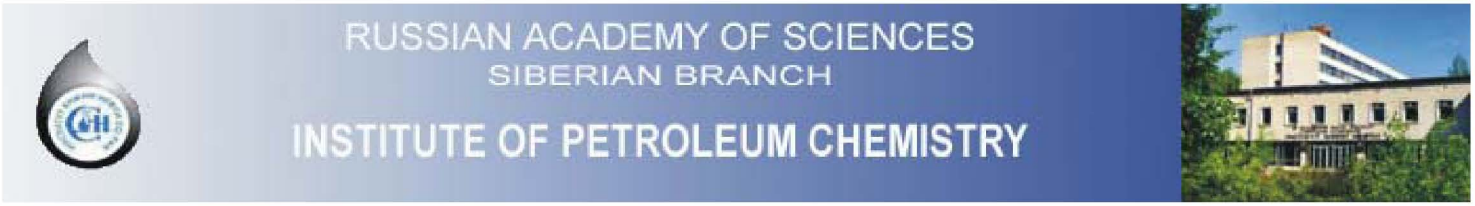

Erythrocyte Reversible Aggregation under the Action of Humic Acids

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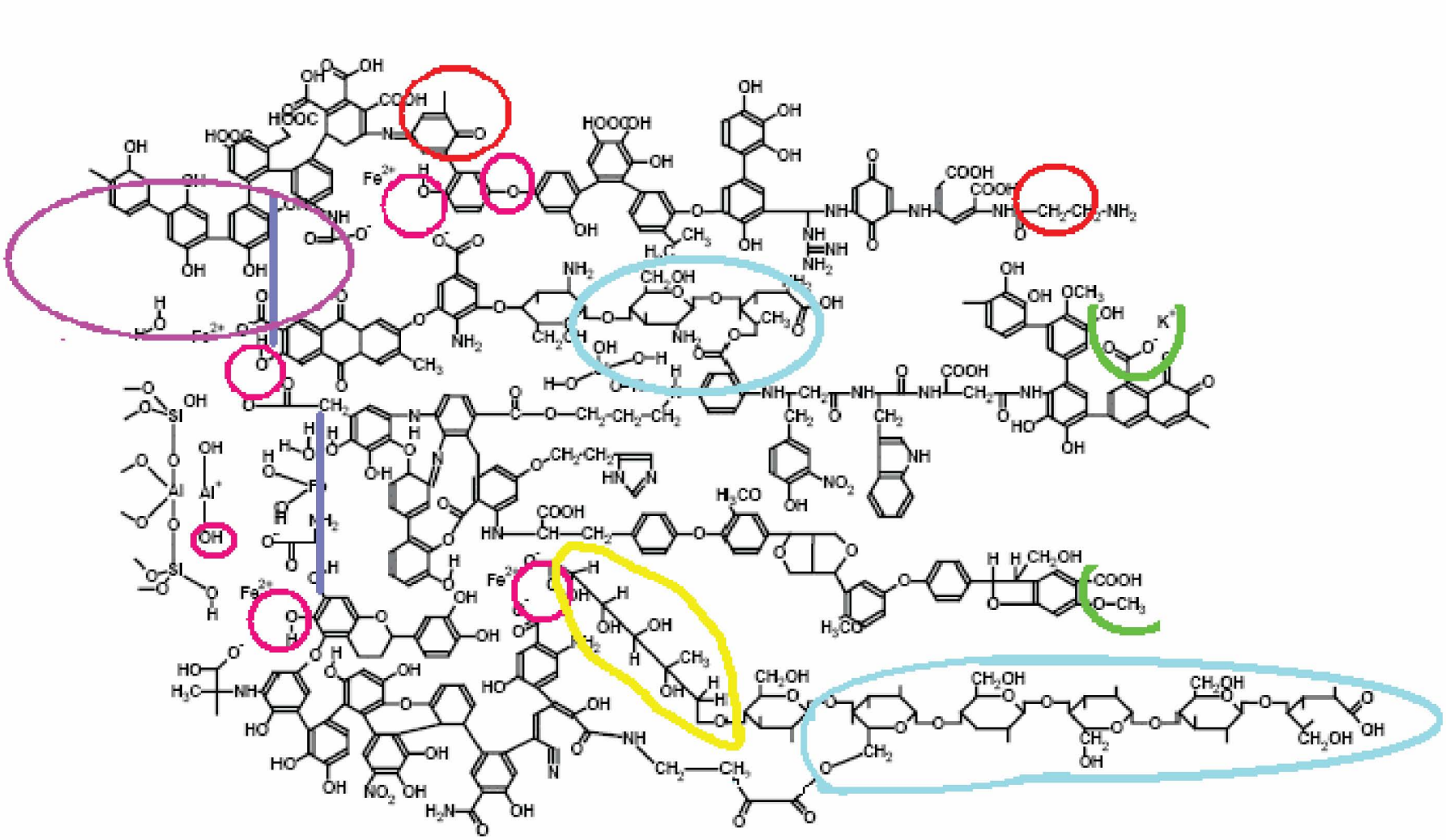
Mechanochemical modification of HAs



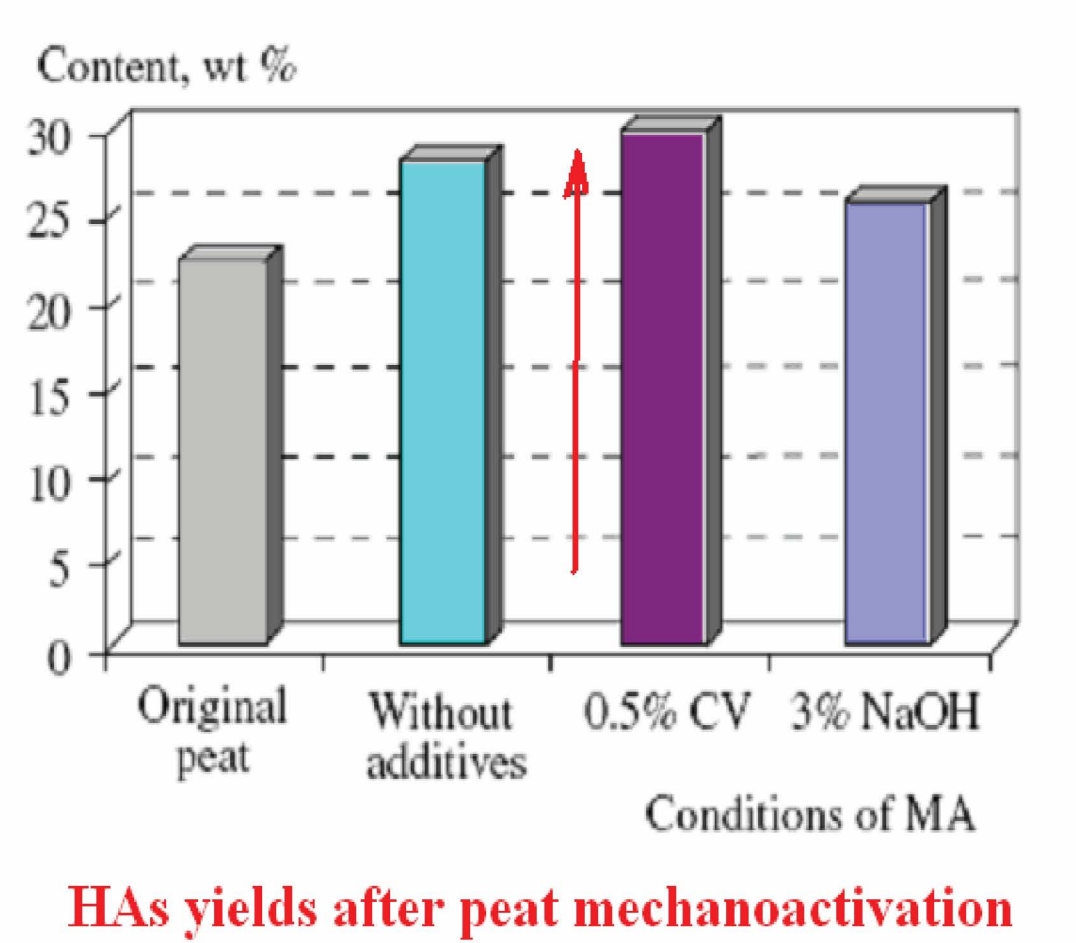
HA1, HA2 – from original peat; HA3, HA4 – peat mechanically treated; HA5, HA6 – peat mechanically treated in the presence of CV; HA7, HA8 - peat mechanically treated in the presence of NaOH

EXPERIMENTAL

- Peat was subjected to a mechanochemical activation (MA) in a VCM-10 flow-through centrifugal vibrating mill (developed at the Institute of Solid-State Chemistry and Mechanochemistry, Siberian Division, Russian Academy of Sciences, Novosibirsk) using 10-mm steel balls at an acceleration of 180 m/s2. Peat was treated both in the absence of chemical reagents and in the presence of NaOH (3%) or celloviridine (CV) (0.5%) as a cellulolytic enzyme. The residence time of the treated materials in the working zone was 2 min.

- **HAs** were isolated from solutions resultant from the extraction of water-soluble components and lipids from peat with a 0.1 N NaOH solution followed by alkali neutralization with a 10% HCl solution. The structure of **HAs** was investigated by the ESR and 13C spectroscopy methods.

The change of HAs yields under MA



HAs yields after peat mechanoactivation

Peat MA in the presence of various reagents changes the yield and characteristics of the basic components. The results obtained suggest that MA increases **HAs** yield by 14.2-33.4% to compare with original peat. **HAs** are isolated from peat in the largest amounts with the use of the enzyme (33.4% increase). This high yield is possible due to the degradation of substances that are difficult to hydrolyze.

The change of HAs structure under MA

The fragment composition of HAs obtained from peat subjected to MA as determined by ^{13}C NMR spectroscopy

Treatment conditions	Carbon atom content in structural fragments, %						
	C=O 220–160 ppm	$C_{ar}O$ 160–140 ppm	$C_{ar}C$, H 40–113 ppm	$C_{alk}O$ 106–93 ppm	$C_{alk}O$, CH_3O 66–54 ppm	C_{alk} 54–0 ppm	degree of aromaticity, f_a
Original peat	12.1	2.3	18.5	20.6	11.3	35.1	21
Without additives	12.9	4.7	16.1	26.9	14.6	27.5	21
0.5% CV	11.0	2.1	16.9	28.4	12.0	29.6	19
3% NaOH	8.9	2.2	19.0	25.2	12.2	32.5	21

The 13C NMR data testify that MA markedly changes the composition of **HAs**; that is, the fraction of alkyl substituents is decreased and the amount of oxygen-containing aromatic (CarO) and aliphatic (CalkO) fragments is increased. The treatment of peat in the presence of CV increases the amount of carbohydrate fragments in **HAs**, likely due to the fracture of glycoside bonds in their macromolecules. Molecules of **HAs** obtained from peat through the treatment with NaOH contain increased fractions of aromatic and carbohydrate moieties. The increase in the total amount of oxygen-containing groups after the mechanical treatment of peat suggests that the oxidation of the preparations with atmospheric oxygen plays a substantial role.

The change of HAs structure under MA

Hydrophilic-to-hydrophobic fragment ratios and the amounts of paramagnetic centers in HAs

Treatment conditions	Ratio	The number of paramagnetic centers, 10^{18}/g
Original peat	0.86	0.87
Without additives	1.35 ↑	0.65 ↓
0.5% CV	1.15	0.72 ↓
3% NaOH	0.94	1.19 !

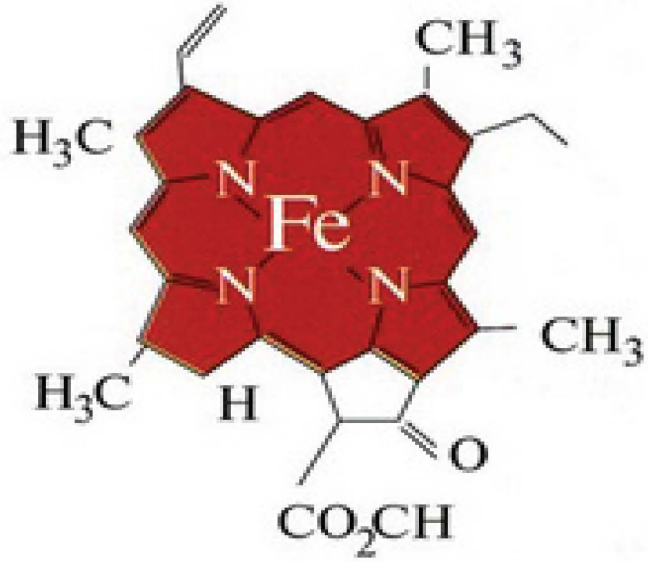
Structural fragments of **HAs** may be conventionally divided into hydrophilic and hydrophobic ones. Their ratio determines the solubility of macromolecules, their spatial organization, and functional properties. For **HAs** isolated from original peat and peat treated with NaOH, hydrophilic-to-hydrophobic ratio is below unity. The MA of peat and its treatment with CV increase the fraction of hydrophilic fragments.

The ESR spectra of **HAs** demonstrated narrow symmetric singlet lines at the g-factor equal to 2, with these lines being typical of unpaired electrons of free radicals. Peat dispersion and treatment with CV decrease the concentration of paramagnetic centers (Table), probably due to the fracture of **HAs** associates and the rupture of hydrogen bonds between polyconjugated systems and functional groups. The treatment with the alkali, on the contrary, increases the number of paramagnetic centers.

The goal of this work is to study erythrocyte reversible coagulation under the effect of humic acids (**HAs**) isolated from peat subjected to a mechanochemical treatment.

The rheological properties of blood are known to depend substantially on the erythrocyte reversible aggregation. Aggregation parameters may be employed as universal indicators for determining the coagulation and stabilization ability of natural preparations. Among many factors providing blood with coagulability, such coagulants as vitamin *K* and naphthoquinone derivatives play an important role.

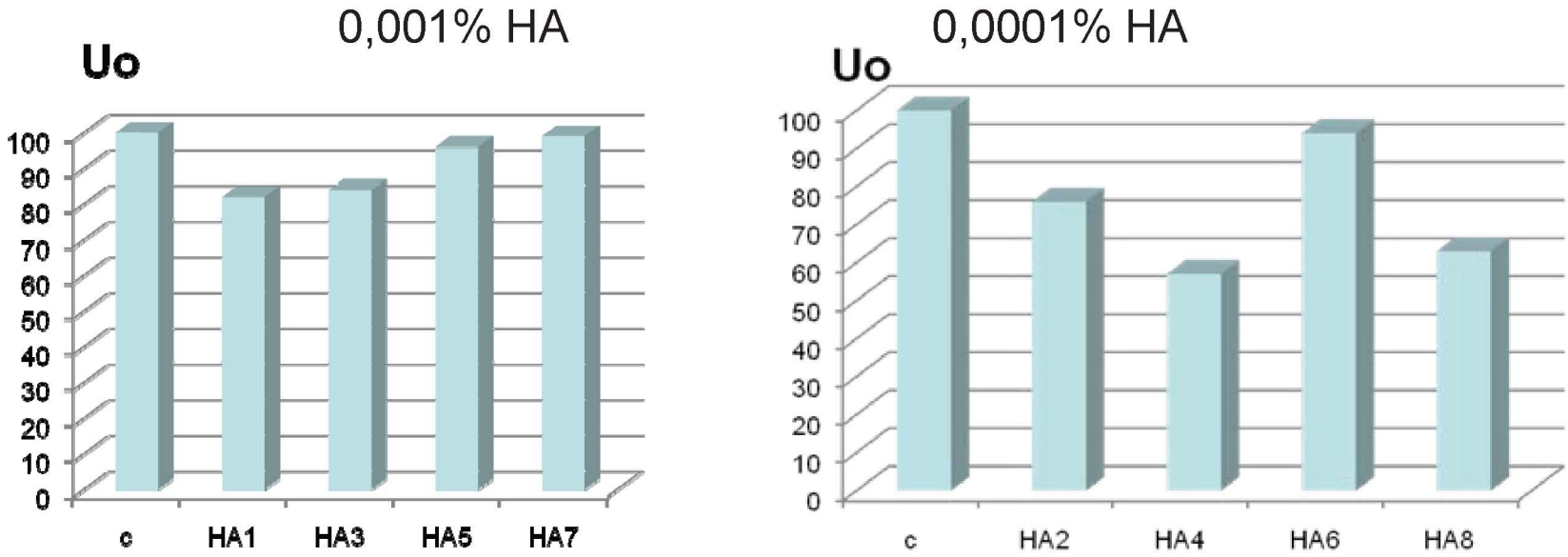
Erythrocyte



Haemoglobin

•Erythrocyte reversible aggregation was studied with an instrument using the vibrational photometric method of measuring the optical density in microscopic volumes of blood. Erythrocyte reversible aggregation was investigated in alkaline solutions at **HAs** concentrations of 0.001 and 0.0001 wt %.

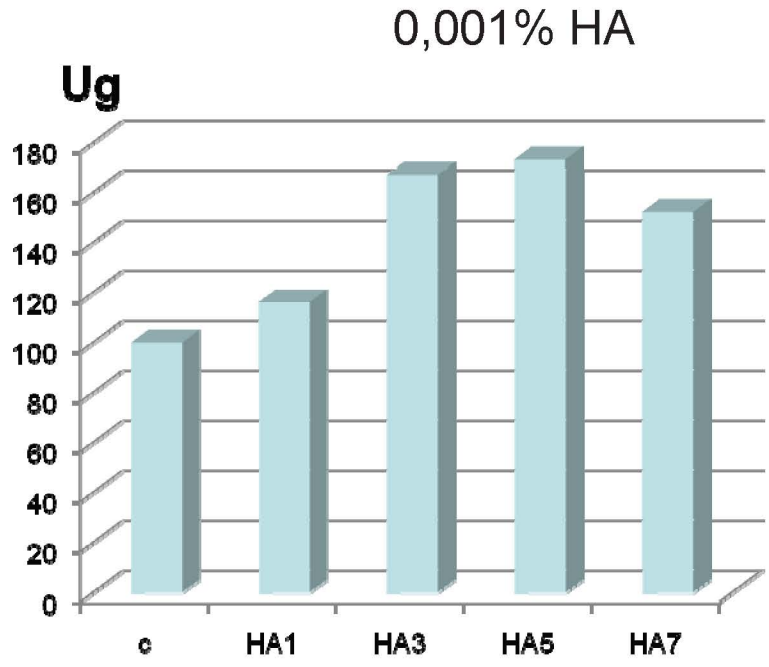
The change of strengths Uo of erythrocyte aggregates

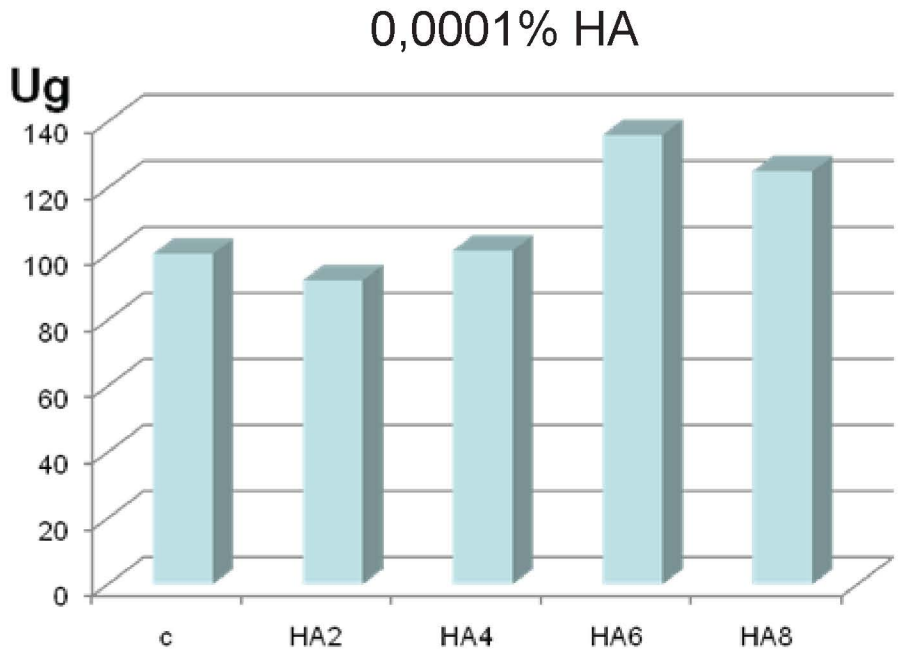


HA1, HA2 – from original peat; HA3, HA4 – peat mechanically treated; HA5, HA6 – peat mechanically treated in the presence of CV; HA7, HA8 - peat mechanically treated in the presence of NaOH

As **HAs** concentration is increased, the minimal strength of erythrocyte aggregates decreases. The largest decrease magnitudes of 42.5 and 36.5% are observed for samples HA4 and HA8 prepared through peat MA and treatment with NaOH. An increase in **HAs** concentration (HA1 and HA3) does not change significantly the minimal strength of aggregates.

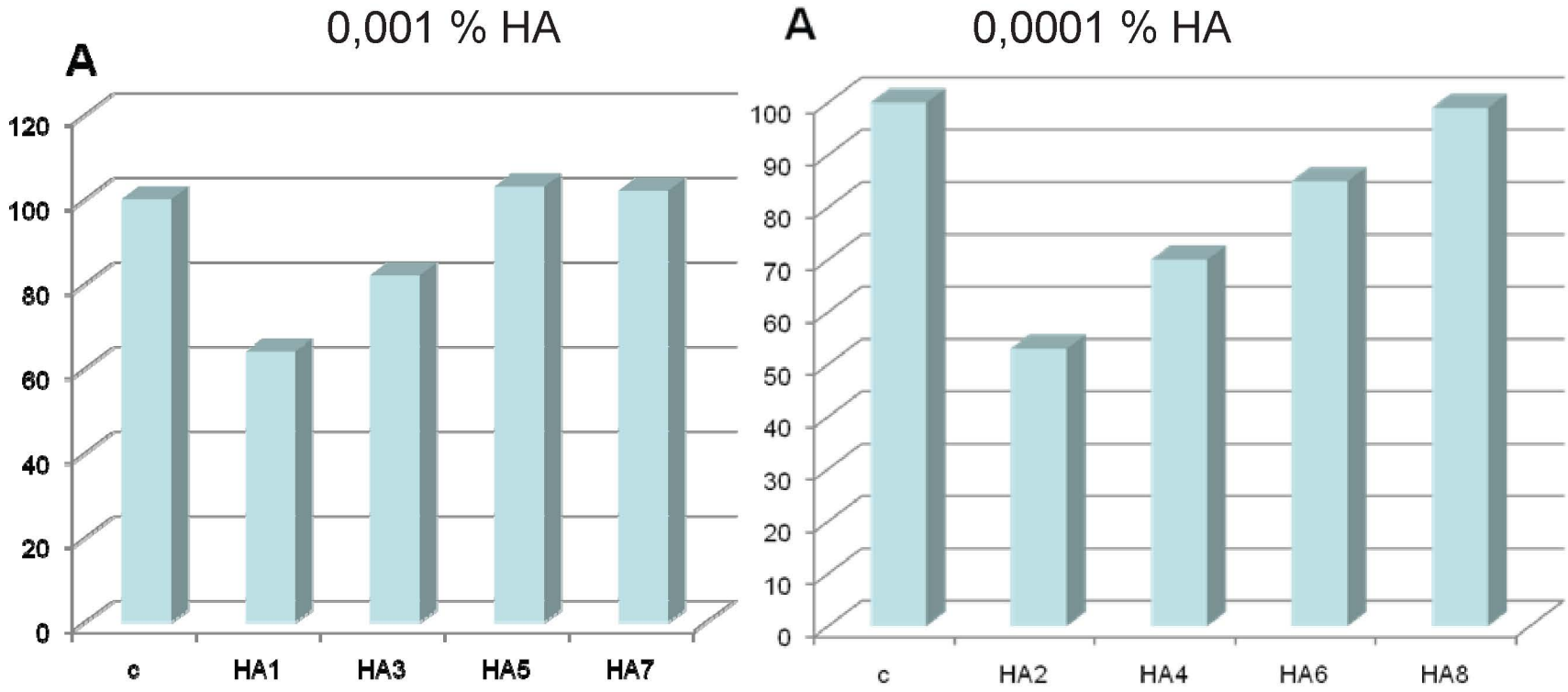
The change of strengths Ug of erythrocyte aggregates





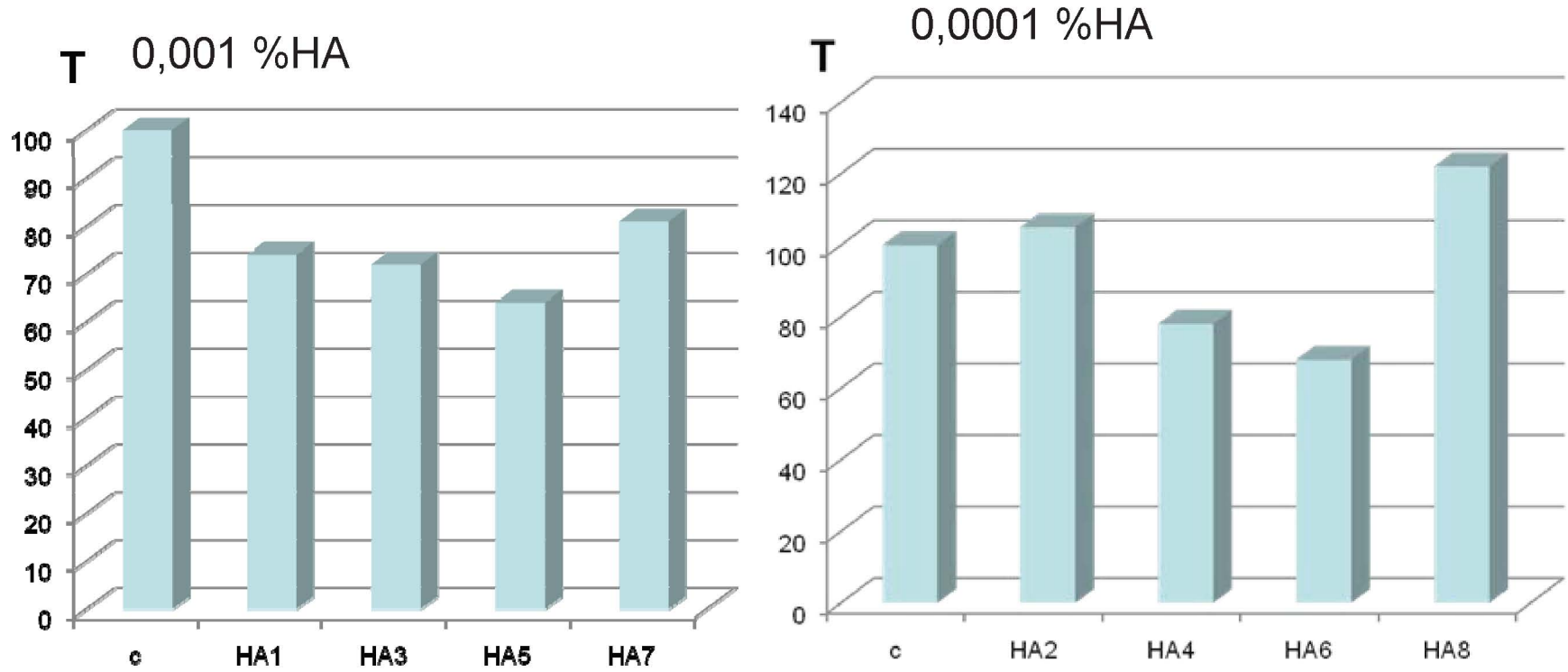
Differences in the maximal strengths of aggregates resultant from the coagulation of erythrocytes with **HAs** isolated under different conditions are more distinct. The value of Ud increases by 73% for HA3.

The change of erythrocyte amount



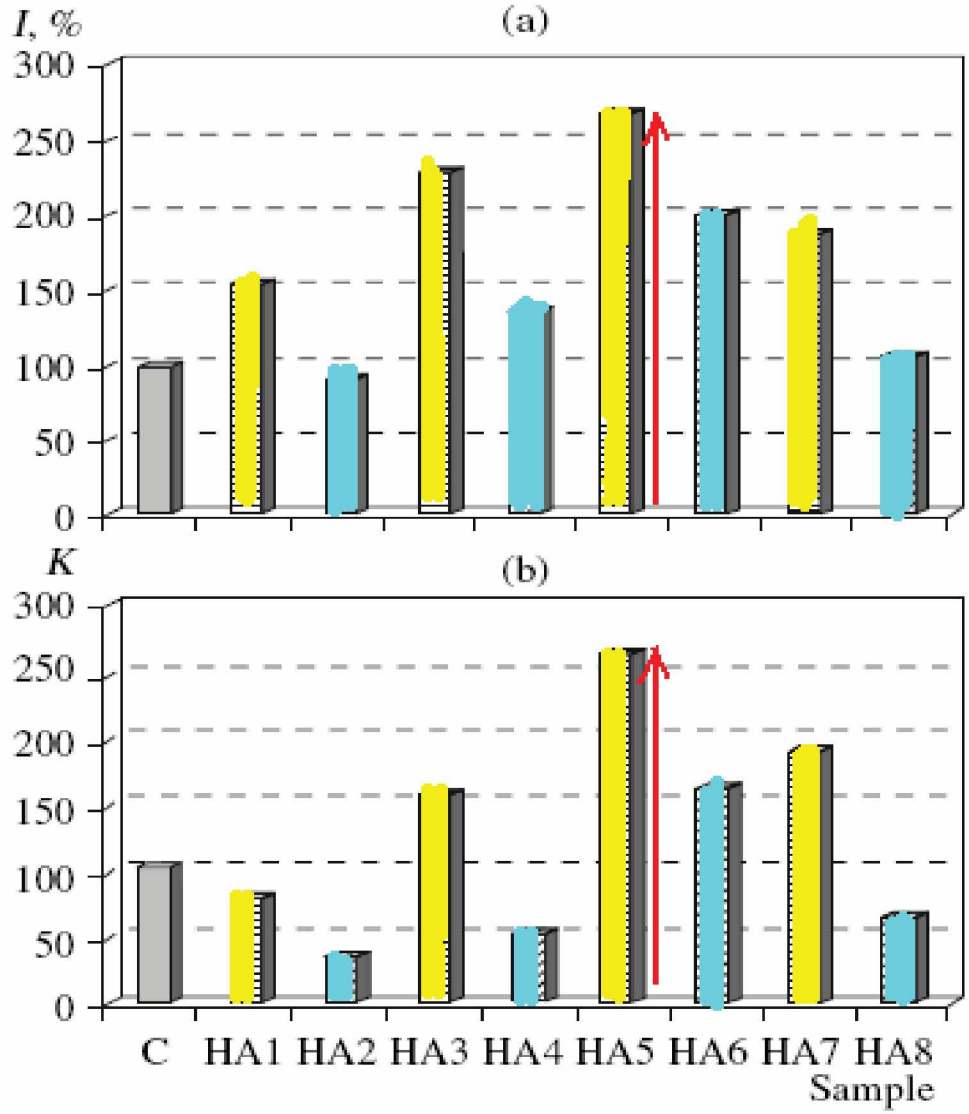
The photometric signal amplitude A, which characterizes the concentration of erythrocytes during their reversible aggregation, appears to be markedly lower than in the control experiment and a 2-3% increase is observed in only two cases.

The change of the spontaneous aggregation period



The time of erythrocyte aggregation τ also decreases upon the addition of **HAs** and amounts to 60-75% of the control experiment result for CV-treated samples. The addition of HA1 (0.0001%) causes the highest (by 46.5%) decrease in the erythrocyte concentration with a slight elongation of aggregation period τ (5.0%).

The change of index and coefficient of aggregation



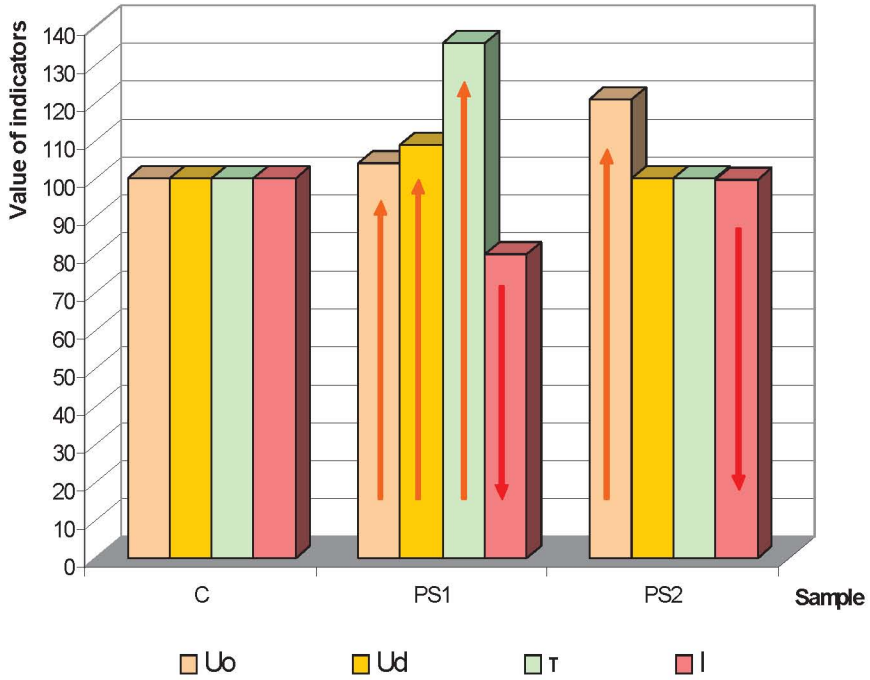
Variations in the (a) index and (b) coefficient of aggregation as depending on HA concentration and the peat MA conditions.

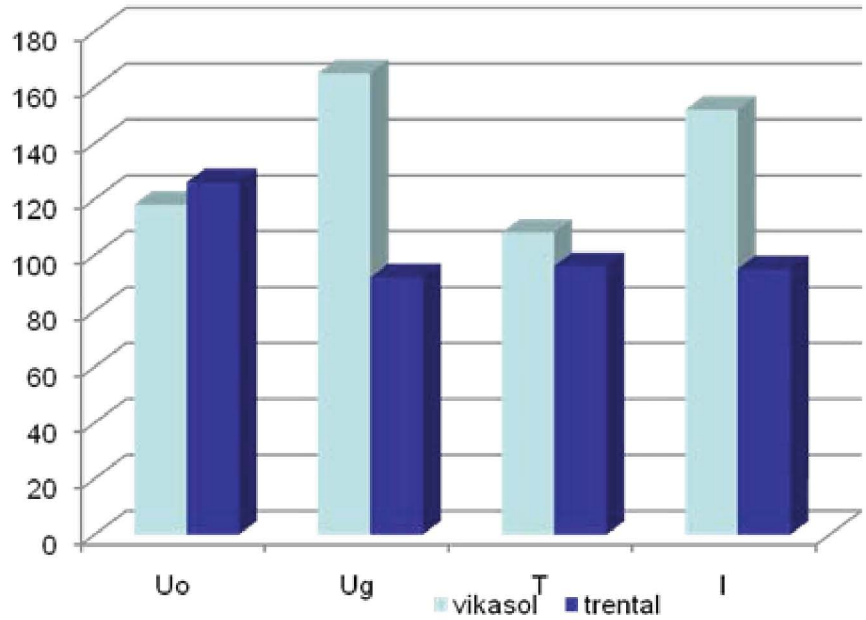
As the **HAs** concentration is increased, the aggregation intensity rises by a factor of 2-3 and appears to be the highest for **HAs** samples extracted from peat subjected to MA. The highest integral aggregation coefficient was observed when HA5 (0.001%) extracted from peat in the presence of CV was added.

Thus, the composition of **HAs** substantially affects their ability to coagulate erythrocytes. The enhancement of erythrocyte coagulation in the presence of **HAs** is probably caused by an increase in the fraction of hydrophilic fragments. The erythrocyte reversible aggregation is always enhanced with an increase in **HAs** concentration.

$$I = Ug / \tau \qquad K = Uo\ Ug\ A / \tau$$

Indicators of erythrocyte reversible aggregation of human blood in the presence of peat polysaccharides (% in relation to the control)





PS1 – extracted from original peat
PS2 – extracted from peat mechanically treated without additives
Vikasol –analog of vitamin K

CONCLUSIONS

Peat mechanical activation increases the yield and solubility of humic preparations. The comparison between results on erythrocyte reversible aggregation testifies that the highest effect is achieved with **HAs** prepared through the peat mechanical activation, either in the absence of additives or in the presence of CV enzyme. A rise in the concentration of **HAs** increases their coagulation ability. Peat polysaccharides exert a multidirectional action - increase the strength of erythrocyte aggregates and reduce the intensity of aggregation processes.The results of the structural analysis enables us to conclude that mechanical treatment enlarges the amount of hydrophilic oxygen-containing fragments and slightly decreases the number of paramagnetic centers in **HAs** molecules.