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Self-assembling nano- and microparticles of chitosan L- and D-aspartate: preparation, structure, and biological activity

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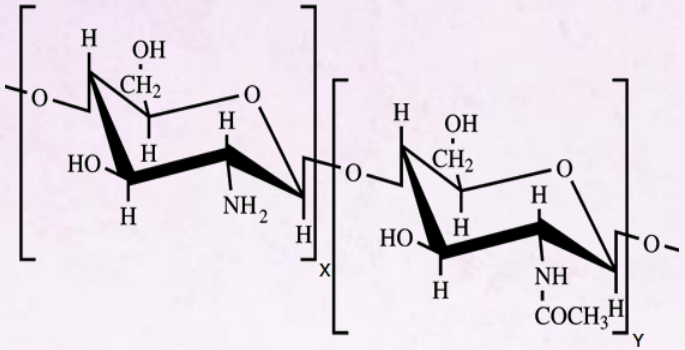
Abstract

Our comprehensive study of solutions of CS D-aminoglucan in L- and D-AspA revealed the effects of counterionic association (self-organization) with the transition of macromolecules to the ionomeric state and phase segregation of the polymer substance into chiral nano- and microparticles. Optimal conditions for stabilizing particle dispersions by functionalizing their surface with a polysiloxane shell have been developed (pharmacologically active silicon tetraglycerolate was used for the first time as a sol-gel precursor). The effect of the L- and D-enantiomer of AspA on the structure, size, shape, and zeta potential of nano(micro)particles was studied by IR spectroscopy, dynamic light scattering, and electron and optical microscopy. It was found that chiral particles of CS·L-AspA and CS·D-AspA are non-toxic, hemo- and biocompatible, and also exhibit high growth-stimulating activity for test plants with the best effect for homochiral D-glucan·D-AspA particles. Experiments in vitro and in vivo revealed that chiral CS·L-(D-)AspA nano(micro)particles are biomimetic of rhizospheric bacteria *Pseudomonas aureofaciens* and could function as an immunizing elicitor, a curing fungicide, and a protective pesticide

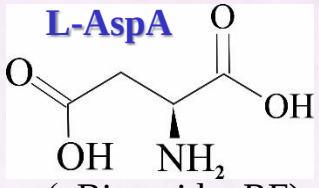
Preparation of chitosan aspartate



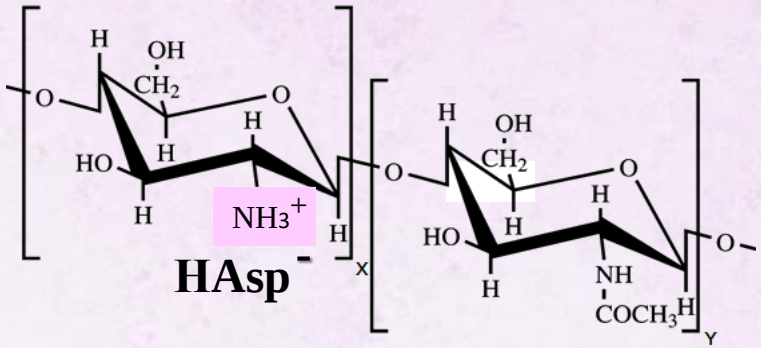
Chitosan (CS)

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

+

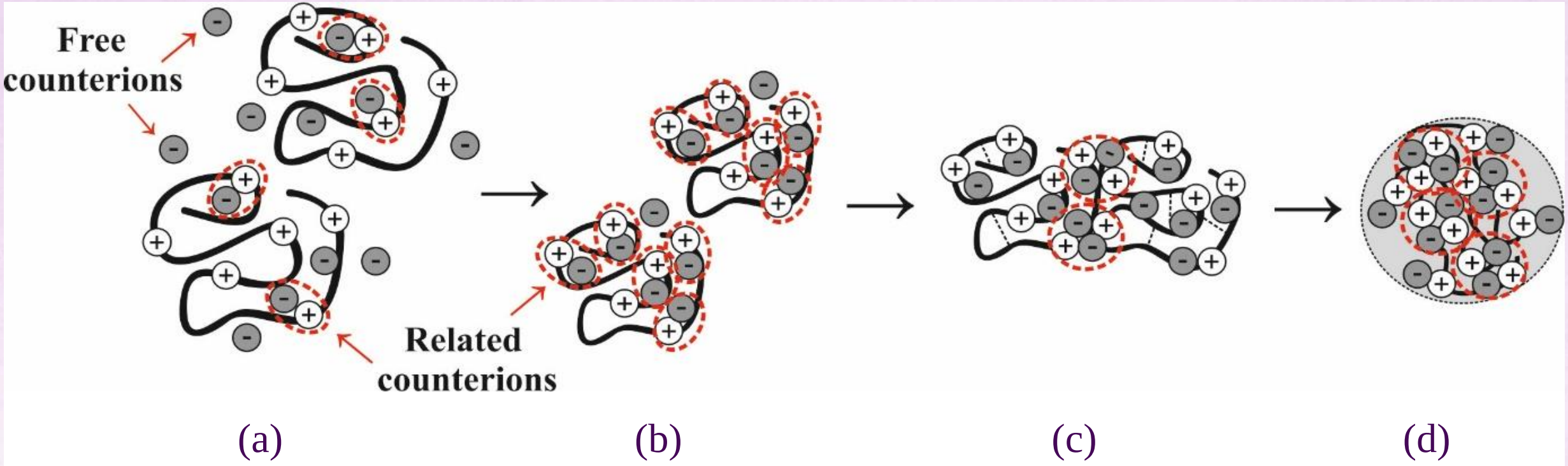


Aspartic acid (AspA)



CS·L-AspA or CS·D-AspA

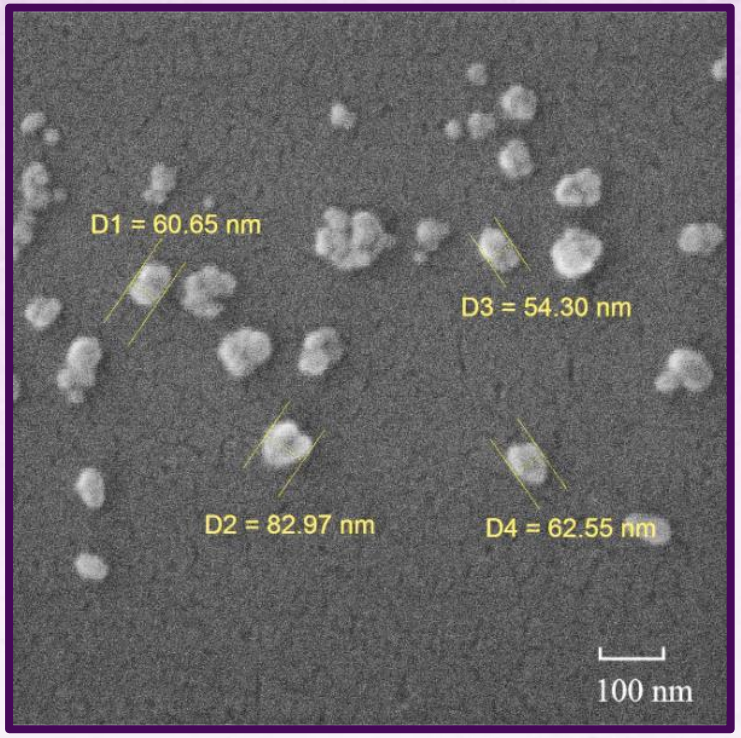
Distribution of free and bound counterions in the CS + AspA + H_2O system during storage: (a) polycation with a partially compensated charge, (b) ion pairs, (c) multiplets, (d) phase segregation of the polymer phase in the form of a nanoparticle*



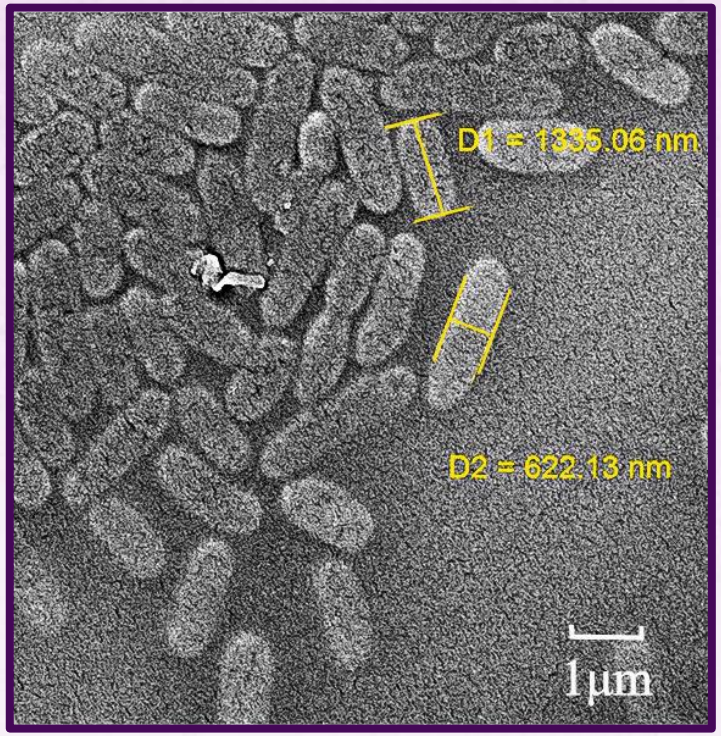
*Lugovitskaya T.N., Shipovskaya A.B., Shmakov S.L., Shipenok X.M. Formation, structure, properties of chitosan aspartate and metastable state of its solutions for obtaining nanoparticles. *Carbohydrate Polymers* 2022, *277*, 118773. https://doi.org/10.1016/j.carbpol.2021.118773

SEM photos of particles isolated from the CS + AspA + H_2O system after 24 (a), 48 (b) and 72 hours (c) of storage*

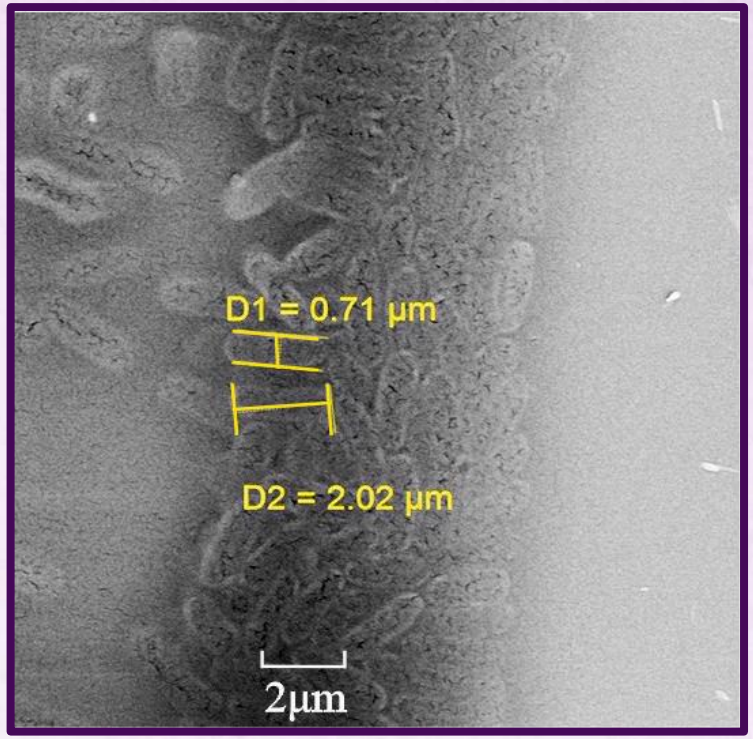
(a)



(b)



(c)



*Lugovitskaya T.N., Shipovskaya A.B., Shmakov S.L., Shipenok X.M. *Carbohydrate Polymers* 2022, *277*, 118773. https://doi.org/10.1016/j.carbpol.2021.118773

Formation of a polysiloxane shell on the surface of particles CS·AspA

Hydrolysis

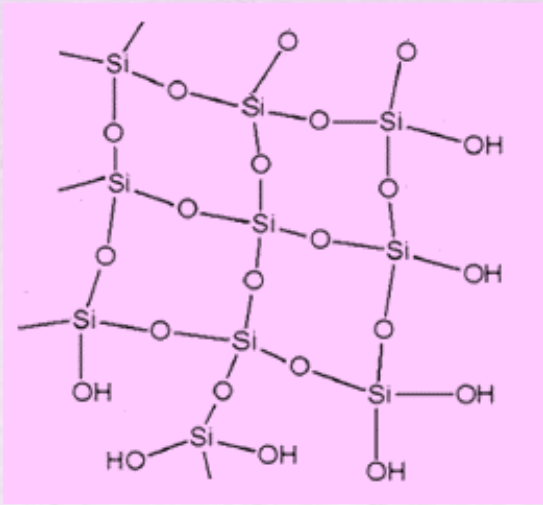
$$\equiv Si-O-R-OH + H_2O \leftrightarrow \equiv Si-OH + HO-R-OH$$

$$\equiv Si-O-R-O-Si\equiv + H_2O \leftrightarrow \equiv Si-O-R-OH + HO-Si\equiv$$

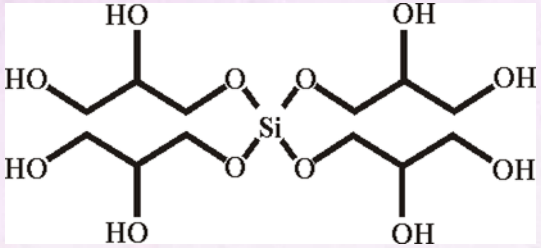
Polycondensation

$$\equiv Si-OH + HO-Si\equiv \leftrightarrow \equiv Si-O-Si\equiv + H_2O$$

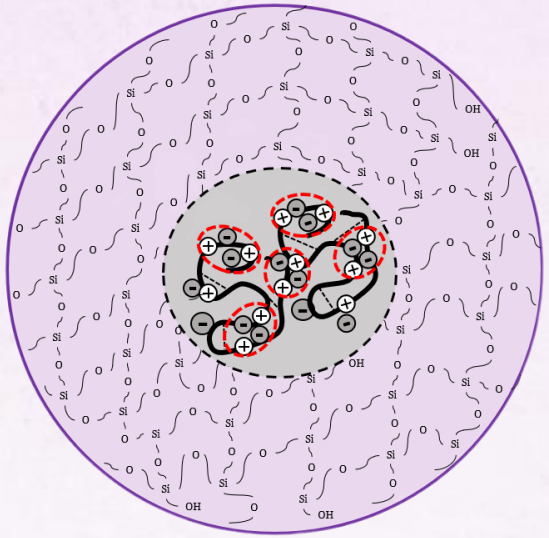
$$\equiv Si-O-R-OH + HO-Si\equiv \leftrightarrow \equiv Si-O-Si\equiv + HO-R-OH$$

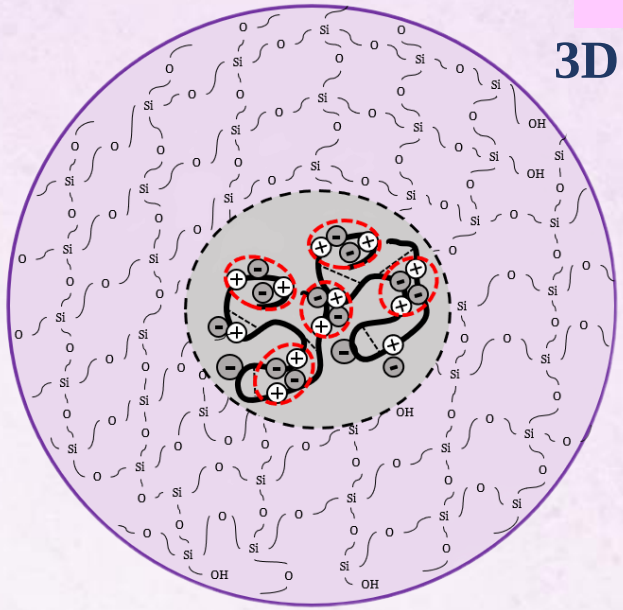


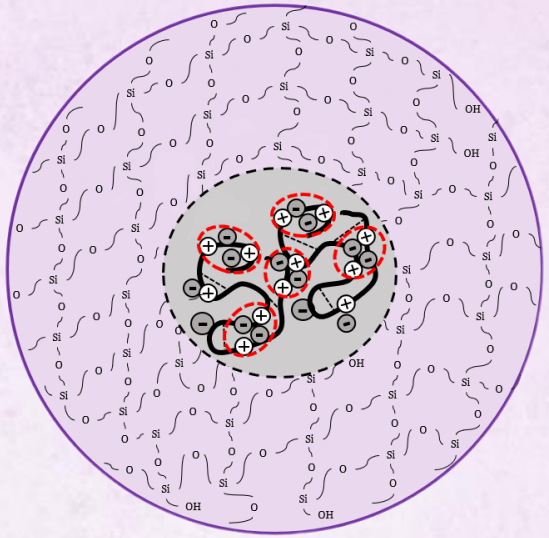
3D network of ≡Si–O–Si≡



Silicon tetraglycerolate*
$Si(OGly)_4$







*Shadrina E.V., Malinkina O.N., Khonina T.G., Shipovskaya A.B., Fomina V.I., Larchenko E.Yu., Popova N.A., Zyryanova I.G., Larionov L.P. *Russian Chemical Bulletin* 2015, *64*(7), 1633–1639. DOI:10.1007/s11172-015-1053-9

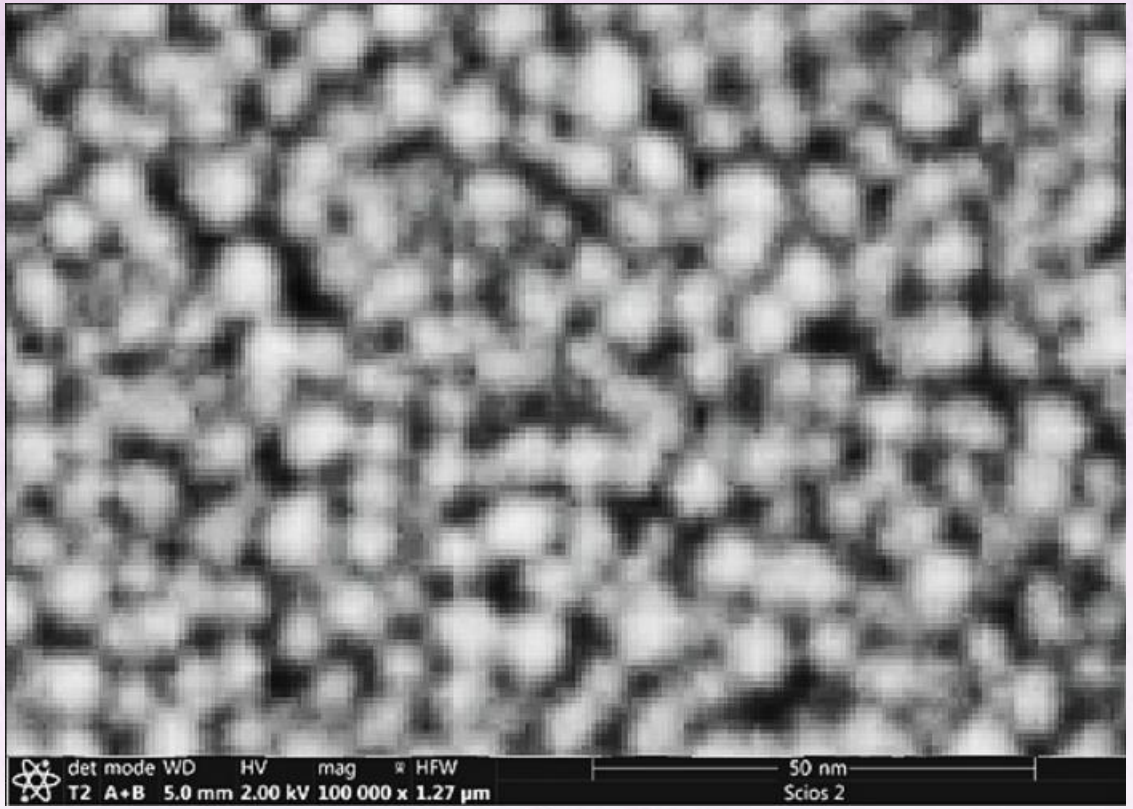
Physicochemical characteristics of the CS + L-(D-)AspA + water system and size characteristics of chiral particles of chitosan L- and D-aspartate obtained by DLS

System			Storage time *t*, days	Conductivity χ, mS/cm	Particle characteristics			
CS salt	Modifier	pH			*d*, nm	Q, %	P_i	ζ, mV
CS + L-AspA	–	4.5	–	0.74	1,200±300	80±8	0.60±0.25	38±3
	$Si(OGly)_4$	3.6	–	0.73	1,500±100	94±2	0.40±0.05	37±2
			14	0.71	1,500±100	92±2	0.40±0.05	37±2
			40	0.70	1,500±100	90±3	0.40±0.05	37±2
			250	0.67	1,100±200	94±2	0.30±0.05	35±2
			300	0.73	1,200±200	95±4	0.40±0.20	36±1
CS + D-AspA	–	4.5	–	0.70	750±150	94±3	0.90±0.10	36±1
	$Si(OGly)_4$	3.5	–	0.70	1,000±100	92±2	0.90±0.20	33±3
			55	0.73	1,200±200	76±2	0.50±0.10	37±1
			125	0.68	1,500±400	66±2	0.60±0.10	31±1

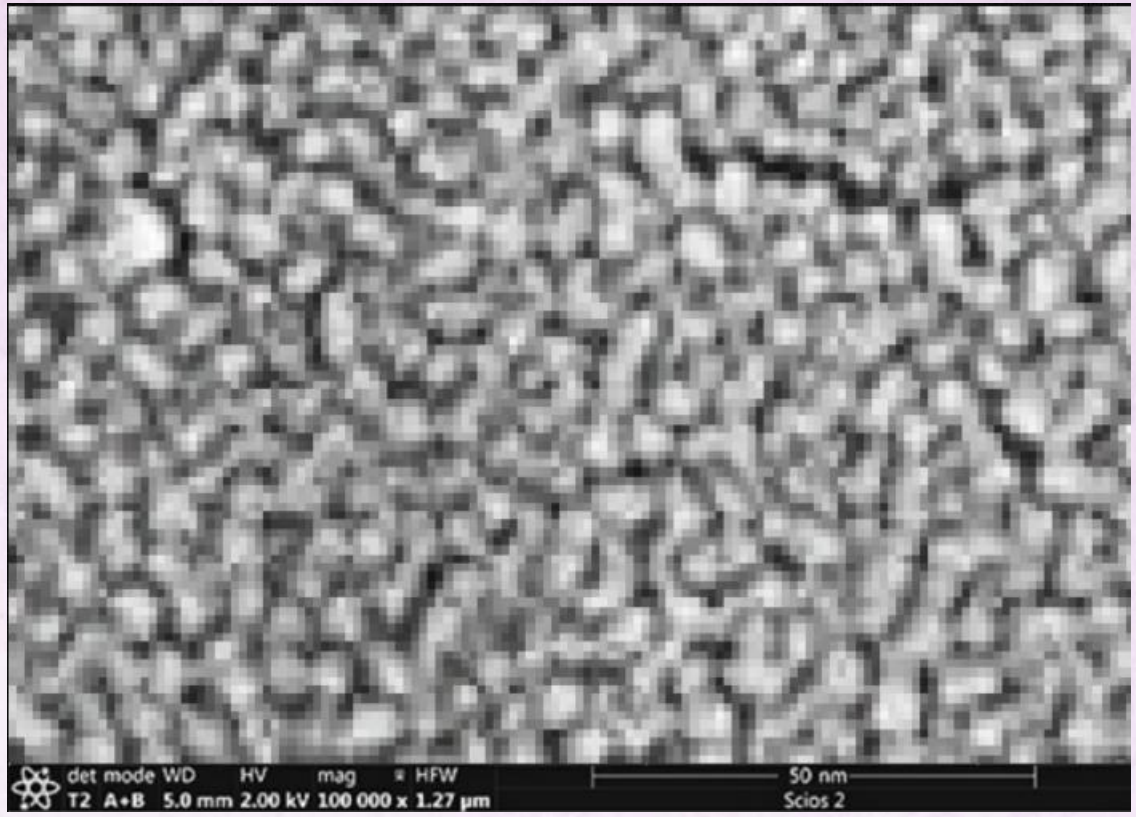
Zetasizer Ultra (Red Label)

FIB-SEM images of chitosan L- (a) and D-aspartate (b) nanoparticles modified with a polysiloxane shell

(a)



(b)



CS·L-AspA **CS·D-AspA**

The largest average characteristic effective diameter (largest transverse size)

17.02 ± 1.25 nm **16.25 ± 3.50 nm**

The smallest average characteristic effective diameter (lsmallest transverse size)

13.65 ± 1.28 nm **14.84 ± 1.50 nm**

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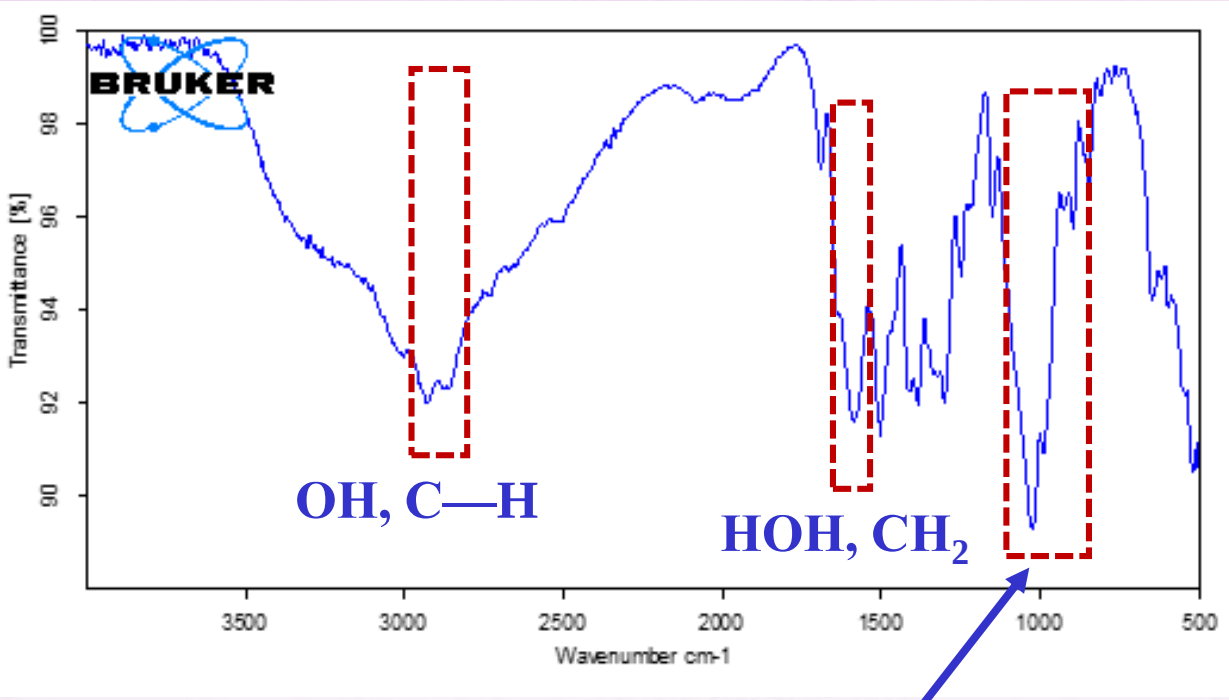
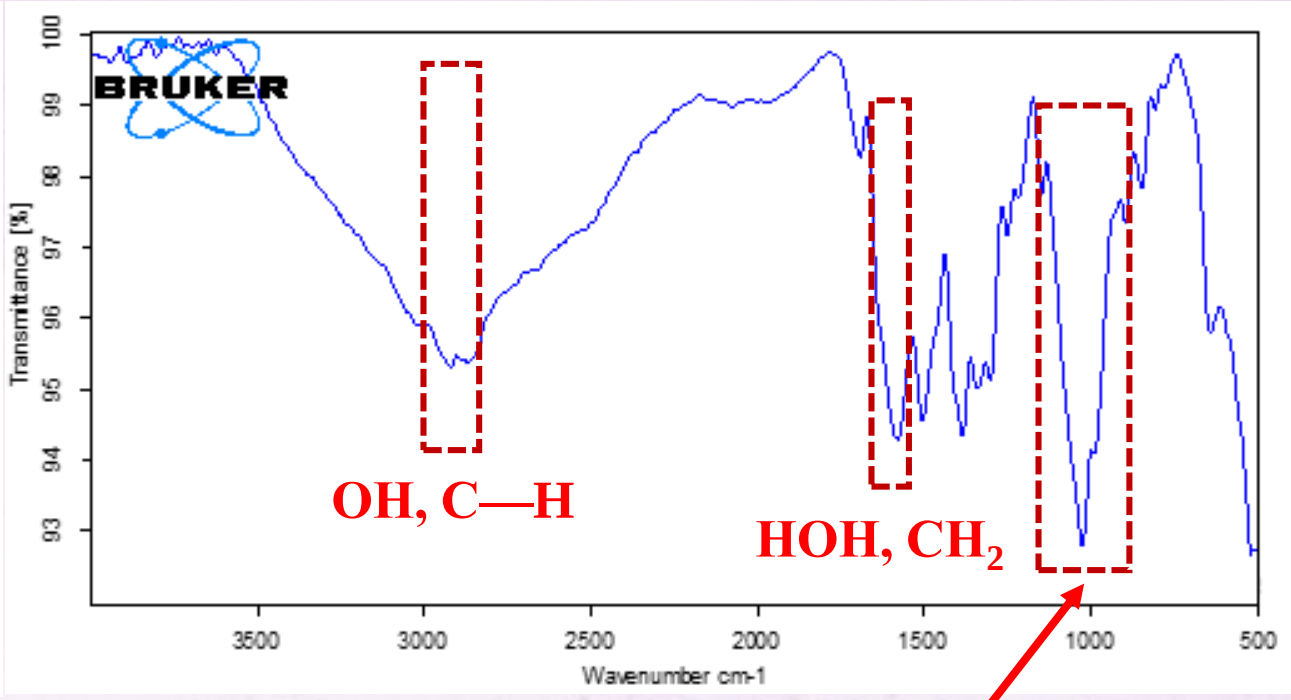
IR spectra of chitosan L- (a) and D-aspartate (b) nanoparticles modified with a polysiloxane shell

(a)



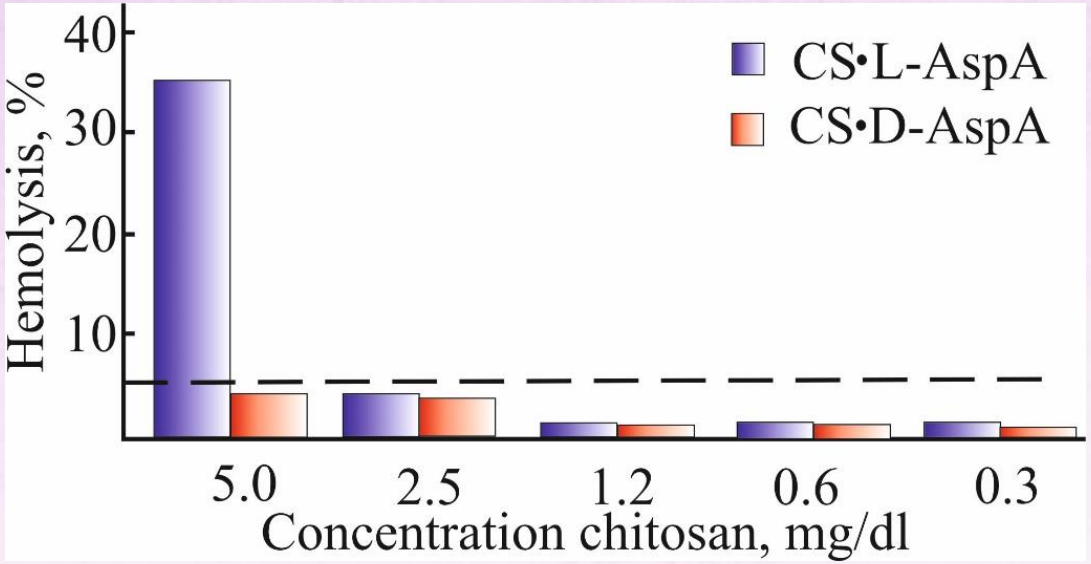
(b)



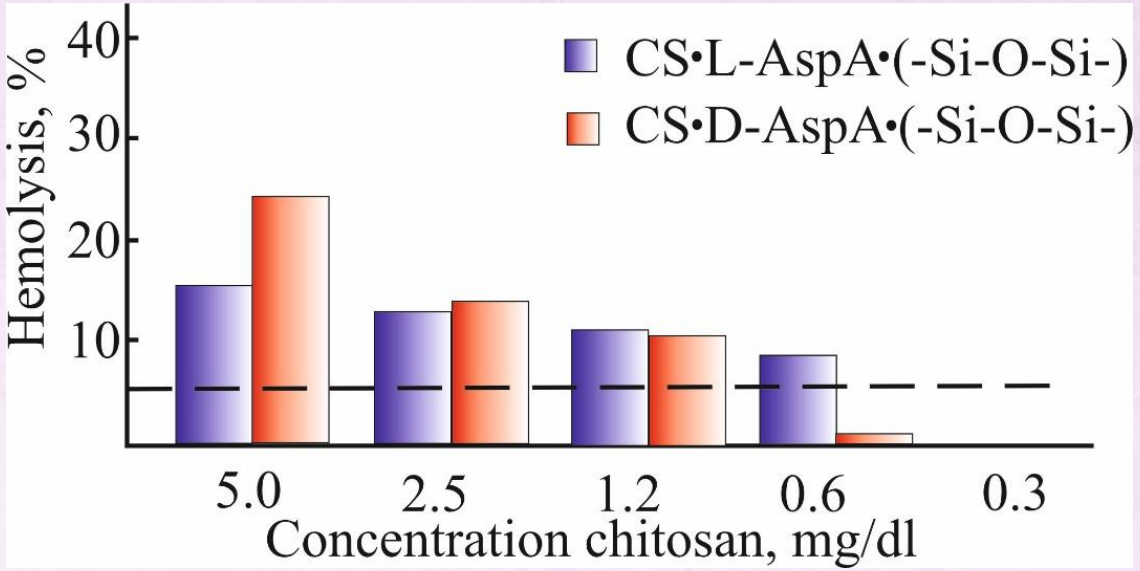
Bruker Alpha IR Fourier spectrometer

Hemolysis of human erythrocytes in the presence of aqueous dispersion of chitosan L- and D-aspartate nanoparticles: basic (a) and modified polysiloxane shells (b)

(a)

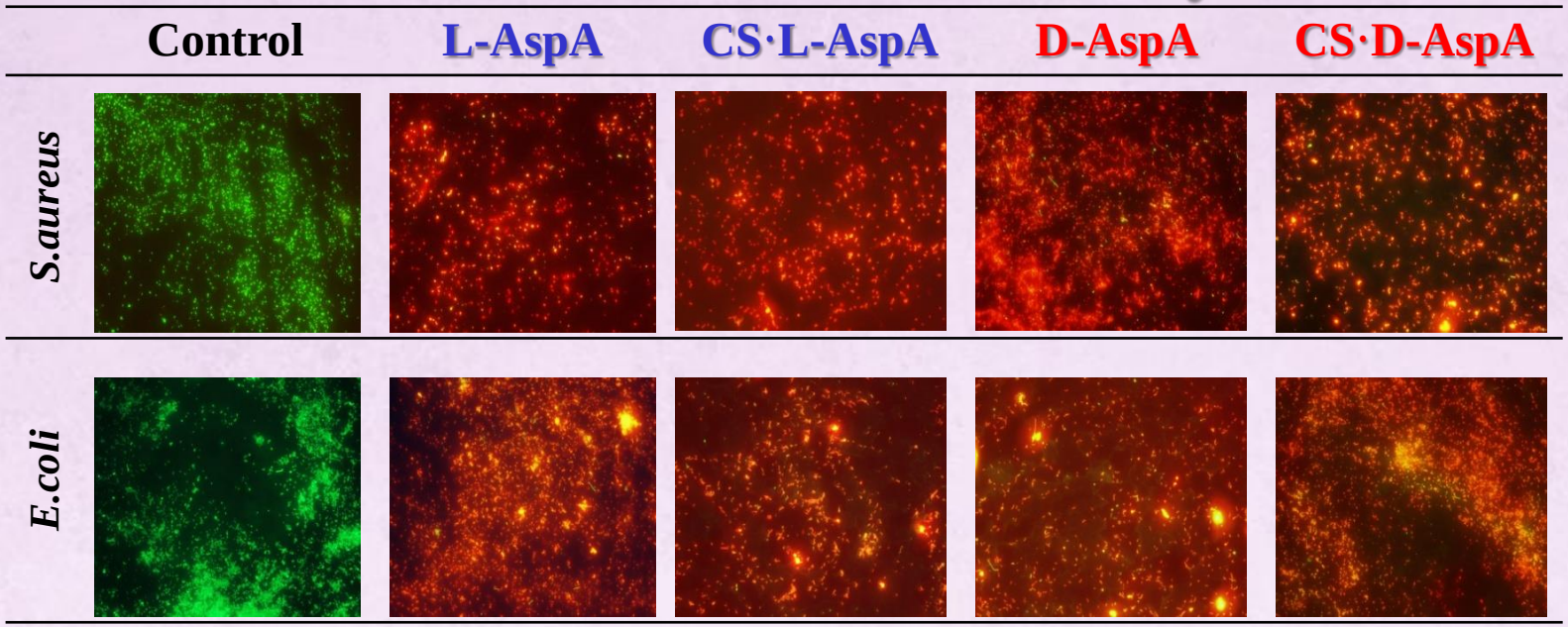


(b)



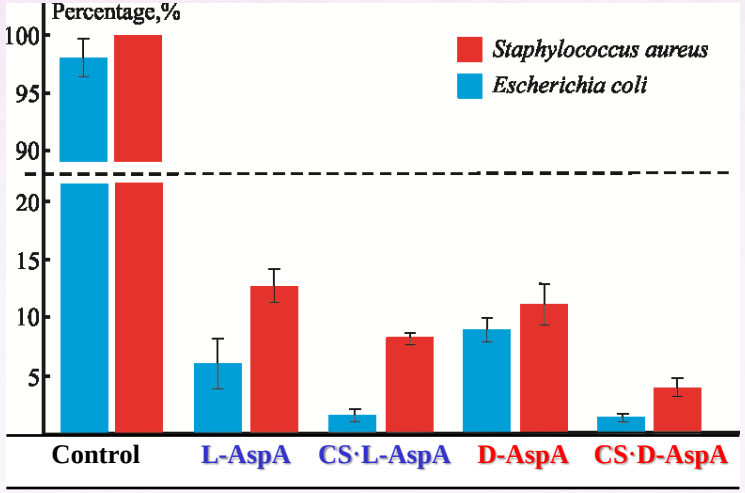
Hemocompatibility was assessed *in vitro* on a model of human erythrocytes under conditions of detection of oxidized hemoglobin form

Antibacterial activity



Green fluorescence – viable cells
Red fluorescence – dead cells
Magnification: x 400

Fluorescent microscopy of cultures of *S. Aureus* 209 P and *E. Coli* 113-13 grown in meat-peptone broth without (control) and with the addition of aqueous solutions of L-AspA, D-AspA, and of aqueous dispersion of nano(micro)particles of CS·L-AspA, CS·D-AspA; 37°C, *t* = 24 hours



The number of living cells in cultures of *S. aureus* 209 P and *E. coli* 113-13 after 24 hours of growth in meat-peptone broth without (control) and with the addition of aqueous solutions of *L*-AspA, *D*-AspA, and of aqueous dispersion of nano(micro)particles of L-AspA, D-AspA, CS·L-AspA, CS·D-AspA; 37°C, *t* = 24 hours

Growth-stimulating activity (in the field)

Sample test plants	Irrigation liquid	Time *t*, days	Germination, %	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

As compared to irrigation with water, the increase in green mass and, accordingly, the yield with the use of our CS·L-AspA was 32.0 ± 2.9% for radish *Raphanus sativus*, 34.4 ± 3.3% for mustard *Sinapis Alba*, and for CS·D-AspA they were 37.9 ± 5.8% and 39.3 ± 1.6%, respectively

Growth-stimulating activity (in the field)

(a)

H_2O CS·L-AspA CS·D-AspA

(b)

H_2O CS·L-AspA CS·D-AspA

Photo of a bed (a) and of shoots (b) of radish *Raphanus sativus* and mustard *Sinapis alba* 25 days after planting in the field (open ground), irrigation with of aqueous dispersion of nano(micro)particles of CS·L-AscA and CS·D-AscA; $C = 0.2 \cdot 10^{-3}$ monomol/dL

Thank you for your attention!

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