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CHEMICAL TECHNOLOGY

Production of Pectin Polysaccharide Complexes with Dicarboxylic Acids

S. T. Minzanova, V. F. Mironov, A. B. Vyshtakalyuk, O. V. Tsepaeva, A. Z. Mindubaev, L. G. Mironova, A. T. Gubaidullin, V. V. Zobov, A. V. Lantsova, G. R. Petrova, F. Kh. Ziatdinova, and Academician A. I. Konovalov

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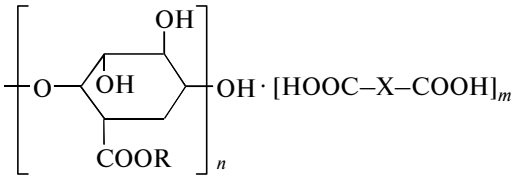
Chemical modification of natural polymers leads to derivatives with new physical, chemical, and biological properties [1, 2]. Acidic pectin polysaccharides, unlike neutral polysaccharides, have been little studied as applied to the preparation of functional derivatives with the retention of the macromolecular chain; therefore, inasmuch as pectins are candidates for use as plant raw material, studying the chemical modification of pectin polysaccharides is an important task [3].

Pectin polysaccharides in various forms, therapeutic preparations, and food stuffs are an efficient natural means of detoxication of the body from the detrimental effect of radionuclides, heavy metals, and other toxic substances. In addition to the radioprotector and hypocholesterinemic activity, pectin suppresses putrefactive microflora in the intestine and is used as the antibacterial remedy for the treatment of gastrointestinal infections. A practically important property of pectin polysaccharides is the ability of their solutions to gelatinize, which is widely used in the dairy and confectionary industries [4, 5]. Pectins are used in increasing frequency as auxiliaries in drug formulations. Numerous domestic and foreign studies (see, e.g., [6]) have addressed the interaction of reactants with pectins and their effect on the pharmacotherapeutical activity of the former. In particular, taking aspirin in the mixture with pectins reduces the irritant action of the preparation on the gastrointestinal tract [6]. Penicillin, tetracycline, and neomycin combined with pectins exhibit the potentiating and detoxic activity of pectins [7]. In the patented antituberculous pharmaceutical composition [8], pectin reduces the toxicity of the composition and ensures its durable action.

The capacity of pectins for complexation is used in medicine practice for the design of prolonged action drugs, the enhancement of their therapeutic activity, and the reduction of their toxicity [9]. It might be expected that a combination of pectins with other biologically active additions (BAAs) will extend the possibility of using pectin in the food and pharmaceutical industries. Succinic and fumaric acids are the most important products and substrates of the tricarboxylic acid cycle [10]. They are also popular BAAs. Saturated and unsaturated dicarboxylic acids activate exchange processes of living organisms, enhancing their energetic possibilities and resistance to various injurious effects. They maintain a high activity of hydrolytic and proteolytic enzymes and improve the safety of vitamins C, P, and PP. They are natural products of metabolism exhibiting enhanced biological activity. According to the results of toxico-hygienic tests of the State Committee of Sanitary-and-Epidemiological Control of the Russian Federation, succinic (E 363) and fumaric (E 297) acids and their salts are allowed to be used as food additives in doses regulated by technological instructions for production of foodstuff and beverages. In contrast to many stimulators, these acids have no doping effect, i.e., exhaustive "whipping up" of some system of the body; rather, they are natural metabolites. However, neat succinic and fumaric acids can irritate the mucous coat of stomach. It might be expected that once complexes of succinic and fumaric acids with pectin get into the stomach, they form gels and are gradually dissolved without producing acidity centers.

In this work, we studied the complexation of pectin polysaccharides with saturated and unsaturated dicarboxylic acids and showed that the latter form molecular complexes **I** with pectin [11].

Arbuzov Institute of Organic and Physical Chemistry, Kazan Research Center, Russian Academy of Sciences, ul. Akademika Arbuzova 8, Kazan, 420083 Tatarstan, Russia



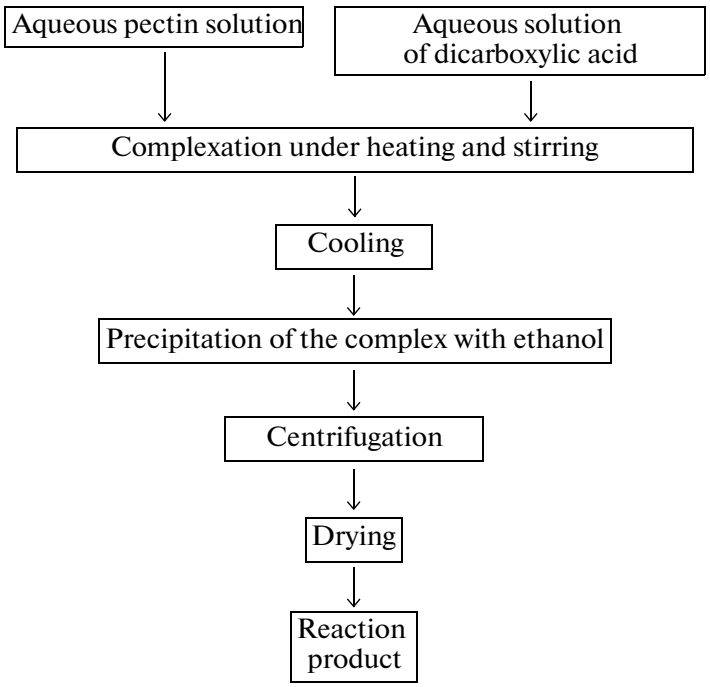
I

[R = H, Me; n = 98; m = 18–50;

X = $(CH_2)_2$ (a), HC=CH (b)].

To prepare complexes, reprecipitated (from water to ethanol) Classic C-401 citrus pectin (Herbstreith & Fox), with an average molecular weight of 17 600 and a degree of esterification of 68%, was used.

The procedure of synthesis of the pectin complex with dicarboxylic acid involves the treatment of an aqueous pectin solution heated to 55–60°C with a solution of succinic or fumaric acid taken in amounts of 0.1–0.8 g acid per 1 g pectin, keeping the mixture (55–60°C) under stirring for 0.5 h, and cooling. Then, complex **I** is precipitated with ethanol, dried, and ground (Scheme 1). The residual content of dicarboxylic acid in the centrifugate after removal of ethanol on a rotary film evaporator is determined by titrimetry.



Scheme 1

We studied the complexation of pectin with succinic acid as a function of temperature (Fig. 1): with increasing temperature, the yield of complex **Ia** increases and achieves a maximum value at 50–60°C. A further increase in temperature does not change the yield; i.e., the above temperature is optimal for complex formation.

To determine the amount of dicarboxylic acid binding to pectin, a series of experiments was run with different pectin-to-acid weight ratios. In the synthesis of complexes **Ia**, the pectin-to-succinic acid ratios were from 10 : 1 to 1.25 : 1. The data are presented as the plot in Fig. 2. As follows from this plot, with an increase in the amount of succinic acid in the reaction mixture, the amount of succinic acid bound to pectin gradually increases, achieves a maximum value at the pectin-to-succinic acid ratio of 2 : 1, and then remains constant. Thus, we determined the maximal amount of succinic acid binding to pectin in the molecular complex through hydrogen bonding.

For analysis of the composition of complex **I**, we suggested a procedure based on the decomposition of the pectin–dicarboxylic acid complex into the components (pectin and acid components): the complex was treated with an alkali on heating for 15 min until the solution became slightly alkaline (pH 7.5).

Sodium pectate was precipitated from the resulting solution with ethyl alcohol, separated by centrifugation, dried, and weighed. From the filtrate, sodium dicarboxylate was obtained by concentration and drying, and the amount of dicarboxylic acid bound in complex **I** was calculated from the amount of this salt. For complex **Ia**, the calculation was performed by the formula

$$\frac{m_1}{M_1} = \frac{m_2}{M_2},$$

where m_1 and m_2 are the weights of sodium succinate and succinic acid, respectively; M_1 and M_2 are the

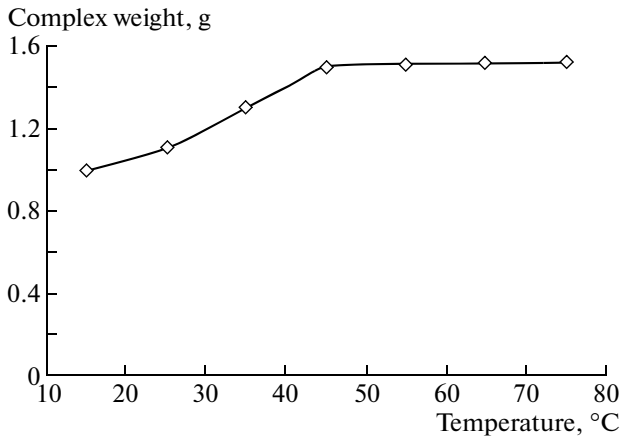


Fig. 1. Complexation of pectin with succinic acid as a function of temperature.

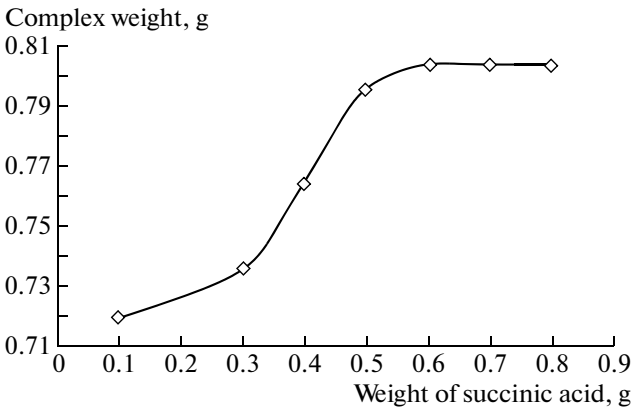


Fig. 2. Weight of complex **Ia** vs. the weight of succinic acid.

molecular weights of sodium succinate and succinic acid, respectively.

To prove the complexation of pectin with succinic acid, rather than formation of their mechanical mixture, the structure of complexes **Ia** and the initial compounds were comparatively studied by X-ray powder diffraction on a Bruker D8 Advance diffractometer (Fig. 3).

Succinic acid powder is characterized by well-defined diffraction peaks corresponding to the crystalline phase. For complex **Ia**, the X-ray diffraction pattern shows broad peaks. Their number and positions somewhat differ from those for pure pectin. The X-ray powder diffraction pattern of a mechanical mixture of two or more substances should show diffraction peaks corresponding to all mixture components. The sensitivity of the X-ray powder diffraction method for detection of the crystalline phase of organic compounds is in the concentration range from 0.5% or higher. At the same time, the complexes under consideration contain more than 10% of dicarboxylic acid. Thus, analysis of all our findings indicates that the modification of pectin with succinic acid leads to a homogeneous distribution of succinic acid over the pectin bulk and is indirect evidence of the formation of complex **Ia**.

The structure of the complexes was confirmed by IR spectroscopy. Comparison of the IR spectra of pectin and complex **Ia** showed that the band of the C=O vibration of the carboxyl group of pectin polysaccharide is shifted from 1745 to 1720 cm^{-1}, which points to the existence of interaction in the pectin–succinic acid system. The low-frequency shift of the bands due to the hydroxyl stretching vibrations ν(OH) in com-

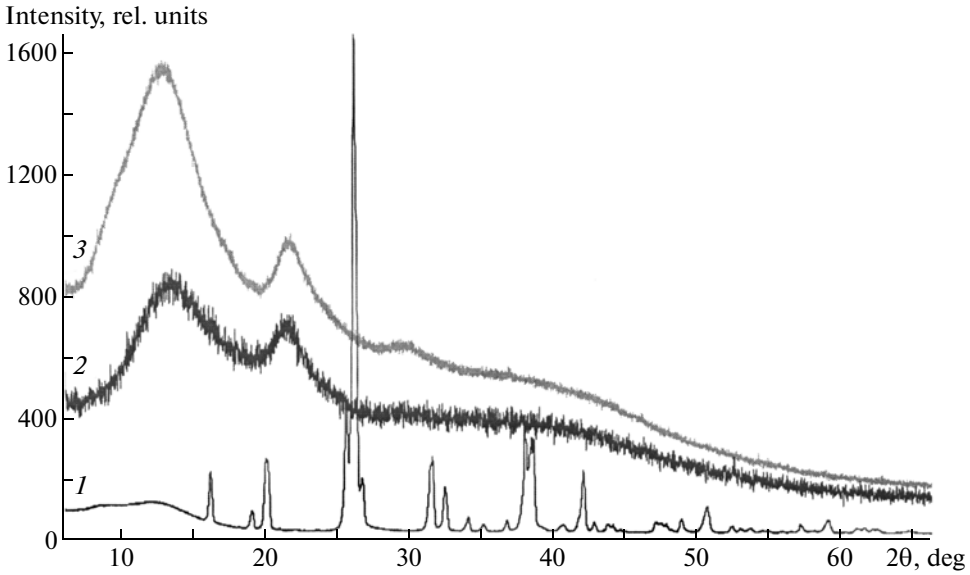


Fig. 3. Experimental X-ray powder diffraction patterns of (*1*) succinic acid, (*2*) complex **Ia**, and (*3*) pectin.

plexes **Ia** from 3448 to 3430 cm^{-1} is caused by the involvement of these groups in the system of hydrogen bonds. We believe that complex formation is through the hydrogen bonds between the carboxyl groups of succinic acid and the carboxyl and hydroxyl groups of pectin. Analogous results were obtained for the pectin–fumaric acid complex.

We studied the biological activity of complexes **Ia**, **Ib**. Their toxicity (LD_{50}) and ulcerogenic action on the mucous coat of stomach was studied in laboratory white mice. The activity of the complexes was compared with the corresponding characteristics for free succinic and fumaric acids taken in equimolar doses. The following results were obtained.

(1) Toxicity. For succinic and fumaric acids, the LD_{50} is 1500 and 1000 mg/kg, respectively. Complexes **Ia** and **Ib** taken in equimolar doses corresponding to the same doses of the organic acids with regard to their content in the complexes (24.5–25.0%) (complex **Ia,** 6000 mg/kg; complex **Ib**, 4000 mg/kg) were not toxic to laboratory mice.

(2) Ulcerogenic action, i.e., injurious action on the mucosa of the gastrointestinal tract. In animals, after the introduction of the acids (1500 and 1000 mg/kg of succinic and fumaric acids, respectively), the mucous coat of stomach showed evidence of the irritant action of the introduced compounds, which was manifested as hyperemia, petechial hemorrhages, and, sometimes, erosion. In animals, after the introduction of complexes **Ia** and **Ib** (6000 mg/kg), no signs of the irritant action on the mucosa were observed. Only in single cases were separate petechial hemorrhages and swelling of the micosa observed. Our findings show that the complexation of dicarboxylic acids—succinic and fumaric acids—with pectin polysaccharides leads to a pronounced decrease in their toxic action on the body and their irritant action on the mucosa of the gastrointestinal tract. Therefore, we may assume that pectin–dicarboxylic acid complexes **I** exhibit synergism of the initial components.

To assess the nutritional value of the pectin complexes with dicarboxylic acids, the in vitro digestibility of the complexes was determined according to the State Standard GOST 24 230-80.

Thus, the complexation of pectins with dicarboxylic acids was studied as a function of temperature and concentration. Procedures of their preparation for technological purposes and analysis were developed. It was shown that the acids incorporated in the complexes have a reduced toxicity and ulcerogenic action on the mucose of the gastrointestinal tract.

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