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Research Article

Humic nanoparticles as a tool for eliminating the toxicity of zinc L-valinate.

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Abstract

Earlier, we showed (Morozova M.A. et al., 2022) that solutions of the humic acids (HAs) and fulvic acids (FAs) complex used as a solvent are applicable for increasing the solubility and colloidal stability of antiviral drugs. In this study, we investigated the ability of humates to form stable colloidal systems with chelated zinc complexes with amino acids. The study of the dispersion properties of the samples was carried out using the dynamic light scattering (DLS) technique. There were obtained HAs and FAs dilutions that form a colloidal solution with zinc chelate complexes with specified characteristics of particle size and zeta potential. The solutions contained mainly 20 nm nanoparticles and their zeta potential was -24 mV. The toxicity was assessed using the Spirotox method. A comparative toxicological analysis of zinc valinate samples dissolved in HAs and water showed that the use of HAs as an adjuvant allows for a radical decrease in the toxicity of zinc chelate.

Keywords: extract of humic substances; fulvic acid; zinc chelate complexes; zincvalinate; toxicity; Spirotox-method

INTRODUCTION

Modification of the dispersion properties of a dosage form has long been the key to changing the pharmacokinetic properties of drugs [1-3]. The inclusion of an active pharmaceutical ingredient (API) in dosage forms of nanoparticles, nanomicelles, microemulsions, and liposomes enables improving the pharmacokinetic parameters (absorption, bioavailability, distribution, targeted delivery) of the drug [4,5].

For example, the inclusion of surface-active lipids in the composition of the drug allowed obtaining liposomal dosage forms that are distinguished by their high therapeutic efficacy rates achieved through stabilization, increased permeability through natural barriers and targeted delivery of the API [6]. An important consequence of targeted delivery of the API in this case is a decrease in the systemic toxicity of the drug. These characteristics of liposomal drugs, together with biodegradability and reduced toxicity of the liposomes themselves, determine the interest in them.

Modification of the physicochemical properties of nanoparticles, such as size, shape, structure, zeta potential and surface functionality, is achieved by obtaining a drug with targeted delivery of the API [7]. Stabilization of dispersed phase particles by regulating the zeta potential allows for an increase in the drug shelf life, as well as an increase in its bioavailability, as has been shown in the example of self-nanoemulsifying drug delivery systems [8].

Natural polyelectrolytes are of interest in the development of new drug delivery systems as a replacement for synthetic carriers, which are more expensive and less environmentally friendly [9]. An example of such natural polyelectrolytes is humic acids (HAs) and fulvic acids (FAs), which have great potential for medical use [10,11]. Humic substances (HS) are heterogeneous, polydisperse mixtures consisting of polymer structures that are formed in soils, bottom sediments, and natural waters during the destruction of microbial and plant residues [12]. HS are characterized by a large number of polar groups in their structures, such as carboxyl, hydroxyl, and phenolic ones; thereby humic

substances are anionic polyelectrolytes. This allows humic substances to chelate metals, forming structures with high bioavailability [13]. As some studies show [14], humic substances are also able to increase the bioavailability of organic molecules. The main components of humic substances are fulvic acids, which are soluble in the entire pH range, while humic acids precipitate at pH <2 [15]. In recent years, various data have appeared on the pharmacological activity of humic and fulvic acids in relation to HIV-1 [16], influenza virus [17], herpes simplex virus-1 [18], tick-borne encephalitis virus [19], Enterococcus faecalis, and Klebsiella pneumoniae [20]. The specific virucidal activity of the HAs and FAs complex against SARS-CoV-2 was also established [21]. Thus, humic and fulvic acids can be used as drug delivery systems and also have their own pharmacological effect: antiviral or antibacterial.

Zinc micronutrient deficiency in the population is a public health problem in a wide range of countries [22]. Zinc deficiency is associated with severe diseases of various etiologies [23,24]. Chelated zinc compounds with amino acids are noteworthy owing to the high bioavailability of the metal and the absence of an irritant effect on the esophagus compared to inorganic salts [25,26]. Therefore, the development of effective, safe, and low-cost approaches to obtain zinc delivery systems is an important task in the fight against zinc deficiency conditions. This study investigated the possibility of using a humic-fulvic acid complex to reduce the toxicity of zinc valinate through the specific interaction with HAs and FAs nanoparticles that changes their dispersion.

MATERIALS AND METHODS

Chemicals

HAs and FAs

We used a liquid concentrated complex of humic and fulvic acids isolated from lowland peat according to the patented technology of the company VimaVita (LLC System-BioTechnologies, Moscow, Russia). Concentrated humic complex (HC) containing purified water and active components HAs, hymatomelanic acids, FAs and structural analogs of humic substances, was obtained by oxidative-hydrolytic degradation of lignin-containing raw materials followed by high-intensity acoustic cleaning. The test solution was a concentrated dark brown viscous liquid with pH = 7.98 ± 0.1 and dry matter content 7.34×10^{-2} g/mL [21]. To study the properties of the humic complex as a delivery system for antiviral drugs the original preparation was not used, but its aqueous dilutions in the ratio from 1:400 to 1:800 by volume. For dilution, highly purified water was used, obtained using the Milli-Q® purification system (Merck, Darmstadt, Germany). All investigated solutions were stored at room temperature for no longer than 24 h.

Reagents

L-valine (98.0%, Sigma-Aldrich Co., Massachusetts, United States), zinc sulfate monohydrate (99.0%, Acros Organics, Barcelona, Spain) and all other chemicals used (potassium hydroxide) were of analytical grade.

Preparation of solutions

Dilutions of humic and fulvic acids were obtained by dissolving the HAs and FAs concentrate (dry matter content 7.34×10^{-2} g/mL [21]) in MQ water (v/v) with constant stirring on a magnetic stirrer.

Zinc valinate solutions were obtained by dissolving L-valine and zinc sulfate (in a molar ratio of 2:1) in the obtained dilutions of HAs and FAs, with constant stirring on a magnetic stirrer. After dissolving the powders of the substances, the pH of the solution was adjusted to a value of 6.00 by adding 0.01 M potassium hydroxide aliquots with constant stirring.

Methods

Dynamic Light Scattering (DLS)

A Zetasizer Nano ZSP (Malvern Panalytical, Worcestershire, UK) based on dynamic light scattering was used to measure the size of nanoparticles in the aqueous solutions of zinc chelate complexes with valine. Disposable polystyrene cuvettes, filled with 1 mL of sample, were used. For each size determination, three replicate measurements were performed, and the average size value was calculated. Each measurement consisted of 12 runs. The refractive index value was 1.3400. To measure the zeta potential, laser Doppler microelectrophoresis was used, based on determining the velocity of movement of nanoparticles using electrophoresis. Each sample was measured 5 times.

Cellular Biosensor *Spirostomum ambiguum* for Testing the Biological Activity

Spirostomum ambiguum is the protozoan ciliate that is widely used as a test culture for toxicological and pharmacological studies of individual and combined biological activity of medicines [27].

The advantage of this model is due to the fact that the sensitivity of the eukaryotic cell makes it possible to interpret the obtained toxicity results in relation to multicellular organisms and humans. Under favorable conditions in a low-mineralized environment, cells do not die for a period exceeding their cell cycle (about 20 h). When it is introduced into an environment with chemical compounds, it dies within a time interval that is a function of concentration and temperature. [28]

The experiment was carried out in a temperature range of 22 – 32 °C (in increments of 2 °C). The experimental installation included a thermostatically controlled 5-hole plate (Lauda Alpha A6 termostat, Göttingen, Germany) and an MBS-10 binocular. Low-power fluorescent daylight lamps (10 W) were used for additional lighting.

One test infusoria *S. ambiguum* and 250 µl of the test solution and were introduced into each of the plate holes. Five measurements were carried out at each test temperature for each solution of the test sample. The cell lifetime was calculated as the interval from the moment of introducing to the solution to cell death. The cell death was determined by immobilization with no contractile reaction to mechanical irritation or by the rupture of the membrane with the release of the contents of the protoplasm outwards.

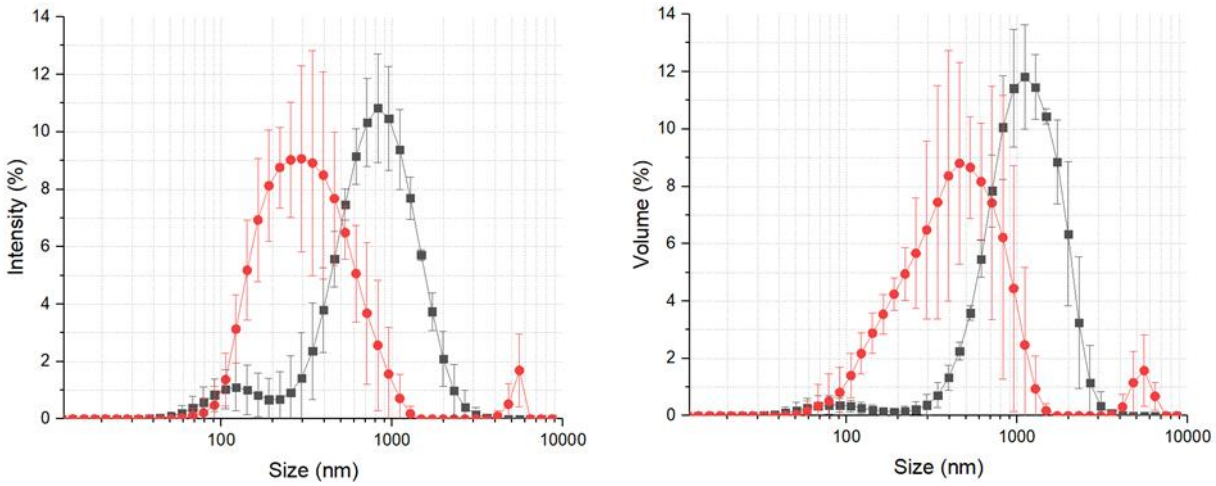
Molecular modeling and data processing

Calculations, statistical processing and visualization of measurements were performed using the OriginPro 2021 software (OriginLab, USA).

RESULTS AND DISCUSSION

Dispersion characteristics of colloids of humic dilutions containing dissolved substances.

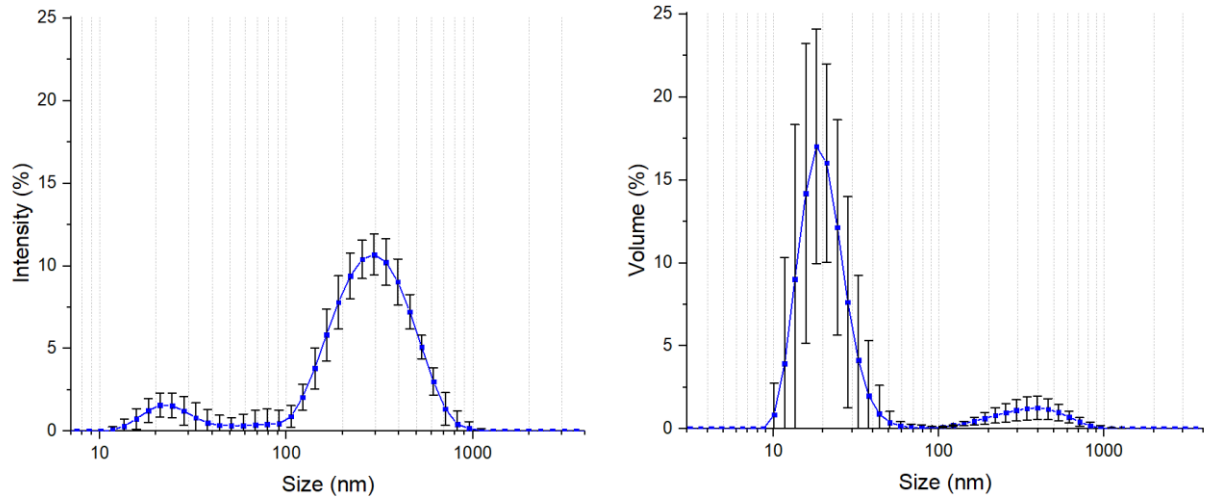
As was shown earlier [29], the humic and fulvic acids complex forms a polydisperse system in water with a tendency to fragment the particles of the dispersed phase upon dilution. Thus, large particles disintegrate upon dilution, releasing smaller particles. We managed to reproduce these results – the results obtained are presented in Figure 1.



(**a**) (**b**)

Figure 1: Size (**a**) and volume (**b**) distributions of particles in nanodispersion of HAs and FAs dilutions (v/v): 1:400 ($18.4*10^{-3}$ g/mL) – black, 1:800 ($9.18*10^{-3}$ g/mL) – red (n=5).

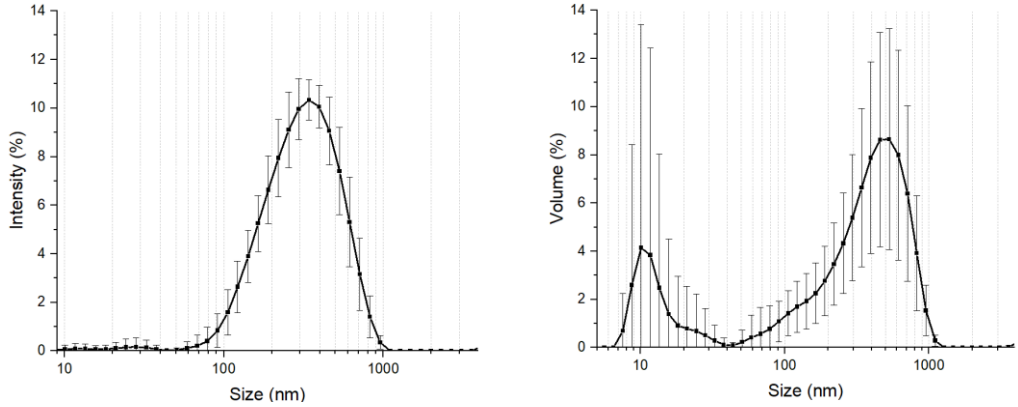
The obtained results characterize the 1:400 and 1:800 HAs and FAs dilutions (obtained from the HS concentrate with a dry residue of 7.34×10^{-2} g/mL) as monodisperse systems with a submicron particle size[21]. The pH of the solvent (HAs and FAs) is 6.00 according to the conditions for the formation of zinc chelates determined in one of the previous studies [49]. The change in the size spectra of HAs and FAs with the formation of zinc chelates in solution is shown in Figure 2.



(**a**) (**b**)

Figure 2: Size (a) and volume (b) distributions of particles of a 20mM zinc valinate solution in HAs and FAs dilution (1:800) (n=5).

As shown in Fig. 2, when zinc valinate is formed in a 1:800 HAs and FAs dilution, the solution has a bimodal distribution of particles: 20 and 300 nm. It is noteworthy that the most abundant particles in the solution, in terms of volume, are formed in the nanoscale range. The dispersion characteristics of the 1:400 dilution were also studied by the DLS method and are presented in Figure 3.



(**a**) (**b**)

Figure 3: Size (a) and volume (b) distributions of particles of a 20mM zinc valinate solution in a HAs and FAs dilution (1:400) (n=5).

According to the results of the dispersion analysis, zinc solution in 1:400 HAs and FAs is a virtually monodisperse colloid with a particle size of about 350 nm. The formation of nanoparticles in it is virtually not observed. Considering the HAs and FAs dilutions as an adjuvant for zinc valinate, it is clear that the 1:800 dilution is more preferable in terms of particle size and the resulting potential for increased bioavailability and colloidal stability.

To analyze the colloidal characteristics and obtained HAs and FAs dilutions, the zeta potential (ζ) of disperse systems was determined. This indicator is the electrokinetic potential arising at the boundary of the sliding layer of a particle. It is used as a measure of colloidal stability of dispersions [30-32]. Since zinc valinate is synthesized in a HAs and FAs dilution, the zeta potential was measured for the aggregate of components of the target solution and for individual substances separately to assess the contribution of each compound to the stability of the entire sample. The results obtained are presented in Table 1.

Table 1: Zeta potential of disperse systems with variable composition based on HAs and FAs dilution (1:800).

GC,dilution (water)	Val, mM	Zn^{2+}, mM	ζ, mV
1:800	20	0.25	-24±9
	20	0	0
	0	0.25	-23±8
	0	0	-32±10

Based on the obtained results, the most stable solution is the 1:800 HAs and FAs dilution. When chelated components are added, the value of the zeta potential decreases. However, although the L-valine solution in HAs and FAs has minimal stability, the value of the zeta potential for zinc valinate is at a level that suggests the stability of the colloidal solution.

Toxicological analysis of zinc valinate in various solvents

A widely studied biological object [33-36], which has proven itself well in the study of acute toxicity, is the cellular biosensor *Spirostomum ambiguum* [37-40]. The study of the death kinetics of this ciliate formed the basis of the Spirotox method. S. ambiguum is an obligate inhabitant of oligotrophic natural reservoirs and is completely insensitive to humates [21], which, in turn, is explained by the origin of humic substances, which are natural components of natural waters. Thus, we were interested in studying the effect of the solvent nature on the acute toxicity of zinc chelate complexes using the Spirotox method. The results obtained are presented in Table 2.

Table 2: Life time of *S. ambiguum* in zinc valinate solutions based on dilution of humic and fulvic acids or MQ water.

Temperature, °C	Mean lifetime±SD, c (n=5)	
	$Zn^{0.25mM}$+Val^{20mM}(HAs and $FAs^{1:800}$)	$Zn^{0.25mM}$+Val^{20mM}(MQ water)
22	>1800	528±51
24	>1800	321±64
26	>1800	205±4
28	>1800	135±21
30	1491±164	99 ±21
32	508±26	-

The study of the *Spirostomum ambiguum* death kinetics revealed a significant difference in the lifetime of the biosensor in solutions containing zinc valinate dissolved in water and in a 1:800 HAs and FAs dilution. The ciliates lived for more than 30 minutes in the zinc valinate medium dissolved in a 1:800 HAs and FAs dilution in the temperature range of 22-28 °C. Whereas the ciliates lived from 1.5 to 8 minutes in the aqueous solution of zinc valinate in the same temperature range. However, *S. ambiguum* died in the aqueous solution at 32 °C too fast to obtain a result with a relatively low error. The lifetime of the cellular biosensor was recorded in the humate solution of zinc valinate at 30-32 °C, but the obtained values were significantly higher in comparison with the control experiment.

CONCLUSIONS

The use of colloids to increase solubility, bioavailability and improve the dispersion characteristics of substances of various natures is widely presented in the literature [41-43]. There are known examples of the use of humic substances as a disperse system that includes various compounds into the particles of the dispersed phase according to the host-guest principle [44]. Such colloids can be used as a drug delivery system [45,46]. An important circumstance is the need to control the particle size and colloidal stability of the resulting disperse systems, which were controlled in this work. A method for obtaining a disperse system with preset colloidal characteristics was shown.

The analysis of the biological activity of the dispersed solutions obtained by the Spirotox method showed a possible way to reduce the acute toxicity of metal chelates, i.e. the use of a HAs and FAs dilution as a solvent. This approach can be used as a potential way to reduce the toxicity of chelating compounds and therefore requires more attention. There are also known examples of the use of humic and fulvic acids to reduce the toxicity and increase the bioavailability of d-metals [47]. Usually, the results obtained by the Spirotox method represent the observation activation energy [28], which is a linear function of LD_{50} for a wide range of orally administered compounds in rats [48]. This toxicological indicator has proven itself as a way to avoid inhumane methods in assessing acute toxicity. However, this study did not allow obtaining $^{obs}E_a$ due to the low toxicity of the studied sample.

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Ethical approvals: This study does not involve experiments on animals or human subjects.

Data Availability Statement: Data supporting reported results can be found on request by e-mail vntumasov@ya.ru.

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REFERENCES:

1. Gaudana R, Ananthula HK, Parenky A, Mitra AK. Ocular drug delivery. AAPS J. 2010 Sep;12(3):348-60. doi: 10.1208/s12248-010-9183-3. Epub 2010 May 1 https://doi.org/10.1208/s12248-010-9183-3 PMid:20437123 PMCid:PMC2895432

2. Gao W, Zhang Y, Zhang Q, Zhang L. Nanoparticle-Hydrogel: A Hybrid Biomaterial System for Local-ized Drug Delivery. Ann Biomed Eng. 2016 Jun;44(6):2049-61. https://doi.org/10.1007/s10439-016-1583-9 PMid:26951462 PMCid:PMC4880511

3. Villarreal-Otalvaro C, Coburn JM. Fabrication Methods and Form Factors of Gellan Gum-Based Materials for Drug De-livery and Anti-Cancer Applications. ACS Biomater Sci Eng. 2023 Jul 10;9(7):3832-3842. https://doi.org/10.1021/acsbiomaterials.1c00685 PMid:34898174

4. Feng T, Wei Y, Lee RJ, Zhao L. Liposomal curcumin and its application in cancer. Int J Nanomedicine. 2017 Aug 21;12:6027-6044. https://doi.org/10.2147/IJN.S132434 PMid:28860764 PMCid:PMC5573051

5. Ding B, Zheng P, Tan J, Chen H, Meng Q, Li J, Li X, Han D, Li Z, Ma X, Ma P, Lin J. Sodium Bicar-bonate Nanoparticles for Amplified Cancer Immunotherapy by Inducing Pyroptosis and Regulating Lactic Acid Metabolism. Angew Chem Int Ed Engl. 2023 Oct 2;62(40):e202307706. https://doi.org/10.1002/anie.202307706 PMid:37587061

6. Guimarães D, Cavaco-Paulo A, Nogueira E. Design of liposomes as drug delivery system for therapeutic applications. Int J Pharm. 2021 May 15;601:120571. https://doi.org/10.1016/j.ijpharm.2021.120571 PMid:33812967

7. Beach MA, Nayanathara U, Gao Y, Zhang C, Xiong Y, Wang Y, Such GK. Polymeric Nanoparticles for Drug Delivery. Chem Rev. 2024 May 8;124(9):5505-5616. https://doi.org/10.1021/acs.chemrev.3c00705 PMid:38626459

8. Arshad A, Arshad S, Alamgeer, Mahmood A, Hussain Asim M, Ijaz M, Muhammad Irfan H, Rubab M, Ali S, Raza Hashmi A. Zeta potential changing self-nanoemulsifying drug delivery systems: A newfangled approach for enhancing oral bioavailability of poorly soluble drugs. Int J Pharm. 2024 Apr 25;655:123998. https://doi.org/10.1016/j.ijpharm.2024.123998 PMid:38490401

9. Lalevee G., David L., Montembault A., Blanchard K., Meadows J., Malaise S., Crepet A., Grillo I., Morfin I., Delair T., et al. Highly stretchable hydrogels from complex coacervation of natural polyelectrolytes. Soft Matter. 2017;13:6594-6605. https://doi.org/10.1039/C7SM01215B PMid:28905969

10. Winkler J., Ghosh S. Therapeutic Potential of Fulvic Acid in Chronic Inflammatory Diseases and Diabetes. J. Diabetes Res. 2018;10:5391014. https://doi.org/10.1155/2018/5391014 PMid:30276216 PMCid:PMC6151376

11. Zamoshchina T.A., Zykova M.V., Gostyukhina A.A., Logvinova L.A., Zaitsev K.V., Lasukova T.V., Svetlik M.V., Kurtsevich E.A., Abdulkina N.G., Belousov M.V., et al. Effect of Humic Acids from Lowland Peat on Endurance of Rats in Forced Swim Test in Relation to Serum Lactate and Corticosterone. Bull. Exp. Biol. 2020 https://doi.org/10.1007/s10517-020-04967-7 PMid:33098504

12. Hou D., He J., Lü C., Wang W., Zhang F. Spatial Distributions of Humic Substances and Evaluation of Sediment Organic Index on Lake Dalinouer, China. J. Geochem.

13. Di Iorio E, Circelli L, Angelico R, Torrent J, Tan W, Colombo C. Environmental implications of interaction between humic substances and iron oxide nanoparticles: A review. Chemosphere. 2022 Sep;303(Pt 2):135172 https://doi.org/10.1016/j.chemosphere.2022.135172 PMid:35649442

14. Fava F, Piccolo A. Effects of humic substances on the bioavailability and aerobic biodegradation of polychlorinated biphenyls in a model soil. Biotechnol Bioeng. 2002 Jan 20;77(2):204-11 https://doi.org/10.1002/bit.10140 PMid:11753927

15. Anielak AM, Kłeczek A. Humus Acids in the Digested Sludge and Their Properties. Materials (Basel). 2022 Feb 16;15(4):1475 https://doi.org/10.3390/ma15041475 PMid:35208014 PMCid:PMC8880807

16. Nosik D.N., Nosik N.N., Teplyakova T.V., Kiseleva I.A., Kondrashina N.G., Bochkova M.S., Ananko G.G. Antiviral activity of extracts of basidiomycetes and humic compounds substances against Human Immunodeficiency Virus (Retroviridae: Orthoretrovirinae: Lentivirus: Human immunodeficiency virus 1) and Herpes Simplex Virus (Herpesviridae: Simplexvirus: Human alphaherpesvirus 1) Vopr Virusol. 2020;65:276-283 https://doi.org/10.36233/0507-4088-2020-65-5-4 PMid:33533211

17. Lu F.J., Tseng S.N., Li M.L., Shih S.R. In vitro anti-influenza virus activity of synthetic humate analogues derived from protocatechuic acid. Arch. Virol. 2002;147:273-284 https://doi.org/10.1007/s705-002-8319-5 PMid:11890523

18. Klocking R., Helbig B., Schotz G., Schacke M., Wutzler P. Anti-HSV-1 activity of synthetic humic acid-like polymers derived from p-diphenolic starting com-pounds. Antivir. Chem. Chemother. 2002;13:241-249. https://doi.org/10.1177/095632020201300405 PMid:12495212

19. Orlov A.A., Zherebker A., Eletskaya A.A., Chernikov V.S., Kozlovskaya L.I., Zhernov Y.V., Kostyukevich Y., Palyulin V.A., Nikolaev E.N., Osolodkin D.I., et al. Examination of molecular space and feasible structures of bioactive components of humic substances by FTICR MS data mining in ChEMBL database. Sci. Rep. 2019;9:12066 https://doi.org/10.1038/s41598-019-48000-y PMid:31427609 PMCid:PMC6700089

20. Rensburg C.E., Dekker A.S. An in vitro investigation of the antimicrobial activity of oxifulvic acid. J. Antimicrob. Chemother. 2000;46:853-854 https://doi.org/10.1093/jac/46.5.853 PMid:11062218

21. Uspenskaya, E.V.; Syroeshkin, A.V.; Pleteneva, T.V.; Kazimova, I.V.; Grebennikova, T.V.; Fedyakina, I.T.; Lebedeva, V.V.; Latyshev, O.E.; Eliseeva, O.V.; Larichev, V.F.; et al. Nanodispersions of polyelectrolytes based on humic substances: Isolation, physicochemical characterization and evaluation of biological activity. Pharmaceutics 2021, 13, 1954 https://doi.org/10.3390/pharmaceutics13111954 PMid:34834368 PMCid:PMC8623726

22. Gupta S, Brazier AKM, Lowe NM. Zinc deficiency in low- and middle-income countries: prevalence and approaches for mitigation. J Hum Nutr Diet. 2020 Oct; 33(5):624-643. https://doi.org/10.1111/jhn.12791 PMid:32627912

23. Chasapis CT, Ntoupa PA, Spiliopoulou CA, Stefanidou ME. Recent aspects of the effects of zinc on human health. Arch Toxicol. 2020 May; 94(5):1443-1460. https://doi.org/10.1007/s00204-020-02702-9 PMid:32394086

24. Roohani N, Hurrell R, Kelishadi R, Schulin R. Zinc and its importance for human health: An integrative review. J Res Med Sci. 2013 Feb;18(2):144-57.

25. Kim YR, Park JI, Lee EJ, Park SH, Seong NW, Kim JH, et al. Toxicity of 100 nm zinc oxide nanoparticles: a report of 90-day repeated oral administration in Sprague Dawley rats. Int J Nanomedicine. 2014 Dec 15; 9 Suppl 2(Suppl 2):109-26. https://doi.org/10.2147/IJN.S57928 PMid:25565830 PMCid:PMC4279774

26. Chang Y, Wang K, Wen M, Wu B, Liu G, Zhao H, Chen X, Cai J, Jia G. Organic zinc glycine chelate is better than inorganic zinc in improving growth performance of cherry valley ducks by regulating intestinal morphology, barrier function, and the gut microbiome. J Anim Sci. 2023 Jan 3;101:skad279. https://doi.org/10.1093/jas/skad279 PMid:37606553 PMCid:PMC10494877

27. O. V. Levitskaya,1 A. V. Syroeshkin,1 and T. V. Pleteneva. Arrhenius kinetics as a bioactivity assessment criterion for drug substances and excipients Pharmaceutical Chemistry Journal, 2015;49(11). https://doi.org/10.1007/s11094-016-1370-9

28. V. V. Goncharuk, A. V. Syroeshkin, I. A. Zlatskiya, E. V. Uspenskaya, A. V. Orekhovab , O. V. Levitskayab , V. I. Dobrovolskiy, and T. V. Pleteneva. Quasichemical Description of the Cell Death Kinetics of Cellular Biosensor Spirostomum Ambigua for Testing the Biological Activity of Aqueous Solutions. Journal of Water Chemistry and Technology · 2017;39(2):178-187. https://doi.org/10.3103/S1063455X17020072

29. Morozova MA, Tumasov VN, Kazimova IV, Maksimova TV, Uspenskaya EV, Syroeshkin AV. Second-Order Scattering Quenching in Fluorescence Spectra of Natural Humates as a Tracer of Formation Stable Supramolecular System for the Delivery of Poorly Soluble Antiviral Drugs on the Example of Mangiferin and Favipiravir. Pharmaceutics. 2022 Mar 31;14(4):767. https://doi.org/10.3390/pharmaceutics14040767 PMid:35456601 PMCid:PMC9030643

30. Sérgio P. Moura and Ana M. Carmona-Ribeiro. Cationic Bilayer Fragments on Silica at Low Ionic Strength: Competitive Adsorption and Colloid Stability. Langmuir 2003 19 (17), 6664-6667 https://doi.org/10.1021/la034334o

31. Fernando P. Araujo, Denise F. S. Petri, and Ana M. Carmona-Ribeiro. Colloid Stability of Sodium Dihexadecyl Phosphate/Poly(diallyldimethylammonium chloride) Decorated Latex. Langmuir 2005 21 (21), 9495-9501 https://doi.org/10.1021/la051052a PMid:16207027

32. Scott A. Bradford, Hyunjung Kim, Chongyang Shen, Salini Sasidharan, and Jianying Shang. Contributions of Nanoscale Roughness to Anomalous Colloid Retention and Stability Behavior. Langmuir 2017 33 (38), 10094-10105 https://doi.org/10.1021/acs.langmuir.7b02445 PMid:28846425

33. Ishida H, Matsumoto C, Shimada M, Suzaki T. SEM observation of non-fixed and water freeze-dried Spirostomum ambiguum. Eur J Protistol. 2022 Aug; 85:125896. https://doi.org/10.1016/j.ejop.2022.125896 PMid:35709567

34. [Shimada M, Hayakawa MM, Suzaki T, Ishida H. Morphological reconstruction during cell regeneration in the ciliate Spirostomum

ambiguum. Eur J Protistol. 2024 Mar 28 https://doi.org/10.1016/j.ejop.2024.126079 PMid:38593565

35. Mathijssen AJTM, Culver J, Bhamla MS, Prakash M. Collective intercellular communication through ultra-fast hydrodynamic trigger waves. Nature. 2019 Jul https://doi.org/10.1101/428573

36. Uskalova DV, Igolkina YV, Sarapultseva EI. Intravital Computer Morphometry on Protozoa: A Method for Monitoring of the Morphofunctional Disorders in Cells Exposed in the Cell Phone Communication Electromagnetic Field. Bull Exp Biol Med. 2016 Aug. https://doi.org/10.1007/s10517-016-3459-2 PMid:27591872

37. Marukhlenko AV, Morozova MA, Mbarga AMJ, Antipova NV, Syroeshkin AV, Podoprigora IV, Maksimova TV. Chelation of Zinc with Biogenic Amino Acids: Description of Properties Using Balaban Index, Assessment of Biological Activity on Spirostomum Ambiguum Cellular Biosensor, Influence on Biofilms and Direct Antibacterial Action. Pharmaceuticals (Basel). 2022 Aug 9 https://doi.org/10.3390/ph15080979 PMid:36015127 PMCid:PMC9415815

38. Syroeshkin AV, Antipova NV, Zlatska AV, Zlatskiy IA, Skylska MD, Grebennikova TV, Goncharuk VV. The effect of the deuterium depleted water on the biological activity of the eukaryotic cells. J Trace Elem Med Biol. 2018 Dec https://doi.org/10.1016/j.jtemb.2018.05.004 PMid:29773469

39. Syroeshkin AV, Uspenskaya EV, Pleteneva TV, Morozova MA, Zlatskiy IA, Koldina AM, Nikiforova MV. Mechanical Transformation of Compounds Leading to Physical, Chemical, and Biological Changes in Pharmaceutical Substances. ScientificWorldJournal. 2018 Dec https://doi.org/10.1155/2018/8905471 PMid:30643492 PMCid:PMC6311245

40. Nałęcz-Jawecki G, Wawryniuk M, Giebułtowicz J, Olkowski A, Drobniewska A. Influence of Selected Antidepressants on the Ciliated Protozoan Spirostomum ambiguum: Toxicity, Bioaccumulation, and Biotransformation Products. Molecules. 2020 Mar 25 https://doi.org/10.3390/molecules25071476 PMid:32218111 PMCid:PMC7180767

41. Rashid I, Murtaza G, Dar AA, Wang Z. The influence of humic and fulvic acids on Cd bioavailability to wheat cultivars grown on sewage irrigated Cd-contaminated soils. Ecotoxicol Environ Saf. 2020 Dec https://doi.org/10.1016/j.ecoenv.2020.111347 PMid:32961489

42. Šebesta M, Koleněcík M, Urík M, Bujdoš M, Vávra I, Dobroečka E, Smilek J, Kalina M, Diviš P, Pavúk M, Miglierini M, Kratošová G, Matúš P. Increased Colloidal Stability and Decreased Solubility-Sol-Gel Synthesis of Zinc Oxide Nanoparticles with Humic Acids. J Nanosci Nanotechnol. 2019 May 1 https://doi.org/10.1166/jnn.2019.15868 PMid:30501816

43. Mohd Omar F, Abdul Aziz H, Stoll S. Aggregation and disaggregation of ZnO nanoparticles: influence of pH and adsorption of Suwannee River humic acid. Sci Total Environ. 2014 Jan 15 https://doi.org/10.1016/j.scitotenv.2013.08.044 PMid:24029691

44. Kowalewska A, Nowacka M. Supramolecular Interactions in Hybrid Polylactide Blends-The Structures, Mechanisms and Properties. Molecules. 2020 Jul 23 https://doi.org/10.3390/molecules25153351 PMid:32718056 PMCid:PMC7435468

45. Mirza MA, Agarwal SP, Rahman MA, Rauf A, Ahmad N, Alam A, Iqbal Z. Role of humic acid on oral drug delivery of an antiepileptic drug. Drug Dev Ind Pharm. 2011 Mar;37(3):310-9. https://doi.org/10.3109/03639045.2010.512011 PMid:20815797

46. Murugesan G, Latha N, Suganya K, Murugan M, Munusamy MA, Rajan M. Stimulus-responsive zinc oxide-functionalized macromolecular humic acid nanocarrier for enhancement of antibacterial activity of ciprofloxacin hydrochloride. Int J Biol Macromol. 2018 Jul 15;114:1109-1116. https://doi.org/10.1016/j.ijbiomac.2018.03.120 PMid:29578024

47. Cuprys A, Pulicharla R, Lecka J, Brar SK, Drogui P, Surampalli RY. Ciprofloxacin-metal complexes -stability and toxicity tests in the presence of humic substances. Chemosphere. 2018 Jul;202:549-559. https://doi.org/10.1016/j.chemosphere.2018.03.117 PMid:29587236

48. Pleteneva, T. V., Galkina, D. A., Fatkulina, O. A., Ogotoeva, D. D., Levitskaya, O. V., Uspenskaya, E. V., & Syroeshkin, A. V. (2023). Arrhenius kinetics in the evaluation of the biological activity of pharmaceutical tinctures. International Journal of Applied Pharmaceutics, 15(4), 277-281. https://doi.org/10.22159/ijap.2023v15i4.48058

49. Tumasov V.N., Marukhlenko A.V., Novikov A.P., Hoang Q.T.N., Koldina A.M., Morozova M.A. Chiral Properties of Zinc Complexes with Bi- and Tridentate Ligands of L- and D-Amino Acids. JAPS, 2025 15 (4). https://doi.org/10.7324/JAPS.2025.209656