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NATIVE AND HYDROLYZED FIBROIN OF NATURAL SILK MODIFIED MECHANOCHEMICALLY BY BENZIMIDAZOLYL-2-METHYLCARBAMATE HYDROCHLORIDE

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Native and hydrolyzed fibroin of natural silk modified by benzimidazolyl-2-methylcarbamate hydrochloride was studied using IR spectroscopy, electron-microscopy, X-ray structure analysis, solubility, and dialysis. Mechanical treatment of a mixture of native and hydrolyzed fibroin of natural silk with benzimidazolyl-2-methtylcarbamate hydrochloride in a 2:1 ratio was shown to form inclusion complexes.

Key words: native fibroin of natural silk, hydrolyzed fibroin of natural silk, enterosorbent, benzimidazolyl-2-methylcarbamate hydrochloride, mechanical treatment, IR spectroscopy, optical microscopy, X-ray structure analysis, solubility, dialysis, inclusion complex.

An enterosorbent consisting of hydrolyzed fibroin of natural silk was previously modified mechanochemically [1] by natural alkaloid, deoxypeganine-hydrochloride, possessed anticholinesterase activity. The formation of inclusion complexes (IC) was demonstrated [2].

Modification of native fibroin of natural silk (FNS) and hydrolyzed fibroin of natural silk (ES) by the benzimidazole anthelmintic preparation benzimidazolyl-2-methylcarbamate hydrochloride (BMCHC) by mechanochemical treatment was studied in order to use FNS and ES as carriers and sources of introducing medicinal substances (MS) into the enteral cavity and to lower the toxicity and increase the bioavailability of MS.

IR spectroscopy, X-ray structure analysis, and optical and electron-microscopy were used in the investigation.

Forms of FNS and ES modified with BMCHC were prepared by grinding in a 2:1 weight ratio in a planetary-centrifugal grinder-activator AGO-2U at various energy levels and activation times.

IR spectra of starting FNS and ES before and after grinding were relatively the same.

The X-ray patterns of FNS reprecipitated from sodium thiocyanate solution and of starting FNS [1] clearly showed diffuse diffraction maxima at $2\Theta = 15\text{-}23°$.

Fibroin became more amorphous after treatment in the AGO-2U if the treatment conditions were more severe. A broad amorphous halo was observed in the diffraction pattern at $2\Theta = 20.5°$.

Analogous results were obtained after grinding the enterosorbent [2].

Optical- and electron-microscopy studies of specimens before and after grinding showed that FNS and ES preparations consisted before treatment in the AGO-2U mainly of large particles several micrometers in size and smaller ones, the dimensions of which were less than fractions of a micrometer. Their structure changed markedly upon dispersion in the activator. Long fiber fragments and large particles with a complicated structure disappeared. The particle size of specimens treated for 10 min at 20 g varied in the range 0.2-0.5 μm. Grinding at 60 g for the same time caused the particle size to decrease to 0.1-0.3 μm (average). Even smaller particles were found.

The results indicate that grinding FNS and ES had no substantial effect on the molecular structure and converted them into an amorphous state.

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TABLE 1. IR Spectra of FNS, ES, BMCHC, and Corresponding Complexes

Compound	ν, cm^{-1}, 1500-1800	ν, cm^{-1}, 1000-1500	ν, cm^{-1}, 2700-3600
1. FNS (60g, 10 min)	1703, 1637, 1523	995, 1003, 1165, 1231, 1264, 1404, 1493	3290, 3078, 2980, 2933
2. ES (60g, 10 min)	1702, 1632, 1519	997, 1005, 1165, 1231, 1262, 1405, 1497	3292, 3078, 2980, 2935
3. BMCHC (60g, 10 min)	1755, 1635, 1611, 1506	1005, 1024, 1088, 1115, 1157, 1199, 1228, 1247, 1349, 1368, 1453, 1473, 1507	3407, 3171, 3021, 2911, 2827
4. FNS:BMCHC, (2:1), 20g, 10 min)	1753, 1699, 1628, 1520	1004, 1093, 1246, 1349, 1146	3280, 3063, 2792
5. FNS:BMCHC, (2:1), 40g, 10 min)	1755, 1630, 1524	1002, 1095, 1105, 1194, 1277, 1287, 1330, 1345, 1367,1497	3290, 3063, 2934, 2960
6. FNS:BMCHC, (2:1), 60g, 10 min)	1755, 1646, 1518	1014, 1093, 1247, 1330, 1348, 1429, 1473	3294, 3067, 2985
7. FNS:BMCHC, (2:1), (60g)- FNS (60g)	1754, 1653, 1598, 1550	1015, 1096,1152, 1195,1246,1267,1285, 1330, 1348, 1419, 1457, 1474	3375, 3048, 2958, 2814
8. ES:BMCHC, (2:1), 20g, 10 min	1754, 1699, 1630, 1524	1004, 1096, 1247, 1349,1146	3281, 3065, 2795
9. ES:BMCHC, (2:1), 40g, 10 min	1755, 1631, 1528	1004, 1096, 1107, 1199, 1277, 1287, 1332, 1348, 1367, 1498	3291, 3063, 2937, 2963
10. ES:BMCHC, (2:1), 60g, 10 min	1755, 1647, 1520	1016, 1096,1248, 1332, 1350, 1429, 1474	3294, 3068, 2987
11. ES:BMCHC, (2:1), (60g) - ES (60g)	1755, 1657, 1599, 1550	1017, 1096, 1154, 1197, 1248, 1268, 1287, 1332, 1350, 1419, 1458, 1474	3376, 3045, 2956, 2816

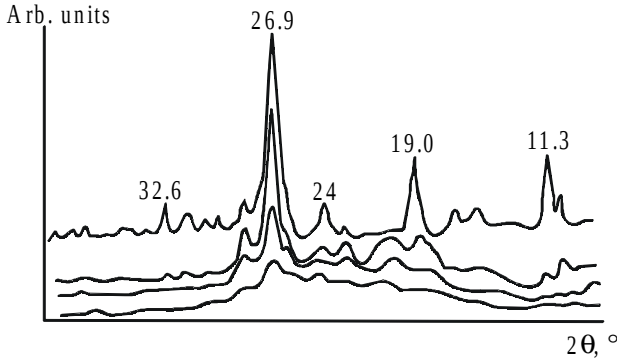

Fig. 1. X-ray diffractogram of FNS:BMCHC mixtures (AGO, 10 min): starting BMCHC (1), FNS:BMCHC (20 g) (2), FNS:BMCHC (40 g) (3), FNS:BMCHC (60 g) (4).

For BMCHC, which is the hydrochloride salt of a benzimidazole base, the IR spectra of the starting and ground specimens also were essentially similar.

X-ray structure analyses of the starting and ground benzimidazole specimens found that BMCHC is a crystalline product. The diffraction pattern of starting BMCHC contained several sharp maxima at 2Θ from 5-10 to 35°. The strongest peak was found at 2Θ = 26.9°. The second strongest peak occurred at significantly smaller angles of 11.3 and 19.0°.

Grinding BMCHC had some specific effects on its crystal structure. The strength of the reflections at 2Θ = 11.3 and 19.0° decreased whereas that of the main reflection at ~26.9° noticeably increased after treatment at 20 g for 10 min. Grinding at 60 g for 10 min further decreased all reflections but the peak at 2Θ = 26.9° practically did not weaken and remained stronger than in untreated BMCHC.

Optical microscopy of the benzimidazoles showed that starting BMCHC was anisodiametric needle-like particles with a bright glow in crossed filters, which also indicates that the MS was crystalline in nature. The BMCHC particles lost their shape after mechanical treatment and stuck together during grinding. Their dimensions decreased significantly (from 80 to 40 μm). They became smaller and more homogeneous as the treatment conditions were intensified. This was consistent with the electron-microscopy investigation.

The IR spectra of FNS:BMCHC and ES:BMCHC taken in 2:1 ratios (Table 1, **4**-**6** and **8**-**10**) changed relative to those of starting FNS (**1**), ES (**2**), and BMCHC (**3**) after joint mechanical treatment at 20, 40, and 60 g (compare frequencies of **4**-**6** with those of **1** and **3**; of **8**-**10**, with **2** and **3**). This leads to the conclusion that IC may be formed. However, the reference spectra (Table 1, **4**-**6** and **8**-**10**) are difficult to interpret because bands overlap.

Difference spectra (Table 1, **7** and **11**) obtained by subtracting spectra of FNS:BMCHC (60 g) - FNS (60 g) and ES:BMCHC (60 g) - ES (60 g) showed unambiguously that IC FNS:BMCHC (2:1, 60 g) and ES:BMCHC (2:1, 60 g) formed because they were not identical to the BMCHC spectrum (Table 1, **3**, compare with **7** and **11**). Furthermore, subtracting the spectra improved the resolution of complicated curves and the interpretation of absorption bands of functional groups.

The spectral changes in the range 1500-1800 cm^{-1} (**1**, **7** and **2**, **11**) consisted of a shift of the absorption maxima for COOH (**7**, 1653 cm^{-1}; **11**, 1657 cm^{-1}) and NHCO (**7**, 1598; **11**, 1599) stretches to low frequency compared with the spectra of starting FNS (**1**, ν_{COOH} 1703 cm^{-1}, ν_{NHCO} 1637 cm^{-1}) and ES (**2**, ν_{COOH} 1702 cm^{-1}, ν_{NHCO} 1632 cm^{-1}). Furthermore, frequency changes in the range 2700-3600 cm^{-1}, which are characteristic of NH^+ vibrations of the BMCHC cation, in the difference spectra (**7** and **11**) relative to those of BMCHC indicate that the intermolecular interactions of FNS and ES with BMCHC occur through H-bonds involving COOH, NHCO, and OH groups of FNS and ES and the BMCHC NH^+ cation. The fact that the spectra of FNS:BMCHC (**4**-**6**) and ES:BMCHC (**8**-**10**) are different with increasing energy level shows that the IC FNS:BMCHC and ES:BMCHC are completely formed at 60 g in the AGO-2U.

The above analysis and the similarity of the difference IR spectra (Table 1, compare **7** and **11**) lead to the conclusion that the complexation mechanism is the same after grinding mixtures of FNS and ES with BMCHC under analogous conditions.

According to the X-ray structure analysis, FNS:BMCHC and ES:BMCHC mixtures underwent extensive amorphization after treatment at 20 g. This increased with increasing severity of the treatment (Fig. 1). Thus, specimens ground at 40 g and especially at 60 g were more likely amorphous than crystalline. Only a weak hump at $2\Theta = 26.9°$ remained of all diffraction maxima for BMCHC. All others disappeared. It must be emphasized that we are talking about amorphization of the MS and destruction of the three-dimensional long-range order and not about chemical changes of the supramolecular structure, which is retained.

Electron-microscopy investigations of joint mechanical treatment of FNS:BMCHC and ES:BMCHC also confirmed that BMCHC crystals were practically completely destroyed after grinding FNS and ES with BMCHC even after 10 min at 20 g. Formless particles of various size, from several micrometers to fractions of a micrometer appeared. Increasing the severity of the grinding (40 and 60 g) decreased further the average particle size at the expense of the larger ones and homogenized the whole mixture.

The IR-spectral, microscopy, and X-ray-structure investigations established that mechanical treatment of FNS and ES with BMCHC caused changes on the supramolecular level (decrease of particle size, formation of associates and H-bonds, etc.), which, in turn, caused changes in the solubility, bioavailability, etc. of the MS.

The formation of IC of FNS and ES with BMCHC was confirmed by studying the dissolution dynamics of BMCHC and its mixtures in HCl (0.1 N). It was previously found that starting BMCHC is very soluble under these conditions whereas the rate of dissolution increased after grinding owing to an increase in the specific surface area [4]. The solubilities of FNS and ES mixtures with BMCHC were less after joint mechanical treatment than that of starting BMCHC (FNS:BMCHC = 2:1 and ES:BMCHC = 2:1 mixtures, ground at the minimal load of 20 g, dissolved ~50% after 50 min whereas 100% of BMCHC dissolved in this same time) (Table 2), i.e., FNS and ES acted as extenders. The solubility of the mixtures increased with increasing severity of treatment, probably due to destruction of FNS and ES.

A study of the dialysis dynamics of a mixture of the studied compounds in HCl (0.1 N), which can evaluate the strength of the IC, showed a slow diffusion of BMCHC through a semipermeable membrane. This indicates that the components are bonded through intermolecular H-bonds, which interferes with diffusion of BMCHC.

Table 3 shows that the desorption of BMCHC from the mixtures is somewhat hindered. This can change the mode of action of the preparation. Thus, desorption of pure BMCHC through a semipermeable barrier is 88.39% after 120 min; from the IC FNS:BMCHC = 2:1 (60 g, 10 min), 77.36%. Studies of the desorption dynamics of BMCHC from mechanically treated mixtures with FNS and ES prepared at various grinding-energy levels confirmed the IR spectral and X-ray structure analyses that determined that the IC of FNS:BMCHC and ES:BMCHC are completely formed at an activator energy of 60 g.

Therefore, joint mechanical treatment with FNS and ES can regulate the desorption rate of low-molecular-weight compounds.

TABLE 2. Dissolution Dynamics of Starting BMCHC and IC FNS:BMCHC = 2:1 after Mechanical Treatment at Various Energy Levels in HCl (0.1 N) (Conc., %)

Energy level, g	Time of dissolution, min								
	0.5	1	2	5	10	20	30	40	50
BMCHC, starting	6.13	8.52	17.93	42.44	65.76	87.73	94.75	97.14	100.28
20	3.55	3.60	3.71	6.97	13.06	23.15	31.90	40.50	48.95
	(3.76)	(3.61)	(3.76)	(7.07)	(13.25)	(23.49)	(32.37)	(41.1)	(49.68)
40	6.47	9.26	13.08	20.58	32.19	46.31	57.92	63.51	69.39
	(6.66)	(9.53)	(13.46)	(21.18)	(33.13)	(47.66)	(59.61)	(65.36)	(71.41)
60	6.27	8.79	10.65	13.89	26.16	44.04	56.02	67.25	69.90
	(6.41)	(8.99)	(10.9)	(14.22)	(26.78)	(45.08)	(57.34)	(68.84)	(71.56)

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Concentrations for IC ES:BMCHC = 2:1 are given in parentheses.

TABLE 3. Desorption Dynamics of BMCHC in HCl (0.1 N) from Mechanically Treated Mixtures FNS:BMCHC = 2:1 at Various Energy Levels (conc., %)

Energy level, g	Time of desorption, min				
	10	20	30	70	120
BMCHC, starting	37.48	48.83	61.65	82.99	88.39
20	30.85 (31.96)	43.11 (44.66)	53.42 (55.34)	73.48 (76.12)	81.83 (84.77)
40	27.94 (28.94)	41.07 (42.54)	50.49 (52.30)	68.42 (70.87)	79.74 (82.60)
60	24.66 (25.53)	37.53 (38.85)	47.40 (49.07)	64.97 (67.26)	77.36 (80.09)

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Concentrations for IC ES:BMCHC = 2:1 are given in parentheses.

Thus, results from the x-ray structure analysis, IR spectroscopy, solubility tests, and dialysis of FNS and ES mixtures with BMCHC proved that mechanical treatment formed IC, which enables FNS and ES to be used as matrices for formulating new preparations. These preparations have a common solubilizing action and are carriers of the MS. After release of the MS from them, they act as adsorbents for microorganisms, metabolites, and toxins.

EXPERIMENTAL

Powdered FNS was prepared by reprecipitation from aqueous sodium thiocyanate solutions.

Mechanical treatment (grinding activation) of FNS, ES, BMCHC, and their mixtures was carried out on a Gefest (Russia) AGO-2U planetary-centrifugal activator.

IR spectra were recorded on a Perkin—Elmer Model 2000 (100 scans, 4 cm^{-1} resolution) single-beam Fourier spectrometer.

X-ray studies were performed on a DRON-3M diffractometer using monochromatized Cu - Kα-radiation at 20-28 kV potential and 15-18 mA current, which was set depending on the preparation. Specimens were prepared by pressing ground preparations into disks. Reflections in the range $2\Theta = 10\text{-}40°$ were recorded.

Dissolution was measured using the literature method [4]. The solutions were analyzed quantitatively using an SF-46 spectrophotometer at $\lambda = 282$ nm.

Equilibrium dialysis was carried out at 37°C in a two-chamber cell divided by a cellophane semipermeable membrane. The membrane was unreactive with the solution components and ensured transport of BMCHC. It was impervious to polyions and counterions bound to them. The dialysis was monitored for 2 h. Samples were taken after set times to monitor the MS concentrations in the dialyzer chambers. The content of MS was determined spectrophotometrically on a SF-46 instrument at $\lambda = 282$ nm.

Microscopy studies were performed using MBI-6 and MBS-1 microscopes. The external appearance, shape, dimensions, presence of pores and defects, degree of swelling, and homogeneity of the specimens was noted. The supramolecular structure of the specimen surfaces was investigated using two-step polystyrene—carbon replicas coated with platinum [5]. The deposition was carried out in a VUP-4K vacuum station. Replicas were examined in a PEM-100 electron microscope. Specimens were sprayed with silver for scanning electron-microscopy investigations and viewed in an REM-200.

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