CHANGES IN MOLECULAR CHARACTERISTICS OF DIBUTYRYLCHITIN UNDER INFLUENCE OF SONIFICATION

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1. Introduction

Several authors have investigated the effect of exposure to ultrasound on polysaccharides like chitin and chitosan [1 - 4]. In recent years a new ester derivative of chitin – dibutyryl-chitin has been studied intensively. Dibutyrylchitin (DBC) shows therapeutic and regenerative activity, is easy processed and has become a substance of numerous applications. It is therefore important to see whether molecular properties of DBC could be changed among others by ultrasound. In first step of our study we used ultrasound for dispersing the dibutyrylchitin in water as a preliminary stage before exposure to enzyme cellulase [5]. The influence of ultrasound treatment in dispersion upon the average molar mass of DBC and on degree of polydispersity was negligible. The reduction in the weight average molar mass did not exceeded the error of the measurement. In further steps investigations have been undertaken to assess the susceptibility of dibutyrylchitin to degradation when treated with ultrasound in solution [6]. We have studied sensitivity of DBC to ultrasound of various characteristics. These were varied both from a standpoint of nominal input power and so called processor cycle that is a ratio of exposure to no exposure intervals (pulsed mode of ultrasound emission). It was established that ultrasound may be used for reduction of average molar mass of dibutyrylchitin and the same applies to the ratio of the weight average to number average molar mass. The observed changes are intensified by increase of processor power and sonification duration. On the other hand there was no effect of processor cycle when effective times of exposure were compared.

The aim of present study was investigation of effects of initial molar mass as well as of DBC concentration upon sensitivity to changes in molecular characteristics at selected parameters of ultrasound generator.

2. Materials and methods

2.1. Polymers

Krill chitin supplied by Sea Fisheries Institute (Gdynia, Poland) was used as raw material for preparation of dibutyrylchitin (DBC). Chitin was purified by treatment with water solution of 2 M HCl in amount of 100 ml for 10 g of chitin during 2 hours at room temperature. Syntheses of dibutyrylchitin were carried out using described and patented method of chitin O-esterification [7] using various ratios of the used components and varying the reaction temperature. The samples were preliminarily characterized by determination of intrinsic viscosity in dimethylacetamide (DMAC) at the temperature of 25 °C:

Series of DBC samples were studied that varied in initial weight average molar mass in the range from 130,000 to 400,000 g/mol.

Sample code	M_w, g/mol	M_n, g/mol	[η] w DMAC (25 °C), 100 cm³/g
DBC-Z1	400,000	199,000	5.25
DBC-Z6	344,200	158,000	4.29
DBC-Z3	221,200	108,500	3.06
DBC-S5	135,900	63,500	2.22

2.2. Condition of exposure to ultrasound

Experiments were performed by using ultrasound generator UP200S (Hielscher GmbH) operating at 24 kHz. A selected nominal input power of 120W was used. Solutions of dibutyrylchitin in dimethylacetamide, at concentration in the range from 0.05/100 cm³ to 2/100 cm³ having a volume of 10 cm³, were used. Each solution was sonificated using a probe of 90 mm length and of 2 mm diameter. The probe was immersed in polymer solution 40 mm below the surface level. The solution was placed in thermostated water bath maintained at 25 °C. A continuous mode of exposure to ultrasound was used in all experiments. Each solution was irradiated for predetermined time interval. After this time the solution was diluted properly and subjected to size exclusion chromatography analysis.

2.3. Determination of polymer average molar masses

The average molar masses of DBC were determined by means of size exclusion chromatography. Measurements were carried out in DMAC-0,5% LiCl with rate flow of 1 ml/min. The Waters Alliance XE HPLC system was equipped with refractive index detector model 410. Volume of injected solution was 100 μl with concentration of 0.1 mg/cm³. Separation was performed on a set of 3 × Plgel Mixed A type supplied by Polymer Laboratory and maintained at 30 °C. The columns were calibrated with a set of pullulan molar mass standards manufactured by Shodex (Japan). Pullulan equivalent molar of DBC samples was evaluated by means of Trisec software from Viscotek.

3. Effects of dbc exposure to the ultrasound

Examples of chromagraphic studies of three polymers differing from the standpoint of their initial molar masses, are presented in Table1. Graphs, demonstrating changes in average

molar masses M_w and M_n, polydispersity Pd, and in form of distribution function of molar masses after varying conditions of ultrasound exposure, are attached too.

Table 1. *Changes in average molar mass and polydispersion of DBC; Sonification conditions: input power 120 W; continuous mode.*

	t_{ef}, min	M_n, c = 0.05 g/100 cm^3	Pd,	M_n, c = 0.3 g/100 cm^3	Pd,	M_n, c = 1 g/100 cm^3	Pd,	M_n, c = 2 g/100 cm^3	Pd,
DBC-Z1	0	199,000	2.01	199,000	2.01	199,000	2.01	199,000	2.01
	5	102,600	1.70	136,300	1.72	144,100	1.93	152,000	1.89
	15	82,700	1.50	92,880	1.70	99,900	1.78	117,900	1.87
	30	68,450	1.51	58,470	2.05	78,800	1.66	79,600	1.93
	45	63,370	1.47	56,930	1.59	64,800	1.61	75,100	1.64
	60	49,400	1.47	53,820	1.56	54,600	1.52	64,666	1.60
	90	39,400	1.50	40,190	1.70	45,000	1.60	51,600	1.57
	120	37,090	1.48	34,450	1.72	40,300	1.52	44,200	1.54
DBC-Z3	0	108,500	2.04	108,500	2.04	108,500	2.04	108,500	2.04
	5	75,700	1.83	80,900	1.91	90,900	2.02	87,800	2.19
	15	68,600	1.55	62,700	1.83	70,300	1.97	79,900	1.98
	30	49,400	1.66	58,200	1.68	62,500	1.76	69,300	1.84
	45	48,200	1.61	48,300	1.62	58,200	1.68	65,000	1.82
	60	42,400	1.58	45,600	1.58	50,900	1.69	58,800	1.78
	90	37,600	1.52	36,300	1.57	41,000	1.74	49,500	1.78
	120	36,400	1.46	34,300	1.55	41,000	1.60	47,700	1.61
DBC-S5	0	63,500	2.14	63,500	2.14	63,500	2.14	63,500	2.14
	5	57,700	1.87	61,000	1.82	61,700	1.97	58,600	2.05
	15	53,600	1.63	54,300	1.72	55,100	1.84	51,700	2.13
	30	49,700	1.53	47,600	1.62	53,000	1.70	47,300	1.95
	45	43,800	1.59	42,900	1.63	42,800	1.74	45,900	1.87
	60	38,000	1.67	42,600	1.54	44,900	1.60	47,900	1.72
	90	37,600	1.52	35,300	1.53	39,900	1.59	42,700	1.68
	120	36,400	1.46	32,300	1.63	34,400	1.64	34,500	1.88

The effect of initial molar mass upon changes of the latter in course of DBC sonification is presented in Figure 1 and Figure 2, for concentrated and diluted solutions, respectively. In each case pronounced reduction of molar mass has bee observed after 5 to 15 min. These changes are more intensive in diluted solutions and for polymers of greater initial

molar masses. However, after prolonged sonification (> 60 min), irrespective of initial concentration and molar mass, in all conditions of exposure of DBC to ultrasound, values of molar mass aim at similar, and perhaps even one limiting value.

The effects of DBC concentration in the solution (0.05; 0.3; 1 and 2 g/100 cm^3) upon average molar masses of DBC are shown in Figure 3. The effects relates to polymers of the highest and lowest initial degree of polymerization. It is clear that degradation process, induced by sonification, is more intensive in a polymer of higher molar mass and in most diluted solutions. A similar phenomenon was noted when sonic degradation of chitosan had been
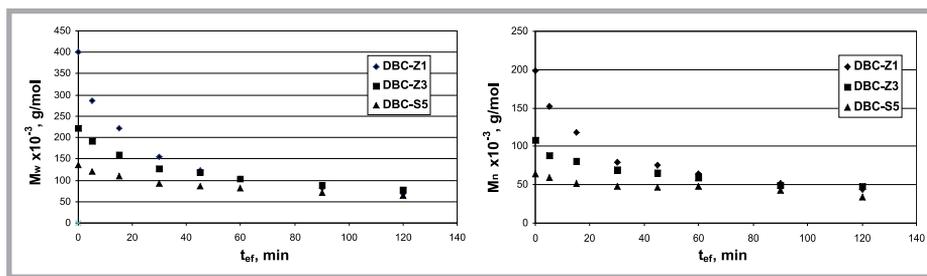
Figure 1. *Effects of initial molar mass on its changes in course of DBC sonification. Sonification conditions: input power 120 W; continuous mode; c =2 g/100 cm³*
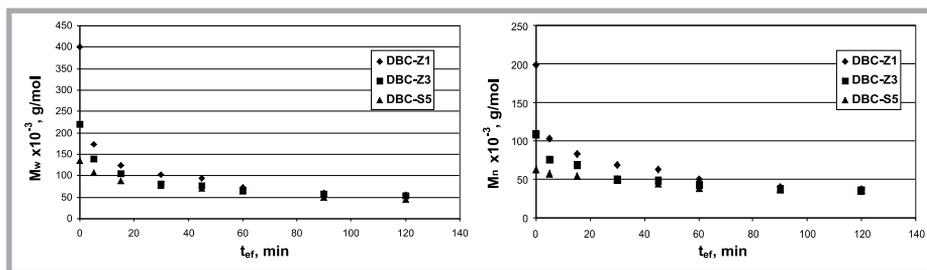

Figure 2. *Effects of initial molar mass on its changes in course of DBC sonification. Sonification conditions: input power 120 W; continuous mode. c =0.05 g/100 cm³.*

followed. It seems likely that in solutions of higher polymer concentration - due to higher viscosity - this is lower intensity of cavitation which leads to less likely disintegration of macromolecules.

The character of molecular changes, induced by ultrasound, is very well illustrated by differential curves of molar mass distribution at various durations of sonification shown on Figure 4. and Figure 5. The curves are selected for the polymer of high and low initial molar mass and at two extreme values of DBC concentration, i.e. 0.05 and 2 g/cm³, respectively.
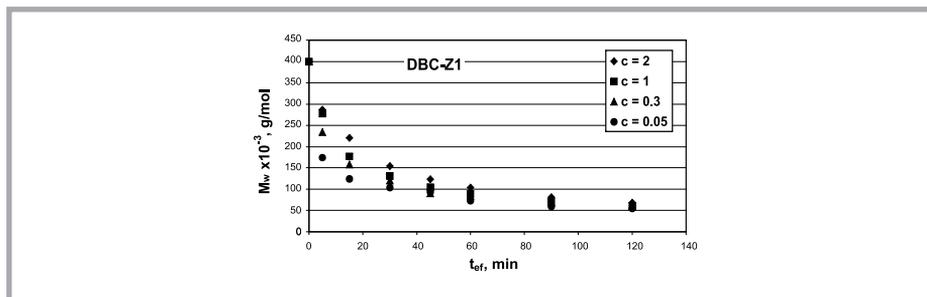

Figure 3. *Influence of DBC concentration upon average molar masses of the polymer as a result of sonification. Sonification conditions: input power 120 W; continuous mode.*
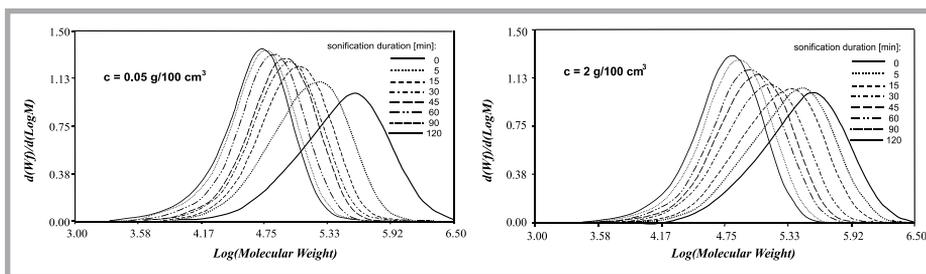
Figure 4. *Differential molar mass distribution curves of DBC Z-1 as results of sonification duration and solution concentration.*
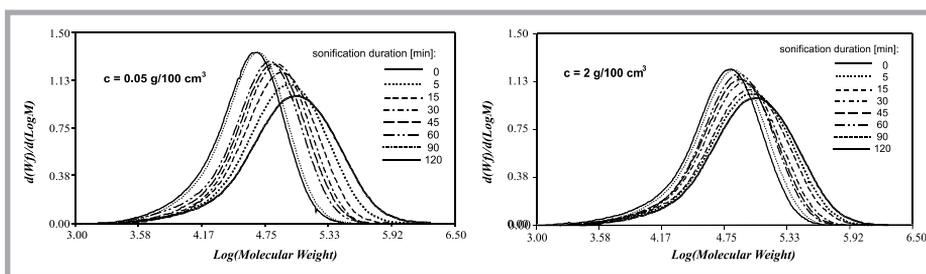

Figure 5. *Differential molar mass distribution curves of DBC S-5 as results of sonification duration and solution concentration.*

Final effect of molar mass changes in polymers undergoing sonic degradation at selected conditions, is presented in Figure 6. In this figure differential molar mass distribution curves are presented for three initial polymers and for polymers after sonification during 120 min. It is clear that, at condition of sonic degradation employed, the distribution curve for each sample becomes narrower and shifted toward lower molar masses. Moreover differences which may be seen between initial distributions are neglible for distributions after sonification (the curves are overlapping).
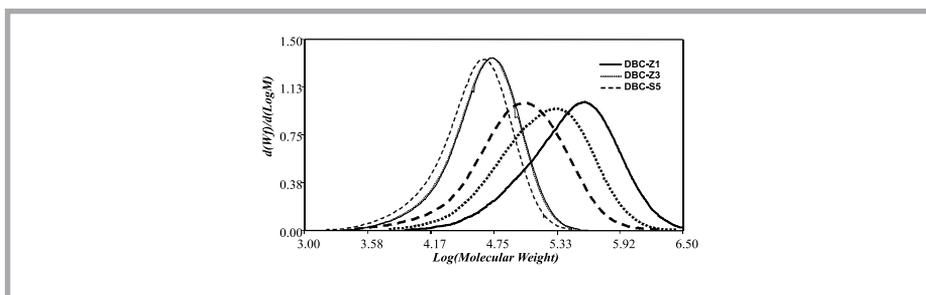

Figure 6. *Differential molar mass distribution curves of initial (heavy lines) and sonificated (fine lines) samples of DBC polymer. Sonification conditions: input power 120W; continuous mode; c =0.05 g/100 cm³; time 120 min.*

In our previous paper [6] we presented the effect of input power on degradation extent on example of DBC S-5. In this work we studied the influence of input power on changes in molar masses of DBC polymer of higher initial molar mass i.e. DBC Z-6. The effect observed after 5 min of exposure in diluted solution is presented in Figure 7. The effect is clearly seen, the molar masses are reduced with increase of the nominal power.

Stability of DBC structure to ultrasonic treatment was also studied by infrared analysis. IR spectra of polymer samples recorded for initial and ultrasound processed DBC are presented in Figure 8. The spectra do not display essential differences. This means that dibutyrylchitin is not destroyed by the ultrasound applied.
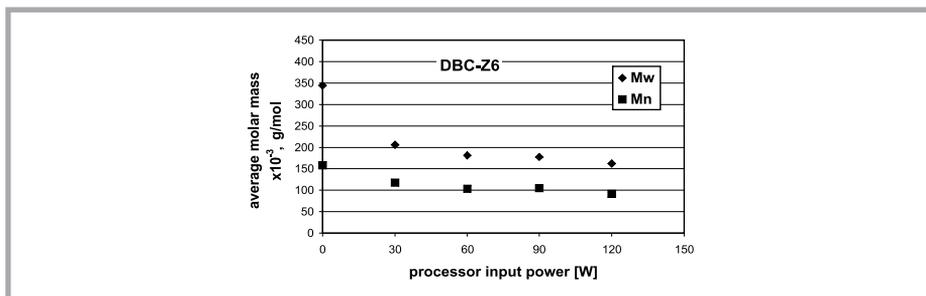

Figure 7. *Effect of processor input power upon average molar masses of DBC-Z6; sonification conditions: continuous mode; c =0.05 g/100 cm³; time 5 min.*
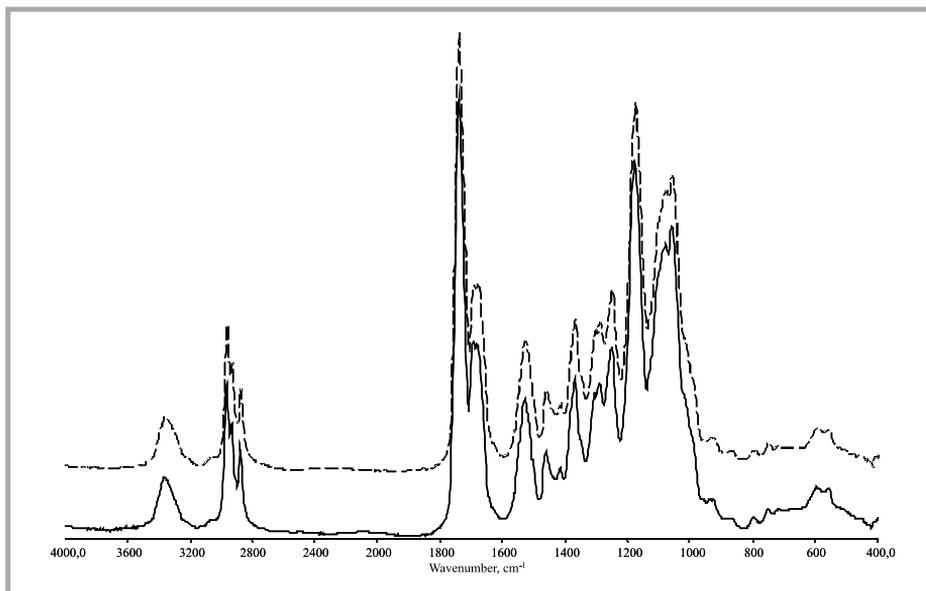

Figure 8. *IR spectra of DBC Z-1 initial (solid line) and sonificated sample (dotted line). Sonification conditions: input power 120 W; continuous mode; t_{ef} =120 min; c = 2 g/100 cm³.*

4. CONCLUSIONS

Studies of ultrasound effects upon molecular characteristics of dibutyrylchitin prove that average molar mass of the polymer is being gradually reduced. In addition the weight average to number average molar mass ratio is also reduced. These effects have a different intensity depending on:

- Initial molar mass
- Concentration of the polymer in solution
- Duration of the exposure
- Delivered power of the ultrasound.

Ultrasounds, when applied in a controlled way under different conditions enables reaching the limiting value of molar mass, below which no further degradation of the polymer by ultrasound (at given power and mode) may be observed. For a number average molar mass the limiting value is ab. 350000 g/mol what corresponds to polymerization degree of ab. 100.

5. REFERENCES

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