Halina Stupińska,
***Edward Iller,**
****Zbigniew Zimek,**
*****Dariusz Wawro,**
*****Danuta Ciechańska,**
Ewa Kopania,
Jarosław Palenik,
Sławomir Milczarek,
*****Włodzimierz Stęplewski,**
*****Grażyna Krzyżanowska**

Pulp and Paper Research Institute,
ul. M. Skłodowskiej-Curie 19/27, 90-570 Łódź, Poland

***Radioisotope Centre POLATOM,**
05-400 Otwock-Świerk, Poland

****Institute of Nuclear Chemistry and Technology,**
ul. Dorodna 16, 03-195 Warszawa, Poland

*****Institute of Biopolimers and Chemical Fibres,**
Member of EPNOE,
European Polysaccharide Network of Excellence
www.epnoe.org
ul. M. Skłodowskiej-Curie 19/27, 90-570 Łódź, Poland

An Environment-Friendly Method to Prepare Microcrystalline Cellulose

Abstract
In this article a method is presented to prepare MCC from cellulose pulp employing an environment-friendly, effective two-step radiation-enzymatic depolymerisation process. The process was grounded on the radiation degradation by means of an electron beam to initially depolymerise the pulp prior to enzymatic hydrolysis. The cellulose pulp and the MCC made of it were estimated with the following quality indices: polymerisation degree, content of crystalline fraction (K_{Wr}), water retention value (WRV), specific volume, whiteness and grain coarseness. The characteristic of the molecular and crystalline structure of MCC as done by gel chromatography (GPC), the microscopic inspection of images and by the wide angle X-ray scattering (WAXS) method. It was documented that the results of preparing MCC by means of two-step depolymerisation depend primarily upon the average cellulose pulp polymerisation degree after enzymatic treatment. MCC preparation conditions recognised as optimal, may be specified as: irradiation of cellulose pulp with a 50 kGy dose followed by enzymatic hydrolysis during 0.5% at 50 °C and module E/S 46 UCMC/g. The depolymerised cellulose pulp is further processed in laboratory scale to form MCC by milling and drying. The MCC obtained is characterised by: DPv 150, a content of crystalline fraction (K_{Wr}) of about 64%, specific volume of 38 g/100 cm³and whiteness of 75%. Mass loss was 4.4% and the content of reducing sugars in the solution was 1.4 mg/cm³. The two-step depolymerisation route makes it possible to prepare MCC with quality indices close to materials used in the pharmaceutical industry, paving the way toward an ecological method of manufacturing microcrystalline cellulose for special uses.

Key words: *radiation-enzymatic depolymerisation, microcrystalline cellulose.*

Introduction

Microcrystalline cellulose (MCC) is a non-fibrous substance widely applied in the paper, pharmaceutical, cosmetics, food, and other industries. Made of either dissolving or paper pulp, it is usually supplied in the form of a white or creamy powder. In the manufacture of MCC the following steps may be distinguished: acid-catalysed hydrolysis of cellulose, the neutraliation, washingand mechanical processing of depolymerised cellulose and drying.

In the framework of the project "Ecological method to prepare microcrystalline cellulose for special uses", a number of methods were investigated with the goal of finding environmentally friendly alternatives to the burdensome acidic hydrolysis of cellulose pulp. The research included:

- Hydrothermal processing of dissolving and paper pulp at 172 – 185 °C
- Radiation degradation with deposited electron energy of 60 -200 kGy [1]
- Enzymatic hydrolysis with the use of enzyme preparations: Econase Ce by AB Enzyme Oy, Finland, xylanase Pulpzyme HA by Novo Nordisk, Finland [2]
- Two-step method: radiation and enzymatic treatment [3]

This paper presents the latter method. Two-step radiation and enzymatic depolymerisation appeared to be an effective way of ecologically preparing MCC.

In investigations concerned with the two-step modification of cellulose pulp in the preparation of MCC, a combination of radiation degradation and enzymatic hydrolysis was chosen [3]. Such a choice was based on the fact it was proven, in the course of research, that single step depolymerisation by enzymes [2] implies a very high E/S module of 184 UCMC/g at 55 °C and 6 hours treatment time. MCC prepared under such conditions reveals a high specific volume of 53 g/100 cm³, polymerisation degree of 230 - 250 and crystalline fraction K_{Wr} of 60 - 61%; however, mass loss is high, exceeding 10%.

A single radiation depolymerisation step in preparing MCC [1] requires a high deposition dose of electron energy of 200 kGy.

On the other hand, only small irradiation doses of 10 – 50 kGy, as was found in earlier research [4], suffice to bring DP down from 821 to 204. It was, therefore, concluded that in such degraded cellulose pulp the action of enzymes on the amorphous phase of elemental fibrils may positively affect the forming of crystallites, and thus the effective forming of MCC under milder conditions.

The research was focused on:

- Initial depolymerisation of the cellulose by irradiation with an electron beam,
- Final depolymerisation by means of enzymes.

An assumption was made to attain a content of the crystalline phase (K_{Wr}) above 60% in the MCC for special uses in the pharmaceutical, food and paper industries. In Table 1 quality requirements of

Table 1. *MCC quality requirements of the pharmaceutical industry.*

Property	MCC type	
	12	302
Polymerisation degree	≤ 350	≤ 350
pH	5 - 7,5	5 - 7,5
Specific volume g/100cm³	> 33	> 36
Grain coarseness sieve analysis, µm	5% > 320	95% < 200
	50%> 160	80% <160
	30%< 100	50% < 100

the pharmaceutical industry concerning MCC (for parameters tested in this work, only) are presented.

Experimental

Materials

A bleached dissolving pulp of type Alicell-Super was used in the investigation. It is made from mountain spruce and supplied by Western Pulp Ltd, Partnership Port Alice Operation, Canada.

The quality indices of the pulp are presented in Table 2.

Cellulase preparation Econase CE made by AB Enzyme Oy , Finland was used in the enzymatic hydrolysis. It is characterised by:

- Endo 1,4 β glucanase activity 2328 U/cm^3
- Paper method activity 137 U/cm^3
- β-glucosidase activity 26 U/cm^3
- Content of reduction sugars 20.43 mg/cm^3.

Research methods

Radiation depolymerisation of the cellulose pulp

Irradiation of the cellulose pulp was accomplished by using an electron beam generated in a LAE 13/9 linear accelerator LAE 13/9. The working parameters of the accelerator were: electron energy – 10 MeV, max. power of the beam – 6 kW, pulse duration – 5.5 μs, scanning frequency – 150 Hz, width of scanning – 60 cm, size of boxes for irradiation – 48 × 56 cm.

125 mm thick CTA (cellulose triacetate) dosimetric foil stripes, made by Fuji Photo Film Co, were used in the measurements of deep dose distribution. The material is characterised by the linear response of the radiation-induced optical absorbance as a function of the dose.

Alicell Super cellulose pulp was irradiated in sheets. The accelerator parameters were suitably adjusted to yield the overall radiation energy doses absorbed by the pulp, equal to 10, 15, 20 and 50 kGy [1]. Next, the irradiated pulp was subjected to enzymatic hydrolysis.

Table 2. *Quality indices of the pulp.*

Average polymerisation degree DP_v	810
α-cellulose content, %	94.2
Whiteness, %	92.5
Extract in dichloromethane, %	0.13
Ash, %	0.05
Silicate content as SiO_2, %	0.001

Enzymatic hydrolysis of the cellulose pulp

The irradiated samples of cellulose pulp were first swollen in an acetate buffer for 2 hours at pH = 4.8 (0.05 M) and 21 °C. The buffer/pulp ratio was 10/1. Prestabitole of 2.0% pulp was added to the buffer. Water was squeezed from the swollen pulp which, still wet, was mechanically processed at 38 °C for 60 min. The pulp was washed with water, squeezed and subjected to the action of enzymes in the buffer. The pulp is then put in glass flasks and placed in an ELPAN water shaker bath. Concentration of the pulp during the process was 5% [1].

The pulp was separated from the enzyme solution in a Büchner funnel at vacuum. Water at 95% was poured onto the pulp and left for 10 min. to deactivate residuum enzymes. The pulp was next filtered and washed three times with cold water [5].

Preparation of MCC from the depolymerised cellulose pulp

A depolymerised pulp of 5 g (an absolute dry substance) was de-fibrillated in 1 l of water in a homogenizer at 900 r.p.m. for 3 minutes. Next, after filtration in a Büchner funnel, it was ground in a porcelain ball mill at 40 - 50 r.p.m. at ambient temperature.

The gel obtained was put into porcelain mortar and dried with intermittent mixing in an open dryer with IR radiators at 50 – 60 °C, and next ground in the mortar for several minutes to prepare a powder. The powder eventually dried up at ambient temperature. Samples of the MCC prepared were stored in polyethylene bags [1, 2].

Analytical methods

Analysis of the enzyme preparation was done according to Good Laboratory Practice (GLP) No G016. Analysed were endo – 1.4, β-gluconase activity (SPR/BBP/8), paper method activity (SPR/BBP/9), and β – glucosidase activity (SPR/BBP/10). The content of reduction sugars was estimated by the colorimetric method with dinitrosalicylic acid (DNS) against the curve prepared for D-glucose (SPR/BBP/15).

Estimation of cellulose pulp and MCC properties

Average polymerisation degree DP was calculated from the limiting viscosity measurements in a cupriethylenediamine (CED) solution of the pulp and MCC according to Standard PN92/P-50101/01 (a translation of international Standard ISO 5351/1:1981).

Calculation of the average polymerisation degree:

$$DP_v^{0.905} = 0.75\,[\eta]$$

where: [η] – limiting viscosity of cellulose in CED.

The content of crystalline cellulose (K_{Wr}) was calculated from the equivalent moisture [6]. Samples of MCC, 1.0 –1.5 g in weight were dried at 105 ^{0}C to constant mass, and then placed for 7 hours in a conditioned chamber at 30 °C and 60% RH. The equivalent moisture content was calculated from :

$$Wr = \frac{m_1 - m_0}{m_0} \times 100,\ \text{g } H_2O/100 \text{ g of dry cellulose}$$

where: m_0 – mass of MCC sample after drying at 105 °C, m_1 – mass of MCC sample after conditioning

The percentage of amorphous cellulose A_{Wr} was calculated from the empirical equation:

$$A_{Wr} = 5.795 + 5.416 \times Wr, \text{ in \%}$$

and the content of crystalline cellulose (K_{Wr}) from:

$$K_{Wr} = 100 - A_{Wr}, \text{ in \%}$$

The Water Retention Value (WRV) was measured according to procedure adopted at the Institute Biopolymers and Chemical Fibres (IBWCh) for microcrystalline chitosan. WRV is/was calculated from:

$$WRV = \frac{m_2 - m_0}{m_0} \times 100, \text{ in \%}$$

where: m_2 – mass of MCC sample after centrifuging at 4000 r.p.m., m_0 – mass of MCC sample after drying at 105 °C.

The whiteness and yellowness of the pulp and MCC was estimated by means of a Zeiss leukometer according to a slight modification of Standard PN-76/P-50169, which applies to pulp and paper. The modification involved using MCC in a transparent film bag instead of a small pulp sheet in the measurements.

Specific volume. A pre-weighed 10 cm^3 glass cylinder was slowly filled up with

air dry MCC, and the cylinder was weighed with the MCC content. The bulk density was calculated from:

$$V_{sp} = (m_{C1} - m_{C0}) \times 10 \text{ g}/100 \text{ cm}^3$$

where: m_{C1} – mass of filled cylinder in g, m_{C0} – mass of empty cylinder in g.

Microscopic measurement of crystallite aggregates of MCC. A NIKON ECLIPSE E 200F microscope with an SSc-DC50 APYC Sony camera was used; magnification - 200 times. The surface, circumference, length and width of the micro-images of the aggregates were measured with the use of computer imaging – a MultiScanBase 14.02 analyser. Average values were taken from each of the 50 measurements.

Molecular weight distribution of cellulose pulp and MCC by gel chromatography method (GPC).
Samples for GPC analysis were prepared according to the Ekmanis method [7] modified by IBWCh.

Chromatography system:
- Columns: 3 × Pigel Mixed A, 20 µm, 300mm, (Polymer Laboratories Ltd.)
- Isocratic pump: HP 1050 (Hewlett Packard)
- Detector: Differential Refractometer HP 1047 (Hewlett Packard)
- Calibration standards: ten "narrow" polystyrene standards with molecular weight within the range of 3790 to 5000000

GPC analysis parameters:
- Continuous phase: Dimethylacetamid (DMAC)/0.5% LiCl
- Column temperature: 80 °C
- Flow speed: 1.0 ml/min
- Concentration of cellulose solution: ~ 0.05%
- Injection volume: 100 µm.

Crystalline structure of MCC by wide angle X-ray scattering (WAXS) method.
Analysis was done for selected pulp and MCC samples at the University of Bielsko-Biała (ATH).

The investigation was carried out on a URD 63 diffractometer, made by Seifert Co., using CuK_α radiation, acceleration voltage - 40 kV and anode amperage - 30 mA. A nickel filter and impulse height analyser provided a monochromatic beam. A scintillating counter was used as a detector.

The diffractograms were analysed according to the Hindeleh and Johnson method [8] and by means of own (THA) software Opti Fit [9].

Grain size distribution in MCC.
The MCC sample was dispersed in water for 5 min., with an addition of $Na_2P_2O_7$, in a LABORETTE 17 ultrasonic bath, and the grain size distribution was estimated by means of an Analysette 22 compact laser grain meter, made by Fritsch Co.

Results of the investigation

Optimisation of the two- step depolymerisation of cellulose pulp

Sheets of cellulose pulp were irradiated with an electron beam according to the method described above, depositing 10, 15, 20 and 50 kGy of energy in the individual trials. In the enzymatic hydrolysis trials, following the irradiation, the impact of hydrolysis time and E/S module on the polymerisation degree of cellulose was investigated . Time in the individual trials was set at 0.5, 1.0 and 4.0 hours, while the E/S module values adopted were 46 and 92 UCMC/g. Mass loss in the cellulose pulp and concentration of reducing sugars were estimated in the solution. The results compiled in Table 3 document that under the mildest enzymatic hydrolysis conditions (0.5 h and E/S module of 46 UCMC/g in trials from R10/AS-19 to R50/AS-21) the attained polymerisation degree DP_v obtained fall within the range of 190 to 322 depending upon the energy dose applied, while relevant DP_v values after the first step-irradiation is within the range of 223 - 464. Mass loss amounts to 3.28 – 5.80% at a levelled concentration of the reduction sugars in a solution of about 1.4 mg/cm^3. A hydrolysis time prolonged to 1.0 hour (trials from R10/AS-24 to R50/AD –22) resulted in a decrease of in polymerisation degree from 2 to 22 units and an increase in the reduction sugar concentration in the solution.

The doubling of the E/S module during half hour hydrolysis of pulps degraded by irradiation with 10 - 20 kGy doses (trials R10/AD-15, R15/AS-16, R20/AS-18) also causes a decrease in the polymerisation degree, with a simultaneous increase in the reduction sugar content in the solution to 2.3 mg/cm^3.

An attempt to attain a deeper depolymerisation of the pulp in which a dose of 10 kGy was first deposited (R10/AS-14) by extending the hydrolysis time at doubled E/S module, failed since an insignificant decrease of the average depolymerisation degree occurred at the cost of mass loss and reduction sugar concentration which climbed to the values of 8.8% and 6.3 mg/cm^3 respectively.

Pondering over the results presented leads to the conclusion that best results in MCC two-step preparation can be achieved in the following processing conditions:
- Irradiation of the pulp with a 50 kGy dose,
- Enzymatic hydrolysis for 0.5 hours at 50 °C with an E/S module of 46 UCMC/g.

Table 3. *Conditions of pulp enzymatic hydrolysis after irradiation and properties of the hydrolysed pulp. Constant hydrolysis conditions: pulp concentration 5%, temperature 50 °C and Enzyme preparation Econase CE.*

Symbol of sample	Radiation dose, kGy	Time of enzymatic hydrolysis, h	Module enzyme/ substrate E/S, UCMC/g	Mass loss, %	DP_V after depolymerisation		Content of reducing sugars in solution, mg/cm^3
					radiation	enzymatic	
R10/AS-14	10	4.0	92	8.80	464	261	6.30
R10/AS-15	10	0.5	92	2.50	464	264	2.21
R15/AS-16	15	0.5	92	5.90	405	271	2.37
R20/AS-18	20	0.5	92	5.08	338	241	2.25
R10/AS-19	10	0.5	46	4.36	464	322	1.44
R15/AS-17	15	0.5	46	5.80	405	291	1.45
R20/AS-20	20	0.5	46	3.28	338	245	1.31
R50/AS-21	50	0.5	46	4.38	223	190	1.39
R10/AS-24	10	1.0	46	6.33	464	302	2.16
R15/AS-23	15	1.0	46	5.14	405	269	2.12
R20/AS-25	20	1.0	46	6.09	338	243	2.00
R50/AS-22	50	1.0	46	5.08	223	178	1.82

Table 4. *Properties of MCC obtained with the two-step radiation/enzymatic degradation; *initial cellulose pulp.*

Symbol of MCC	MCC propereties							
	DP_V	WRV, %	Equivalent moisture content Wr, $gH_2O/100g$ dry pulp	Cellulose content		Bulk density, $g/100cm^3$	White-ness (FN), %	Yellow-ness, %
				amorph-ous (A_{Wr}), %	crysta-line (K_{Wr}), %			
Alicell Super*	–	79	7,34	45,5	54,4	–	92,4	–
MC/R10/AS-14	210	61	5.88	37.6	62.4	57.38	77.4	1.42
MC/R10/AS-15	230	59	6.06	38.6	61.4	–	76.9	1.32
MC/R15/AS-16	225	77	6.09	38.8	61.2	31.24	78.0	1.54
MC/R20/AS-18	205	58	5.98	38.2	61.8	35.61	76.5	2.61
MC/R10/AS-19	265	69	6.18	39.3	60.7	20.15	78.8	1.02
MC/R15/AS-17	240	60	6.30	39.9	60.1	24.22	79.7	1.00
MC/R20/AS-20	196	70	5.90	37.8	62.3	34.66	76.5	2.61
MC/R50/AS-21	148	67	5.60	36.1	63.9	38.42	75.3	7.17
MC/R10/AS-24	275	92	6.47	40.8	59.2	24.97	74.0	9.05
MC/R15/AS-23	216	51	5.89	37.7	62.3	34.27	74.3	8.08
MC/R20/AS-25	213	58	6.13	39.0	61.0	29.15	–	–
MC/R50/AS-22	145	58	5.74	36.9	63.1	34.42	–	–

Such conditions provide a high content of the crystalline phase, average polymerisation degree in the assumed range, high MCC bulk density and comparatively low mass loss. The latter amounts to 4.4 % with the reduction sugar content in the solution being 1.4 mg/cm3.

Preparation of MCC from cellulose pulp after the two-step depolymerisation

According to the methodology described above, MCC was prepared from the cellulose pulp first depolymerised in the two-step process.

As can be read in Table 4, the MCC samples obtained are characterised by DP_V within the range of 145 – 275, and save trial MC/R10/AS-24 and a crystalline phase percentage (K_{Wr}) at the required level. The MCC with the highest crystallinity K_{Wr} of 63.1 and 63.9% was prepared from cellulose pulp that was first radiation-degraded with a 50 kGy dose, and next enzymatically hydrolysed for 0.5 – 1.0 hours at module E/S of 46 UCMC/g. These MCCs also was characterized by the lowest average polymerisation degree DP_v of 145 and 148.

Satisfactory results were also attained with pre-depolymerised pulp with a 20 kGy dose. A dose of 10 kGy combined with the same enzymatic hydrolysis conditions caused a too mild MCC yield (MC/R10/AS-24 and MC/R10/AS-19) with crystallinity K_{Wr} within the range of 59.2 – 60.7%, and a specific volume of 25.2 and 20.2 g/100 cm3 ,respectively. Such low values are probably related to the comparatively high DP, amounting to 265 and 275. The same applies to MCC made of pulp irradiated with a 15 kGy dose and subjected to a 0.5 hour enzymatic hydrolysis (MC/R15/AS-17). Much better MCC properties could be achieved after prolonging the enzymatic hydrolysis to 1 hour (MC/R/AS-23) and by lifting the E/S module to a level of 92 UCMC/g for all pulps after initial radiation depolymerisation. A 4 hour hydrolysis time for the pulp after the initial deposition of a 10 kGy dose is not recommendable, though good MCC properties could be achieved. The reason is a high mass loss of 8.9% and high concentration of reduction sugars in the solution (see Table 3).

From the above explanation of the results attained from preparing MCC by means of two-step radiation' enzymatic depolymerisation, it appears that the properties of MCC depend, to a certain degree, on its average polymerisation degree (Figure 1).

From Figure 2 it may, with high probability, be deduced that in the investigated range of MCC preparation parameters, a linear coordination exists between the DP_v of MCC and its content of crystalline phase (K_{Wr}) and specific volume.

The high probability of the existence of a linear function between basic MCC properties and its average polymerisation degree, and between the MCC's DP_v and the pulp's DP_v after the two-step radiation-enzymatic modification offers, in our opinion, a good future chance of steering the MCC manufacturing process toward materials with tailored properties. However, careful verification of the equations is still needed.

Molecular and crystalline structure, size of crystallite aggregates and grain coarseness of MCC

The MCC from trials MC/R-15/AS-23 and MC/R-50/AS-21 were analyzed with respect to:

- Distribution of molecular weight by GPC method,
- Crystalline structure by WAXS method,
- Dimensions of crystallite aggregates by microscopic inspection,
- Grain coarseness by laser method.

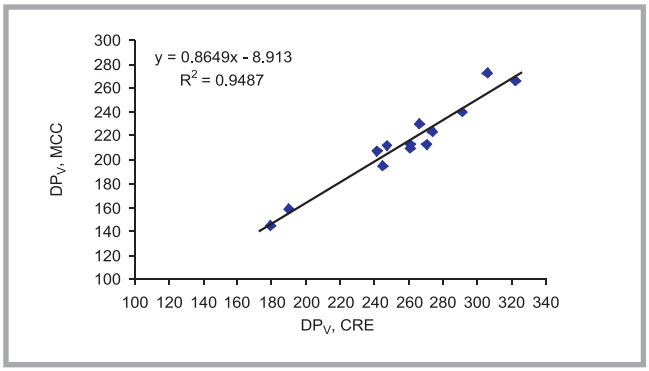

Figure 1. *Dependence of MCC average DP on the average DP of the pulp after radiation-enzymatic depolymerisation.*

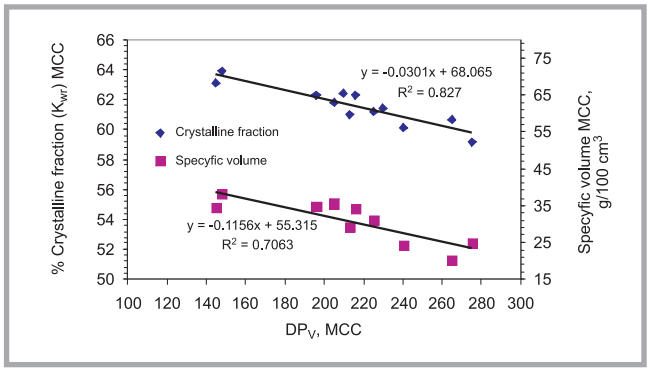

Figure 2. *Dependence of crystalline phase content and specific volume of MCC on its average polymerisation degree.*

The two MCC samples above with DP_v 216 and 148 respectively, were obtained as a result of two-step depolymerisation: radiation with a dose of 15 and 50 kGy, and enzymatic at E/S module of 46 UCMC/g 1 and 0.5 hours (see Tables 3 and 4).

Distribution of molecular weight by GPC method

Results of the GPC investigations, presented in Table 5 and Figure 3, give evidence of the cracking of the cellulose chain occurring in the pulp subjected to radiation and enzymatic degradation. The extent of the chain cutting depends on the DP and polydispersity Pd of cellulose after radiation-degradation. A 15 kGy dose irradiation halved the content of long chains and caused a respective increase of the short chain fraction. The average polymerisation degree $DP_{w,\ GPC}$ amounts to 406.

An 11% content of long chains with $DP > 550$ remained in the MCC denoted as MC/R-15/AS-23, and this is the reason why polydisperisity *Pd* remained at 2.5 and $DP_{w,\ GPC}$ at 262.

The use of a 50 kGy dose had the immediate effect of a 9-fold decrease in the DP > 550 fraction to 5% content, and a 2.5-fold increase in the $DP < 200$, causing a reduction in $DP_{w,\ GPC}$ to a value of 205. As a result, MCC denoted as MC/R-50/AS-21 was obtained with the $DP_{w,\ GPC}$, which about 180 with a 70% content of the DP < 200 fraction, while the long chain fraction represented merely 3%, and polydispersity *Pd* was at the level of 1.94.

Estimation of MCC crystalline structure by wide angle x-ray scattering (WAXS) method

Table 6 presents measurement results for the MCC samples prepared in comparison with the initial cellulose pulp and commercial MCC of Vivapur types 101, 102 and 105, made by Rettenmaier Co. The samples marked MC/R-50/AS-21 and MC/R-15/AS-23 are characterised by a crystallinity degree 6 - 8% lower than for Vivapur and 10% lower when compared with the initial pulp. Unknown is the crystallinity degree of the starting pulp used for the preparation of the Vivapur material.

The dimensions of our MCC crystallites in the determined directions, which are perpendicular to planes (110), (200) and (004), are close to those of Vivapur. In particular, the diagonal sizes of crystallites $D_{(110)}$ and $D_{(200)}$ in sample MC/15/AS-23 with $DP_{w,GPC} = 260$ fall in the range found in Vivapur and are identical with those of the initial cellulose pulp. On the other hand, the dimension of crystallites in a perpendicular direction to plane (004), in other words lengthwise, is by 2 – 2.5 times bigger than those of the cellulose pulp for all the MCC samples tested. The biggest crystallites are found in the MC/R-50/AS-21 sample made of

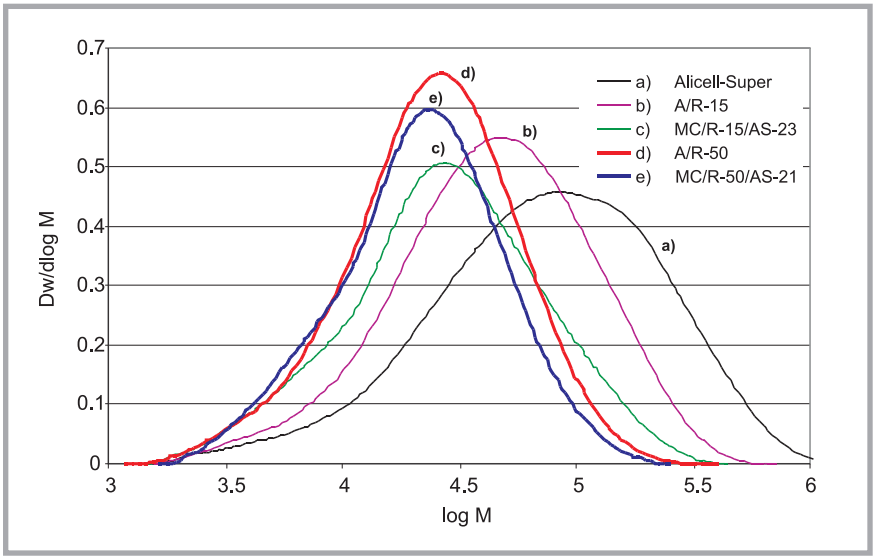

Figure 3. *Molecular weight distribution curves of cellulose pulp and MCC obtained with two-step radiation/ enzymatic degradation.*

Table 5. *Impact of depolymerisation conditions on the molecular parameters of cellulose pulp and MCC.*

Type of cellulose	Dose EB, kGy	Mn, kD	Mw, kD	Mw/ Mn Pd	$DP_{w,\ GPC}$	Percentage of fraction with polymerisation degree DP		
						<200	200-550	>550
Alicell super	0	23.28	85.20	3.66	776	24	31	45
A/R-15	15	18.44	48.30	2.62	406	37	40	23
MC/R-15/AS-23	15	16.94	42.39	2.50	262	57	32	11
A/R-50	50	13.22	26.70	2.02	205	63	32	5
MC/R-50/AS-21	50	15.12	29.33	1.94	181	70	27	3

Table 6. *Characteristic of MCC crystalline structure by WAXS method.*

Symbol of sample	Crystallinity degree, %	Dimensions of crystallites, nm		
		$D_{(110)}$	$D_{(200)}$	$D_{(004)}$
Alicell-Super	58.9	5.5	5.5	3.2
MC/R-50/AS-21	46.0	6.2	5.3	7.6
MC/R-15/AS-23	45.8	5.5	5.5	6.2
Vivapur 101	54.2	5.6	5.7	6.5
Vivapur 102	53.6	5.1	5.5	6.8
Vivapur 105	52.0	5.5	5.4	6.8

Table 7. *Sizes of MCC crystallites aggregates.*

Symbol of sample	MC/R-15/AS-23	MC/R-50/AS-21	Vivapur 101	Vivapur 102	Vivapur 105
Circumference, µm (average value)	75.60	73.80	66.10	128.50	51.20
Standard deviation	40.32	63.45	45.62	80.78	18.62
Length, µm (average value)	22.70	20.60	23.50	35.50	17.40
Standard deviation	12.07	16.49	17.24	20.72	6.85
Width, µm (average value)	13.50	11.40	9.40	17.50	8.70
Standard deviation	6.28	10.17	4.54	11.16	2.86

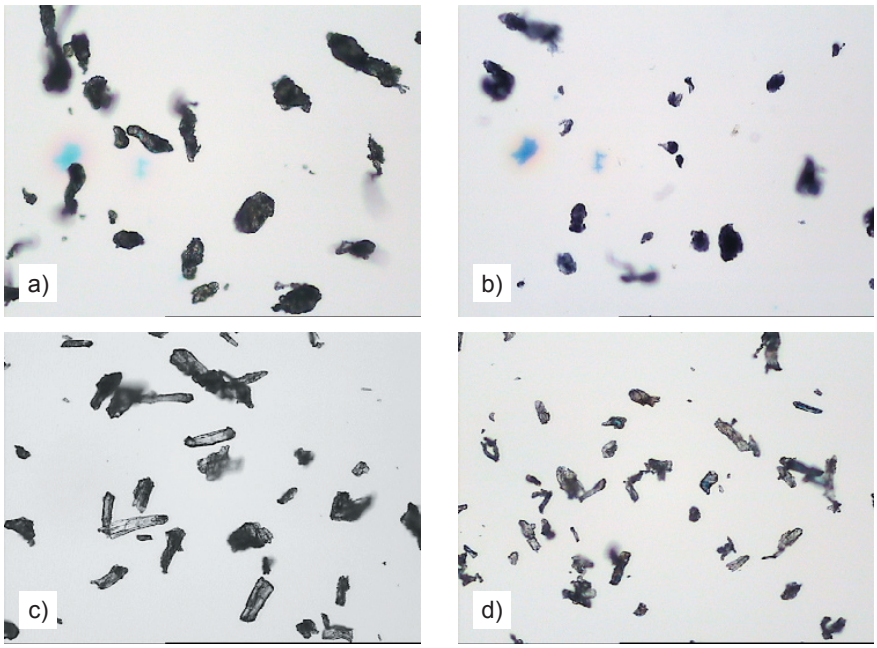

Figure 4. *Microscopic images of crystallite aggregates of MCC samples:a) MC/R-15/AS-23, b) MC/R-50/AS-21, c) Vivapur 101, d) Vivapur 105.*

cellulose pulp pre-depolymerised with a 50 kGy radiation dose, and next enzymatically degraded for 0.5 hour.

Microscopic inspection of crystallite sizes of MCC

The average dimensions Of the crystallite aggregates of the MCC samples (Table 7, see page 171), estimated with respect to the images in Figure 4, are close to the average size of crystallite aggregates of the commercial MCC - Vivapur 101. Standard deviations are similar, too.

Grain coarseness estimation of selected MCC samples done by the laser method revealed>> a higher coarseness in the MCC samples tested (see Table 8.) than that required by the pharmaceutical industry (see Table1.)

It must, however, be pointed out that the unsorted MCC sample obtained after radiation (15 kGy dose) and enzymatic (1 hour) degradation is characterised by particle distribution: about 14% bigger than 320 µm, about 60% bigger than 160 µm and about 22% smaller than 100 µm, which is somewhat closer to the requirement for MCC type 12 (Table 1).

Table 8. *Grain coarseness of MCC.*

Grain size, µm	Grain distribution, %	
	MC/R-50/AS-21	MC/R-15/AS-23
1-10	1.86	1.78
10-50	14.24	9.52
50-100	6.30	11.51
100-150	10.04	15.70
150-200	12.77	15.25
200-250	13.44	13.37
250-300	15.13	12.84
300-320	6.00	4.50
320-350	6.25	5.04
350-500	12.32	9.21

It may be expected that the sorting of the MCC powder to remove the coarsest fraction > 350 would greatly improve the granulation.

Summary

The research results presented here lead to the conclusion that microcrystalline cellulose for special uses, including pharmaceutical, can be prepared by dissolving pulp and employing a two-step radiation-enzymatic depolymerisation of the pulp. The method involves the irradiation of cellulose pulp with an electron beam and depositing a 50 kGy dose in the pulp which is next subjected to enzymatic hydrolysis by means of Econase CE cellulolytic enzymes. The hydrolysis proceeds for 0.5 hour at 50 °C with the enzyme, to substrate E/S module amounting to 46 UCMC/g.

The unsorted microcrystalline cellulose prepared in laboratory scale from such depolymerised cellulose pulp is characterized by:

- average polymerisation degree *DPv* 150
- content of crystalline phase K_{Wr} about 64%
- specific volume 38 g/100 cm^3
- grain coarseness similar to the pharmaceutical requirement for MCC type 12.

The material contains merely 3% of long-chain cellulose with *DP* > 500, and its crystallite dimensions are close to those of the commercial Vivapur MCC, which is applied in the pharmaceutical industry.

References

1. *Stupińska H., Iller E., Zimek Z., Kopania E., Palenik J., Milczarek S.: Preparation of microcrystalline cellulose employing ecological depolymerisation methods of cellulose pulp. Part 1. Radiation degradation (in Polish). Przegląd Papierniczy, Vol. 8(2006) p. 475.*
2. *Stupińska H., Wawro D., Ciechańska D., Krzyżanowska G., Stęplewski W., Kopania E., Palenik J., Milczarek S.: Preparation of microcrystalline cellulose employing ecological depolymerisation methods of cellulose pulp. Part 2 Enzymatic hydrolysis (in Polish). Przegląd Papierniczy, Vol. 9(2006) p. 543.*
3. *Stupińska H., Palenik J., Kopania E., Wawro D., Ciechańska D., Krzyżanowska G., Iller E., Zimek Z.: Method to prepare microcrystalline cellulose. Polish patent Appl. P-380328, 2006.*
4. *Iller E., Kukiełka A., Chmielewski A. G., Zimek Z., Michalik J., Perlińska J., Stupińska H., Mikołajczyk W., Starostka P., Strobin G.: Radiation stimulation of various cellulose pulps for the preparation of derivatives (in Polish). Przegląd Papierniczy, Vol. 8(2000) p. 672.*
5. *Struszczyk H., Ciechańska D., Wawaro D., Urbanowski A., Guzińska K., Wrześniewska-Tosik K.: „Method for the manufacture of fibres, film and another products from modified soluble Cellulose" EP 122 80 98.*
6. *Bikales N. M., Regal L.: „Cellulose and Cellulose Derivatives", Russian edition, editor Z. A. Rocovin, t. 2, Roz. VI A, red. O. A. Battista, pp. 412-421, Moscov 1974.*
7. *Ekmanis J. L.: Am. Lab. News. Jan/Feb., 10 1987.*
8. *Hindeleh A. M., Johnson D. J.: J. Phys. D: Apel. Phys. Vol. 4(1971) p. 259.*
9. *Rabiej M.: Polimery, Vol. 47(2002) No. 6, p. 423; Polimery Vol. 48(2003) No.4, p. 288.*

Received 05.11.2007 Reviewed 10.12.2007