA water suspension (0.1 %) was initially adjusted to pH 7, stirred overnight at 25±2 °C and pumped through a column at a rate of 4 mL $min^{-1}$. The effluent collected is termed as fraction 1 (F1). After rinsing with deionized water, the resin was eluted with 1 M NaOH solution until the discoloration and fraction 2 (F2) were obtained. The resin was washed again with deionized water and as HA was not completely eluted, 10 % NaCl solution was pumped through the column eluting fraction 3 (F3). To precipitate all the HA fractions obtained, pH was adjusted to <1, centrifuged (4000 rpm) washing it with 0.1 M HCl until $Na^+$ concentration in filtrates was less than 0.1 ppm (determined by atomic atomic absorption spectrometry

(AAS), AAnalyst 700, Perkin Elmer, USA). Precipitates were dried at 38 °C and used for further analyses.

*Humic acid esterification*

To block HA functional groups, the esterification procedure proposed by Andjelkovic *et al.*[21] was modified as follows: 5 ml of thionyl chloride (Merck, Germany) was added dropwise using a dropping funnel to a stirred HA solution (300 mg of HA in 12 ml of methanol (Merck) at approximately 5 °C (ice-cooling) and the mixture was stirred for 3 hours at the same temperature. Afterwards, the reaction mixture was left overnight at 25±2 °C to decompose excess thionyl chloride. The HA suspension was centrifuged at 4000 rpm for 10 min and the precipitated ester was washed using distilled water and 0.1 M HCl until the test for sulphates was negative. The esterification procedure was repeated twice, and the final esterified products (HA E) were dried at 38 °C overnight.

*Elemental analysis (C, H, N and S)*

The C content of HA samples was determined by the elemental analyzer (CHNS 628, LECO Corporation) after drying the samples over $P_2O_5$ under vacuum, carboxyl and phenolic group contents were calculated.

*Attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR)*

ATR-FTIR spectra of HAs in the 4000–400 $cm^{-1}$ range were recorded by an Alpha spectrometer (Bruker, Germany, 4 $cm^{-1}$ resolution, 64 scans). The air spectrum was used as background. Peak intensities were determined relative to the baseline dependent on the spectral region. Baselines in the 3700–1800 and 3000–2800 $cm^{-1}$ range were used for 3283 and 2920 $cm^{-1}$ bands, respectively. Intensities of 1705, 1620, 1520, 1080 and 1030 $cm^{-1}$ bands were determined using the baseline between 1830 and 400 $cm^{-1}$. Relative peak intensities of 3283, 2920, 1705, 1620, 1080 and 1030 $cm^{-1}$ bands were calculated by dividing peak intensity values by that for the 1520 $cm^{-1}$ band.[22] Each peak height was calculated as an average of two replicates.

*Acid–base titrations*

The modified procedure of Ritchie and Perdue[23] was used for acid–base titrations of HA functional groups. HA suspensions (0.36 g $L^{-1}$) were prepared in 10 mL of 0.1 M NaCl, after which 0.2 mL of 0.1 M NaOH were added and left overnight to be completely dissolved. To neutralize the added NaOH, 0.2 mL of 0.1 M HCl was added, and titrations performed using the automatic titrator (Radiometer TTT85, Denmark). The previously calibrated (pH 4, 7 and 10 standard buffers) combined pH electrode (Radiometer PHC2601-8, France) was used to monitor pH. Temperature was maintained at 25.00±0.02 °C and the sample continually stirred under a nitrogen atmosphere. The initial pH suspension was recorded. The NaOH titrant was added in 7 μL increments (titration rate 12.5 μL $min^{-1}$) and the next titration step was not initiated until a pH value stable for 7 s (with drift of no more than 0.001 pH unit) was obtained. Each sample was titrated from its initial pH (3.0 to 3.3) to maximum 10.5-10.7 pH within 25 to 35 min. Three replicate titrations were performed for each HA sample, fourth titration done only if unusual pH behavior or equipment malfunction were noticed.

*Dynamic light scattering*

To perform dynamic light scattering (DLS) measurements, the HA suspensions (0.02 g $dm^{-3}$) were prepared in 0.1 M NaCl to maintain the ionic strength constant. Their pH value was adjusted to pH 10 using 0.1 and 1.0 M NaOH solutions and suspensions were equilibrated for 72 h at 25±2 °C and their pH values checked prior to the measurement. Due to pronounced

HA aggregation at low pH values, alkaline suspensions were acidified to pH 3 by HCl (0.1 and 1.0 M) for further size measurements.

Size measurements were performed using a Zeta-sizer Nano ZS with 633 nm He–Ne laser (Malvern Panalytical, Malvern, UK), and data analyzed by the Zetasizer Software version 6.20 (Malvern Panalytical, Malvern, UK). Measurement details are presented in Jovanović *et al.*[4] Absorbances of alkaline and acid suspensions at 633 nm, needed for size measurements, were recorded by UV–Vis spectroscopy (Evolution 60s, Thermo Fisher Scientific, Waltham, MA, USA).

The aggregate size for both HAs, esters and fractions was correlated to carboxyl and phenolic group content (obtained by the titration method) using Origin 8.5.1 software.

## RESULTS AND DISCUSSION

### *HA carboxyl and phenolic functional groups content*

Carboxyl and phenolic group content, obtained by titration method, as well as the most intensive peak in the PSD (d), are summarized in Table I. With a goal to separate carboxyl and phenolic functional groups, HAs studied were fractionated using the secondary amine weak base resin. According to Lin *et al.*,[16] HA solution, after passing through the resin column, should contain a higher content of carboxyl group (F1), while the phenolic group should be retained at the resin and eluted with 1 M NaOH solution (F2). But, as in this study, HA was not completely eluted (the resin remained dark colored), 10 % NaCl solution was pumped through the column obtaining fraction F3. It is evident from functional group contents obtained by the titration method that the separation procedure used did not completely fractionate humic acids into phenolic and carboxyl groups, as already concluded by Lin *et al.*[16]

TABLE I. Functional groups content and the most intensive peak in particle size distribution (d) of Rendzic Calcaric Leptosol (RCLHA), Leptic Calcaric Regosol (LCRHA) humic acids, their fractions (F1-F3) and esterified forms (E), and IHSS standard Elliott Soil humic acid (EHA)

| HA sample | Functional group content, mqe $gC^{-1}$ | | $d$ / nm | log ($d$ / nm) |
|---|---|---|---|---|
| | Carboxyl | Phenolic | | |
| RCLHA | 10.8±0.2 | 2.8±0.7 | 515.0 | 2.712 |
| RCLHA E | 9.7±0.3 | 2.1±0.2 | 3265 | 3.514 |
| RCLHA F1 | 10.4±0.4 | 2.71±0.04 | 873.7 | 2.941 |
| RCLHA F2 | 14.8±4.3 | 4.8±1.2 | 31.71 | 1.501 |
| RCLHA F3 | 15.5±0.7 | 4.6±0.7 | 9.290 | 0.968 |
| LCRHA | 8.2±1.2 | 3.0±1.1 | 32.66 | 1.514 |
| LCRHA E | 4.0±0.5 | 4.0±1.3 | 506.3 | 2.704 |
| LCRHA F1 | 8.3±1.0 | 3.3±1.1 | 997.4 | 2.999 |
| LCRHA F2 | 13.9[a] | 5.4[a] | 39.44 | 1.596 |
| LCRHA F3 | 11.9±0.2 | 3.02±0.06 | 9.483 | 0.977 |
| EHA | 7.5±0.5 | 2.7±0.1 | 5180 | 3.714 |

[a]One measurement due to small F2 quantity

By comparing carboxyl and phenolic groups content in fractions and unfractionated RCLHA, a difference was noticed within measurement uncertainty for F1, while increase was obvious for F2 and F3. Both functional group contents for LCRHA and F1, within measurement uncertainty, remained unchanged. LCRHA F2 and F3 revealed carboxyl group content increase in comparison to unfractionated HA and F1, while the phenolic group content was higher in F2 only. The differences noticed between unfractionated RCLHA and LCRHA and their fractions, as well as among fractions themselves, were even less significantly pronounced in comparison to Lin *et al.*[16]

RCLHA E and LCRHA E were obtained using the esterification procedure. According to Andjelkovic *et al.*[21] the esterification resulted in carboxyl group blocking. It is obvious that carboxyl group content was slightly lower in RCLHA E, but remarkably lower in LCRHA E in comparison with non-esterified forms.

*HA ATR-FTIR spectra*

ATR-FTIR spectra are illustrated in Fig. 1, while some relative band intensities (*I*) and aromaticity index values obtained by ATR-FTIR are depicted in Table II. ATR-FTIR spectra of RCLHA, LCRHA, their fractions and esters as well as EHA, with absorption bands typical of humic acids,[24] had most of the peaks in the same position but with different intensities. Spectra of RCLHA, LCRHA and EHA were quite alike suggesting their similar structure.

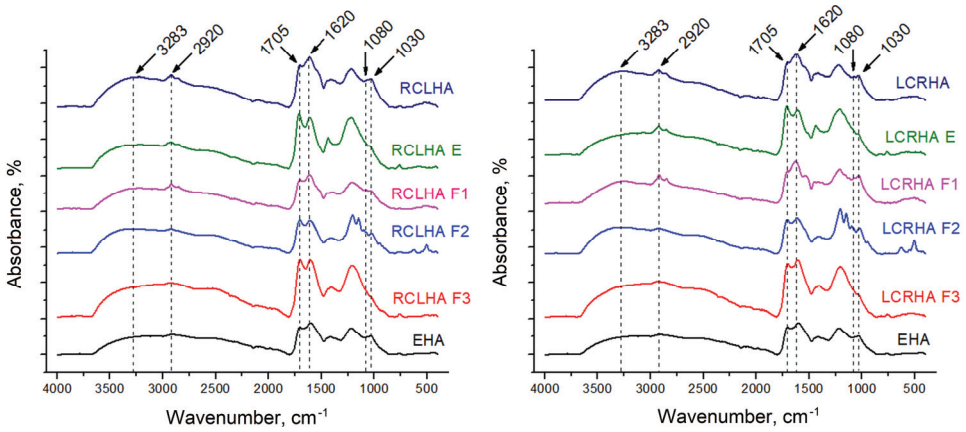


Fig. 1. ATR-FTIR spectra of Rendzic Calcaric Leptosol (RCLHA) and Leptic Calcaric Regosol (LCRHA) humic acids, their fractions (F1–F3) and esterified forms (E), and IHSS standard Elliott Soil humic acid (EHA).

The applied fractionation and esterification procedures obviously led to structure changes and resulted in different band intensities of fraction and ester spectra. A mutual comparison of the recorded spectra was done using relative band intensities. Both F1 fractions did not undergo any changes in the relative

abundances of either phenolic ($I_{3273}$) or carboxyl C ($I_{1705}$). In F2 fractions there was an obvious increase of both functional group relative abundances, while in both F3 fractions the carboxyl C relative abundance was higher. Both esters underwent an evident increase in $I_{1705}$ relative intensity due to the stretching vibration of ester groups formed. For both HAs the aromatic C ($I_{1620}$) relative abundance increased in fractions and especially in esters. Regarding the aliphatic C ($I_{2923}$), it was noticed that relative abundance values are higher in esters and F1, while a decrease was obvious in F2, and particularly in the F3 fraction. Regarding polysaccharide C ($I_{1080}$ and $I_{1030}$), the relative abundance increased in order F2 > E > F1 and decreased in F3 fraction.

TABLE II. Some relative band intensities ($I$) (to intensity of C=C aromatic band at 1520 $cm^{-1}$) and aromaticity index values of Rendzic Calcaric Leptosol (RCLHA), Leptic Calcaric Regosol (LCRHA) humic acids, their fractions (F1–F3) and esterified forms (E), and IHSS standard Elliott Soil humic acid (EHA), obtained by ATR-FTIR (measurement uncertainty < 5 %)

| HA sample | $I_{3273}$ | $I_{2923}$ | $I_{1705}$ | $I_{1620}$ | $I_{1080}$ | $I_{1030}$ |
|---|---|---|---|---|---|---|
| RCLHA | 0.877 | 0.181 | 1.262 | 1.518 | 0.828 | 0.880 |
| RCLHA E | 1.029 | 0.205 | 2.481 | 2.321 | 1.190 | 1.055 |
| RCLHA F1 | 1.092 | 0.300 | 1.550 | 1.810 | 1.006 | 0.986 |
| RCLHA F2 | 1.378 | 0.139 | 1.953 | 1.924 | 1.287 | 1.187 |
| RCLHA F3 | 0.986 | 0.083 | 1.975 | 1.957 | 0.871 | 0.657 |
| LCRHA | 0.928 | 0.204 | 1.258 | 1.531 | 0.797 | 0.840 |
| LCRHA E | 0.862 | 0.310 | 2.303 | 2.078 | 1.192 | 1.036 |
| LCRHA F1 | 0.864 | 0.380 | 1.249 | 1.590 | 0.914 | 1.008 |
| LCRHA F2 | 1.256 | 0.126 | 1.669 | 1.748 | 1.337 | 1.322 |
| LCRHA F3 | 0.948 | 0.089 | 1.628 | 1.734 | 0.800 | 0.664 |
| EHA | 0.897 | 0.110 | 1.352 | 1.523 | 0.864 | 0.907 |

*HA self-aggregation*

The volume-based particle size distribution (PSD) of unfractionated HAs, fractions and esters is depicted in Fig. 2, while the most intensive peak in their PSD ($d$) is summarized in Table I. PSD was obviously different for RCLHA, LCRHA and EHA. Also, RCLHA E and LCRHA E differed in their PSD significantly, while their fractions revealed quite similar PSD. It is clear that, after the fractionation procedure had been performed, both HAs aggregated in a similar way. Opposite to other authors,[25–28] who detected three particle size populations, PSDs obtained in this study did not show defined particle size regions.

It is obvious from Table I that the decreased content of both carboxyl and phenolic groups in RCLHA F1 and E, in comparison to RCLHA, resulted in a pronounced aggregate size ($d$ = 873.7, 3265 and 515.0 nm, respectively). Vice versa, for a higher functional groups content in both RCLHA F2 and F3, a lower aggregate particle size was noticed ($d$ = 31.71 and 9.290 nm, respectively). Although both functional group contents were changed within measurement

uncertainty, F1 and E aggregate sizes were remarkably higher compared to LCRHA ($d$ = 997.4, 506.3 and 32.66 nm, respectively). Both F2 and F3, having higher carboxyl, and F2 with higher phenolic concentration, showed a less pronounced aggregation ($d$ = 39.44 and 9.483 nm, respectively) than LCRHA and F1. Although the LCRHA aggregate size was lower than for RCLHA, the particle size of its fractions retained the same order as for RCLHA (F1 > F2 > F3).

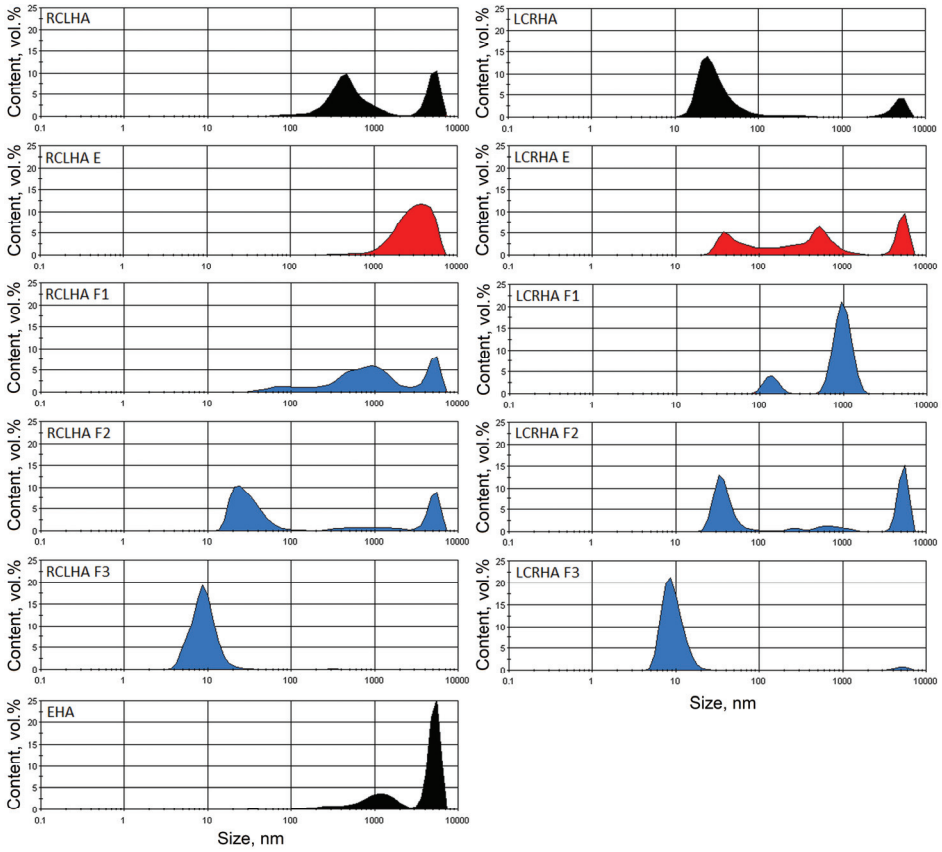


Fig. 2. Particle size distribution by volume (particle size content, vol.%) of Rendzic Calcaric Leptosol humic acid (RCLHA) and Leptic Calcaric Regosol humic acid (LCRHA), their fractions (F1-F3) and esterified forms (E), and IHSS standard Elliott Soil humic acid (EHA) at pH 3 after 3 days.

To confirm dependence observed, the aggregate size for both HAs, esters and fractions was correlated to carboxyl and phenolic group contents obtained by the titration method and correlation presented in Fig. 3. Although the correlations obtained were not strong ($R$ = 0.588, $p$ = 0.073 and $R$ = 0.574, $p$ = 0.083 for

carboxylic and phenolic groups, respectively), it is apparent that, at low pH, the higher groups content bring about the less pronounced HA aggregation.

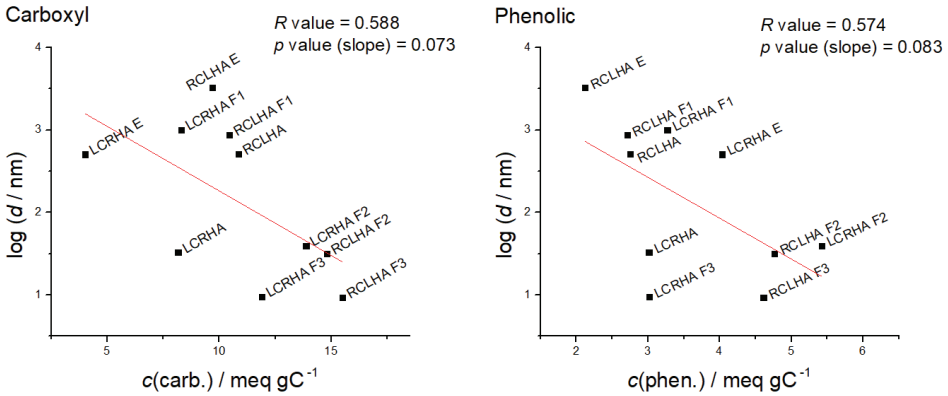


Fig. 3. The most intensive peak in particle size distribution (particle size log *d*) *versus* carboxyl and phenolic group content (RCLHA - Rendzic Calcaric Leptosol humic acid, LCRHA – Leptic Calcaric Regosol humic acid, F1–F3 – fractions; E – esterified form).

As the applied HA fractionation and esterification procedures led to structure changes resulted in different ATR-FTIR band intensities, the aggregate size for both HAs, esters and fractions was correlated to relative band intensities from FTIR spectra. A significant positive correlation ($R = 0.76$), depicted in Fig. 4, was obtained for the 2923 $cm^{-1}$ band only, indicating that the higher aliphatic C components content influences the more pronounced HA aggregation.

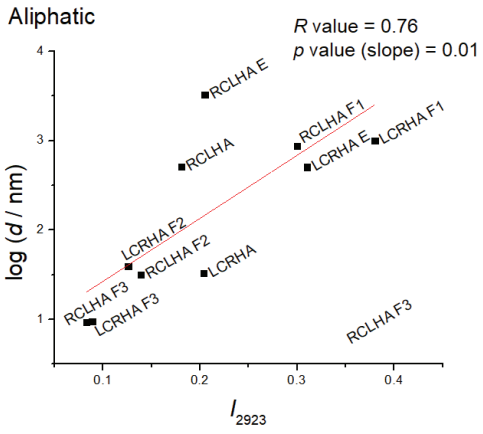


Fig. 4. The most intensive peak in particle size distribution (particle size log *d*) versus 2923 $cm^{-1}$ relative FTIR band intensity (RCLHA – Rendzic Calcaric Leptosol humic acid, LCRHA – Leptic Calcaric Regosol humic acid, F1–F3 – fractions; E – esterified form).

As already emphasized, fractionation and esterification procedures performed led to structure changes, consequently influencing HA self-aggregation process. Functional group contents indicated that the separation procedure used

did not completely fractionate humic acids into phenolic and carboxyl groups. Nevertheless, change of functional groups content in fractions was achieved. Additionally, esterification led to change of functional groups content, both resulting in different HA aggregate sizes. The correlation analysis indicated that with the higher group content the HA aggregation is less pronounced, and one can assume that both carboxyl and phenolic group are not predominant in the HA self-aggregation process at low pH. According to the literature data,[4,9] the hydrogen from functional groups, forming hydrogen bonds at low pH, is likely responsible for HA self-aggregation. Besides functional groups, from this study arises that other HA components also participate in various phases of HA self-aggregation process, as already suggested by Mecozzi and Pietrantonio.[10]

As previously mentioned, correlation coefficient between aggregate size and aliphatic, in comparison to other components, was obviously the highest. Since the correlation for aliphatic components is stronger than for carboxylic and phenolic groups, it can be concluded that they strongly influence the self-aggregation process in comparison to functional groups. Thus, F2 and particularly F3 fractions revealed the less intense self-aggregation. It can be seen that aromatic C was slightly higher and aliphatic C relative abundance profoundly lower in F2, and especially F3. Some literature data[10–12] have suggested that carbohydrates and proteins, as well as lipids, play a facilitative role in formation of HS aggregates. Hence, the lower abundance of aliphatic C in F2 and F3 could be considered as insufficient to start the self-aggregation process. Regarding polysaccharide relative abundance, no obvious regularity and influence to HA self-aggregation was observed.

The largest aggregates were measured for esterified forms with blocked carboxyl groups. Although the particle size vs. aromatic C correlation was not high, the increased relative abundance of aromatic C in esters is obvious, assuming their participation in hydrophobic bonding and consequent influence to more pronounced aggregation. Hakima and Kobayash[13] already emphasized that higher hydrophobicity, *i.e.*, aromaticity induces stronger hydrophobic interactions and more pronounced HA self-aggregation.

The relation of HA self-aggregate size with carboxyl and phenolic group content, as well as aliphatic C relative abundance, at low pH, could be considered universal regardless of the structural characteristics of original or modified HA forms.

## CONCLUSION

To investigate the influence of structural components on the HA self-aggregation process, soil humic acids were fractionated, using the secondary amine weak base resin, and esterified to selectively block carboxyl groups. Both the fractionation and esterification processes, herein applied, contribute to the HA

structural changes which resulted in the content of functional groups determined by titration, as well as from the ATR-FTIR spectra intensities. The performed modifications influence the HA self-aggregation process by giving different particle size distributions.

According to the not strong negative correlations, between the carboxylic and phenolic group content and aggregate size at low pH, the higher the groups content is, the less pronounced is the HAs aggregation. It can be assumed that functional group content, both carboxyl and phenolic, is not predominant in the HA self-aggregation process.

Based on the ATR-FTIR data, the significant positive correlation of aliphatic C components and aggregate size could indicate a dominant influence of these components in the HA self-aggregation process. A lower abundance of aliphatic C components in HA fractions could be considered as not sufficient to start the self-aggregation process. Obviously, the increased relative abundance of aromatic C in esters likely points to its participation in hydrophobic bonding and, consequently, more pronounced aggregation. The influence of polysaccharide C relative abundance to HA self-aggregation was not observed.

The relation of HA self-aggregate size with carboxyl and phenolic group content, as well as aliphatic C relative abundance at low pH, could be considered universal regardless of the structural characteristics of original or modified HA forms.

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ИЗВОД

## САМОАГРЕГАЦИЈА ЗЕМЉИШНИХ ХУМИНСКИХ КИСЕЛИНА У ЗАВИСНОСТИ ОД ЊИХОВИХ СТРУКТУРНИХ КАРАКТЕРИСТИКА

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Основни циљ овог рада је испитивање утицаја карбоксилних и фенолних група, као и ароматичних, алифатичних и полисахаридних компоненти, на самоагрегацију земљишних хуминских киселина (HA). Да би се добиле фракције са различитим садржајем функционалних група, земљишне HA (Leptosol и Regosol) су фракционисане коришћењем јоноизмењивачке смоле. Да би се проценио утицај сваке функционалне групе на самоагрегацију, естерификацијом су блокиране карбоксилне групе. Присуство структурних компоненти у HA је одређено потенциометријском титрацијом и ATR-FTIR спектроскопијом. Процес агрегације на pH 3 је праћен техником динамичког расејања светлости. Резултати указују да је агрегација HA слабије изражена што је већи садржај функционалних група. Значајна позитивна корелација алифатичног C и величине агрегата указује на њихов доминантан утицај на самоагрегацију HA. Постоји могућност да је,

због ниске заступљености алифатичног C, започињање процеса агрегације отежано. Повећање присуства ароматичног C у естрима вероватно указује на њихово учешће у хидрофобним везама, услед чега је израженија агрегација. Однос величине агрегата и карбоксилне и фенолне групе, као и алифатичног C, на ниском pH, може се сматрати универзалним без обзира да ли се ради о структурним особинама изворне или модификоване форме HA.

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