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Polymer Composite Films Based on Citrus Pectin for Controlled Delivery of Ceftriaxone

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Abstract—Interaction of citrus pectin with Ceftriaxone has been studied by UV, IR, and NMR spectroscopy. Polymer films capable of controlled Ceftriaxone release have been prepared based on the citrus pectin mixture with polyvinyl alcohol. The decisive role of supramolecular structure of the polymeric matrix on the films transport properties has been demonstrated.

Keywords: citrus pectin, Ceftriaxone, complex formation, polymer film

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Incorporation of a drug into a polymer matrix allows for its prolonged action and the optimized pharmacokinetics [1, 2]. Moreover, using polymer film as immobilization matrix facilitates targeted delivery of the drug [3, 4]. Among various types of polymeric carriers, pectins (natural polysaccharides with the main chain composed of 1,4-connected units of α-D-galacturonic acid with partially esterified carboxy groups) are of definite fundamental and applied interest [5]. It has been suggested that modification of citrus pectin (CP) with polyvinyl alcohol (PVA) can afford fabrication of stronger elastic films revealing certain therapeutic effect due to incorporation of an appropriate drug. Further advantage of such composition is its biodegradability under the application conditions: the polymers constituting the film are water soluble, and the low molecular mass of PVA makes its excretion easy [6].

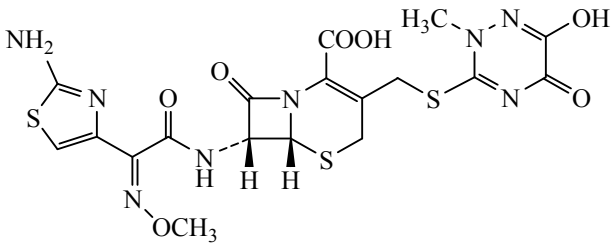
This work was aimed to investigate the possibilities of complex formation between citrus pectin with antibiotic Ceftriaxone (Ctx) and of model composite films formation (Scheme 1).

The formation of the complex with Ceftriaxone is possible due to the presence of hydroxy, carboxy, and methoxy groups in pectin molecules. The interaction of citrus pectin with Ceftriaxone was studied by UV spectroscopy. UV absorption spectrum of Ceftriaxone solution (1×10^{-4} mol/L at pH 7) contained two absorption bands with maxima at 245 and 280 nm (Fig. 1, curve *2*).

Electron absorption spectrum of aqueous solutions of a mixture of citrus pectin with Ceftriaxone (Fig. 1, curve *3*) differed from that of pure Ctx: the addition of citrus pectin caused a red shift of the absorption band maximum by 5–7 nm, accompanied by its intensity increase and some smoothing. The said changes point at possible complex formation between citrus pectin and Ceftriaxone leading to the changes in the electronic structure of the latter [7] (the absorption spectrum of pure aqueous citrus pectin revealed weak UV absorption, Fig. 1, curve *1*).

The composition and stability constant of the formed complex was determined applying isomolar series and molar ratios methods, taking the absorbance at 245 as the signal. The data processing of both

Scheme 1.



Ceftriaxone

methods revealed that the complex composition equaled 1 : 1 (the polymer amount being expressed as number of the repeating units). The complex stability constant β_k was calculated from the slope of $[CP]_0/(A - A_0)$ as a function of $[Ctx]^{-1}$ ($[CP]_0$, initial concentration of pectin; [Ctx], Ceftriaxone concentration; A and A_0; absorbances of the solution in the presence and in the absence of Ctx); $\beta_k = 38.6 \times 10^4$ L/mol, showing a moderate stability of the complex.

The complex formation was further confirmed by ^{1}H and ^{13}C NMR spectra of aqueous solutions of Ctx and of its mixtures with CP.

^{13}C NMR spectrum of the complex contained the signal of Ctx methyl group (characteristic quartet at 42.43 ppm) close to the signal of another quartet signal, of methyl group at oxytriazinone cycle (44.21 ppm). ^{1}H NMR spectrum revealed duplication of singlet signals of the methyl groups as well. According to the two-dimensional heteronuclear correlation spectra HSQC, the signals of methyl groups carbon atoms were correlated with the singlet signals of methyl groups in ^{1}H NMR spectrum (at 3.56 and 3.64 ppm) (Table 1).

Other signals in the Ctx ^{13}C NMR spectrum did not change upon the complex formation. That led to a conclusion that the complex was formed via the oxytriazinone ring of Ceftriaxone. It was further confirmed by the ^{1}H–^{15}N HMBC spectrum (Table 1; Figs. 2 and 3). The spectrum fragment is given in Fig. 3; in the complex spectrum the correlation was observed between the signal of methyl group at 3.56 ppm and those of two nitrogen atoms of oxytriazinone ring (–108.05 and –206.10 ppm); similar cross-peaks were found for the signal of methyl group at 3.64 ppm.

The OH stretching vibrations band at 3600–3050 cm^{-1} was significantly weakened and smoothed in the IR spectrum of the complex as compared with the spectrum of pure CP; that could point at association of the free hydroxy groups through hydrogen bonding [8]. The absorption band at 1732 cm^{-1} (assigned to stretching vibrations of the C=O group in carboxyl or ester fragment of pectin) was shifted to longer wavelength by 27 cm^{-1} upon the complex formation. Characteristic groups of the bands assigned to vibration of pyranose rings of pectin (at 1000–1200 cm^{-1}) was weakened and smoothed in the complex spectrum as well, evidencing the participation of those oxygen-containing groups in the complex formation.

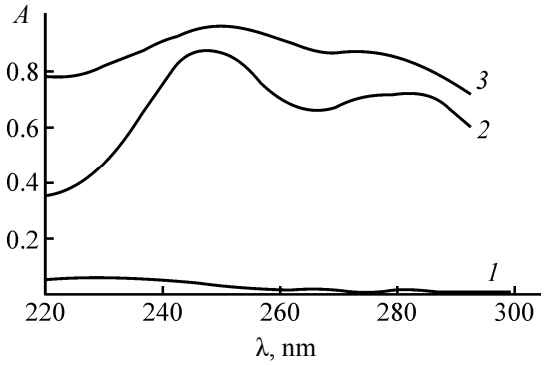


Fig. 1. Electron absorption spectra of aqueous solutions of (*1*) citrus pectin, (*2*) Ceftriaxone, and (*3*) a mixture of citrus pectin with PVA.

To summarize, the observed spectral changes demonstrated that the complex between Ceftriaxone and citrus pectin was formed via coordination of the oxytriazinone ring of Ctx with the carbonyl and hydroxy groups of CP to form the intermolecular hydrogen bonds.

Modification of polymer films accompanied with the changes of supramolecular structural fragments in size is known among the most important factors to control the films transport properties [9–11]. Altering the amount of the modifier introduced at the stage of the film fabrication allows for efficient control of the matrix properties.

We determined the size of supramolecular structural elements present in solutions of pure citrus pectin

Table 1. Correlations observed in ^{1}H–^{13}C HSQC, ^{1}H–^{13}C HMBC, and ^{1}H–^{15}N HMBC spectra of Ceftriaxone

δ_H, ppm	δ_C, ppm		δ_N, ppm
	HSQC	HMBC	HMBC
3.42; 3.68	26.42	33.54; 119.01; 130.10; 57.07	–227.02
3.56	42.43	156.31	–108.05; –206.10
3.91	62.47	147.62	–9.62
3.99; 4.38	33.54	26.42; 119.01	–149.32
5.16	57.07	58.53	–227.41
5.76	58.54	57.07	–227.41; –265.08
6.94	112.87	140.27; 147.62; 170.31	–9.62; –146.03

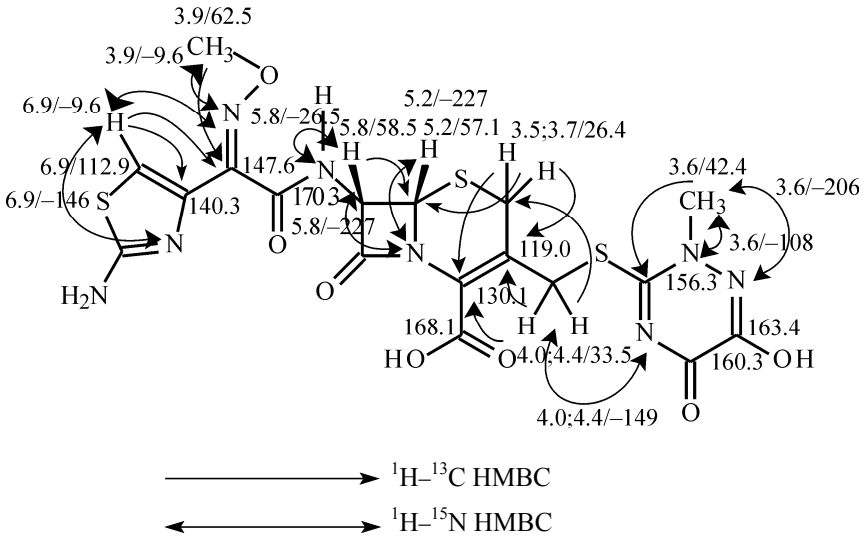


Fig. 2. Main correlations and signals assignment (δ_H/δ_N, δ_C ppm) in ^{1}H, ^{15}N, and ^{13}C spectra of Ceftriaxone.

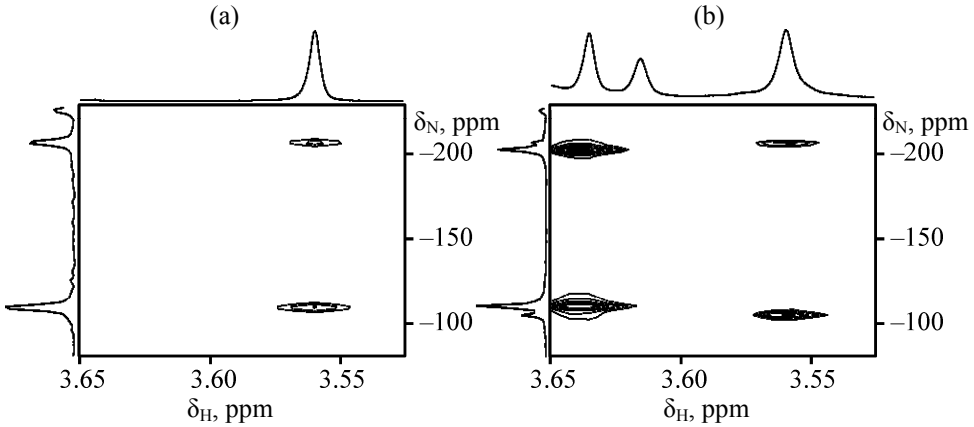


Fig. 3. Fragments of ^{1}H–^{15}N HMBC spectra of (a) Ceftriaxone and (b) a mixture of citrus pectin with PVA.

and of its mixtures with polyvinyl alcohol (CP : PVA = 1 : 0.2, 1 : 0.4, and 1 : 0.6) by laser light scattering. Figure 4 shows the corresponding differential distribution of the particle sizes. The pure pectin revealed relatively narrow distribution of the particle sizes (0.15–0.2 μm). Addition of the increasing amount of PVA to the solution of CP led to broadening of the distribution profiles; the particles as large as 0.1 to 1.0 μm were observed in the mixed solutions (Table 2).

Increase of PVA load in the mixture led to the growing polydispersity of the particles (Table 2). Pectin molecules are known for efficient self-association [12]. Likely, the growing polydispersity with increasing PVA concentration was due to re-organization of macromolecular associates of citrus pectin and to formation of larger aggregates incorporating polyvinyl alcohol macromolecules (hydrogen bonding between PVA and CP was seemingly stronger that that at CP self-association). Indeed, according to the data presented in [13], PVA hydroxy groups form hydrogen bonds with both hydroxy and carboxy groups of D-galacturonic acid units of CP.

We expected that changes in the aggregates size could alter the transport properties of the films prepared from the CP–PVA solutions of different composition.

Figure 5 displays kinetic curves of Ceftriaxone release from the prepared polymeric films. The fastest release was observed in the case of pure CP film, whereas it was slowed down approximately twofold with increasing the PVA load up to the CP : PVA ratio

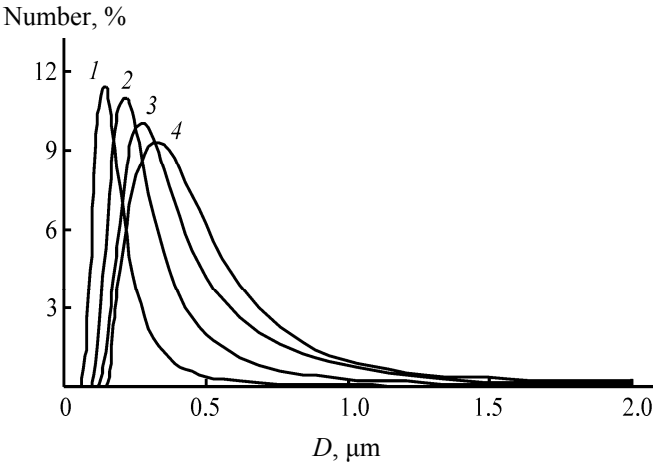


Fig. 4. Differential distribution of the macromolecules size in pure citrus pectin (*1*) and in the mixtures with CP : PVA ratio of 1 : 0.2 (*2*), 1 : 0.4 (*3*), and 1 : 0.6 (*4*).

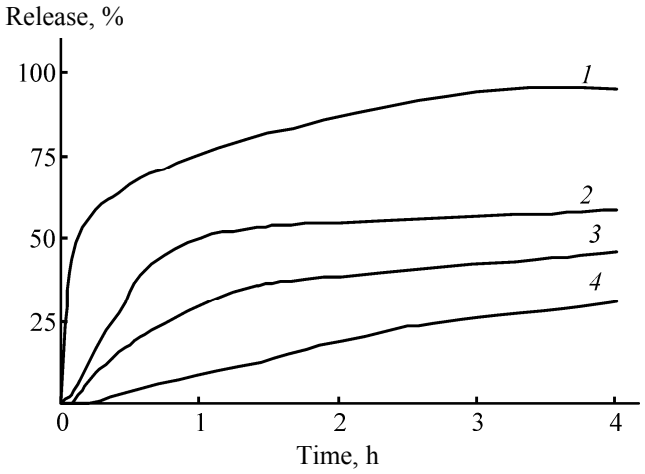


Fig. 5. Kinetics of Ceftriaxone release at different concentrations of PVA in the polymeric matrix. (*1*) pure citrus pectin; (*2–4*) the mixtures with CP:PVA ratio of 1 : 0.2 (*2*), 1 : 0.4 (*3*), 1 : 0.6 *(4)*.

of 1 : 0.6. The release deceleration was likely connected with the changes of the supramolecular structure of the films due to modification with PVA. In particular, introduction of PVA could facilitate formation of hydrophobic aggregates.

Kinetic data of prolonged (up to 7 days) release of Ctx from the prepared films are summarized in Table 3. Ctx release from the film based on pure CP was complete within several hours, whereas modification with PVA could prolong the release to up to a week.

To conclude, changing polyvinyl alcohol concentration in its mixture with citrus pectin allowed control of the supramolecular structure of the formed polymeric films and of release kinetics of a model drug forming a complex with pectin.

EXPERIMENTAL

We used citrus pectin with average molecular mass of 162 kDa (esterification degree of 55%) and polyvinyl alcohol with average molecular mass of 48 kDa (acetylation degree of 0.8–2.0%). Ceftriaxone (Deko, Russia) was used as received.

UV spectra of aqueous solutions were recorded using a UV-Vis SPECOR DM-40 spectrophotometer at 220−350 nm. IR spectra of suspensions in mineral oil were registered with a Shimadzu IR Prestige-21 spectrophotometer at 700−3600 cm^{-1}. ^{1}H, ^{13}C, and ^{15}N NMR spectra of solutions in D_2O were recorded with a Bruker Avance III 500 spectrometer.

Composition of the formed complexes was determined by means of isomolar series and molar ratios

Table 2. Parameters of integral macromolecules size distribution in the solutions of CP–PVA mixtures

CP : PVA	D_{10}[a], μm	D_{50}[a], μm	D_{90}[a], μm	P[b]
1:0	0.095	0.144	0.245	1.04
1:0.2	0.142	0.222	0.420	1.25
1:0.4	0.180	0.290	0.580	1.38
1:0.6	0.200	0.330	0.680	1.45

[a] D_{10}, D_{50}, and D_{90} are diameters of particles corresponding to 10, 50, and 90% of the integral distribution curve.
[b] P (polydispersity) = $(D_{90} - D_{10})/D_{50}$.

Table 3. Release (% of the total load) of Ceftriaxone from the composite films

Time, days	No CP	CP : PVA		
		0.2	0.4	0.6
1	97.2	80.1	53.5	48.1
2		64.3	57.7	51.3
3		79.2	68.8	57.2
5		89.6	73.6	64.2
7		97.7	79.2	69.2

methods using the absorbance as an indicator [14]. The total concentration of Ctx and CP in the isomolar series was kept constant at 1×10^{-4} mol/L; the CP : Ctx molar ratio was varied from 50 : 1 to 1 : 20. In the series of solutions with constant Ctx concentration of 1×10^{-4} mol/L, CP concentration was varied from 0.25×10^{-4} to 1×10^{-2} mol/L. The polysaccharide concentration was expressed as number of the repeating units per unit volume. Ionic strength was kept constant at 0.1 mol/L with NaCl ("chemically pure" grade).

Supramolecular particle sizes in the aqueous solution were determined by laser light scattering (Sald 7101, Shimadzu; semiconductor laser at 375 nm, accessible particle sizes of 10 nm to 300 μm). The measurement was performed in Sald-BC quartz cells with mechanical vertical stirring. The measurement was performed under inert atmosphere of dry purified argon.

Polymeric films to be studied were formed as follows. 70 mL of 2 wt % solution of CP was put into a flask, and then 0.5 g of Ctx was added (higher loads of Ctx resulted in brittle films). The mixture was stirred during 1 h at 25°C, and then the required amount of 5 wt % PVA solution and 0.1 g of glycerol (the latter making the formed film elastic) were added. The CP : PVA ratios were of 1 : 0, 1 : 0.2, 1 : 0.4, and 1 : 0.6 (w/w). Kinetics of Ctx release into the surrounding solution was determined by measuring the solution absorbance at 245 nm.

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REFERENCES

1. Mudarisova, R.Kh. and Badykova, L.A., *Polym. Sci. (A),* 2012, vol. 54, no. 2, p. 106. DOI: 10.1134/S0965545X12020083.
2. Ivantsova, E.L., Kosenko, R.Yu., Iordanskii, A.L., Rogovina, S.Z., Prut, E.V., Filatova, A.G., Gumargalieva, K.Z., Novikova, S.P., and Berlin, A.A., *Polym. Sci. (A),* 2012, vol. 54, no. 2, p. 87. DOI: 10.1134/S0965545X12020058.
3. Siepmann, F., Siepmann, J., Walther, M., MacRae, R.J., and Bodmeirer, R., *J. Control. Release*, 2008, vol. 125, p. 1. DOI: 10.1016/j.jconrel.2007.09.012.
4. Bierhalz, A., Silva, M., Sousa, H., Braga, M., and Kieckbusch, T., *J. Supercritical Fluids*, 2013, vol. 76, p. 74. DOI: 10.1016/j.supflu.2013.01.014.
5. Ovodov, Yu.S., *Chem. Nat. Comp.,* 1975, vol. 11, no. 3, p. 319. DOI: 10.1007/BF00571199..
6. *Polymery v farmatsii* (Polymers in Farmaceutical Industry), Tentsova, A.A. and Alyushin, M.T., Eds., Moscow: Meditsina, 1985.
7. Andrews, L.J.. and Kifer, R., *Molecular Complexes in Organic Chemistry*, San Francisco: Golden, Day, 1964.
8. Ioffe, B.V., Kostikov, R.R., and Razin, V.V., *Fizicheskie metody opredeleniya stroeniya organicheskikh molekul* (Physical Methods of Determonation Structure of Organic Molecules), Leningrad: Leningrad. Univ., 1976.
9. Kulish, E.I. and Kolesov, S.V., *Russ. J. Appl. Chem*., 2005, vol. 78, no. 9, p. 1486. DOI: 10.1007/s11167-005-0543-1.
10. Mudarisova, R.Kh., Badykova, L.A., Koptyaeva, E.I., and Monakov, Yu.B., *Izv. Vuzov, Ser. Khim. Khim. Tekhnol*., 2011, vol. 54, no. 5, p. 78.
11. Novac, O., Liza, G., Profire, L., Tuchilus, C., and Popa, M.I., *Mater. Sci. Eng. (C),* 2014, vol. 35, p. 291. DOI: 10.1016/j.msec., 2013.11.016.
12. Shelukhina, N.P., *Nauchnye osnovy tekhnologii pektina* (Scientifical Bases of Pectine Technology), Frunze: Ilim, 1988.
13. Rashidova, S.Sh., Voropaeva, I.L., Mukhamedzhanova, M.Yu., Reshetnikova, I.V., and Ruban, I.N., *Russ. J. Appl. Chem.*, 2002, vol. 75, no. 7, p. 1136. DOI: 10.1023/A: 1020728716425.
14. Bulatov, I.P. and Kalinkin, M.I., *Prakticheskoe rukovodstvo po fotometricheskim metodam analiza* (Practical Guide of Photometric Analysis Methods), Leningrad: Khimiya, 1986.