CHITOSAN AS AN ACTIVE FACTOR IN MULTIFUNCTIONAL MEDICAL WOUND COVERINGS

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Abstract

In melt-blow nonwovens, achieved via blowing with compressed hot air stream, the strings of molten thermoplastic polymer form the base of developed multifunctional medical wound covers. Depending on the conditions of process parameters it is possible to produce monofilaments of different thickness, including superfine ones having the diameter below 1μm. Applied polymer type decides upon the properties of formed fibres. One of the advantages of using melt-blow technique is the possibility of modifying the properties of produced nonwovens by incorporation of additional powdered substances to their structure. Such modifiers can be introduced directly by "powdering" polymer granulate. The condition determining the application of a given powder modifier in melt-blow technology is its sufficiently high disintegration (grinding).

The aim of the presented work was to develop wound covers that are sorptive and aseptic and provide granulation tissue growth. Biodegradable aliphatic polyesters covered with microbial chitosan or medical active carbon (product of PICA) of grain diameter 6-8 μm were used in this research work. Chitosan was separated from the cell walls of the *Absidia orchidis* NCAIM F00642 fungus. The produced composite materials based on biodegradable polymers contained 10% of powdered modifier. Additionally, medical wound covers in the form of nonwoven matrix from biodegradable polymers modified with chitosan in gel form were produced as well.

Introduction

Melt-blow method is an integrated technology combining processes of fibre formation with web production. It consists in blowing - with compressed hot air stream - the strings of molten thermoplastic polymer coming from the extruder head through multi-hole/orifice die and in receiving produced fibres on a take-up device. Depending on the construction of a take-up device it can be in a form of a drum or a tape (specified width).

In nonwoven manufacturing process three phases can be distinguished:
- polymer melting and extruding,
- blowing molten polymer with compressed hot air stream,
- fibre collection in a form of web.

This is presented in Fig. 1. Processing scheme is presented in Fig. 2

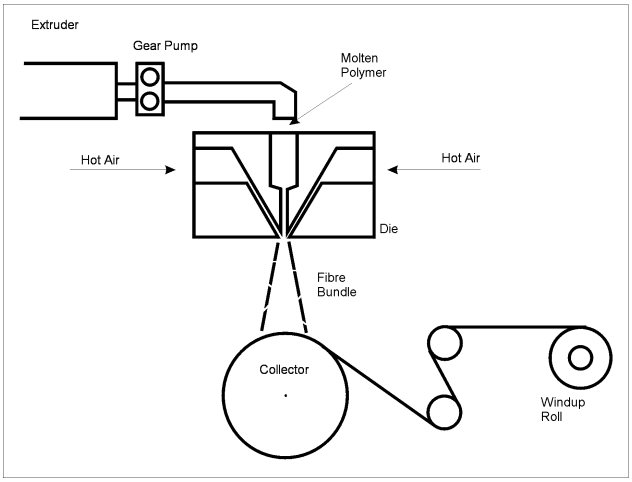

Figure 1. Fibre formation according to melt-blow method

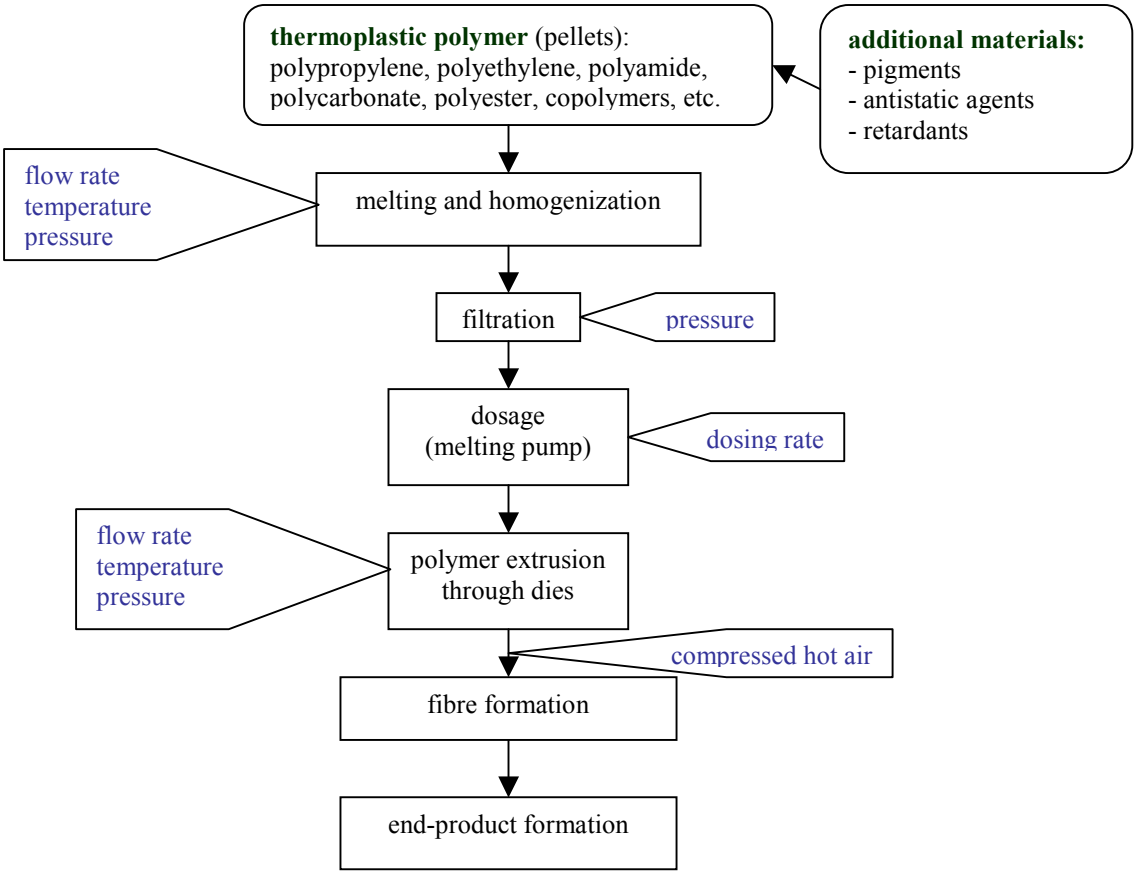

Figure 2. Nonwoven production according to melt-blow technique – processing scheme

In melt-blow method several parameters have the influence on polymer processing and on the properties of end-products; the most important factors are:

1. polymer type (molecular mass, polydispersity, melt flow rate),
2. processing temperature,
3. polymer flow rate (output)
4. ratio of hot air mass to polymer mass,
5. temperature and flow rate of compressed air,
6. spinning die diameters,

7. method of produced web collection,
8. distance of fibre forming head from the surface of take-up device.

One of the advantages of melt blow technique is the possibility of modifying the properties of produced nonwovens by incorporation of additional powdered substances to their structure. Such modifiers can be introduced directly by "powdering" polymer granulate. The condition determining the application of a given powder modifier in melt-blow technology is its sufficiently high disintegration (grinding).

Chitosan, a copolymer of glucosamine and N-acetyloglucosamine, is one of the most important derivatives of chitin. Chitosan is a biopolymer whose importance increases from year to year. The areas of its possible application are wide: medicine (wound healing), diatry, stomatology, agriculture (plant protection), environmental protection (wastes purification) or biotechnology (immobilization of enzymes and cells).

Chitosan is also a natural component of cell walls of fungi belonging to the class of Zygomycetes (e.g. *Absidia, Mucor, Rhizopus, Gongronella*) and can be produced by extraction from their cell walls. This kind of chitosan has several advantages in comparison to chitosan produced from chitin by chemical deacetylation. It can be produced independently of chitin production in fishery; the production is dependent only on the scale of bioreactor. It has no fishy odour which is substantial in cosmetics and medicine and is thought to have a higher bioactivity than chitosan from chitin. Additionally fungal chitosan has repeatable and unique properties: low acetylation degree (approx. 5-15%) and high molecular weight (up to few millions of Da). Such chitosan can be hardly obtained in chemical processes.

Material and Methods

Polymers Melt-blow nonwovens made of biodegradable co-polyesters: Eastar BIO GP and Eastar BIO ULTRA form a base for wound coverings. Processing parameters of these polymers were chosen with reference to their characteristics and producers recommendations as well as with reference to authors know-how and experience in the area of melt-blow nonwovens production. According to the recommendations of polymer producers, both polymers were dried for 3 hours at the temperature of 65-66ºC before pouring to the extruder funnel.

Active carbon In research works active carbon (PICA product) of grain diameter 8-10μm and specific surface area 1500 m²/g were used.

Amorphous chitosan of krill origin (Euphasia superba), DP=52.25.104 (produced by Sea Fisheries Institute, Gdynia (Poland))

Chitosan from fungi – chitosan of microbiological origin isolated from *Absidia glauca* fungus. The fungi were cultivated onYPG medium enriched with mineral salts in a submerged cultures carried out in a bioreactor Biostat ED (B.Braun, Germany). Culture medium (6.5 L) was added to the bioreactor, sterilized (121^0C, 15 min) and inoculated with 500 mL of inoculum (total volume - 7L). The fungi were incubated at 26^0C, pH = 5.5, aerated and mixed. 1N NaOH and 1N HCl solutions were used to stabilize the pH value. After 40-48 hours fungal biomass was separated from the broth by centrifugation (6 000 rpm, 20 min) and washed twice with deionized water. Next it was treated with 1 N NaOH solution, in the ratio 1 g biomass: 20 cm^3 NaOH solution, at 121^oC, (20 min). In this way the proteins were removed. The alkali insoluble fraction (cell walls) was then centrifuged (6 000 rpm, 20 min). The fungal cell walls were treated with 1 % acetic acid in the ratio 1 g dcw : 100 ml HCl and mixed. The solution was centrifuged and an acid soluble fraction was collected. The liquid was mixed and alkalized to pH 10.0 with 1 N NaOH - biopolymer was precipitated. Biopolymer was separated from the liquid by centrifugation (20 000 rpm, 20 min), washed with deionized water, lyophilized (-24^oC) and ground down to obtain a fraction below 28μm. The biopolymer can be stored at room temperature in a closed vessel.

Mesurement of IR spectrum of the chitosan The evaluation of applied chitosan was done under infrared conditions in the wavelength of 600-4000 cm^{-1}. The IR spectra of chitosan were carried out using the KBr disco method in In Broker IFS 66 spectrometer. Based on the infrared spectrum the degree of acetylation was determined, using absorbance ratio A_{1655}/ A_{3450} and calculated according the following equation: DA [%]= (A_{1655}/ A_{3450}) 100 /1,33

The thermogravimetric (TG) investigations were performed using a TGA-7 termobalance of Perkin-Elmer in a nitrogen atmosphere (sample of about 5 mg, a heating rate of 15 C min-1 within the temperature range from 50 to 550 C).

The Differential Scanning Calorimetry analysis – DSC. The method bases on the fact that the energy is supplied in the same way to tested sample and to reference material. The areas under peaks result directly from changes of samples enthalpy. This analysis was done using DSC XP-10. The measurement principle is the well known heat flux DSC. It measures the temperature difference between the sample and the reference platform.

Scanning Electron Microscope (SEM) All images were achieved using microscope Joel JSM-5500 LU where the voltage of current flowing through the fibre was 10 kV.
The samples for testing were prepared in the following way: nonwoven sample in a square form of 3mm was fixed to metal table and then sprayed with gold – thickness of 30nm. Spraying system – a product Joel JFC-1200 was used. Matrixes evaluation was performed using computer program SIGMA.

Results and Discussion

Evaluation of the properties of chitosan

IR spectrum of the chitosan are presented in Figures 3 and 4.

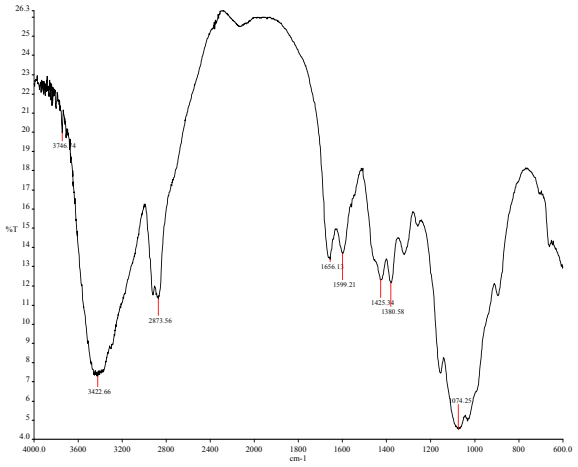

Figure 3. The infrared spectra of amorphous chitosan

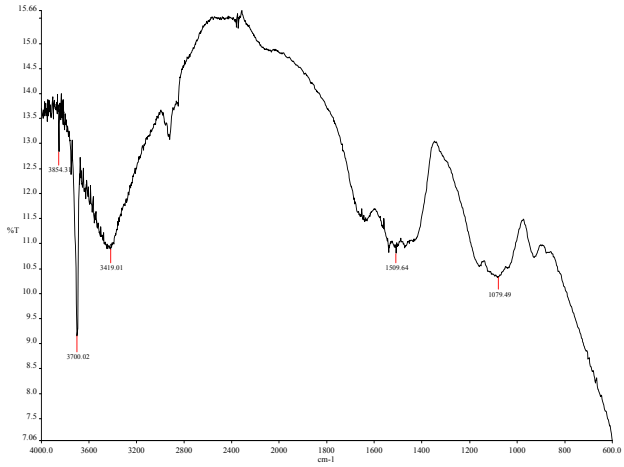

Figure 4. The infrared spectra of microbiological chitosan

Characteristic temperatures of chitosan samples:

Ample type	T 5% [^{o}C]	T 50% [^{o}C]
chitozan bio d > 0.028	102,5	------
chitozan bio d < 0.028	97,9	------
Process parameters: gas: nitrogen heating rate:15 ^{o}C/min;		

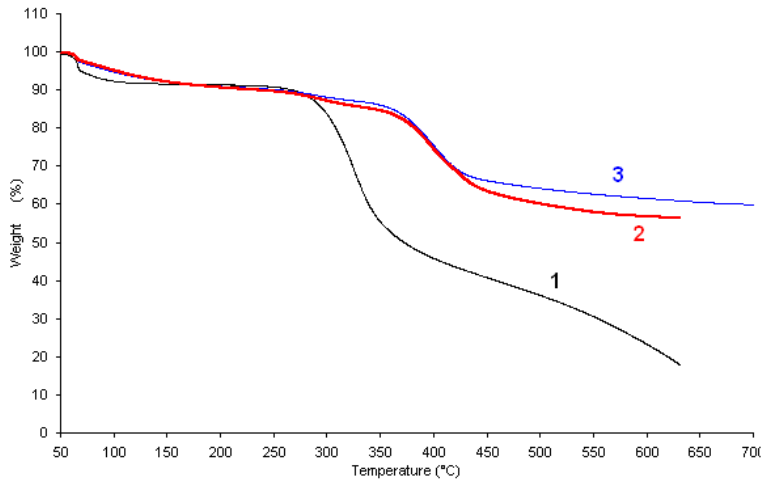

Figure 5. Thermogravimetric analysis of chitosan; 1- amorphous chitosan , 2, 3 – microbiological chitosan

To assess the possibilities of chitosan application as co-polyesters modifiers in melt-blow technique, thermogravimetric analysis for selected chitosans was done (Fig 5). After initial water evaporation both chitosan types maintain thermal stability – amorphous chitosan (krill chitosan) is thermally stable up to 300°C while microbilogical chitosan is stable up to the temperature of 370°C. Hence, chitosan composites production should be carried out below specified temperatures.

To obtain the complete picture of thermal resistance the Differential Scanning Calorimetry analysis – DSC was also performed. Other measurement conditions are presented in Figure 6.

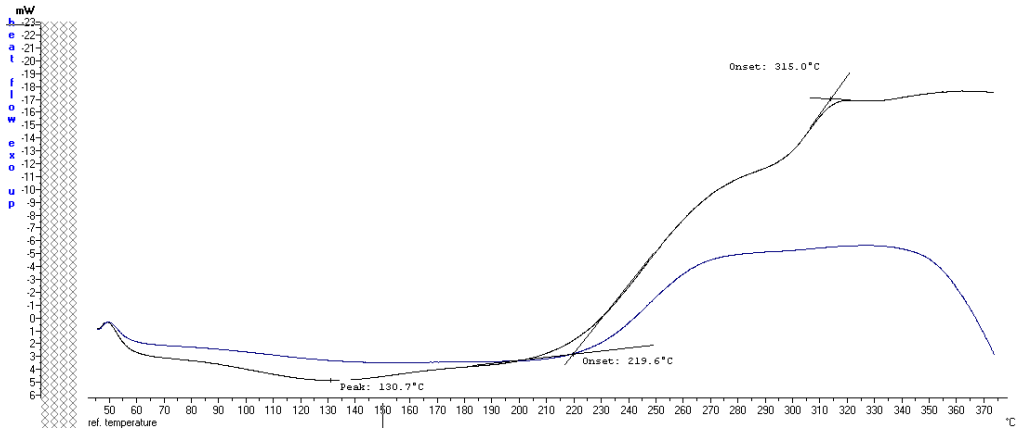

Figure 6. DSC analysis - comparison of microbiological and amorphous chitosan

Studying the results obtained in DSC analysis it should be noted that structural changes in both chitosan types occur much earlier – at the temperature of 265°C than it is indicated in

thermogravimetric method. It also should be emphasized that fungal chitosan is much more thermally stable than krill chitosan – amorphous one. Change occurrence stops at the temperature of 315°C for amorphous chitosan while in case of microbiological chitosan at the temperature of 350°C. In both cases all changes have exothermic character.

The initial phase of the diagram is connected with water evaporation process. The temperature of 120°C results from high value of specific surface area of each type of chitosan.

Matrix production from biodegradable co-polyesters according to melt-blow technique

Multifunctional wound coverings (inner and outer) were formed on the basis of melt blow nonwovens. Achievement of nonwovens made of microfibres – superfine fibres of average thickness in the range of 1-2 μm was the prerequisite for the development of such wound coverings. For this purpose, fibre forming head with dies of Ø = 0.375 mm was built. In fibre forming process the ratio of applied compressed air mass to polymer mass exceeded 75.

Processing of both polymers was carried out at the similar temperature of polymer extrusion and temperature of compressed air (232°C) while the flow rates of applied polymer (3.2 ÷ 4.5 g/min) and compressed air (5 ÷ 10 m³/h) were changing.

Specific temperatures of copolymer samples

copolymer type	T 5% [°C]	T 50% [°C]
EGBP/2/7 25	394.6	431.2
ULTRA 3/8 25	397.1	435.2
Process parameters: gas: nitrogen heating rate: 15 °C/min		

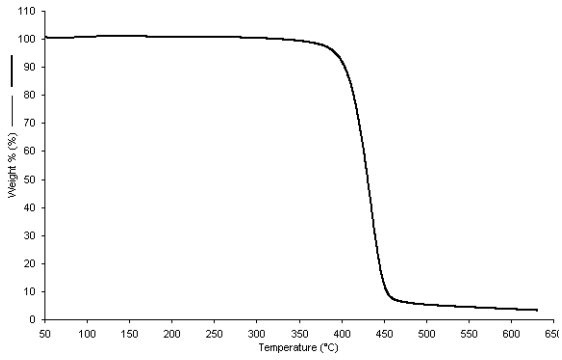

Figure7. Thermogravimetric analysis of Eastar Bio GP – co-polyester

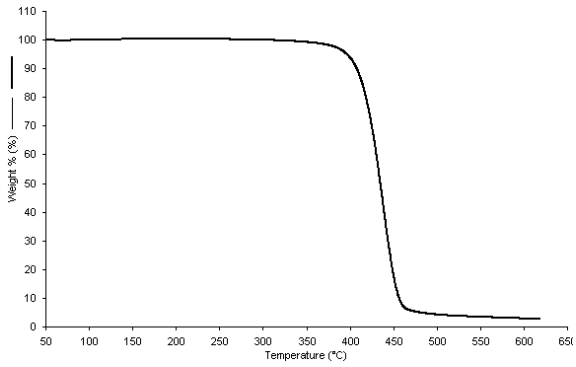

Figure 8. Thermogravimetric analysis of Eastar Bio Ultra – co-polyester

Applied co-polyesters are thermally stable and maintain high value of thermal resistance - in case of Easter Bio GP co-polyester the temperature up to 400°C and for Easter Bio Ultra co-polyester the temperature up to 410°C. Matrix production process should be carried out below these temperatures.

Wound coverings for outer usage were obtained as the result of powdered modifiers introduction to base nonwoven structures. Composite materials (co-polyester-chitosan and co-polyester-active carbon) were prepared before the very defibering process in solid-solution system at the temperature of granulate melting while continuous stirring. Produced matrixes were not always uniform. It is supposed that re-granulation process can affect this and give better results. The following products were used in research works: medical carbon of mass per unit area 1500 m²/g and chitosan of microbiological origin isolated from *Absidia* type fungi.

The structure of achieved composite nonwovens was evaluated using microscopic technique. Presented photos illustrate the structure of produced polymer matrix (Fig. 11 and Fig.12). Produced nonwovens play the role of a matrix for multifunctional wound coverings and exhibit required sorption properties. Elementary fibres had the diameter d< 2μm. Figures 13 and14 present obtained composite materials.

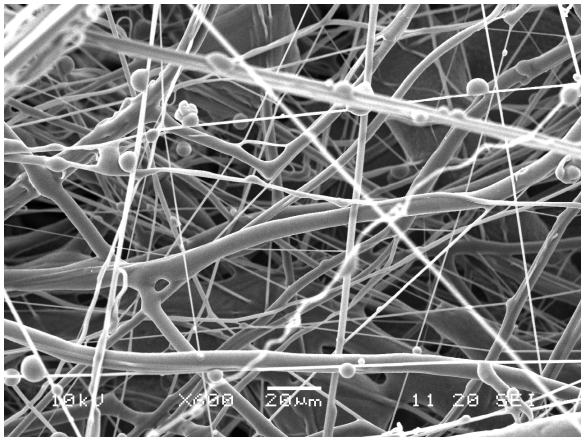

Figure 11. Photo of matrix surface made of Bio Ultra co-polymer

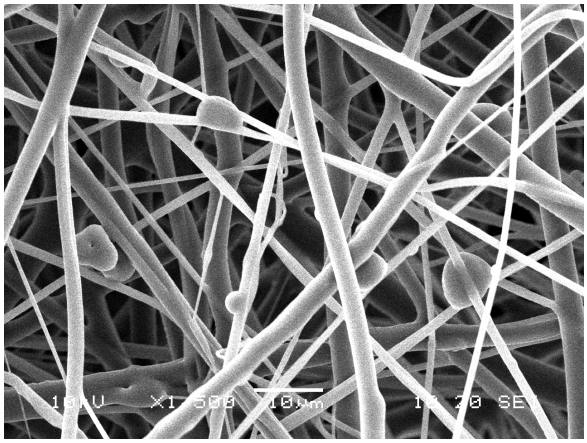

Figure 12. Photo of matrix surface made of Bio GP co-polymer

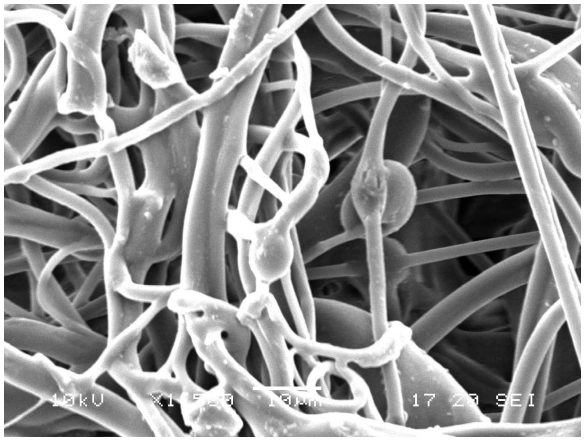

Figure13. Photo of matrix surface of composite material (Eastar Bio Ultra co-polyester and chitosan)

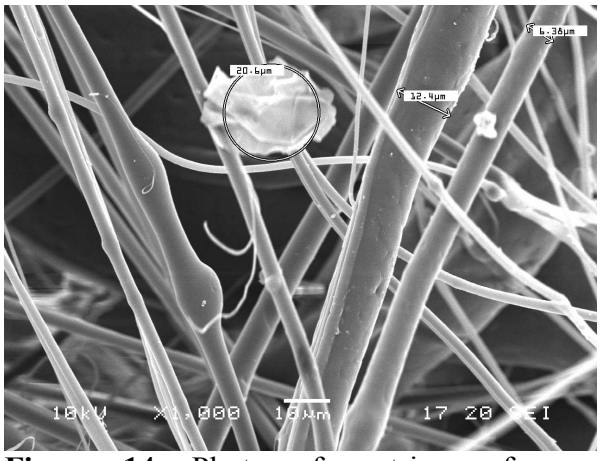

Figure 14. Photo of matrix surface of composite material (Eastar Bio Ultra co-polyester and medical active carbon)

Performed research works led to the following conclusions:

1. In the process of producing composite materials according to melt-blow technique it is possible to introduce – directly or indirectly – powdered modifiers.
2. In direct method it is advisable to use powdered products below 8 μm.
3. The contents of applied modifiers in composite nonwoven is limited; the amounts above 10% result in process disturbance and the strength parameters of the end-product decrease.
4. The condition limiting the application of the direct method is different thermal resistance of individual components.
5. To achieve composite material: co-polyester-chitosan, it is necessary to perform the process at the temperature below 265°C.
6. More uniform product can be achieved applying initial re-granulation process of co-polyester-chitosan.

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References

[1] Buntin R.R, Lohkamp D.T., TAPPI, 56 (1973), 7.

[2] Wente Van A.. Ind. and Eng. Chem., 8 (1956), 1342.

[3] Schwartz E.: Pat.US 3 806 289, (1974).

[4] Pat.GB 1432 864, (1976),

[5] Dever M., Davis W.T., Nowicka Cz., International Nonwovens Journal, 2 (1994), 26.

[6] Nowicka Cz., Rakowski W., Wcisło P., Nonwovens Industry,11 (1995), 50.

[7] Anonim, Nonwovens Industry, 2 (1996), 98.

[8] Wcisło P., KałużkaJ., Pęczkowska B.,Przegląd Włókienniczy, 1 (2006), 28.

[9] Sparkers B.G., Murray D,G., Pat. US 4 572 906, (1986).

[10] Kifune K.,Tanae H.,Yamaguchi Y.,Pat. US 4 651 725, (1987).

[11] Biagini G.,Bertani A. inni, Biomaterials, 12 (1991), 281.

[12] High Performance Biomaterials, ED. M. Szychter, Technomic Publ. Co., Lancaster, USA, (1991).

[13] Aiba S., Int.J.Biol.Macromol. 13 (1991), 40.

[14] Varum K.M., Smidsrod O.; In: Advances in Chitin Sci., A.Domard, G.A.F.Roberts, K.M.Varum (eds.), Jacques Andre Pub., Lyon, France, (1997), 168.

[15] Roberts G.A.F., Chitin chemistry, The MacMillan Press LTD, London, Great Britain, (1992) 1.

[16] White S.A., Farina P.R., Fulton I., App. Environ. Microbiol. 38 (1979), 323-328.