Characterization of Starch from *Amaranthus cruentus* L.

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The starch isolated from seeds of *Amaranthus cruentus* L. has been characterized by physicochemical and pasting properties as well as by determination of starch-granule distribution and enzyme susceptibility to α-, β-, and γ-amylases in comparison with those of wheat, maize, waxy maize, and potato starches as references.

Amaranthus cruentus L. (purple amaranth) is grown in the United States of America and since amaranth grains contain 62 % of starch and about 14—16 % of proteins it is used as an ingredient in some foods such as puddings, soups, salad dressings. The nutrient composition of amaranth grains — total protein, its amino acids, as well as minerals and vitamins, has been comparable or even better than that of common cereal grains [1].

There are only a few reports on isolation and some of the properties of starch from amaranthus grains, therefore, as a part of our studies on alternative starch sources, investigation on the physicochemical and pasting properties and amylolytic susceptibility monitoring the structure of branching part of starch seemed to be worthwhile.

EXPERIMENTAL

Mature seeds of *Amaranthus cruentus* L. (No. 29 grain type), morphological group "Guatemalan" were used. Sample was grown on experimental plots at the Research Institute of Plant Cultivation in Piešťany in 1989. The colour of the seeds obtained was characterized as golden-brown and the perisperm of the seeds stained red-brown with iodine solution.

The enzyme preparations were tested on a soluble starch of anal. grade (Lachema, Brno; characterized in the laboratory by the iodine test and by M_r found to be 54 000, *i.e.* DP = 33.3). Potato starch (Slovenské škrobárne, Spišská Nová Ves), wheat starch (Slovenské škrobárne, Štúrovo), maize starch (Slovenské škrobárne, Boleráz), and waxy maize starch (No. 418, Slovenské škrobárne, Trnava) were used as standard polysaccharides.

α-Amylase from *Bacillus subtilis* (specific activity 127 nkat mg^{-1}), β-amylase from barley (41 nkat mg^{-1}), and γ-amylase (82 nkat mg^{-1}) were commercial products (Koch-Light, Colnbrook); the activities were determined at working pH of 5.8, temperature 25 °C, and substrate concentration 1 mg cm^{-3}. All chemicals used were of anal. grade.

The quantitative estimations of moisture, ash, protein, and fat of seeds and starch of amaranth were done by standard procedures of AOAC [2]. The starch content was determined polarimetrically [3].

The size of the starch granules was established by fully automatic analysis of particle size with Laser Sizer Particle Analyser (Fritsch-Analysette 22). Starch samples for scanning electron microscopy were dried to the critical point, mounted on stubs with double sticks and coated with 60 nm of gold. These were viewed and photographed on a Jeol JSM-840 A scanning electron microscope at an accelerating voltage of 25 kV and magnification 7000 x.

The amylose content was established from the iodine sorption biamperometrically [4] and parallelly photometrically [5]. The amount of reducing saccharides was determined by *Somogyi* [6] and *Nelson* [7].

Gelatinization temperature ranges were monitored following [8] using a microscope equipped with a hot stage. Water-binding capacity, swelling power, and solubility of the amaranth starch were established at the temperature 50 °C, 60 °C, 70 °C, and 80 °C [9].

Pasting properties were studied in aqueous system of the starch paste (6.25 mass %) [10] using the Brabender Viscograph equipped with 700 cm^3 sensitivity cartridge.

Isolation of Starch

The seeds of amaranth were steeped in distilled water (6 °C; 24 h), then wet-milled. The dispersion of perisperm was screened through 150 μm and 170 μm screen to remove fibre. The crude starch slurry was centrifuged (1500 *g*; 20 min).

Sediment was washed with distilled water repeatedly and the resulting starch slurry was treated with 0.01 M-NaOH, then with 1 M-NaCl (6 °C; 24 h). The extraction of proteins decreased the content of pro-

tein in sample to 0.5—1 %. So purified starch was suspended in 80 % aqueous methanol, heated (40 °C; 1 h) in a water bath, centrifuged and freeze-dried.

The sample was defatted by extraction with 75 vol. % aqueous solution of 1-propanol (70 °C; 3 h). Defatted starch was filtered, washed with methanol repeatedly and dried *in vacuo* at 60 °C over P_2O_5.

Fractionation of Starch

At the separation of the linear and branching part amaranth starch was suspended in 15-fold excess of 2 M-KOH. After 30 min of mixing, neutralization with 2 M-HCl to pH 5.2, dilution of the solution to 2 % of starch mass fraction, and centrifugation (1500 *g*; 15 min) the supernatant was heated in Erlenmayer flask to 80 °C and 1/5 of the volume of 1-butanol was added. After cooling and standing for 2 d, the solution was centrifuged and the sediment of insoluble butanol complex was obtained. Sediment was washed with 1-butanol-saturated water, then with methanol and dried in desiccator over P_2O_5 at laboratory temperature. The amylopectin was precipitated from the transparent supernatant by adding to the equal volume of methanol under strong stirring. The precipitate was washed with methanol repeatedly, filtered and dried as usual. Yield of the butanol complex was 4.5 % and of amylopectin 89.5 %.

Preparation of β-Limit Dextrin

The β-limit dextrin was obtained from the solution of starch, prepared as by fractionation with the exception that neutralization was done to pH 5.7 and without the next dilution. β-Amylase in the ratio E : S = 1 : 50 was added to this solution. The β-amylolysis was monitored by determination of the amount of released maltose. After 2 d the addition of β-amylase was repeated and after another 3 d the mixture was centrifuged (1500 *g*; 30 min), shortly boiled and again centrifuged. The solution was dialyzed against water for 3 d and freeze-dried. Yield of dextrin obtained was 47.5 %.

Retrogradation

Retrogradation of starch was determined [11] without stirring of its 2.5 % solution obtained by dissolution of the sample in 2 M-NaOH and neutralization with 2 M-HCl to pH 5.2. The aliquots withdrawn at time intervals were centrifuged and the supernatant was used to measure the iodine sorption.

Maximum of Absorption

At the determination of the absorption maximum of the complex starch—iodine 75 mg of starch was dissolved in 5 cm^3 of 2 M-KOH, neutralized after 0.5 h with 2 M-HCl and diluted with water to 150 cm^3 after adding of 1.75 cm^3 of 0.01 M-I. The spectral photometer UV VIS Specord M-40 (Zeiss, Jena) was used. The iodine absorption spectrum of amaranth starch revealed the absorption maximum at λ = 540 nm.

Periodate Oxidation

The periodate oxidation was established in suspension with concentration of polysaccharide 1.5 mg cm^{-3} and $NaIO_4$ 3.0 mg cm^{-3}. The mixture was kept in refrigerator for 2 d and the consumption of the oxidation reagent and content of formic acid formed were determined according to [12].

Enzyme Hydrolysis

In α-, β-, and γ-amylolyses the enzyme solutions (according to their activities) were added into the substrate solution, so that the reducing power did not exceed 10 % of its maximum value within the first 15 min (at conditions as in the determination of enzyme activities). The progress of hydrolysis was monitored photometrically by determination of the amount of reducing sugars in time intervals, using the calibration graph for D-glucose, measured at pH 5.8. The enzymes characterized by their specific activities determined on the soluble starch as the substrate were used also on potato and wheat starch which served as reference samples for amaranth starch.

RESULTS AND DISCUSSION

The chemical composition of amaranth seeds and isolated starch is presented in Table 1. From the whole seeds 59 % of the determined amaranth starch with the high purity (95.56 %) was isolated. After purification the resulting starch still contained traces of residual protein.

Distribution of particles of amaranth starch according to their size is presented in Fig. 1. The sample contains two fractions of grains. The small grains with

Table 1. Proximate Composition of Amaranth Seeds and Starch

Sample	*w* Crude protein %	*w* Crude fat %	*w* Starch %	*w* Ash %
Seeds	15.68	7.63	55.41	2.96
Starch	0.85	0.22	95.56	0.20

Values are calculated to dry basic mass and are the average of five measurements, protein (w(N) × 6.25).

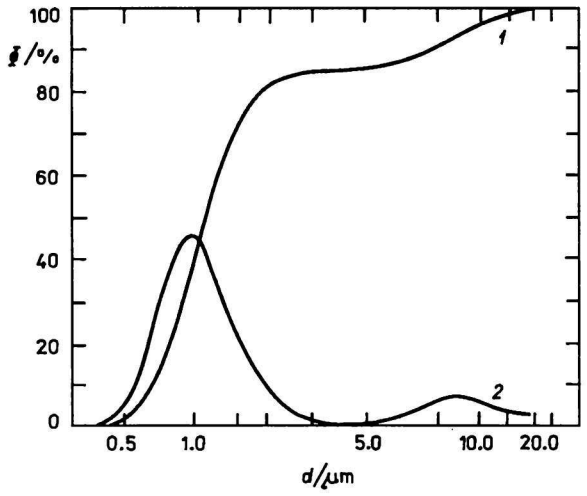

Fig. 1. Particle size distribution curves of *Amaranthus cruentus* L. *1*. Integration curve; *2*. derivation curve.

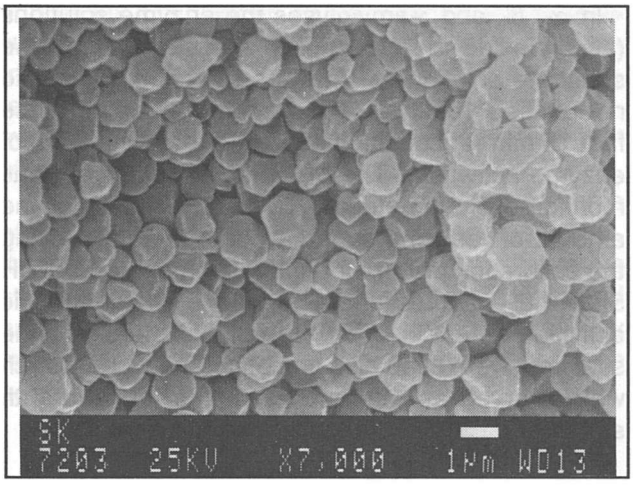

Fig. 2. Scanning electronic microscopy photomicrograph of amaranth starch (magnification more than 7000 ×).

the diameter 0.5—1.40 μm (maximum of grains have the size about 1 μm) present 85 % and the large ones with the diameter 5—25 μm (maximum 10—15 μm) 15 % of the starch. The results obtained are comparable with the literature data presented for other types of amaranth starch, 1—3.5 μm [13, 14] and 0.75—1.25 μm [15].

The shape of granules, which are isolated from starchy perisperm cells of the seeds is illustrated in Fig. 2, and exhibits polygonal character.

The physicochemical properties of the wheat, maize, and amaranth starch are shown in Table 2. Following the data the amaranth starch has a much higher solubility, a higher water-binding capacity, and greater swelling power. These differences might be attributed to the differences in the degree of intermolecular association between starch polymers due to associative forces, such as hydrogen and covalent bonding.

Table 2. Physicochemical Properties of Starches

Properties	Conditions	Starch		
		Amaranth	Maize	Wheat
S^a / %	50 °C	3.2	3.6	4.8
	60 °C	5.6	5.4	7.5
	70 °C	85.7	8.2	11.3
	80 °C	91.3	13.2	11.4
SW^b / g g^{-1}	50 °C	3.7	1.4	2.9
	60 °C	7.1	3.2	7.2
	70 °C	22.1	10.0	8.0
	80 °C	26.9	14.9	10.0
WBC^c / g g^{-1}	50 °C	2.6	0.3	1.9
	60 °C	6.1	2.2	6.1
	70 °C	15.1	8.9	7.0
	80 °C	15.4	13.7	8.8
GTR^d / °C	from	59.0	55.0	65.0
	to	70.0	63.0	68.0
	max	935.0	645.0	260.0
AV^e / B.U.	92.5 °C	875.0	590.0	200.0
	30 min at 92.5 °C	820.0	540.0	160.0
	cooling to 50.0 °C	900.0	1260.0	410.0
	30 min at 50.0 °C	940.0	1070.0	320.0
	cooling to 25.0 °C	960.0	1280.0	380.0
	30 min at 25.0 °C	980.0	1330.0	380.0

a) Solubility, *b*) swelling power, *c*) water-binding capacity, *d*) gelatinization temperature range, *e*) amylograph viscosity.

Swelling and solubility are useful for understanding the nature of associative bonding forces within the granules. The higher values for both properties obtained at higher temperatures elucidate the relaxation of the homogeneous and strong bonding forces within the granules. Starch from *Amaranthus cruentus* L. has no amylose and a very small size of particles and therefore the associative forces within granules are less stronger than those in corn granules according to [16].

Amylose content and starch granule size have been reported as factors influencing starch gelatinization. The values of initial and final temperature of gelatinization obtained for amaranth starch are in good agreement with literature data [17] and are lower than those for corn starch. The measurements with partially purified samples have shown a dependence on the content of protein and lipids in the starch.

Pasting properties were determined by using of the Brabender Visco-Amylograph. The data on pasting behaviour indicated the peak viscosity 935 B.U. (conventional Brabender Unit, 1 B.U. = 9.80 x 10^3 N m) at the content of starch 6.3 mass % and showed a very little thinning of the hot paste on cooking at 92.5 °C. Amaranth starch shows almost no viscosity peak, *i.e.* the curve for initial 92.5 °C viscosity is very little lower than that for the peak viscosity. This behaviour reflects the greater stability of the swollen amaranth starch granules against mechanical dis-

integration, and is very similar to corn starch. The small increase in viscosity on cooling to 50 °C reflects very small retrogradation tendency of the amaranth starch (shows minimum set-back, similarly as waxy sorghum starch) [10]. That confirms the knowledge about weaker intermolecular forces within the amaranth starch granules [16, 17].

The absorption maximum of the iodine—starch complex (λ = 540 nm) showed a downward shift towards lower wavelengths. It is known that this shift in λ_{max} is indicative of the nature of starch, particularly of the content of linear fraction in a particular starch. Normal starch exhibits λ_{max} of 580—600 nm. However, waxy-maize starches showed λ_{max} 520—550 nm indicating higher content of amylopectin fraction. The results confirm the waxy-type character [18—20] of amaranth starch.

Iodometric titration of starch with biamperometric indication provides information on iodine sorption, expressed as amylose content [4]. Amylose binds iodine on forming the known blue complex of specific physicochemical properties. Contrary to common, commercially available starches, the course of titration of the amaranth starch sample indicates the absence of amylose (Fig. 3). The curve deviates only negligibly from that of the blank experiment, and no part of it reflects iodine sorption, this being a case similar to corn starch isolated from the waxy hybrid, which behaves as an amylose-free one.

It is interesting that though amylopectin and the amylopectin-type starch generate during titration the

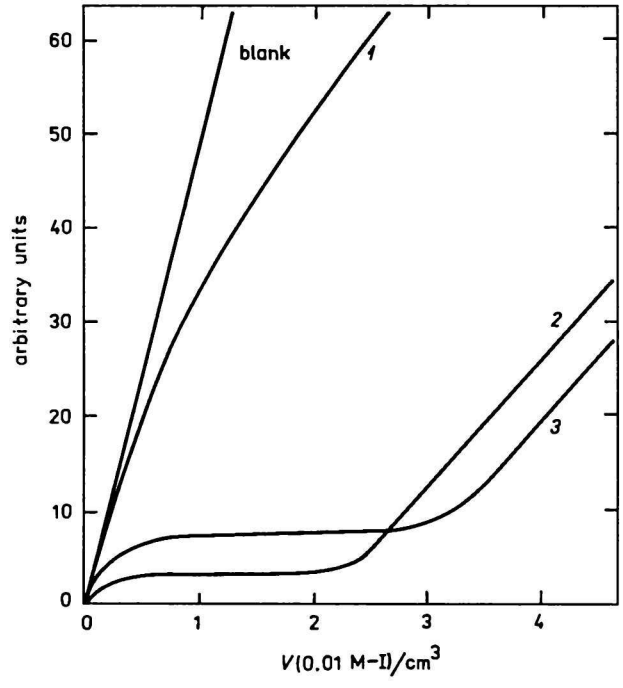

Fig. 3. Biamperometric determination of iodine adsorption. *1*. Amaranth starch; *2*. wheat starch; *3*. potato starch.

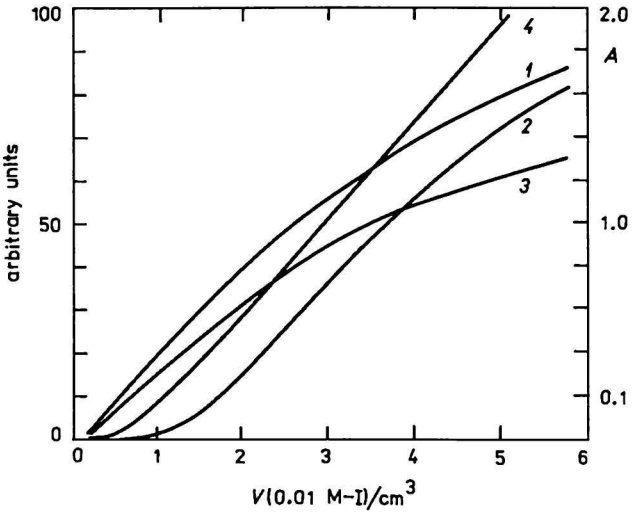

Fig. 4. Titration of amaranth starch with iodine using biamperometric and photometric indication. *1*. Biamperometry of amaranth starch; *2*. photometry of amaranth starch; *3*. biamperometry of waxy maize starch; *4*. photometry of waxy maize starch.

characteristic purple shade of the solution, in the first stage of titration of amaranth starch the solution remained colourless and became weakly coloured only in the later stages, *i.e.* after addition of higher portion of iodine. The difference course of biamperometric titration and photometric indication is presented in Fig. 4.

The absence of the linear component, usually present in other starches, was proved also by fractionation procedures, whereby the starches are resolved to amylose and amylopectin. Though there was obtained a negligible portion of the first fraction, which should represent amylose, it showed no iodine adsorption, either.

The structures of polysaccharides are studied mainly by using specific enzymes. Measurement of the amylase susceptibility and its comparison to other substances points to differences in the detailed structures of the starch components. α-Amylase (Fig. 5) degrades the amaranth starch somewhat slower but deeper than potato or wheat starch. As α-amylase is an endo enzyme, it indicates sufficiently long inner chains in the molecule, where the attack is easily realizable. On the other hand, β-amylase (Fig. 6) is less effective on amaranth starch than on other starches. Since β-amylase is an exo enzyme, it means that amaranth starch possesses short external chains. This fact was proved also by the yield of β-limit dextrin obtained in preparative β-amylolysis. The course of degradation with γ-amylase (glucoamylase) (Fig. 7) was similar as in the case of wheat starch.

The prepared β-limit dextrin was characterized by periodate oxidation [12]. It is a specific reaction, the result of which provides information on the degree

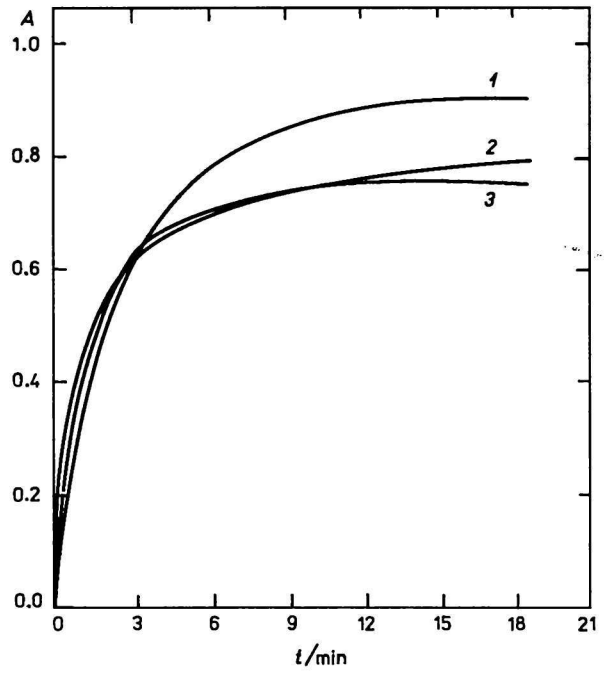

Fig. 5. α-Amylolysis of starches. The curves as in Fig. 3.

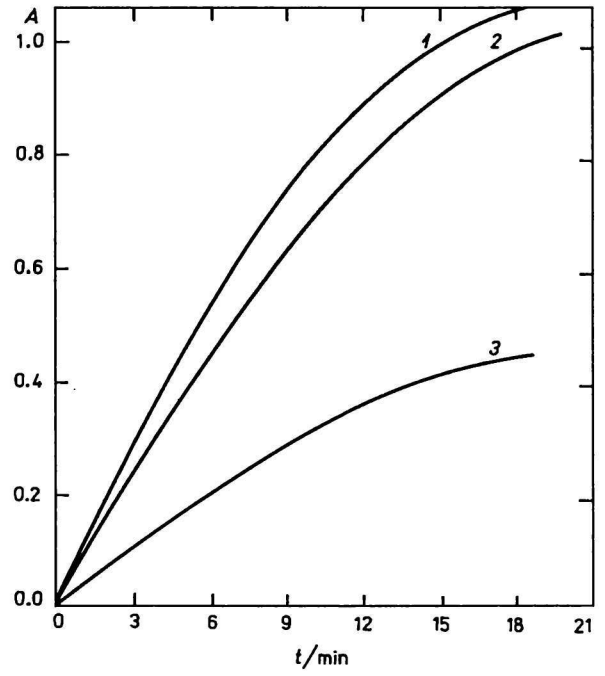

Fig. 7. γ-Amylolysis of starches. The curves as in Fig. 3.

of branching of starch polysaccharides, because formic acid is released only from the terminal D-glucose units. The found low degree of branching confirmed the longer inner chains established by α-amylase.

Retrogradation is a significant property of starch, representing instability of its solutions, and is induced by transition of amylose into the insoluble form. Although the generally used iodometric determination was not successful in this case due to low iodine adsorption, the sample of amaranth starch apparently had no tendency to spontaneous retrogradation.

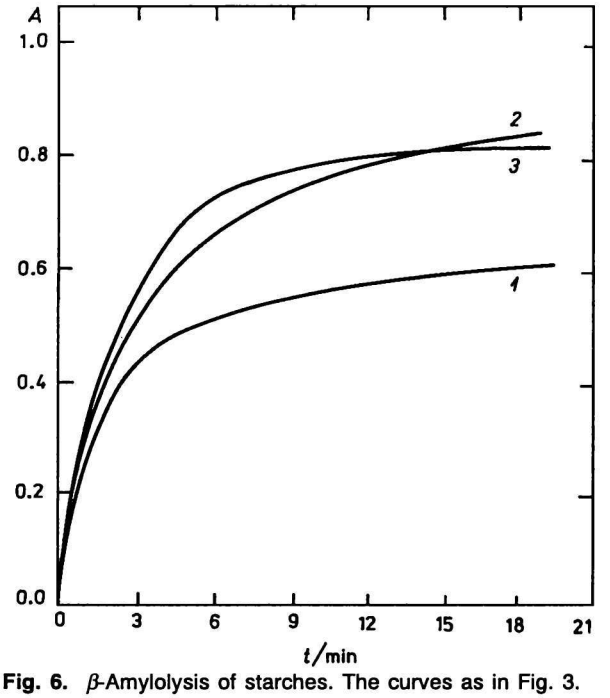

Fig. 6. β-Amylolysis of starches. The curves as in Fig. 3.

From the determinations presented above, the results of which well correlate, the following conclusions can be drawn. The studied amaranth starch does not contain the linear amylose fraction and consequently, it is an amylopectin-type starch. This statement is supported also by formation of stable, not retrograding solutions. The molecule does not contain longer external chains or longer inner chains which would adsorb iodine. Since the inner chains are long enough to enable good α-amylolysis, the branching must be relatively regular and the distribution of inner and external chains should be homogeneous.

These observations clarify the details of the structure of *Amaranthus cruentus* L. starch. However, it is noteworthy that the solutions of this starch do not generate with iodine an adequate colour which would correspond to amylopectin. This fact might point to some peculiarity in the structure of this polysaccharide.

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Plasma Polymerized Chlorobenzene

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Polymer films using chlorobenzene as monomer were prepared by the method of plasma enhanced chemical vapour deposition (PECVD). IR absorption spectra of products of polymerization reaction were studied in the wavenumber range from 400 cm^{-1} to 4000 cm^{-1}. The spectra confirmed the presence of polymer product but on the other hand showed that plasma polymerization of chlorobenzene leads to the breaking of some bonds in the substituted benzene ring resulting in the appearance of conjugate alkenes of different lengths.

Polymer films are used extensively also in integrated circuit fabrication and in the fabrication of solid state sensing devices. Especially several kinds of hydrophilic polymers have been used as materials for humidity sensors [1—3]. One of the broad variety of potential monomers is chlorobenzene — an accessible organic halo compound.

Lot of methods are suitable for polymerization of this monomer. Chlorobenzene has been polymerized [4, 5] with aluminium chloride—cupric chloride to produce materials that consist mainly of poly(*o*-phenylene) structures with the chloride atom situated at the position 4 (Fig. 1). The product of polymerization reaction is red, with average degree of polymerization of 10—12. Chlorobenzene polymerized in a fairly low yield (from 6 to 14 %). Antimony pentafluoride converted chlorobenzene to low yields of polymeric species [6] presumably of polyphenyl-like structure.

Our attention has been directed to the method of plasma enhanced chemical vapour deposition.

Organic substance in the vapour phase being excited into luminescence by an electric discharge, a solid film tends to deposit on all surfaces exposed to the luminous plasma. Depending on the conditions and the mechanisms of plasma polymerization the solid deposit from the plasma need not be formed in the conventional manner of functional groups uniting with each other in a repeating chain reaction. It is believed that ionic and ion-radical fragments are formed under the intense electron bombardment and recombine as they accumulate on the electrodes. The result is a complex intermolecular rearrangement of chemical bonds at more or less specific locations

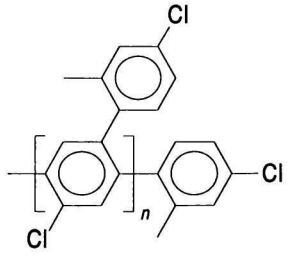

Fig. 1. Polymer structure of chlorobenzene.