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NANOSTRUCTURES OF PECTIN AND ITS METAL COMPLEXES

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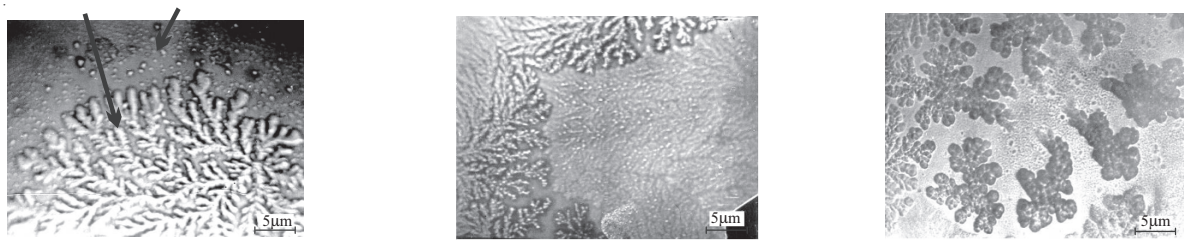
Complexes with metal ions and nanostructures of pectin from Yalongoch lemon were prepared by precipitation. The structural organization and characteristics of the resulting nanostructures were studied by electron microscopy, rheology, and potentiometric titration. A growth-stimulating effect on cotton seeds was found for pectin nanostructures containing biogenic cations.

Keywords: pectin, nanostructures, metal complexes, electron microscopy, rheology, biological activity, seed treatment.

Pectin (PC) is a water-soluble biologically active polymer that is widely used in medicine, the food industry, agriculture, etc. [1]. Its biological activity is clearly pronounced in the nanostructural form (certain type of supramolecular structures) or as nanostructured metal complexes [2]. The formation of one PC supramolecular structure or another depends greatly on the initial state and conformational rotation of its molecules in the solutions and the activity of the carboxyl and methoxyl groups in the chains. The preparation of nanostructures based on citric PC, which is used more widely than other types of PC for medical, food, and preventative purposes, is of great interest. Our goal was to study the formation of precipitated PC nanostructures and its complexes with metal ions (microelements) in addition to finding their biological activity.

PC molecules typically are highly structured in solutions and can produce nanometer-sized particles depending on the precipitation method. Preliminary experiments showed that acetone was the most effective precipitant for producing PC nanostructures. Electron-microscopy images showed the formation of nanometer-sized PC particles in addition to dendritic crystals upon precipitation by acetone, especially for PC:acetone = 1:1.8 (Fig. 1). Increasing the amount of acetone in the mixture enlarged the crystallites and was accompanied in parallel by the formation of larger particles and dendritic structures.

The formation of PC structures in solutions and their subsequent precipitation can be controlled using metal ions, which form polymer:metal complexes of various dimensions, including nanometer-sized ones. Changing the PC concentration favors the formation of certain particle shapes and sizes regardless of the type of Me^{2+}. Electron-microscopy studies showed that nanostructures with particle sizes 10–50 nm were formed for all samples in the studied PC concentration range. Figure 2 shows that the solution with concentration C = 0.1% gave the most uniform structure with respect to distribution and size, i.e., the lower the solution concentration C < 0.1% was, the smaller were the dimensions of the nanoparticles.



a *b* *c*

Fig. 1. Electron-microscopy images of PC precipitated by acetone for PC:acetone = 1:1.8 (a), 1:2.3 (b), and 1:2.6 (c).

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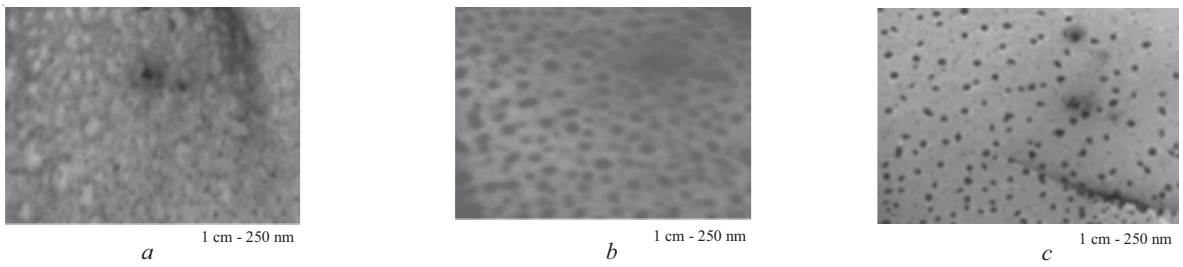
TABLE 1. Viscous Flow Activation Energy (E_a) for Solutions (2%) of PC with Co^{2+} and PC with Cu^{2+} in Shift Flow with Various PC:M^{2+} Ratios

System	Ratio, g/g	E_a for various parts of the curves, kJ/mol		
		initial	inflection	final
PC		33.9	52.7	56.6
PC + Cu^{2+}	40:1	18.7	55.1	27.0
	20:1	14.5	53.0	30.04
	10:1	13.5	44.7	42.6
PC + Co^{2+}	40:1	11.4	44.6	24.1
	20:1	13.4	49.9	39.5
	10:1	22.8	58.2	37.6



a *b* *c*

Fig. 2. Electron-microscopy images of PC:Co^{2+} metal-complex nanostructures for PC:Co^{2+} = 0.58:0.02 mol/mol from 16 to 50 nm (a); 0.29:0.02 mol/mol from 25 to 50 nm (b); 0.058:0.02 mol/mol from 10 to 30 nm (c).

Effect of PC Ordering on Formation of PC:metal-complex Nanostructures. Polymer molecules are effectively ordered when intermolecular contacts occur in flowing solutions. PC molecules in the range C > 1% do not exist in an isolated state, i.e., conditions are favorable for interaction. This can be seen in the deviation from a straight line of the reduced viscosity as a function of concentration (Fig. 3) according to Huggins ($\eta_{sp}/C = [\eta] + K[\eta]^2C$) because of the increase of K > 2.5 [3]. This condition complicates mixing of PC with metal ions and requires the complexation to be carried out in a shift field where disruption of the intermolecular interactions creates conditions for effective PC:Me^{2+} interaction. Figure 4 shows the results for PC:Co^{2+} = 20:1 and PC:Cu^{2+} = 20:1. Increasing the temperature (T) decreased the effective viscosity ($\ln\eta_{eff}$) of the solutions. The rheograms contain three sections, initial, inflection, and final. The difference is especially noticeable in the middle section where the strong deformations of the shift field and the temperature increase destroy PC intermolecular interactions and create conditions for effective reaction with metal ions in the descending flow. The formation of polymer:metal complexes is evident in the sharp increase of the effective viscosity curves up to a ceratin plateau value. The PC:Me^{2+} interaction was estimated from these characteristic sections by constructing plots of $\ln\eta_{eff}$ as functions of inverse temperature (1/T). The viscous flow activation energy (E_a) of the PC:Me-complex solutions was calculated from the slope using the formula [4]

$$\ln \eta_{eff} \approx \ln A_r + E_a R/T,$$

where A_r is a pre-exponential factor and R is the universal gas constant.

Table 1 lists the resulting viscous flow activation energies for PC and its complexes with Co^{2+} and Cu^{2+} at various PC:Me ratios. Compared with the initial PC solution, solutions containing metal ions typically had much lower E_a values. This was observed for all PC:Me^{2+} ratios and is indicative of the formation of PC:Me complexes in the flow.

The precipitated PC:Me complexes were powders with a characteristic color for each complex, i.e., lilac for Co^{2+}, blue for Cu^{2+}, beige for Mn^{2+}, and greenish for Ni^{2+}. The ratio of reacted PC carboxyls ($COOH_{PC}$) and metal cations (Me^{2+}) was calculated using data from potentiometric titrations and the formula [5]

$$COOH_{PC}:Me^{2+} \approx C_{PC} \times V_{PC}/C_{Me^{2+}} \times V_{Me^{2+}},$$

where C_{PC} and C_{Me2+} are the concentrations of PC carboxyls and the corresponding metal cations (mol) and V_{PC} and V_{Me2+} are the solution volumes of the PC and metal cations, respectively (mL).

TABLE 2. Complexes of PC Polymer with Various Metal Ions ($COOH_{PC}$ is the PC Carboxyl Group)

Complex	Mass ratio, mol	
	$COOH_{PC}:Me^{2+}$, initial	$COOH_{PC}:Me^{2+}$, in complex
PC + Mn^{2+}	2.9	1.95
PC + Cu^{2+}	2.9	2.00
PC + Ni^{2+}	2.9	2.05
PC + Co^{2+}	2.9	2.12

TABLE 3. Sorptive Properties of PC Metal Complexes for Divalent Biogenic Metal Cations

Parameters	PC + Mn^{2+}	PC + Cu^{2+}	PC + Ni^{2+}	PC + Co^{2+}
X_m, g/g	0.0165	0.0209	0.0259	0.0295
S_{sp}, m^2/g	52.343	73.508	90.512	103.692

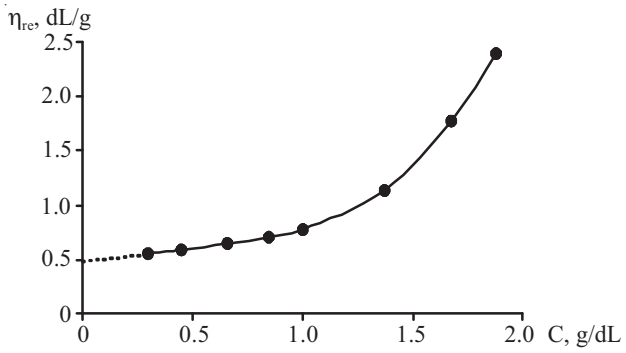


Fig. 3. Reduced viscosity (η_{re}) as a function of concentration (C) for aqueous PC solutions.

Table 2 shows that two PC carboxyls are coordinated to a single metal cation. The interaction of PC with different liquids, including water, changes as a result of the formation of the metal complexes. This is clearly evident in the sorptive properties of the metal complexes. Depending on the type of metal cation, the sorptive properties of the resulting PC:Me complexes can be controlled. The sorption parameters, in particular the sorption capacity (X_m) and specific surface area (S_{sp}), are placed in the following order for the PC:Me complexes: $Mn^{2+} < Cu^{2+} < Ni^{2+} < Co^{2+}$ (Table 3). This result suggested that the reduction in the sorptivity and sorption parameters as a function of the metal cations was due to more effective linking of PC molecules through these ions, as a result of which the density of the PC:Me complex increased.

The PC and PMC nanoparticles exhibited biological activity for encapsulated cotton seeds. The experimental results showed that biological indicators such as the sprouting energy, germination, and sprout length of treated cotton seeds were greater for seeds treated with nanoparticles (50–60 nm) than control seeds (Table 4).

Table 4 presents results from analogous experiments on the effect of PMC nanoparticles. Seeds treated with PC:Co^{2+} PMC solutions had higher parameters than the controls. The solution of PC:Co^{2+} (0.1%) had a noticeable stimulating effect on cotton seeds, increasing the sprouting energy by 20% and the germination by 26% compared with the controls. The length of the sprouts were measured at certain time intervals. The average length of the cotton seed sprouts in all versions of the experiments increased at 5–12 days. These parameters increased in seeds treated with PMC of a different concentration, in contrast with the control. The best result was achieved for treatment with PC:Co^{2+} solution (0.1%).

Thus, the results showed that PC nanostructures can be produced by complexation with metal ions (biogenic microelements) in various ratios and precipitation. Studies of the biological activity [6] of nanoparticles of PC:Me complexes showed that systems containing biogenic cations [7] exhibited high sensitivity and ability to stimulate the growth of encapsulated cotton seeds. Nanometer-sized PC:Co^{2+} complexes at the studied concentrations had a positive effect on the sprouting energy, germination, and cotton seed growth dynamics, which provided early growth and development.

TABLE 4. Effect of Metal-Complex:Polymer Nanostructures (25–50 nm) on Development of Cotton Seed Sprouts for PC–Co^{2+} 1:0.5

PC concentration, %	Sprouting energy, %	Germination, %	Cotton seed sprout average length over days, cm				Raw matl. mass, g	Dry mass, g
			5	7	10	12		
Control	68	72	2.8	4.38	6.5	8.6	4.99	0.66
1*	70	82	2.8	5.0	7.0	9.9	5.69	0.96
1	72	80	2.9	5.4	8.8	10.5	8.50	1.27
0.5	76	85	3.3	6.4	9.4	10.3	12.03	1.35
0.25	80	96	3.0	5.5	9.5	10.8	12.56	1.67
0.1	88	98	3.3	5.6	9.6	10.9	12.13	1.53

*PC was used.

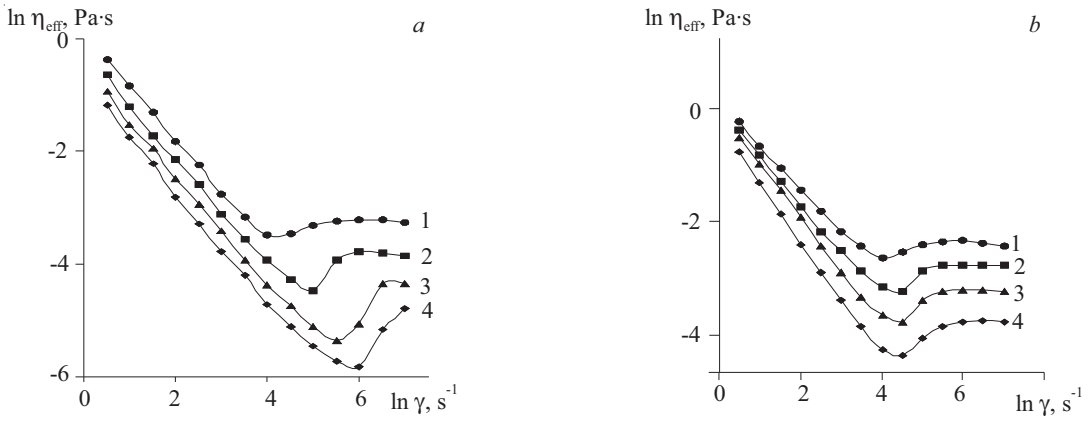


Fig. 4. Logarithm of effective viscosity ($\ln \eta_{eff}$) as a function of logarithm of velocity gradient ($\ln \gamma$) for PC:Co^{2+} (a) and PC:Cu^{2+} (b) at T = 25°C (1), 40 (2), 55 (3), 70 (4).

EXPERIMENTAL

PC extracted by acid hydrolysis [1] from Yalongoch lemon was investigated. This sample typically had etherification degree $a_{et} \approx 73.46\%$ and contained 0.00585 mol of carboxyls, 0.01620 mol of methoxyls, and 0.00331 mol of L-rhamnopyranose and had molecular weight 33,200. The average chain contour length L ≈ 80 nm; the number of chain segments, N ≈ 3.

We used biogenic metals and microelements Cu^{2+}, Co^{2+}, Mn^{2+}, and Ni^{2+} to produce the nanostructured metal complexes. The PMC were formed in a shift field of PC solutions (C > 1%) that was generated on a Rheotest-2 instrument. The formation of the PC nanostructures was studied using electron microscopy (resolving power 3 Å) and rheology.

The biological activity of PC PMC nanoparticles for encapsulated cotton seeds was determined by the literature method [8]. We studied cotton seeds of variety Bukhara 102 that were treated with PC and PMC:Co^{2+} nanoparticles before planting. We used PC solutions of different concentrations (from 0.1 to 1%) that contained Co^{2+} (0.2 M) at a 1:0.5 component ratio and precipitation by acetone.

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