The 2-nd International Electronic Conference on Biomolecules: Biomacromolecules and the Modern World Challenges

Structure, properties and biological activity of chitosan salts with *L*- and *D*-aspartic acid

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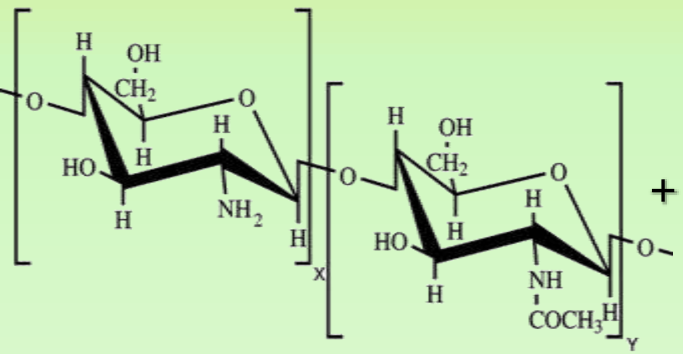
1–15 November 2022

Abstract

A comprehensive study of the structure, properties and biological functionality of salt chitosan complexes with *L*- and *D*-aspartic acid (AspA) was carried out. It has been established that these polymer salts differ in spatial organization, chirooptic characteristics, surface charge, and macrocoil size. In experiments *in vitro* on a wide range of biological objects (unicellular algae, planktonic crustaceans, aerobic bacterial microorganisms, cell cultures, and test plants) it was found that the chitosan salt with D-AspA exhibited the best biological activity. The results obtained confirm our hypothesis that the biological homochiral hierarchy principles are most consistent with chitosan (D-aminoglucan) derivatives with the D-antipode of the acid.

Preparation of chitosan aspartate

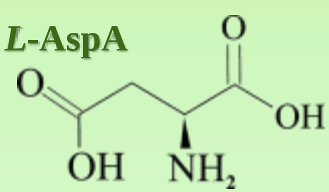


Chitosan (CS)

Molecular weight of 200 kDa Degree of deceleration DD = 80 mol.% («Bioprogress Ltd.», RF)

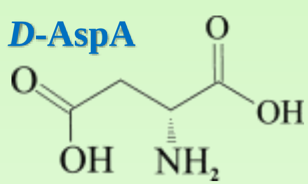
+

***L*-AspA**



(«Bioamid», RF)

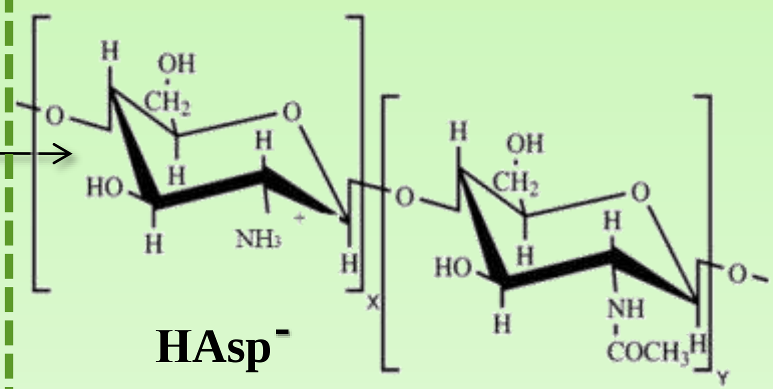
***D*-AspA**



(«Vekton», RF)

Aspartic acid (AspA)

→



HAsp$^-$

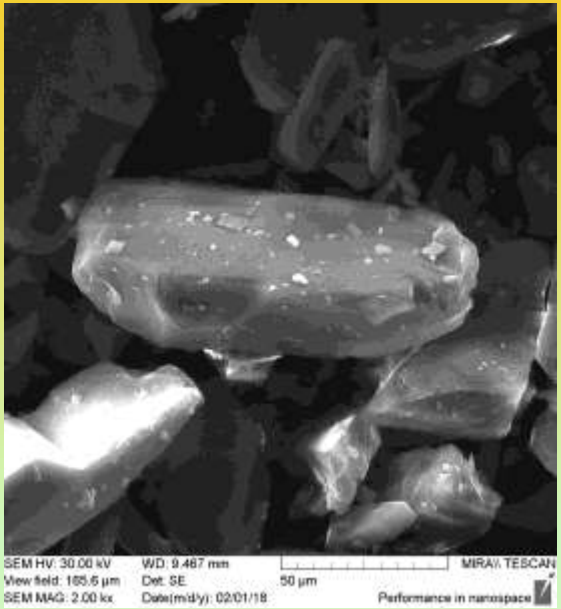
CS·*L*-AspA

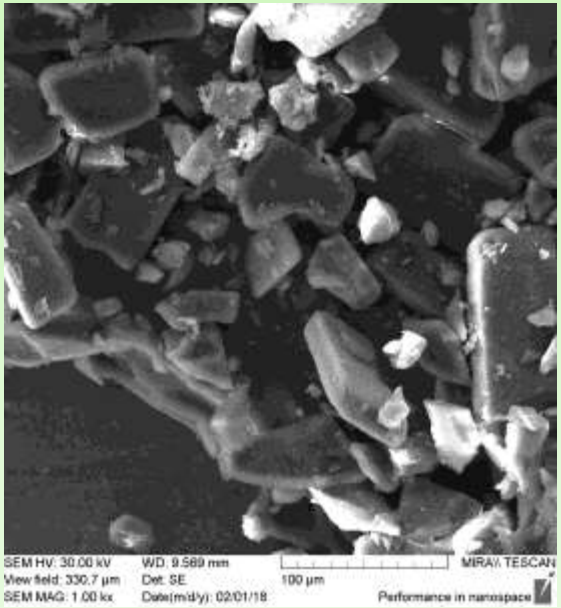
or

CS·*D*-AspA

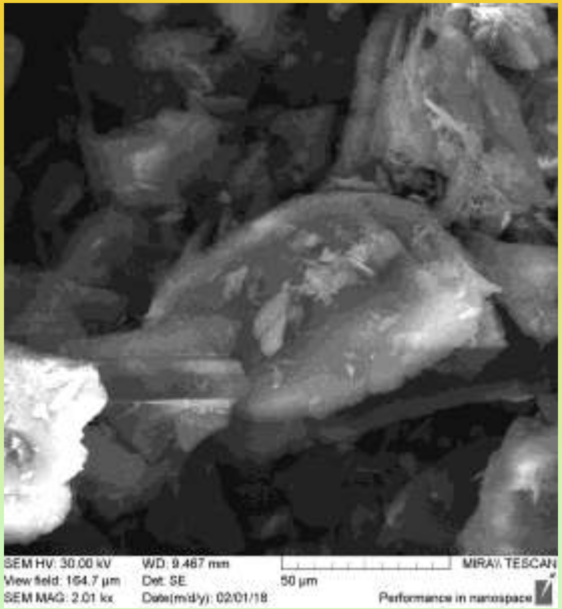
SEM photos of the powders AspA

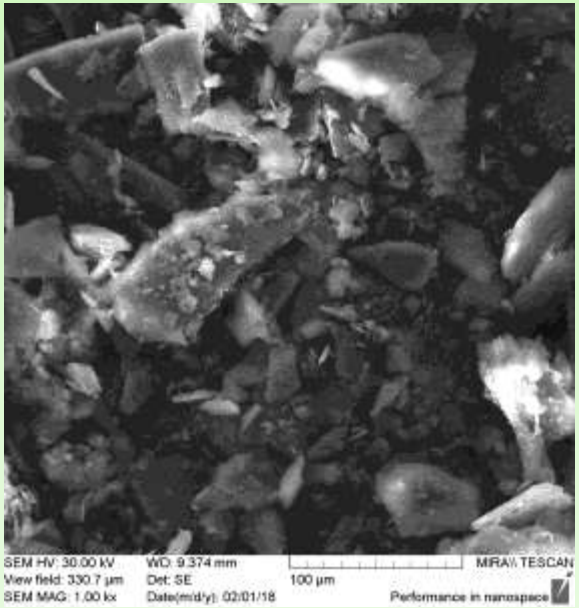
L-AspA





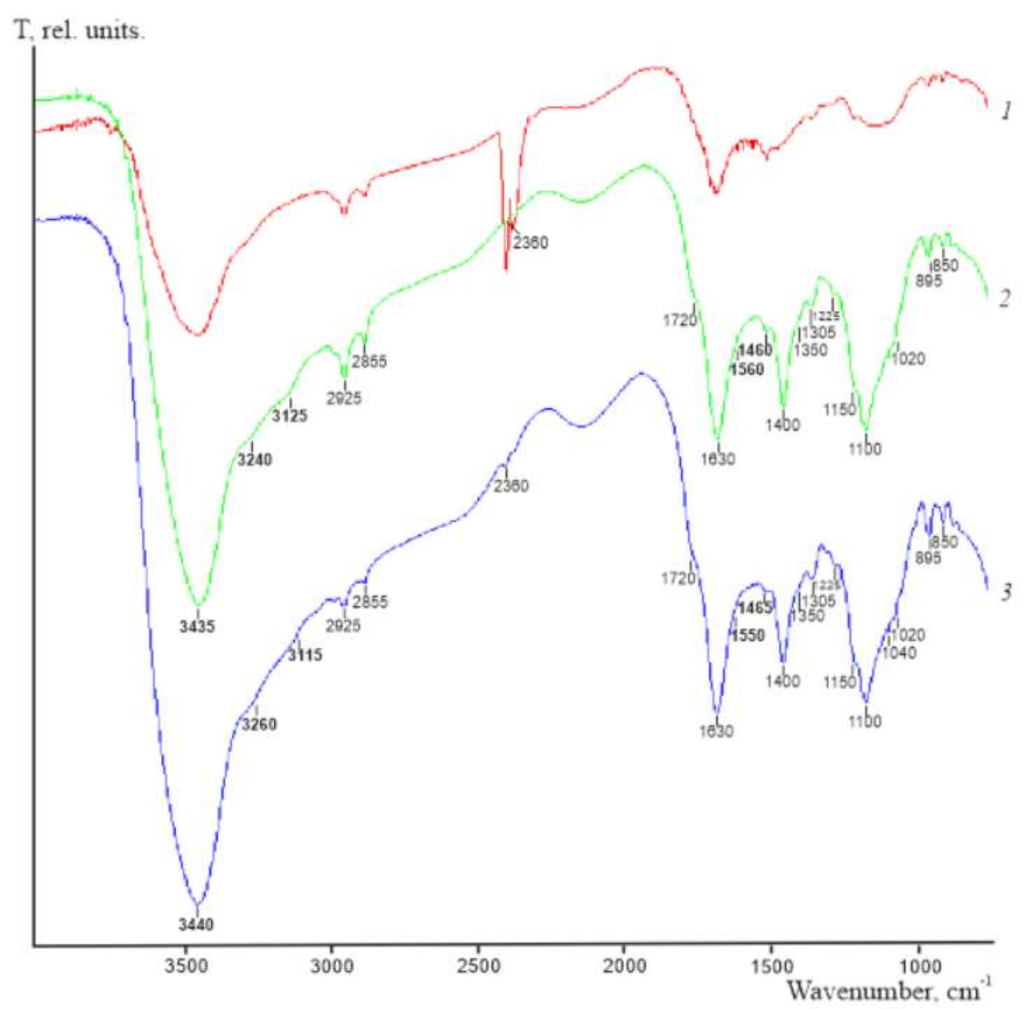
D-AspA





IF spectroscopy

Oscillation types	Wave numbers ν, cм$^{-1}$	
	CS·*L*-AspA	CS·*D*-AspA
ν_{O-H}	**3435**	**3440**
ν_{N-H}	**3240**	**3260**
ν_{N-H} (NH_3^+)	**3125**	**3115**
ν_{as}(CH)	2925	
ν_s(CH)	2855	
ν_{as}(C-N)	2360	
$\nu_{C=O}$ (COOH)	1720	
$\delta_{C=O}$ (Amide I)	1630	
δ_{N-H} (Amide II)	**1560**	**1550**
ν_{C-O} (COO^-)	**1460**	**1465**
δ_{as} (CH)	1400	
ν_{C-N} (Amide III)	1350, 1305	
δ(OH)	1225	
ν(C−C), ν(C−O), ν(C−N), δ(C−H) Glucopyranose ring	1150	
	1100	
	1020	1040, 1020
	895	

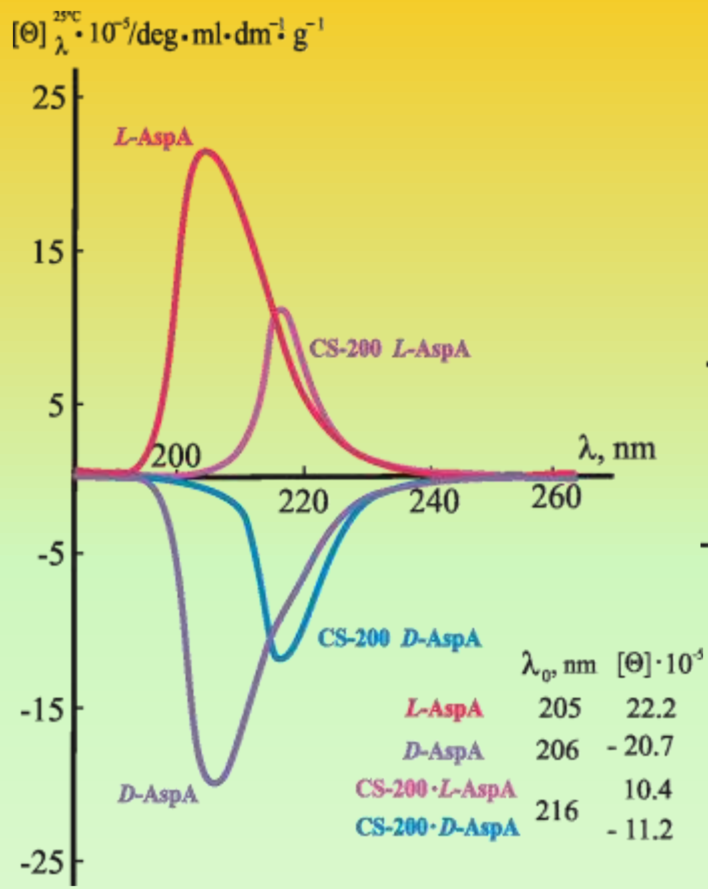


IR spectra of CS powders (*1*), CS·*L*-AspA (*2*) and CS·*D*-AspA (*3*)

Vertex 70v vacuum FTIR spectrometer (Billerica, MA, USA)

Chirooptic properties of solutions

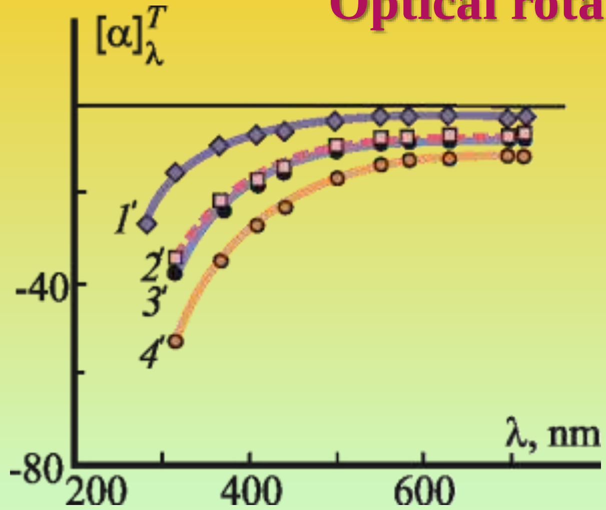
Circular dichroism (CD)

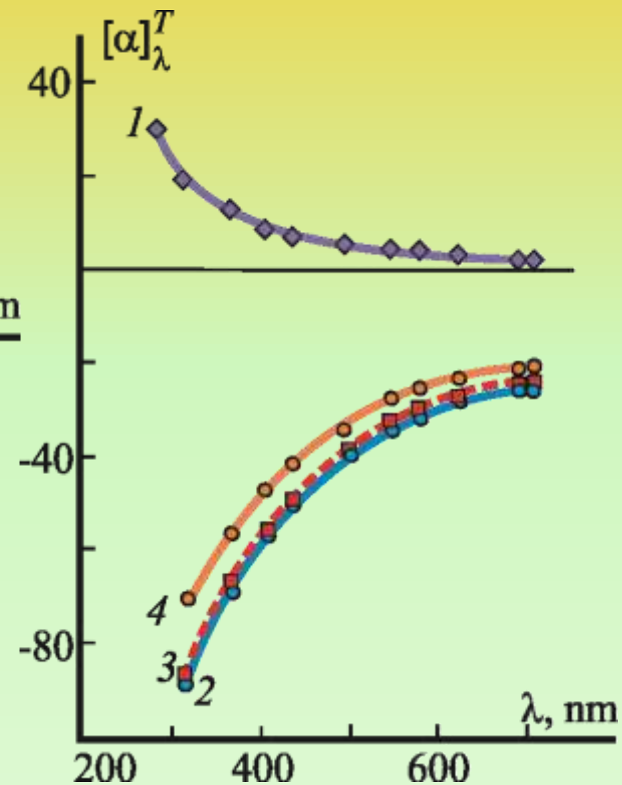


CD spectra of aqueous solutions of *L*-AspA (*1*), *D*-AspA (*2*), CS in *L*-AspA (*3*), and CS in *D*-AspA (4); the molar ratio [AspA]/[~-NH_2] = 0.85 mol/monomol.

Chirascan™ Spectrometer (Applied Photophysics Ltd, USA)

Optical rotation dispersion (ORD)

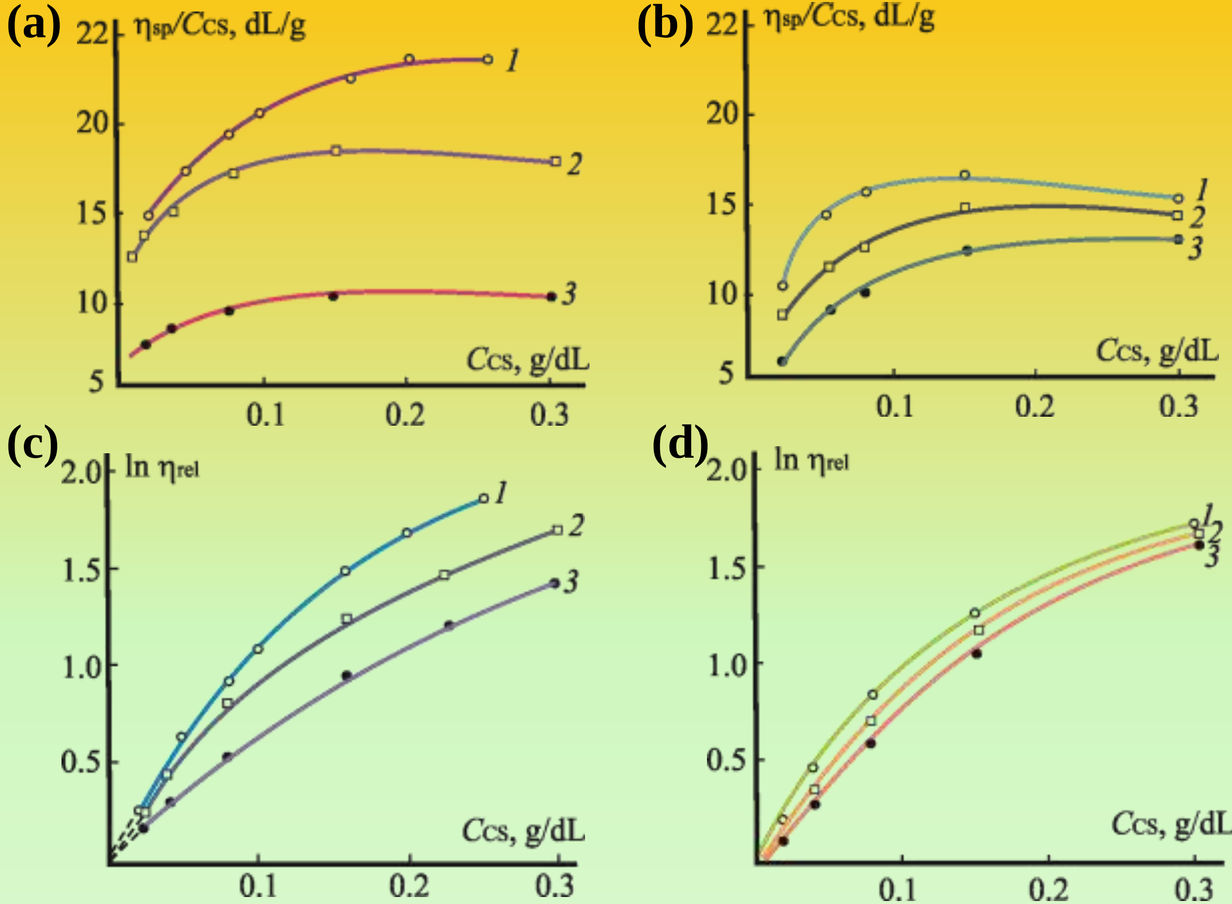




ORD curves for aqueous solutions of *L*-AspA (*1*), *D*-AspA (*1*′), CS in *L*-AspA (*2*– *4*), and CS in *D*-AspA (*2*′– *4*′); the molar ratio [AspA]/[~-NH_2] = 0.85 (*2*, *2*′), 1.0 (*3*, *3*′) and 2.0 mol/monomol (*4*, *4*′).

Automatic SPU-E spectropolarimeter (RF)

Hydrodynamic properties of solutions



Concentration dependences of the viscosity number (a, b) and the logarithm of the relative viscosity (c, d) of CS solutions in *L*-AspA (a, c) and *D*-AspA (b, d) with $C_{AspA} = 1.5 \cdot 10^{-3}$ (*1*), $3.0 \cdot 10^{-3}$ (*2*) and $6.0 \cdot 10^{-3}$ mol/dL (*3*).

Ubbelohde capillary viscometer (Cannon Instrument Company", USA) *d* = 0.56 mm

$C_{AspA} \cdot 10^3$, mol/dL	[η], dL/g		R_{ef}, нм	
	CS·*L*-AspA	CS·*D*-AspA	CS·*L*-AspA	CS·*D*-AspA
1.5	12.5	8.8	73	65
3.0	11.1	8.4	71	64
	3.5*	2.1*	48*	41*
6.0	7.5	6.8	62	60

* *I* = *const* (0.17 M NaCl)

Limit number of viscosity

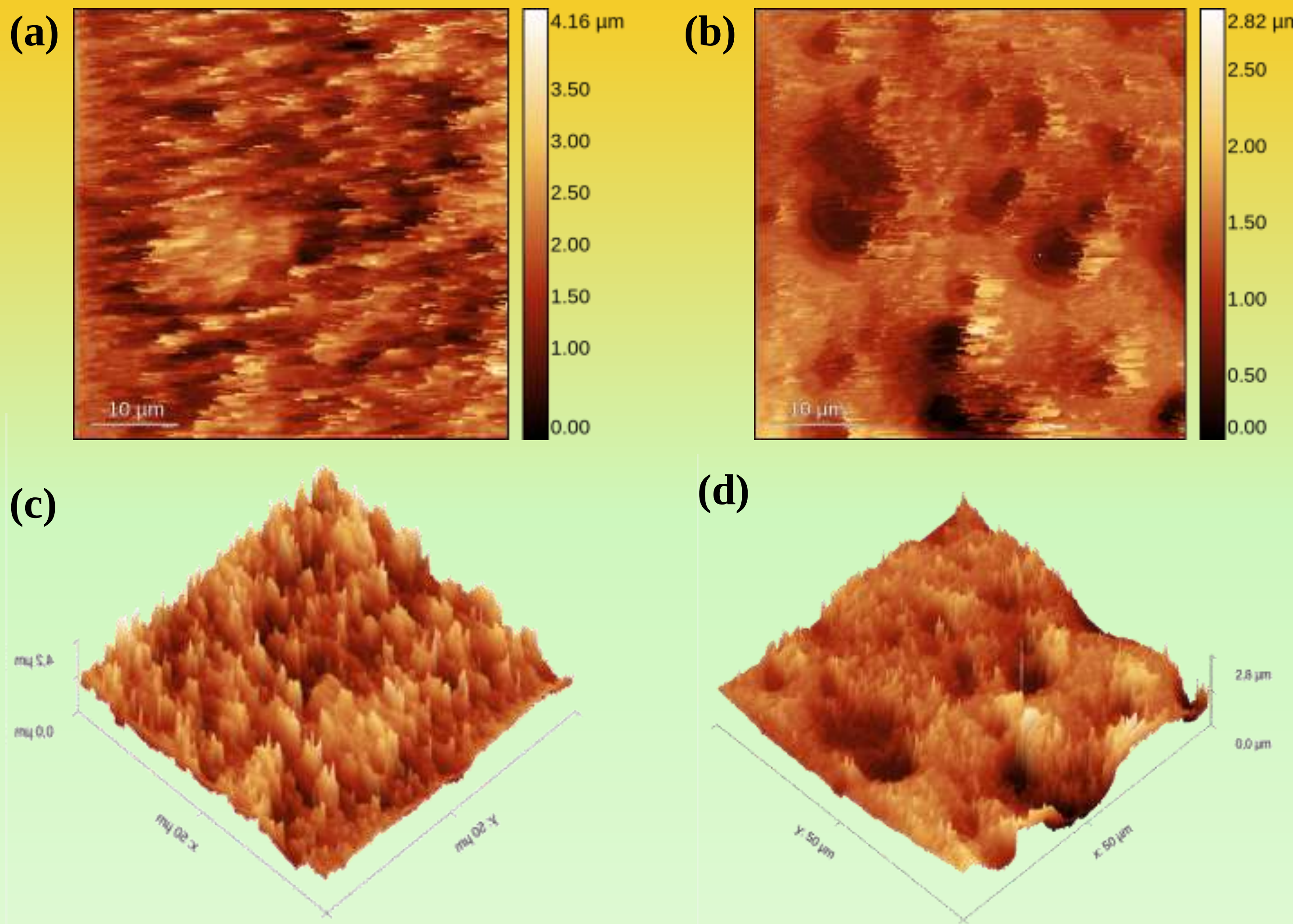
$$[\eta] = \partial \ln\eta_{rel} / \partial C_{CS}$$

[Wolf B.A. // *Macromolecular Rapid Communications*, 2007. Vol.28. No.2. P.164-170]

Effective radius of a macromolecular coil

$$R_{ef} = \sqrt[3]{\frac{3[\eta]\,\overline{M}_\eta}{10\pi N_a}}$$

AFM of the surface of hydrogel plates



AFM images of the surface of hydrogel plates made of CS•*L*-AspA (a, c) and CS•*D*-AspA(b, d) obtained at the molar ratio of [AspA]/[~ -NH_2] = 0.85 mol/monomol.

Ntegra Spectra (NTMDT, Zelenograd)

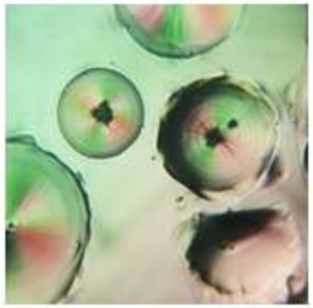
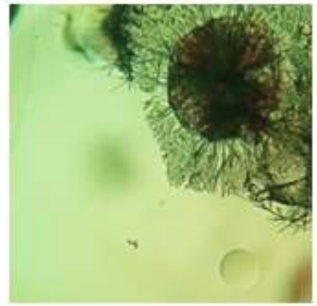
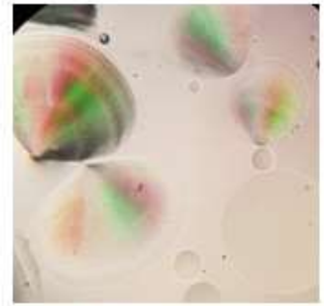
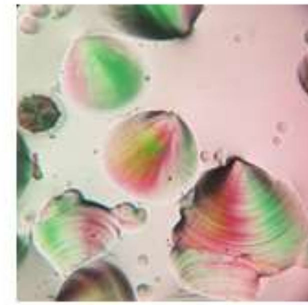
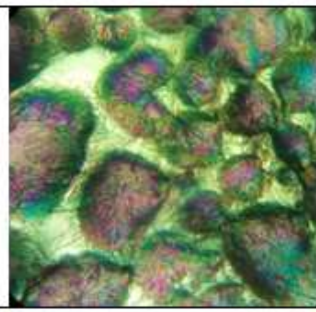
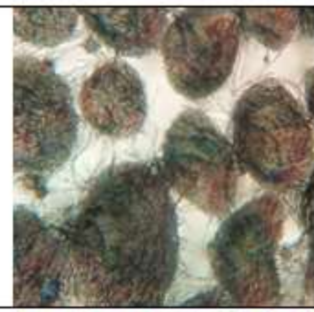
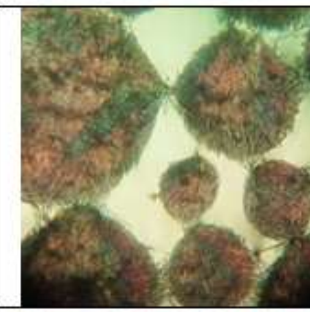
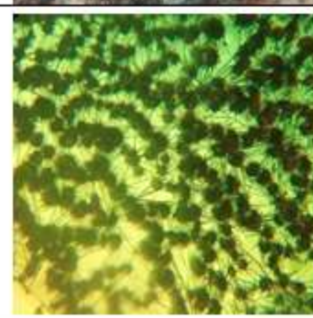
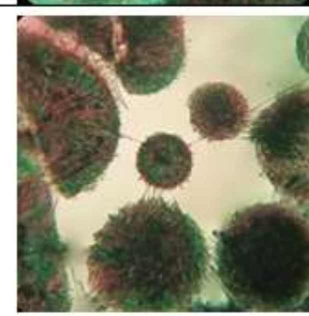
Multi-angle dynamic light scattering

Parameter	$C_{CS}\cdot10^3$, monomol/dL	Temperature, ºC					
		25			37		
		Molar ratio [AspA]/[~-NH_2], mol/monomol					
		0.85	1.0	2.0	0.85	1.0	2.0
CS·*L*-AspA							
ζ-potential, mV	0.9	34.2	39.3	40.5	37.1	38.2	38.9
	2.4	39.3	39.3	35.6	33.3	37.9	35.9
	4.7	37.0	39.6	–	29.2	35.4	–
Wall ζ-potential, mV	0.9	51.0	51.8	41.5	52.2	53.6	49.5
	2.4	44.9	38.9	42.5	38.6	41.5	48.6
	4.7	37.2	29.6	–	28.6	37.3	–
Conductivity, mS/cm	0.9	0.40	0.39	0.51	0.47	0.46	0.61
	2.4	0.71	0.67	0.84	0.85	0.80	1.01
	4.7	1.30	1.24	–	1.56	1.50	–
CS·*D*-AspA							
ζ-potential, mV	0.9	36.5	32.3	33.7	38.0	31.0	36.2
	2.4	37.9	39.4	42.7	37.6	36.4	37.6
	4.7	42.3	33.3	–	34.8	35.5	–
Wall ζ-potential, mV	0.9	36.2	52.6	59.5	45.5	46.7	47.9
	2.4	37.3	40.2	36.0	39.0	39.0	42.9
	4.7	27.9	26.3	–	21.9	29.9	–
Conductivity, mS/cm	0.9	0.44	0.39	0.51	0.52	0.46	0.61
	2.4	0.83	0.76	0.85	0.99	0.76	1.02
	4.7	1.68	1.19	–	2.02	1.44	–

Zetasizer Ultra/Pro (Malvern Panalytical, England)

Polarizing microscopy

Kinetics of crystallization of *L*-menthol in aqueous solutions of CS•*L*-AspA and CS•*D*-AspA

$C_{CS}\cdot10^3$,monomol/dL*	CS·*L*-AspA		CS·*D*-AspA	
	Extractive crystallization time, min			
	5	30	5	30
1.8				
3.5				
4.7				

* [AspA]/[~-NH_2] = 0.85 mol/monomol

Polarization microscope LaboPol-2 (RF), magnification ×10

Complex formation with metal ions

System	Multiplicity of breeding, times	pH	Complex formation ***, %
L-AspA*	10	5.5	47.9±0.7
	100	6.4	21.4±1.1
	1000	6.7	12.4±0.9
D-AspA*	10	5.6	39.0±1.2
	100	6.5	10.1±0.8
	1000	6.7	6.40±0.7
CS·*L*-AspA**	10	5.9	89.6±1.4
	100	6.8	62.6±1.3
	1000	6.9	37.4±2.1
CS·*D*-AspA**	10	5.7	97.6±1.2
	100	6.6	92.2±0.9
	1000	6.8	79.8±1.7

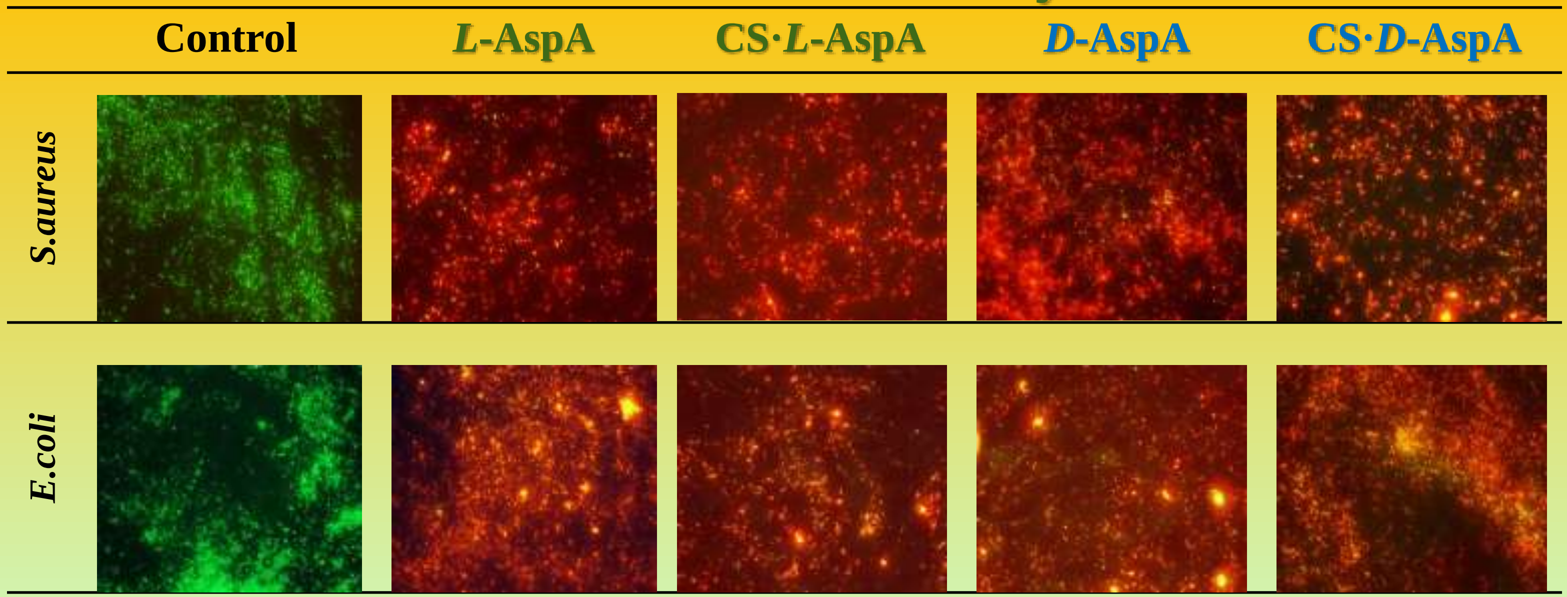
* $C_{AspA} = 0.8 \cdot 10^{-3}$mol/dL

** $C_{CS} = 0.8 \cdot 10^{-3}$monomol/dL, [AspA]/[~-$NH_2$] = 1.0 mol/monomol

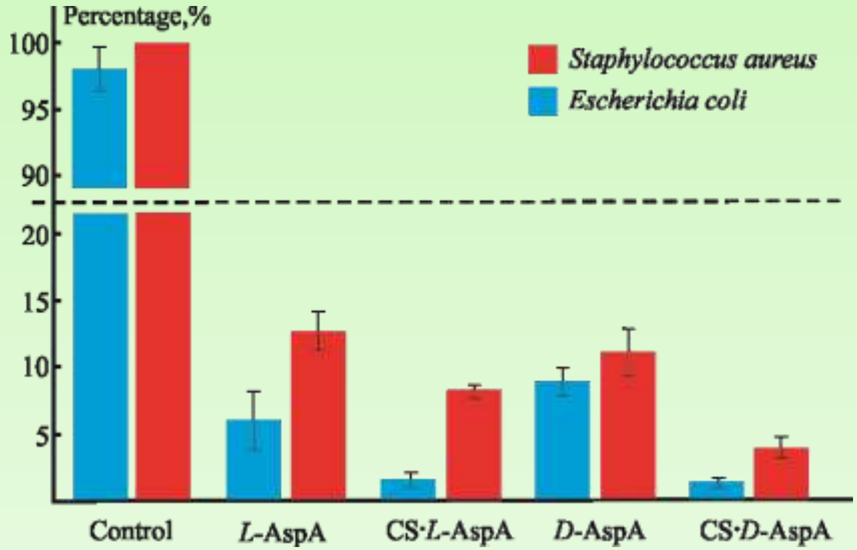
*** Complex formation was assessed by the change in the chlorophyll fluorescence intensity of *S. quadricauda* microalgae cultivated in Prat's medium, t = 4 days.

Reagent	Concentration, g/dm^3
Nutrient medium Prat	
Potassium nitrate, KNO_3	0.1
Potassium phosphate disubstituted, $K_2HPO_4 \cdot 3H_2O$	0.01
Magnesium sulfate, $Mg_2SO_4 \cdot 7H_2O$	0.01
Iron chloride, $FeCl_3 \cdot 6H_2O$	0.001
Bioobject	
S. quadricauda	30 thousand $cells/cm^3$

Antibacterial activity

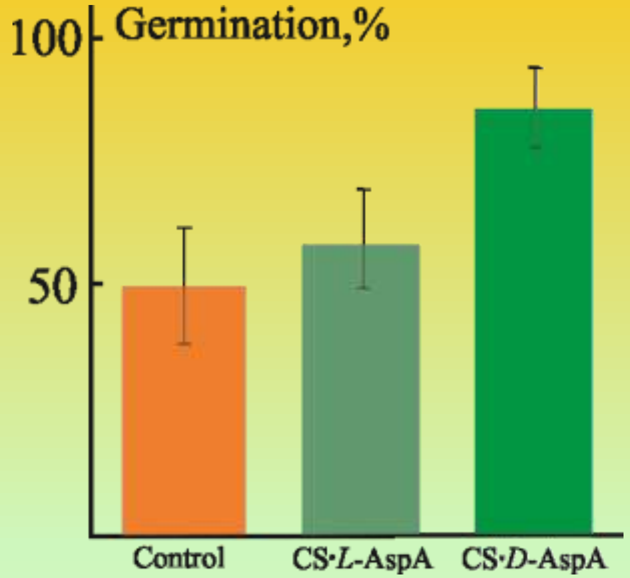


Fluorescent microscopy of cultures of *S. Aureus* 209 P and *E. Coli* 113-13 cultures in meat-peptone broth without (control) and with the addition of aqueous solutions of *L*-AspA, *D*-AspA, CS·*L*-AspA and CS·*D*-AspA; the molar ratio [AspA]/[~-NH_2] = 0.85 mol/monomol; 37°C, t = 24 hours. Green fluorescence - viable cells; red fluorescence - dead cells. Magnification: x 400.



Number of living cells in cultures of *S. aureus* 209 P and *E. coli* 113-13 cultures after 24 hours of growth in meat-peptone broth without (control) and with the addition of aqueous solutions of *L*-AspA, *D*-AspA, CS·*L*-AspA and CS·*D*-AspA; the molar ratio [AspA]/[~-NH_2] = 0.85 mol/monomol; 37°C.

Biostimulatory activity (laboratory conditions)



Germination of test seeds of *Lunum usitatissimum* in laboratory conditions on aqueous extract from the initial soil-soil (control) and from the soil-soil after biodegradation (t = 40 days) of hydrogel plates made from CS·*L*-AspA and CS·*D*-AspA obtained with the molar ratio [AspA]/[~-NH_2] = 1.0 mol/monomol.

Photo of germinated test-seed of *Linum usitatissimum*

CS·*L*-AspA **H_2O** **CS·*D*-AspA**

Biostimulatory activity (field conditions)

Sample test plants	Irrigation liquid	Time, days	Germination (field), %	Growth stimulating activity	
				Shoot height, mm	Mass of shoots, kg
Radish *Raphanus sativus*	H_2O	6	64±2	2.5-3.0	–
		10	76±4	3.8-4.2	–
		25	–	95-100	1.03±0.04
	CS·*L*-Asp +H_2O*	6	90±3	3.5-4.0	–
		10	93±2	6.0-8.0	–
		25	–	105-115	1.36±0.03
	CS·*D*-Asp+H_2O*	6	92±2	3.4-4.5	–
		10	96±2	6.5-8.3	–
		25	–	110-120	1.42±0.06
Mustard *Sinapis alba*	H_2O	6	20±5	4.5-5.0	–
		10	75±5	7.5-8.5	–
		25	–	130-140	0.61±0.03
	CS·*L*-Asp+H_2O*	6	40±4	5.0-7.0	–
		10	85±3	10.0-10.5	–
		25	–	150-170	0.82±0.02
	CS·*D*-Asp+H_2O*	6	60±3	6.0-8.0	–
		10	90±1	11.5-12.5	–
		25	–	170-190	0.85±0.01

* $C_{CS} = 0.2\cdot10^{-3}$ monomol/dL, [AspA]/[~-NH_2] = 1.0 mol/monomol

Raphanus sativus

Sinapis alba

Photo of a bed of radish (*Raphanus sativus*) and mustard (*Sinapis alba*) 25 days after planting in the field conditions (open ground), watered and sprinkled with a solution of CS•*L*-AscA and CS•*D*-AscA with $C_{CS} = 0.2\cdot10^{-3}$ monomol/dL and the molar ratio composition [AspA] / [~-NH_2] = 1.0 mol/monomol.

Biostimulatory activity (field conditions)

H_2O CS·*L*-AspA CS·*D*-AspA

H_2O CS·*L*-AspA CS·*D*-AspA

Photos of shoots of radish (*Raphanus sativus*) and mustard (*Sinapi salba*), cut off 25 days after planting in open ground, watered and sprinkled with a solution of CS•*L*-AscA and CS•*D*-AscA with C_{CS} = 0.2·10 $^{-3}$ monomol/dL and the molar ratio [AspA]/[~-NH_2] = 1.0 mol/monomol.

Thank you for your attention!

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