ISSN 1064-2293, Eurasian Soil Science, 2015, Vol. 48, No. 12, pp. 1292–1299. © Pleiades Publishing, Ltd., 2015.
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SOIL CHEMISTRY

On the Nature of Humic Substances

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Received August 13, 2014

Abstract—It is argued that the isolation of low-molecular-weight compounds from humic substances does not prove their supramolecular nature, because small molecules can be sorbed on macromolecules by interacting with them due to noncovalent bonds. The relative mobility of molecular segments in humic substances has been proposed to be used as a criterion for the discrimination between the humic substances of supra- and macromolecular nature. The macromolecules are characterized by mobility of their segments, whereas supramolecular systems have stiff structure. This difference between macro- and supramolecules results in different behaviors of the matrices (gels) formed from them in the processes of segregation. In the macromolecules, the formations of a new phase appearing at the segregation (microphase separation) are of nano size, at least in one dimension. They are incapable of moving within the matrix and form a well-known, limited set of systems. In the supramolecular matrices, the new-phase formations should have higher mobility and ability to move within the matrix with the formation of particles and zones of not only nano, but also micro sizes, as well as a significantly larger set of systems, including fractal configurations. The experimental electron microscopic study of the humic matrices of soil gels shows that the new-phase formations in the matrix of humic substances have not only nano, but also micro sizes and are capable of moving within the matrix, which confirms the supramolecular nature of humic substances. The proposed method has allowed generalizing the supra- and macromolecular approaches, because macromolecules can enter into the composition of supramolecular systems. It is no less important that the behavior of HSs can be perceived as the behavior of stiff impenetrable particles that may compose the structures of different types and sizes.

Keywords: soils, humic substances, humic matrix of soil gels, macromolecular and supramolecular organizations

DOI: 10.1134/S1064229315120066

FORMULATION OF THE PROBLEM

Two approaches are usually contrasted to each other at the consideration of the nature and structure of humic substances (HSs): when HSs are regarded as macromolecules [1, 3, 5, 9, 23] or supramolecular systems composed of molecules with noncovalent bonds [4, 21, 22, 24]. From these contrast positions, studies are performed, and the obtained results are considered.

Supramolecular systems consist of low-molecular-weight organic compounds bound by numerous noncovalent (weak) bonds; therefore, when low-molecular-weight compounds are detected in the composition of humic acids, a conclusion is drawn about their supramolecular nature [10, 21]. However, the actual situation is more complex. Along with the extreme systems (macromolecular and supramolecular), the existence of some intermediate systems with specific structures can be supposed.

This is related to the fact that soils and other environments in which HSs are formed contain many low-molecular-weight organic compounds and oligomers resulting from the degradation of biopolymers. These are sugars, amino acids, peptides, fats, low-molecular-weight acids, lignin transformation products, etc. It is unlikely that these substances will not be sorbed on large HS molecules (regardless of their macro- or supramolecular nature), which presumably have a large amount of different active sites. In our opinion, this supposition is well justified, because the complementarity principle lies behind the structure of biopolymers.

This paper is aimed at the

(1) analysis of the possible nature and structure of HSs,

(2) determination of the properties of the systems formed from HSs with due account for the nature and properties of the latter and the choice of the criteria of supramolecular or macromolecular nature HSs,

(3) comparison of the behavior of gels composed of macromolecules and of supramolecular formations,
(4) analysis of experimental data on the structures of the new phase formed in the gels of HSs, and

(5) determination of the nature and structure of HSs as based on the properties of the systems formed from these substances.

Possible options for the nature and structure of humic substances

Variant	Nature and structure	Structural organization and behavior of fragments of large molecules
1	Macromolecules	Macromolecular segments freely move with respect to one another
2	Macromolecules with sorbed low-molecular-weight substances	Macromolecular segments with sorbed low-molecular-weight substances freely move with respect to one another
3	Macromolecules whose segments are cross-linked by low-molecular-weight substances	Rigid system with definite structure; macromolecular segments lost mobility and do not move with respect to one another
4	Molecular assemblies	Systems with clear composition and structure

RESULTS AND DISCUSSION

Theoretical Analysis of Possible Structure of HSs

Let us analyze the situation[1] resting upon the experimental results of earlier HS studies and the known properties of high-molecular-weight compounds and supramolecular systems.

The existence of several types of these systems may be supposed (table). The proposed classification was based on the structure and behavior of segments of large (macro- and supra-) molecules of HSs. A gradual transition from the macromolecular to the supramolecular systems is traced in the table. From the physicochemical positions, this transition can be realized at the gradual substitution of covalent bonds by noncovalent bonds. We consider the stability of the position of separate fragments (segments of macromolecules and low-molecular-weight molecules in supermolecules) in the space as the main criterion.

First option. Let us suppose that HSs are macromolecules. Then, they should roll in a coil, where the segments permanently change their position in the space [7, 8]. We consider "pure" HS macromolecules and hold that low-molecular-weight organic molecules cannot be relatively strongly fixed on macromolecules. However, this is obviously too ideal a picture.

Second option. It is known that HSs contain different functional groups, hydrophobic sites, aromatic rings, etc., i.e., macromolecular segments [5] capable of interacting due to noncovalent (supramolecular) bonds [4]. They can interact within one or different molecules. These processes are well known; they form the secondary, tertiary, and quaternary protein structures and a DNA helix. It may be also supposed that the HS coil (particle) includes low-molecular-weight substances complementary to the segments of HS macromolecules, which can be fixed on them due to noncovalent interactions. Their fixation strength can strongly vary and depend, first, on the size of these molecules and, hence, the number of bonds linking them to the macromolecules, and, second, on the mutual arrangement of functional groups and hydrophobic sites on the macromolecules and sorbed small molecules.

Thus, we may suppose the existence of HS macromolecules, whose segments did not lose ability to move in the space but have sorbed organic molecules. We consider this macromolecular structure of HSs as more realistic.

Third option, which logically follows from the aforesaid, is that molecules of low-molecular-weight organic substances are sorbed and bind macromolecular segments to one another due to numerous weak interactions. The macromolecular segments lose their mobility in this case.

In this approach to the structure of HSs, as well as in the second option, the macromolecule includes low-molecular-weight substances but retains its macromolecular nature: macromolecular segments move with respect to one another. In the other case, a rigid construction based on HS macromolecules with unchanged structure, which represents a supramolecular formation, should appear.

Other ways for the realization of the third option cannot be excluded. It may be supposed that oligomeric molecules[2] act as a multicentric basis for the formation of supramolecular systems.[3]

Fourth option. The appearance of HS molecules from low-molecular-weight organic compounds is due to the formation of classical supermolecules with clear composition and architecture. Only the HSs corresponding to option 1 contain no low-molecular-weight substances. This can be the least probable option for the existence of HSs in soils, because it is unlikely that no low-molecular-weight compounds

[1] It should be noted that the current work deals with the study and analysis of bound disperse structures (gels) based on HSs rather than free disperse HS-based structures traditionally considered by soil scientists.

[2] Oligomeric molecules represent organic molecules with repeated links and a molecular weight of 1000–10000 Da, all parts of which are bound together by covalent bonds.

[3] If the single supramolecular system based on an oligomeric molecule is considered as fulvic acid, this option is closer to the reality, because it explains the experimental data on the mutual transition of humic and fulvic acids in soils in the annual cycle [13].

would be sorbed on HS macromolecules in such a complex multicomponent mixture as the soil solution.

The humic substances in the other three options (2–4) contain low-molecular-weight molecules detectable by the methods used to confirm the supramolecular nature of HSs.

Thus, the existence of several types of HS structure (nature) can be supposed, but only the first of them is purely macromolecular, and the fourth of them is purely supramolecular. From the general physicochemical positions, the existence of intermediate types (options 2 and 3) may be supposed.

The key problem is to find the means to distinguish between options 1 and 2, on one hand, and 3 and 4, on the other hand.

Criteria of Supramolecular and Macromolecular Natures of HSs

The existence of more than two types of HS nature and structure raises the question of criteria. When should such formations be considered as macromolecules and when as supramolecular systems?

The possibility of isolating low-molecular-weight organic substances is absent only in option 1. Therefore, the presence of low-molecular-weight organic substances in HSs is not a proof of the supramolecular nature of HSs.

Theoretically, HSs can be subdivided into supramolecular and macromolecular systems depending on the proportions of molecules bound together by noncovalent and covalent bonds within the particle. However, the methods based on the quantitative determination of the proportion of organic matter bound by the supramolecular or covalent bonds within an HS particle are unrealizable at present.

An analogous problem was faced in the study of caoutchouc elasticity mechanism in the early 1930s. An attempt to explain it in terms of the chemical structure of molecules was failed. However, the consideration of caoutchouc, along with other polymers, from the positions of the behavior of macromolecules and their spatial arrangement allowed explaining the elasticity of polymers. Relying on this analogy, one should select criteria based on the comparison of nonchemical properties of macromolecules and supramolecular systems, because they can provide indications about the structure of HSs.

The detection of differences in the physical and physicochemical properties of supra- and macromolecular bodies is a difficult problem. The matrices of supramolecular formations are called suprapolymers because of the similarity of their properties to those of polymer matrices. Therefore, the quantitative differences between these matrices in density, elasticity, transition temperatures, and many other properties are hardly indicative.

As follows from the discussion above, the major qualitative difference between macromolecular and supramolecular systems is that the former consist of relatively mobile segments, whereas the latter consist of stiff segments. We have tried to identify the property that might be diagnostic of this difference.

A Comparison of the Behavior of Gels Composed of Macromolecules and Supramolecules

It was noted that the relative mobility of the system components (segments in macromolecular coils) is an essential qualitative difference between the macromolecular and supramolecular systems. Because of the low filling density of the coil space with macromolecular segments (it rarely exceeds 1%), the coils mutually penetrate one another [7, 8]. The local zones of macromolecules interact and hamper the relative translocation of the entire macromolecules in the matrix. The macromolecular segments retain mobility and change their position in the space. In such matrices, the interaction between segments of one macromolecule is replaced by the interaction between segments of different macromolecules [7].

The supramolecular systems are characterized by the presence of clear architecture, the absence of free internal space, and the impossibility of their mutual penetration [4].

Consequently, the polymer matrix (gel) consists of mutually penetrating macromolecular coils, and the supramolecular matrix is composed of particles from supramolecular systems in mutual contact. It follows that this difference in the structural organization of matrices should be manifested in the difference of structures resulting from segregation processes in bound disperse systems of macromolecules [6, 15–20] and supramolecular systems.

Let us analyze the behavior of the new phase in the macro- and supramolecular systems. We consider segregation in the macromolecular systems during the well-studied process occurring in polyelectrolyte gels at the change in the number of dissociated groups (degree of hydrophobicity) due to the pH change or the entry of ions forming low-dissociated compounds with these ions into the system. Interaction between hydrophobic segments (from the same or different macromolecules of the gel matrix) with the formation of hydrophobic phase nanoareas is observed in this case [19, 20]. This process is termed as microphase separation [6, 15–20] (Fig. 1). The new-phase nanoareas can include segments of different macromolecules; therefore, the translocation of these hydrophobic nanoareas within the gel matrix is impossible, because different macromolecules are fixed with respect to one another by the formed nanoareas. These areas are extremely small in size, because they can be formed only at the conformational rapprochement between macromolecular segments, and the conformational translocations of segments are low.

Because of the immobility of whole macromolecules relative to one another within the polymer matrix, these

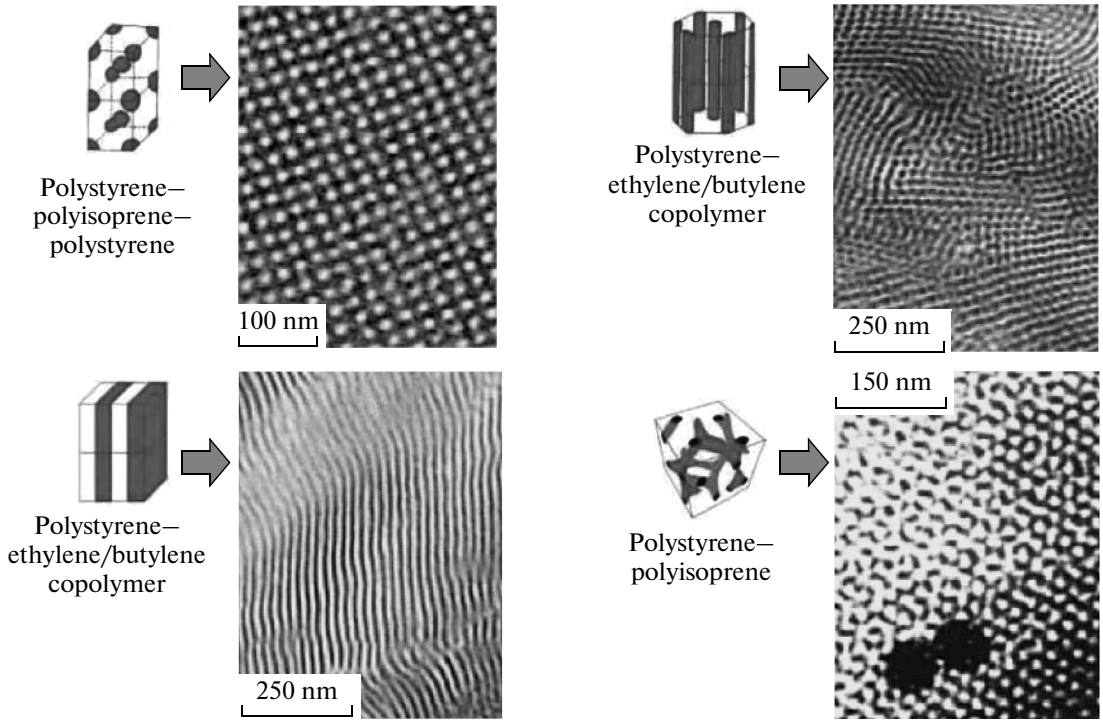

Fig. 1. Nanostructures formed at the microphase separation of polymers.

formations are of nano size and form a limited range of structures: nanodots, nanorods, nanolamellas, and diamond-like and gyroid-like formations.

A radically different situation should be observed for an organic matrix based on supramolecular particles with dissociated groups. When their degree of dissociation decreases, their hydrophilic–hydrophobic properties change: the surface hydrophobicity of these particles increases. Such processes, as well as the distribution of ionized groups on the surface of supramolecular structures, are of random character; therefore, a distribution of the particles of supramolecular systems depending on the hydrophilic–hydrophobic properties of their surface (the degree of hydrophilicity) may be expected. As a result, more hydrophobic nanoparticles will move within the matrix,[4] which they form by combining with one another due to the hydrophobic effect (entropy factor) to create areas of a new, more hydrophobic phase. Due to the freedom of movement, such areas can have a significantly larger size and a relatively complex structure, because they do not have to be limited to the structure typical for microphase-separated polymers: nanoparticles, nanorods, nanolamellas, and gyroid-like structures.

The performed analysis allowed development of another criterion for the determination of the HS nature (supra or macro). This is a significantly more universal criterion.

Let us envisage a macromolecule of some prohumic substances, on which low-molecular-weight organic substances are sorbed. It cannot be classified into the supra or macro structures from its content of low-molecular-weight organic substances, but its classification using the criterion of the relative mobility of segments is reasonably easy.

Analysis of Experimental Data on the Structures of the New Phase Forming in HS-Based Gels

If the sorption of low-molecular-weight organic substances on HS macromolecules ensures the crosslinking and loss of mobility of macromolecular segments, then macromolecules are converted to structures with supramolecular properties. Analogously, supramolecular systems are large molecules containing one or several oligomeric molecules, if their segments are not mobile with respect to one another.

For the HS-based systems, structures resulting from the segregation of the humic matrix were detected and studied by electron microscopy [11–14]. It may be supposed that the analysis of the obtained results will elucidate the nature of HSs.

[4] The matrix of HS particles is not an impenetrable continuum, but it consists of HS particles. This structure has a free space, where HS particles can move. This approach uses the analogy between the humic and polymer matrices. They have a free space, which allows the translocation of macromolecular segments or the entry of low-molecular-weight substances into and translocation within the matrices [7]. Even the crystal lattices have a free space, where atoms or ions can move (diffuse).

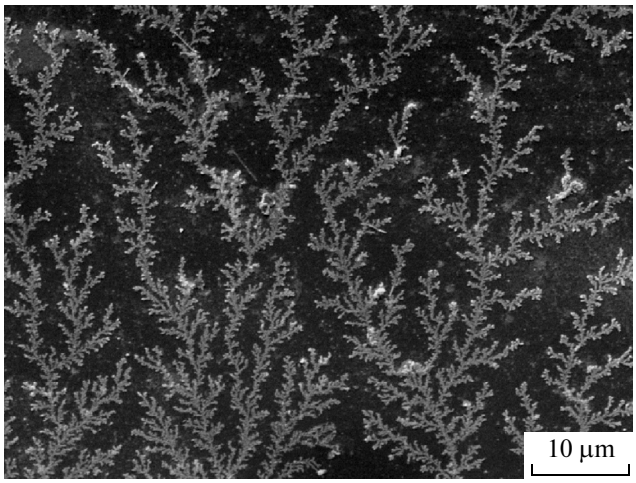

Fig. 2. Dendrite structures observed during the segregation of organic matter in humic systems at the addition of small amount of aluminum (2.81%), magnification ×2000.

The study of HS matrices appearing in the Al-containing alkaline extracts from peat neutralized by an H-cationite [14] revealed that segregation with the release of new-phase nanoareas 20–30 nm in size (very similar to those formed during microphase separation) is observed in the humic material at a low aluminum content. However, when the aluminum concentration increases, the released particles increase in number and begin to interact. This results in the formation of dendrite structures composed by fine particles 100–200 nm in size, as seen in a photomicrograph (Fig. 2).

It should be noted that the possibility of conversion of free disperse systems to bound disperse systems was studied in this case, rather than changes in the bound disperse systems under specific impacts. However, bound disperse systems probably appeared due to drying (the increase in the concentration of aluminum in the peat extract did not result in coagulation) rather than coagulation in this case. This was related to the experimental conditions, when the decrease in pH was accompanied by a decrease in ionic strength rather than its increase, as generally observed during neutralization. For this purpose, alkaline extracts from peat, to which aluminum was added as an alkaline aluminate solution if necessary, were treated with an H-cationite. The alkali metal cations were substituted by hydrogen. As a result, the system pH decreased simultaneously with the solution ionic strength, which significantly stabilized the colloidal systems.

The stability of such HS solutions and the absence of coagulation in them suggested that the transition of these free disperse systems to the bound disperse state occurs after the deposition of solution drops onto a substrate for drying. The above structures appear under drying.

Consequently, the obtained results indicate the existence of HS particles no more than 10–20 nm in size, which form a humic matrix and have different properties. Some of them are deposited in the humic matrix as new-phase particles 10–20 nm in size, which are combined into particles 100–200 nm in size with their dendrites when the concentration of aluminum increases.

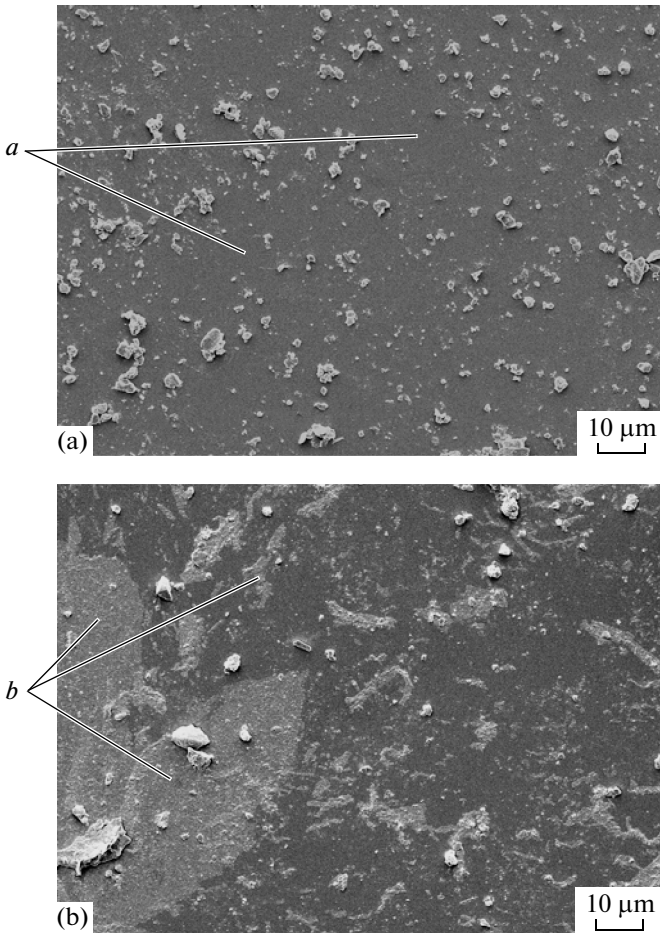

Fig. 3. Electron microscopic image of gel films isolated from (a) the original soddy-podzolic soil and (b) that modified with zinc (1/3 mg zinc/g soil); magnification ×1000 (explanation in text).

An unambiguous conclusion about the movement of the new-phase particles within the humic matrix cannot be drawn from these data, because a free disperse system–bound disperse system transition occurred in the drying experiments. However, these results indicate the formation of a new phase in HSs and the tendency of the HS new-phase particles to be combined.

The separation of large new-phase blocks of tens of microns in size in the humic matrix is observed at the addition of zinc in trace amounts to a soddy-podzolic soil [22]. The gel film from the original soil is presented in the photomicrograph of Fig. 3a. Mineral particles entering in the organic matrix are visible, and the matrix remains invisible in this case because of the absence of appreciable segregation (*a*). The gel film

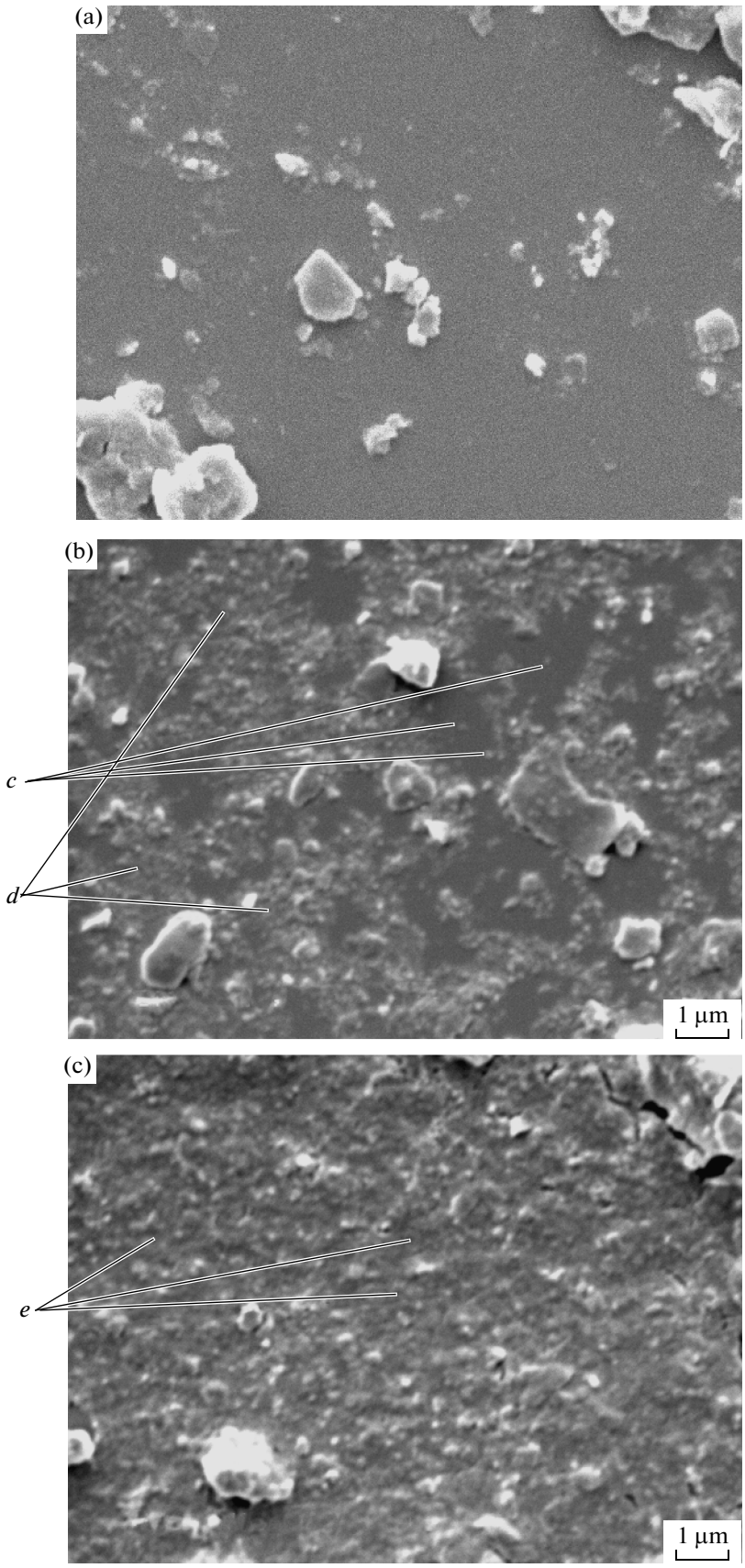

Fig. 4. Electron-microscopic photos of soil gels at the concentration of modifiers in soddy-podzolic soil corresponding to 4–5 μg/g: (a) original soil without additions; (b) Span 80; (c) Span 80; magnification ×10000.

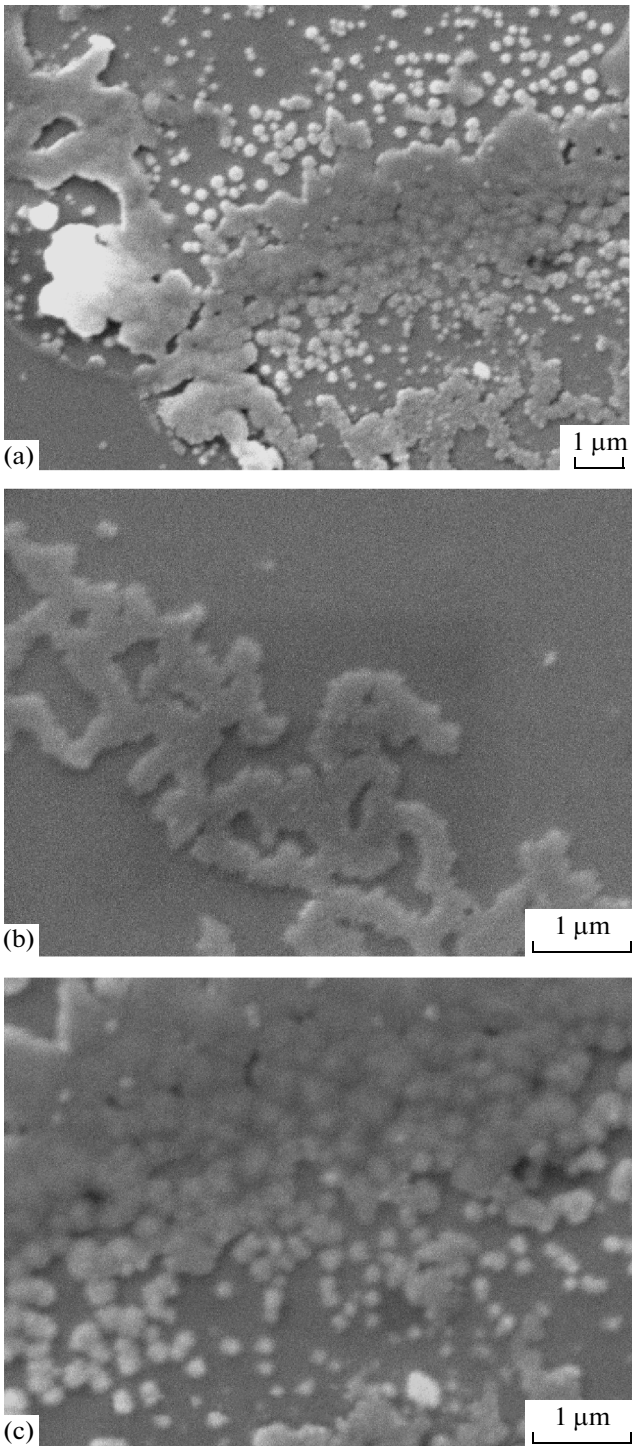

Fig. 5. Electron-microscopic photos of soil gels from a brown semidesert soil at different magnifications.

from the soil after the addition of zinc is presented in the photomicrograph of Fig. 3b, where not only mineral particles, but also heterogeneities in the organic matrix, are well visible (*b*).

In this case, the soil was treated with zinc-containing solutions to the level of field capacity. Under these experimental conditions, zinc ions interacted with HSs occurring in the bound disperse state, and the existence of such structures of the HS new phase is difficult to explain without the translocation of new-phase particles within the humic matrix. Furthermore, both the new-phase particles of several hundreds of nm and their aggregates of micron size are observed in the photomicrograph.

Analogous aggregates of the new-phase particles were detected in soil gels at the addition of some surfactants [23] (Fig. 4). The gel film isolated from a sample of the original soddy-podzolic soil is presented in the photomicrograph. The image is analogous to that in Fig. 3a, although at a larger magnification (Fig. 4a). The precipitates of the new phase in the organic matrix are almost imperceptible. At the addition of Span 80 to the soil (Fig. 4b), abundant precipitates of new-phase particles 100–200 nm in size are observed (*c*), which are accumulated and form areas of several microns in size (*d*). At the addition of Span 20 to the soil (Fig. 4c), segregation is enhanced and covers the entire organic matter (*e*). In these cases, surfactants also interact with the bound disperse HSs, which requires the concept of the free movement of new-phase particles to be used for explaining the obtained results.

An even more convincing proof for the formation of nanoareas of new organic phase in the humic matrix of soil gels, their translocation within the matrix, and aggregation was found in the electron microscopic study of gel films isolated from a brown semidesert soil [24] (Fig. 5). In the presented electron microscopic images, the forming particles of the new HS phase are clearly distinguished; they move within the humic matrix and combine to form either extended areas of the new HS phase or complex patterned structures.

It should be noted that the moving of the new phase took place in the gels of wet soils. The removal of the water "fixed" the state typical of these systems at the moment of their drying.

Numerous electron microscopic images, which confirm the translocation of the distinguished new-phase areas within the humic matrix, are accumulated in the study of humic gel systems isolated from different soils.

CONCLUSIONS

Thus, the analysis of the behavior of bound disperse systems formed by macromolecules and supermolecules, as well as the experimental electron microscopic study of the humic matrix of soil gels, allows a conclusion to be drawn that the formed areas of the new HS phase are capable of moving within the humic matrix and interacting with one another, which argues for the supramolecular nature of HS particles.

Actually, the proposed approach resulted in the generalization of the supra- and macromolecular approaches and eliminated some contradictions in the consideration of HSs from the positions of their

supramolecular organization, because macromolecules can enter into the composition of supramolecular systems.

A no less important result of our study is that the behavior of HSs can be perceived as the behavior of stiff impenetrable particles that may compose the structures of different types and sizes.

ACKNOWLEDGMENTS

This work was supported in part by the Russian Foundation for Basic Research (project no. 13-04-00140) and the Russian Ministry of Education and Science (project no. 37.2486.2014/K).

REFERENCES

1. L. N. Aleksandrova, "Humus as a system of polymeric compounds," in *Transactions of the Jubilee Session Dedicated to the 100th Anniversary of V.V. Dokuchaev* (Academy of Sciences of the Soviet Union, Moscow, 1949), pp. 225–232.
2. M. I. Dergacheva, *Soil Organic Matter: Statics and Dynamics (by Example of Western Siberia)* (Nauka, Novosibirsk, 1984) [in Russian].
3. M. M. Kononova, *Soil Organic Matter* (Academy of Sciences of the Soviet Union, Moscow, 1963) [in Russian].
4. J.-M. Lehn, *Supramolecular Chemistry Concepts and Perspectives* (Wiley, New York, 1995).
5. D. S. Orlov, *Humic Acids of Soils and General Theory of Humification* (Moscow State University, Moscow, 1990) [in Russian].
6. N. G. Rambidi and A. V. Berezkin, *Physical and Chemical Basis of Nanotechnologies* (Fizmatlit, Moscow, 2008) [in Russian].
7. Yu. D. Semchikov, *High Molecular Compounds* (Akademiya, Moscow, 2006) [in Russian].
8. A. A. Tager, *Physical Chemistry of Polymers* (Khimiya, Moscow, 1978) [in Russian].
9. R. L. Tate, *Soil Organic Matter* (Wiley, New York, 1987).
10. O. A. Trubetskoi and O. E. Trubetskaya, "Soil humic acids: macromolecules or supramolecular complex," in *The International Conference "Role of Biodiagnostics Ecological Assessment of Soils and Connecting Media," Abstracts of Papers* (Binom. Laboratoriya Znanii, Moscow, 2013), p. 217.
11. G. N. Fedotov, G. V. Dobrovolskii, and T. F. Rudometkina, "Influence of nanostructure organization of soil gels on the water stability of soil structure," Dokl. Biol. Sci. **441** (1), 377–380 (2011).
12. G. N. Fedotov, G. V. Dobrovolskii, and T. F. Rudometkina, "Influence of microphase stratification of soil gels on catalase activity of soils," Dokl. Biol. Sci. **438** (1), 179–181 (2011).
13. G. N. Fedotov, G. V. Dobrovol'skii, and S. A. Shoba, "On the mechanism of nanostructural organization of soil gels," Dokl. Biol. Sci. **445** (1), 268–271 (2012).
14. G. N. Fedotov, G. V. Dobrovol'skii, S. A. Shoba, T. F. Rudometkina, M. S. Cherich, and V. S. Shalaev, "Microphase segregation in humus systems," Dokl. Chem. **429** (1), 283–285 (2009).
15. P. G. Khalatur, "Self-organization of polymers," Soros. Obraz. Zh. **7** (4), 36–43 (2001).
16. V. S. Khmelevskaya, "Self-organization in the solid object," Soros. Obraz. Zh. **6** (6), 85–91 (2000).
17. A. R. Khokhlov, "Sensitive gels," Soros. Obraz. Zh. **4** (11), 138–142 (1998).
18. A. R. Khokhlov, "Design of AB-co-polymers," Soros. Obraz. Zh. **6** (10), 108–115 (2000).
19. A. R. Khokhlov and E. E. Dormidontova, "Self-organization in ion-containing polymer systems," Usp. Fiz. Nauk **167** (2), 113–128 (1997).
20. A. R. Khokhlov and S. I. Kuchanov, *Lectures on Physical Chemistry of Polymers* (Mir, Moscow, 2000) [in Russian].
21. A. Piccolo, "The supramolecular structure of humic substances," Soil Sci. **166** (11), 810–832 (2001).
22. G. E. Schaumann, "Review of Article soil organic matter beyond molecular structure Part I: Macromolecular and supramolecular characteristics," J. Plant Nutr. Soil Sci. **169**, 145–156 (2006).
23. F. J. Stevenson, *Humus Chemistry. Genesis, Composition, Reactions* (Wiley, New York, 1994).
24. R. Sutton and G. Sposito, "Molecular structure in soil humic substances: the new view," Environ. Sci. Technol. **39**, 9009–9015 (2005).

Translated by K. Pankratova