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PROPERTIES OF THE POLYCOMPLEX OF APPLE PECTIN WITH BENZIMIDAZOLYL-2-METHYLCARBAMATE HYDROCHLORIDE AFTER MICRONIZATION

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X-ray diffraction, microscopic, and sorption studies of benzimidazolyl-2-methylcarbamate hydrochloride (BMCHC) with various types of pectin showed that the structure of the mixtures depends on the composition, grinding conditions, and pectin type.

Key words: micronization, enzymatic apple pectin, benzimidazolyl-2-methylcarbamate hydrochloride, structure, medicinal substances.

One of the most promising methods for manufacturing medicinal preparations (MP) with improved efficacy is micronization [1-4]. MP are used in mixtures with various polymers, including polysaccharides.

We used micronization to prepare model systems based on benzimidazolyl-2-methylcarbamate hydrochloride (BMCHC) and enzymatic apple pectin (EAP) that have increased activity, prolonged properties, and lower toxicity [4].

It seemed interesting to study systems based on BMCHC and EAP and samples of pectin with various degrees of demethoxylation (λ, %) using a combination of physical and physicochemical methods.

We carried out microscopy studies of EAP and BMCHC samples, the mechanical mixture of which is a combination on the optical level of separate elongated crystals of BMCHC that glow in polarized light and shapeless amorphous particles of EAP of various sizes.

After micronization of this mixture at 40 g for 10 min, aggregates of platelike pectin pieces and shortened BMCHC crystals adhering to each other appear.

If the micronization conditions are intensified (60 g/10 min), the BMCHC crystals appear in polarized light as very fine glowing particles that form aglomerates with flattened platelike pieces of EAP, obviously due to impact and shear forces.

BMCHC:EAP mixtures with 41.0 and 65% methoxylation give the same aggregates as with EAP (λ = 80%) after micronization under the same conditions (40 g/10 min) because there are more OH groups to carry out the physicochemical reaction with MP and to form aglomerates that differ little on the optical level. It should be noted that the anisodiametric nature of the BMCHC crystals is less clearly defined.

The BMCHC:EAP mixture with the highest level of methoxylation (λ = 92%) is minaly fine shapeless particles of various brightness in polarized light that do not aggregate to each other, evidently because of the lower reactivity of the components.

An estimate of the particle sizes for the BMCHC:pectin mixtures (Table 1) showed that the average particle sizes after micronization decrease more with increased grinding. This is clearly seen by comparing these parameters for BMCHC:EAP mixtures (λ = 80%) as a physical mixture and after grinding at 40 and 60 g for 10 min.

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TABLE 1. Particle Sizes in the BMCHC:EAP System

Sample No.	Sample (conditions)	Sizes, μm	
		max.	aver.
1	BNCHC:EAP (λ = 80%), phys. mixt. (1:2)	192.0	65.5
2	BNCHC:EAP (λ = 41%), 40 g/10 min (1:2)	284.9	74.3
3	BNCHC:EAP (λ = 65%), 40 g/10 min (1:2)	269.5	41.2
4	BNCHC:EAP (λ = 80%), 40 g/10 min (1:2)	184.8	60.4
5	BNCHC:EAP (λ = 92%), 40 g/10 min (1:2)	115.0	43.5
6	BNCHC:EAP (λ = 80%), 60 g/10 min (1:2)	169.4	58.8

Minimal particle sizes, 3.5 μm.

TABLE 2. Sorption of Water Vapor at 25°C by BMCHC and Pectin Samples

Rel. humidity, %	BMCHC:EAP				
	(λ = 80%) (1:2) phys. mixt.	(λ = 41%) (1:2) 40g/10 min	(λ = 65%) (1:2) 40g/10 min	(λ = 92%) (1:2) 40g/10 min	(λ = 80%) (1:2) 60g/10 min
	1	2	3	4	5
10	0.20	0.50	0.50	0.15	0.90
30	0.50	1.20	1.30	0.35	2.30
50	0.70	1.80	2.10	0.55	3.30
65	1.30	2.70	2.50	1.00	4.60
80	2.50	4.20	3.30	2.00	6.90
90	4.30	5.90	4.70	3.30	10.80
100	13.8	14.80	10.40	12.50	27.00

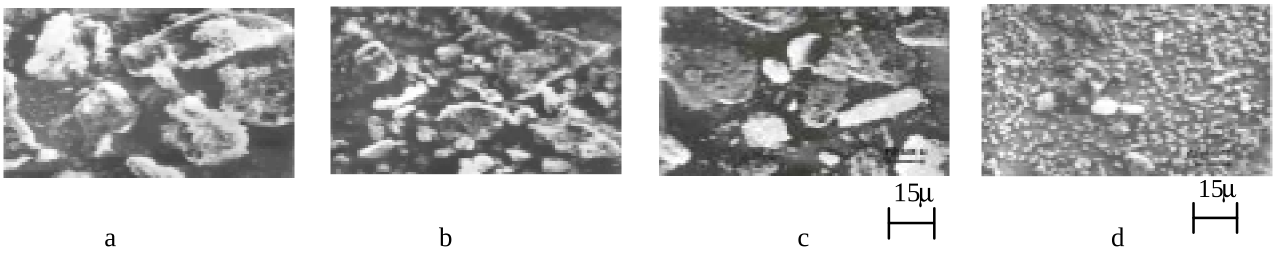

Fig. 1. SEM images of BMCHC:EAP mixtures: λ = 80% (phys. mixt.) (a), λ = 65%, 60 g/10 min (b), λ = 80%, 60 g/10 min (c), and λ = 92%, 60 g/10 min (d).

Under identical conditions and with a decreased content of methoxyls in the BMCHC:pectin mixtures, a tendency toward increased average and maximum sizes is seen, especially for EAP (λ = 41%). This is consistent with a more extensive reaction and a tendency to aggregate.

On the other hand, the range and average particle size decrease significantly for the sample with the maximum level of methoxylation. This indicates a lower tendency toward physicochemical reaction and, therefore, formation of aggregates and correlates with IR spectroscopic data obtained earlier that showed the formation of H-bonds in the mixture after micronization [4].

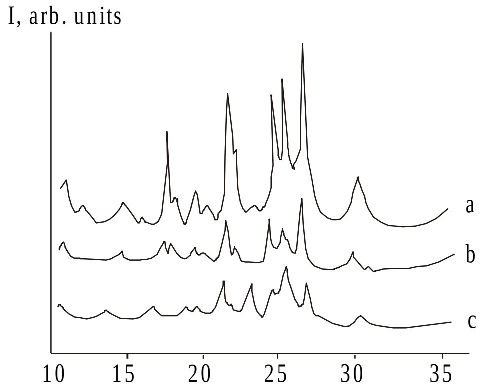

Fig. 2

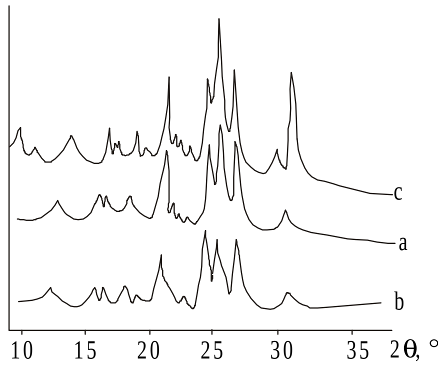

Fig. 3

Fig. 2. X-ray diffraction patterns of BMCHC (a) and BMCHC:EAP mixtures (b, c). Starting BMCHC (a), BMCHC:EAP physical mixture, λ = 80% (b), and BMCHC:EAP, λ = 80%, 60 g/10 min (c).

Fig. 3. X-ray diffraction patters of BMCHC:EAP mixtures: λ = 41%, 40 g/10 min (a), λ = 65%, 40 g/10 min (b), and λ = 92%, 40 g/10 min (c).

It was shown that the OH (3280 cm^{-1}) and C=O (1736 cm^{-1}) absorption bands weaken after joint mechanical treatment of BMCHC:EAP (λ = 80%, 1:2). Furthermore, the NH absorption band of BMCHC shifts from 3404 to 3343 cm^{-1} and that of the carbomethoxy group changes slightly. This is consistent with complex formation through H-bonds between NH of BMCHC and C=O of pectin and NH (BMCHC) and OH of EAP [4]. However, IR spectra of mechanically treated BMCHC:EAP mixtures (λ = 92%, 1:2) change insignificantly in the region of NH and C=O absorptions. Therefore, the ability of EAP to form complexes after additional esterification decreases because the hydroxyl of the galacturonic ring is excluded from the reaction sphere and all carboxyls are esterified.

At higher magnifications on an electron microscope (Fig. 1), the finer structure of the samples is visible. Rod-like crystals of MP and platelike EAP pieces (λ = 80%) with finely dispersed structure are clearly visible in the BMCHC:EAP (λ = 80%) physical mixture.

After micronization (40 g/10 min), the BMCHC crystals are difficult to identify, the EAP plates become thinner, and the graininess is less noticeable. However, more densely packed particles of MP are visible.

The structures of the starting compounds are even more evened out after increasing the micronization energy (60 g/10 min). Only aggregates of very fine component particles sticking to each other are observed as a result of the reaction. Pieces of this same type are typical of BMCHC:EAP mixtures (λ = 41 and 65%).

Mixtures of BMCHC with fully methoxylated EAP (λ = 92%) produce only fine separate particles that retain their integrity, evidently due to a decrease in the extent of the reaction and a weakening of the H-bonds as a result of the lower number of OH groups.

X-ray diffraction studies of the BMCHC:pectin samples were also performed.

The MP typically give a large number of reflections that is indicative of a complex crystal structure. The strongest reflections in the diffraction patterns of BMCHC appear at 2θ = 18.8, 23, 26, 27, 28.2, and 31.7 (Fig. 2, a).

Pectin is known to be an amorphous substance and gives no maxima in the diffraction pattern. Therefore, only BMCHC reflections can be interpreted in the BMCHC:pectin mixtures. These appear weaker than in starting MP owing to the lower content.

The principal MP reflections become even weaker after grinding the BMCHC:EAP model mixture (1:2) at 60 g for 10 min. The ratio of the heights changes. The half-width of the reflections increases. This is consistent with a significant structural change for BMCHC owing to amorphization under the severe micronization conditions. Diffraction patterns of the same type are obtained for BMCHC mixed with low-methoxylated pectins (λ = 41 and 65%). The mixture of BMCHC with fully methoxylated pectin (λ = 92%) gives stronger reflections. This is consistent with a higher degree of MP crystallinity (Fig. 3).

TABLE 3. Sorption Properties of BMCHC:EAP Samples

Sample	Parameters			
	λ_m (g/g)	S_{sp} (m^2/g)	W_0 (cm^3/g)	r_c, Å
1	0.0052	18.377	0.138	150.0
2	0.246	86.488	0.270	62.0
3	0.0120	42.381	1.148	70.0
4	0.0142	49.805	0.104	42.0
5	0.0034	12.047	0.125	20.7

It was found that the sorptivity of BMCHC:EAP (1:2) increases significantly as a result of micronization at 60 g for 10 min (Tables 2 and 3). Thus, whereas the BMCHC:EAP physical mixture (λ = 80%) at 65% relative humidity contains 1.30% sorbed moisture, the amount increases to 4.60% after micronization. This is due to a significant decrease in the particle size, which gives the sample a high specific surface area (86.488 m^2/g instead of 18.377 m^2/g) although the average pore size decreases (62 Å instead of 150 Å). The total pore volume in the mixture increases (0.270 cm^3/g instead of 0.138 cm^3/g).

The sorptivity of the BMCHC mixture with methoxylated pectin decreases more as the level of methoxylation increases. The sorptivity at 65% relative humidity for pectins with λ = 41, 65, and 92% is 2.7, 2.5, and 1.0%, respectively. This occurs because the samples are more hydrophobic. The specific surface area increases slightly (49.805 m^2/g instead of 42.381 m^2/g) owing to the decreased particle size. It then decreases significantly (to 12.047 m^2/g) because of the high hydrophobicity with full methoxylation. This is accompanied by a substantial decrease of pore size.

The decreased specific surface area degrades the medicinal effect of the mixture as the level of pectin methoxylation increases. This may be due to a decrease in the reaction between the components because the probability of forming H-bonds decreases.

Thus, model systems based on BMCHC and EAP were studied. The structural features of these depend substantially on the level of pectin methoxylation.

EXPERIMENTAL

BMCHC [5, 6], EAP [3], and samples of low-methoxylated pectin from EAP with λ = 41, 65, and 80% [3, 7] were prepared as before.

Grinding and activation of BMCHC with EAP was carried out in an AGO-2U (Gefest, St. Petersburg, Russia) planetary-centrifugal grinder-activator in metallic drums lined with Teflon. The grinders were agate spheres. BMCHC (0.5 g), EAP (1.0 g) of various methoxylation levels, and spheres (10.5 g, 1:7 ratio) were loaded successively into the drum. The mixture was ground for 10 min at 40 or 60 g.

X-ray diffraction studies were performed on a Dron-3M diffractometer using monochromatized Cu Kα-radiation at 20 kV and 12 mA. Samples were prepared as ground preparations in pressed disks. Reflections in the range 2θ = 10-30° were recorded by powder diffraction in the equatorial plane.

Microscopy investigations were carried out using an MBI-6 optical microscope in transmitted and polarized light. The minimum, maximum, and average particle sizes were estimated from two parallel estimates of 30 measurements each.

Electron-microscopy investigations were carried out in scanning mode on an REM-200 microscope. Samples were coated beforehand with Ag in a VUP-4K vacuum chamber.

Sorption studies were performed in a vacuum chamber on a Mc Bain balance over a wide range of relative humidities (10-100%) at 25°C. The specific surface area (S_{sp}), monolayer volume (λ_m) in grams of adsorbent per gram of solid, and pore volume (W_0) and average size (r_c) were estimated using the sorption data and the BET equation [8].

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