PREPARATION AND STUDY OF CARBOXYMETHYLSTARCH

G. A. Fedorova and N. T. Bondarenko

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It has been shown that when a mixture of starch with sodium hydroxide and monochloroacetic acid is irradiated, the carboxymethylstarch (CMS) obtained consists of fractions with high levels of carboxymethyl groups and fractions with a predominant content of carbonyl groups, and when this mixture is subjected to mechanical treatment a far-reaching rearrangement of the structure of the starch takes place with its homogeneous interaction with the reagents to form CMS.

In connection with the possible use of carboxymethylstarch (CMS) as a blood substitute [1] and a polymeric support for drugs [2], investigations in a technological direction are of great practical importance.

A polymer support must dissolve in water, contain functional groups to which the drug substance can be attached, be compatible with blood, not cause toxic effects, and not be antigenic. The time of its circulation in the blood stream is determined by its molecular weight (MW) and molecular weight distribution (MWD). Where it is necessary for a drug to penetrate into cells by endocytosis, the support must have a fairly high MW. At the same time, for excretion through the kidneys the MW should be fairly low. This contradiction can be resolved by the use of starch with definite molecular weight parameters and a definite content of carboxymethyl groups regulating the depth of its cleavage under the action of amylase and capable of adding a drug substance. The choice of the optimum technology for the preparation of such a support requires experimental investigation.

The activation of the carboxymethylation of starch has been carried out by irradiation and by a mechanical method, and a comparative investigation of the starch products has been made. The main parameters of the CMS obtained are given in Tables 1 and 2.

The radiation degradation taking place on the irradiation of polysaccharides is accompanied by the formation of carbonyl and carboxy groups which have a favorable influence on their rheological and aggregation properties, but these groups are distributed nonuniformly when a polysaccharide is irradiated both in the dry state and in solution (Fig. 1). It is obvious that when a definite size of the macromolecule is reached it passes into a qualitatively new physicochemical state at which the absorption of the energy of the radiation is realized mainly in reactions involving the radiation-chemical transformation of the monomeric units with no appreciable fall in the MW.

When potato starch is irradiated, oxidative-destructive processes take place primarily in the amorphous regions of the amylopectin fraction [3], since amylose, with strong intramolecular interaction, is located in the zone of the nucleus [4], and in view of the considerably "cage" effect and slow diffusion of oxygen within the grain it undergoes degradation to a small degree. The microheterogeneity of the structure of starch in the absence of dispersion mixing of the components, in which their relative amounts in any small volume of the mixture will be the same as in the mixture as a whole, leads to the situation that the CMS obtained on irradiation actually consists of fractions with high contents of carboxymethyl groups and fractions with predominant contents of carbonyl groups, and its resistance to amylolytic attack is determined not only by the mean degree of substitution but also by the nature of the distribution of the substituents.

On diffractograms of mechanically activated mixture, the pattern of a deep disordering of the starch structure is observed, with reflections characterizing monochloroacetic acid and sodium hydroxide disappearing and new reflections characteristic for sodium chloride (d (Å) = 3.23 (15), 2.78 (100), 1.61 (18), 1.40 (9)) appearing, which indicates a chemical interaction of the components. Correspondingly, the hygroscopicity of the preparations in-

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TABLE 1. Conditions for Obtaining CMS and the Main Parameters of the CMS Obtained on Irradiation

Monochloroacetic acid, wt. %	Sodium hydroxide, weight, %	Irradiation dose, Mrad	Cleavability by enzymes, %	Solubility in water at 20°C, %	$\overline{M}_w \cdot 10^3$, daltons
0	0	30	65,0	15,8	84,4
4	4	30	44,5	63,1	108,4
8	8	30	32,5	93,4	98,1
12	12	30	24,0	95,9	86,6
16	16	30	18,0	97,5	80,6
22	22	30	12,5	97,6	72,2
30	30	30	9,5	97,5	65,0
16	16	5	18,5	63,9	575,3
16	16	10	17,5	81,0	281,7
16	16	20	17,0	82,6	131,6
16	16	30	17,0	95,5	80,6
16	16	40	17,0	98,5	55,7

TABLE 2. Results of Analyses of CMS Obtained on Dispersion

Type of mill	Amounts of reactant, wt. %	Time of dispersion, h	Degree of substitution	pH of a 1% solution	D of the molar complexes, λ 570 nm	Cleavability by enzymes, %
Ball mill	18	1	0,16	8,4	1,450	37,5
		3	0,25	7,2	1,080	20,0
		5	0,27	6,8	1,050	17,5
		10	0,28	6,5	0,990	17,5
		20	0,28	6,5	0,920	17,0
		40	0,28	6,2	0,830	17,0
Vibromill	0	20	0,00	6,0	1,710	74,0
	24	1	0,24	10,9	0,900	18,5
		5	0,37	8,6	0,565	14,5
		10	0,39	7,3	0,420	12,5
		20	0,40	6,8	0,350	12,0
		40	0,40	6,8	0,300	12,0
	40	5	0,36	11,9	0,375	14,5
		10	0,43	11,2	0,290	7,8
		20	0,52	9,8	0,000	5,5
		40	0,56	9,0	0,000	3,5

creases with dispersion (Table 3), and the pH of the solutions falls (Table 2). The purification of the preparations on ion-exchange resins followed by freeze-drying or thermal drying does not lead to an appreciable change in the degree of orderedness, since voluminous carboxymethyl groups appear in the macromolecules, these groups being identified by chemical methods [5] and by the appearance in the IR spectra of intense bands (1620 and 1430 cm^{-1}) belonging to the stretching vibrations of the carboxylate ion COO^- [6] with a corresponding decrease in the intensity of the bands of the stretching vibrations of OH groups (3450 cm^{-1}). The capacity for binding iodine and the cleavability of the starch by enzymes fall (Table 2).

The performance of an analogous modification in a relatively dry medium, when the adsorbed moisture was eliminated by drying the polymer at 105°C, showed that even on prolonged dispersion (70 h) with a 50% content of modifying agents the dry samples swelled poorly in water and their enzyme cleavability was 40%.

Measurement of the temperature of the mixtures (with the aid of a chromel-copel thermocouple with a temperature of the free end of 0°C) directly in the dispersion process and analysis of these mixtures by the DTA method permitted the conclusion that the presence of adsorbed moisture, which is capable of breaking down the structure of starch along the line of hydrogen bonds, accelerated grinding and determined the direction of the degradation process exclusively along the lines of this weakening. With the superposition of a short-time rise in the pressure and temperature on contacting surfaces, this led to the realization in the mechanochemical apparatus of the conditions of a hydrothermal system and, through the formation of active surface atoms at the moment of dispersion, the possibility of the synthesis of CMS. Under these conditions, the heterogeneous interactions approximate in essence to homogeneous reactions, which permits a product fairly homogeneous with respect to the degree of substitution to be obtained (Fig. 2).

In the initial stage of mechanical treatment, when the degree of substitution increased, the viscosity of the aqueous solutions rose, and as disaggregation proceeded and the mobility of the particles increased, it fell (Fig. 3). Since under these conditions dispersion takes

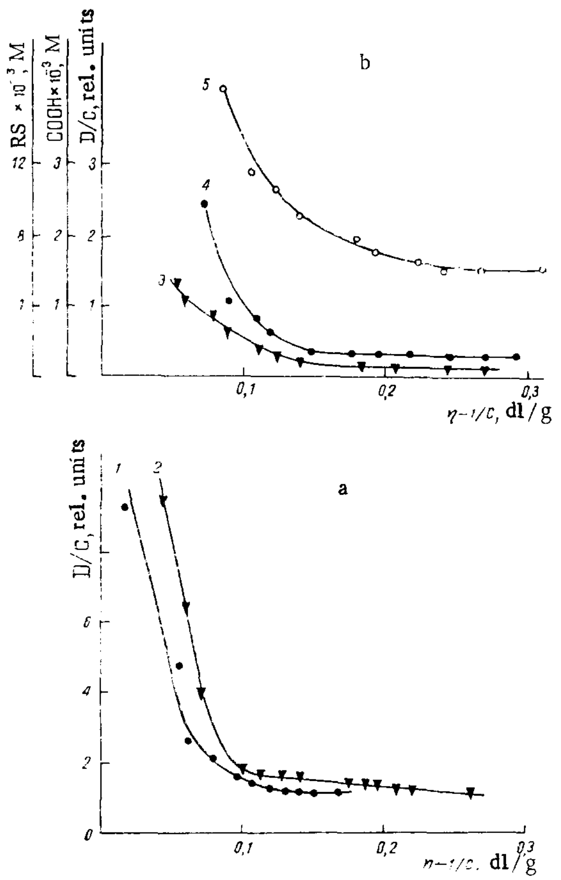

Fig. 1. Change in the intensity of UV absorption (1, 2, 4), reducing capacity (5), and acidity (3) of irradiated polysaccharides according to fractions: a) on irradiation in the dry state; b) in solution [1] starch, 40 Mrad; 2) CMS, 10 Mrad, degree of substitution 0.6; 3, 4, 5) 6% aqueous solution of dextran, 4 Mrad, pH 9.0].

place through the breakdown of supermolecular formations, the CMS retained the high MW that was characteristic for the initial starch.

EXPERIMENTAL

The IR spectra of films deposited on a KRS-5 crystals were taken on a UR-20 instrument, and UV spectra on an SF-4a spectrophotometer. X-ray phase analysis was performed on a DRON-1 diffractometer with CuK_α radiation.

The irradiation of a mixture of native starch (moisture content 14%, R_W $4.5 \cdot 10^5$) with powdered sodium hydroxide and powdered monochloroacetic acid was carried out in Cellophane bags in a RKhM-γ-20 apparatus at a power of the ^{60}Co source of 300 rad/sec with doses of 5-40 Mrad.

The mechanical activation of the carboxymethylation process was carried out in a vibromill (steel chamber and balls, diameter of the balls 7 mm, volume of the chamber 1 liter, amplitude of the vibrations 1.5 mm, speed of the motor 1390 rpm), and also in a ball mill (porcelain chamber and balls, weight of one ball 8.7 g, volume of the drum 5 liters, speed 41 rpm).

The amount of modifying agents in the mixture was varied from 0 to 50% with respect to the weight of starch. For each mole of acid, 2 moles of hydroxide were used. Samples were taken for analysis after predetermined intervals of time.

Enzymatic hydrolysis was performed by the action of diastase. After the preparations had been incubated with the enzymes for 4 hours, the degree of conversion into maltose was determined by a standard method [7]. The preparation (100 μg) was incubated with the diastase (activity 12.3 units/ml) in an isotonic solution of sodium chloride (total volume 100 ml) with pH 7 and a temperature of 35°C maintained by an ultrathermostat.

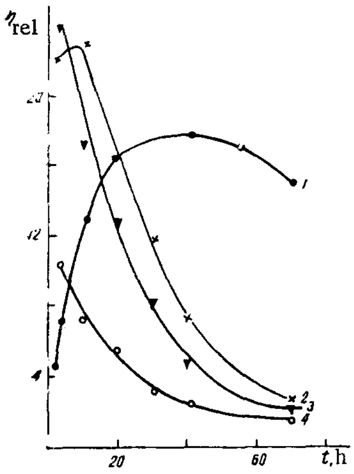

Fig. 2

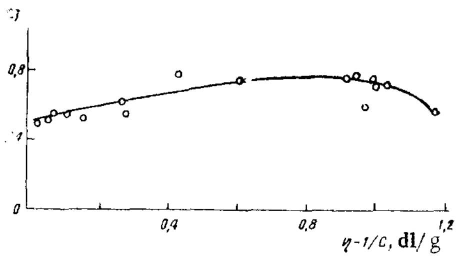

Fig. 3

Fig. 2. Dependence of the viscosity of 1% solutions of CMS on the time of dispersion: 1) ball mill, content of modifying agents 18%; 2) vibromill, 24%; 3) 40%; 4) 50%.

Fig. 3. Distribution of carboxymethyl groups over the fractions for CMS with a degree of substitution (DS) of 0.65.

TABLE 3. Sorption of Moisture by Dispersed Starch

Atmospheric humidity, %	Equilibrium moisture content of the starch (%), dispersed			
	without the reagents	with the reagents (28 wt. %)		
	25 h	5 h	25 h	70 h
21	0,0741	0 0782	0,0790	0,0823
30	0,0785	0,0955	0,0972	0,0991
45	0.1160	0 1314	0 1365	0,1421
60	0 1538	0.2042	0.2156	0,2399
100	0.4086	1,5670	1,6515	1,9500

Viscosities were determined on a Ubbelohde viscometer at 20°C, the absorption of moisture from the air gravimetrically, and the capacity for binding iodine as described by Bailey and Whelan [8].

Fractionation was carried out by gel filtration on Sephadex G-200. The solution under investigation, containing 10 g of the dry matter of the polymer in 150 ml of distilled water or a 0.9% solution of sodium chloride was deposited on a column (5 × 150 cm) . Fractions with a volume of 50 ml each were collected automatically in the collector of a KhKOF-1 comparative chromatograph. The concentrations of the polymer in the fractions were determined on a previously calibrated ITR-2 interferometer.

SUMMARY

The irradiation of starch together with sodium hydroxide and monochloroacetic acid permits the diminution in the molecular weight of the polymer and its carboxymethylation to be carried out in one stage, but the CMS obtained consists of fractions with high contents of carbomethyl groups and fractions with a predominant content of carbonyl groups.

When the same mixture is subjected to mechanical treatment, the far-reaching reordering of the supermolecular structure of the starch takes place with its simultaneous interaction with the reagents to form CMS, but the high molecular weight that is characteristic for the initial polymer is retained.

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ISOLATION OF RAFFINOSE FROM COTTONSEED MEAL

I. A. Ivanova, M. T. Turakhozhaev,
and T. T. Shakirov

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The solubility of raffinose in various solvents has been studied. The influence of several factors on the process of extracting raffinose from cottonseed meal has been investigated. The optimum conditions of the extraction process have been established.

Raffinose is a nonreducing trisaccharide consisting of D-galactose, D-glucose, and D-fructose residues [1]. It is used in agriculture for the development of pedigree stock breeding and as a reagent for scientific-research purposes.

Raffinose is present in sugar beet at a level of 0.01-0.02% [2, 3] and also in the manna of several Australian eucalyptuses [4], and in rice, wheat, millet, soybean, pea, and jute seeds [5, 6]. The level of raffinose in jute amounts to 2%, which has permitted the seeds of this technical crop to be used as a raw material on the industrial scale [6]. The most accessible source of raffinose consists of cotton seeds and cottonseed meal.

There is contradictory information in the literature concerning the amount of raffinose in cotton seeds: It ranges from 1.5 to 4.0% by weight or, according to some reports, to 9%, which is apparently due to a dependence on the amount of carbohydrate synthesized on the variety of cotton plant and the soil and climatic conditions [7-9].

We have adopted a method of obtaining raffinose from cottonseed meal [10]. The method is based on the extraction of the raw material with aqueous ethanol with elimination of resinous substances from the concentrated extract, and the subsequent crystallization of the raffinose.

In order to select solvents for the extraction and further purification of the raffinose, we studied its solubility in organic solvents and aqueous organic mixtures (Table 1). For the experiment we used raffinose corresponding to TU [Technical Specification] 6-09-50-2374-81.

As can be seen from Table 1 the best solvents for extracting raffinose from the meal are aqueous ethanols, and for freeing it from ballast substances those of hydrophilic nature, such as a mixture of acetone, ethanol, and water. In order to determine the optimum concentration of ethanol for extracting raffinose, we performed a series of experiments in which 0.5-kg portions of ground cottonseed meal were extracted with ethanol of various concentrations at room temperature. Meal from cotton seeds of the 1981 harvest produced by the Kokand oils and fats combined was used.

Extraction was performed by the steeping method, and a total of these extractions, each of 3 h, was carried out. The combined extract was concentrated to 1/10 of its initial volume and was treated with gasoline to eliminate resinous substances, after which it was diluted with a twofold amount of acetone and was left for crystallization. The crystals that deposited were filtered off with suction and recrystallized from aqueous ethanol (Table 2).

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