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Studies on the Structure of Semi-permeable Membranes by Means of SEM Problems and Potential Sources of Errors

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The effect of sputtering with a conductor of the semi-permeable membranes surface on SEM pictures obtained is presented. On the example of photomicrographs of several different types of semi-permeable membranes, changes in the appearance of various membrane surfaces, uncovered and sputtered with thicker and thicker layers of the conductor are presented. It has been shown, how essential differences in the appearance of the studied material can be caused by the deposited conductor. It has been shown what errors in the interpretation of SEM images can be caused by applying the sputtered conductor layer with a thickness insufficient to the structure and properties of the studied material. Necessity of minimizing the layer thickness of the sputtered conductor and experimental determination of the sputtered layer thickness was found. Appropriateness of taking the pictures in the mode without sputtering and necessity of comparing the pictures with and without sputtering have been suggested. The useful way of carrying out magnifications' of membranes made of polymers of low melting points has been also presented.

K e y w o r d s: SEM, semi-permeable membranes, polymeric microcapsules, membrane studies

1. Introduction

Scanning electron microscopy (SEM) is at present one of the basic methods used for the characterization of semi-permeable membranes [1–4]. It is also often utilized not only in studies of the membrane structure, but also for other purposes, e.g. for

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fouling studies [5, 6]. Simultaneously with SEM, more and more often atomic force microscopy (AFM) is applied [7, 8] permitting estimation of the studied membrane's structure [9]. However, SEM remains still one of the basic experimental techniques. Since nearly all the semi-permeable membranes are made of materials being either very bad electric conductors or isolators, to take pictures of the membranes it is necessary to take advantage of one of the two techniques: sputter the studied object with a conductor or make magnification by means of apparatuses adapted to make magnification at some higher pressure then commonly used, often called "biological" apparatuses. Unfortunately, authors often do not state the technique with which the picture was taken [2, 3]. They most often only inform of the conductor with which the sample was sputtered [1, 4, 6], and only a very few present the thickness of the deposited conductor layer [10, 11]. For this are used commercial sputter coaters. The sputters for coating the investigated surfaces by conductance layer used low temperature plasma, most often argon plasma. It is well known that the plasma treatment can alter the physicochemical properties of polymer surface. The different low temperature plasmas are used for modification of membrane's surface [12–17], for grafting polymerisation on membrane surface [18–21], for modification of additives used in membrane formation [22] and coating of polymeric membranes with hydrogel [23]. It has been stated that the argon-oxygen plasma smooth out the surface of the polysulfone membrane [12]. These properties of low temperature plasma should be take into consideration.

Membranes designed for medical and biotechnological applications, due to obvious reasons, undergo much stricter discipline than membranes for other applications and must be thoroughly studied. First of all, they must be bio-conforming, cannot emit harmful degradation products or cause undesirable tissue-cell reactions. The shape of the membranes surface, its structure, porosity and coarseness are of importance in contact with live cells or simply with live tissue [24, 25]. Therefore, when developing and studying semi-permeable membranes for biomedical purposes, scanning electron microscopy is commonly utilized [2–5].

In fact, necessity of evaluating the surface of the developed by us membranes for contact with live cells induced us to take up the problem of correct sample preparation for the studies of the membrane surface. Due to the fact that often pictures of membranes taken without spraying were out of focus or unclear, we decided to check how the appearance of the membrane surface is affected by spraying of them with a conductor.

2. Experimental Part

2.1. Materials and Methods

Polysulfone (PSf) Udel 1700 NT LCD (Dow Corning) of m.w. 70000, polyethersulfone (PES), Ultrason E6020P (BASF) of m.w. 58000, glicolid/ε-caprolac-

tone/L-lactyd tertpolymere of m.w. 48000 (Centre of Polymer Chemistry, Zabrze, Poland) respectively were used for the obtaining of semi-permeable membranes. Polyvinylpyrrolidones (PVP) of m.w. 10000 and 40000 (Aldrich) were used as pore precursors. N-Methylpyrrolidone (NMP) (Fluka), trichloromethane (TCM) and dimethylformamide (DMF; Polskie 0dczynniki Chemiczne – POCh) were used as solvents. Calciun chloride (POCh) was used as additive for microcapsiles gelation bath. Demineralized water of 18.2 MΩcm resistance, obtained by means of a Mili Q system (Millipore) and ethyl alcohol (POCh) were used as non-solvents.

Hollow fibre membranes from polysulfone and polyethersulfone were made by the well known dry-wet spinning method [26] from DMF solutions containing 18% of membrane forming polymer and 10% of PVP m.w. 40000. (for both hollow fibers the outer diameters were about 850 μm; wall thickness 100 μm; cut off 68 kD). The flat membranes from PSf and PES were made by dry-wet method too [27] from NMP solutions containing 20% of membrane forming polymer and 15% of PVP m.w. 10000. The both membranes cut of was 20 kD and their wall thickness about 300 μm. The flat membranes made from tertpolymer were obtained from 15% solution in TCM. The porous membrane from tertpolymer was obtained by phase inversion method in ethanol (thickness 150 μm cut off 48–50 kD). The membrane with closed skin layer was made by evaporation technique (thickness 100 μm). Microcapsules (average diameter 1.79 ± 0.07 mm) were made from NMP solution containing 7%, of PES supplemented with 5.3% PVP 10000 by the electrostatic extrusion technique combined with the wet phase inversion method using mixture of 1.1% calcium chloride in demineralized water solution and methanol in a ratio of 2:1. [28, 29].

All of the examined membranes were manufactured in our laboratories. Before the microscopic examinations the hollow fibre membranes and the microcapsules were frozen in liquid nitrogen, fractured and stuck to a supporting table. Scanning electron microscope, type TM 1000 of Hitachi was used for recording magnifications. For sample sputtering with a layer of gold (Au), the K550X sputter coater was utilized.

2.2. Recording of Magnifications

In order to compare the appearance of the sputtered and non-sputtered surface, the membranes were placed on a supporting table. Then, half of the table was covered with aluminum foil and sputtering was performed controlling the thickness of the layer applied by means of the sputter adjustment device according to the producer's instructions. The picture of thus prepared sample is shown in Fig. 1.

Succeeding layers of gold were sputtered on the same sample, or on consecutive supporting tables the same hollow fiber was mounted in such a way that on all tables the same hollow fiber side was exposed to the top. Due to this methodology we were certain that we always look at the same surfaces. The border of sputtering

seen with the means of an electron microscope is sufficiently sharp, and this solution excludes making a coincidental mistake in the identification of the surface looked at (Fig. 2).

Fig. 1. Picture of the supporting table with hollow fiber membranes. Upper half (darker hollow fibers) is sputtered with a layer of gold of 10 nm thickness

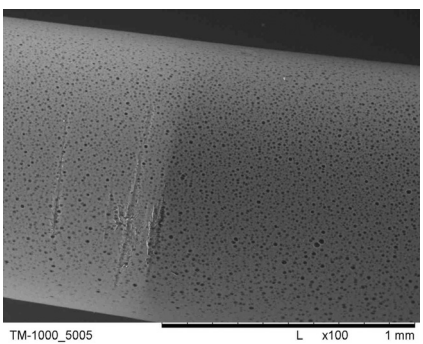

Fig. 2. View of the border of sputtering with a gold layer of 5–6 nm thickness. The hollow fiber part sputtered with the conductor is lighter in the SEM picture. Magnification 100×

3. Results and Discussion

3.1. The Influence of Thickness of the Sputtering Layer on the Appearance of the Membrane Sample

In order to evaluate the structure of the external skin layer of the membranes with the developed internal surface, predicted for medical and biotechnological purposes [25], a number of pictures of the surface were taken. One of the characteristic elements observed in some membranes is presence of the relatively large pores covered with a thin and subtly built cuticle layer (Fig. 3). Since these membranes were selected for further modification, it was decided to study in detail this external cuticle layer.

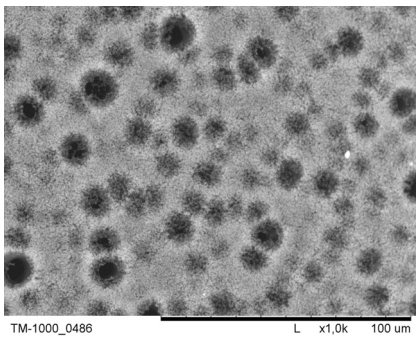

Fig. 3. Picture taken without sputtering with a conductor. Magnification ×1000

The pictures taken without sputtering have somewhat turned-up edges and are often out of focus (especially at magnifications above ×3000). To improve focusing we decided to cover the membranes with a conductor. When experimentally selecting the thickness of the conductor we carried out a series of magnifications of the same sample sputtered with thicker and thicker layer of the conductor (gold). The sets of images of the same magnifications of the same membrane differing only in the thickness of the conductor allowed to notice interesting results. Therefore, we carried out a series of systematic studies showing how the surface view changes after sputtering of subsequent layers of the conductor onto the membrane surface. The results obtained are presented in Figs 4 and 5.

Figure 4 clearly shows how the view of the membrane surface changes. Picture a) was taken without sputtering with charge transfer/removal by means of elevated air pressure. Picture b) presents the membrane sputtered with a very thin layer of gold (the 5–6 nm range). This picture was also taken with charge transfer by means of elevated air pressure. Pictures c) and d) show the membranes sputtered with thicker layers of gold (12–14 and 25 nm, respectively). These pictures were taken in standard conditions and the charge was transferred by the conductor layer. When comparing the pictures it can be observed how the consecutive layers of the sputtered conductor change the membrane surface appearance. Picture a) is as if slightly blurred, but despite this, macropores covered with a delicate cuticle layer, which put on the form of a delicate net, are clearly visible. The thin conductor layer (Picture b) causes blurring of the net structure and exposes micropores present on the surface. The next thicker layer further smoothens the membrane surface (Picture c). The micropores are visible here as depressions in the membrane surface. If the previous images had not been taken it would not be possible to suspect their existence. At the same time, this picture suggests a decrease in the membrane porosity. Complete "pouring" over of the membrane surface took place at the golden layer of 25 nm, causing invisibility of the macropores. On the basis of this picture it can be assumed that the membrane contains on its surface only rare pores of the dimensions of single microns. Crackings in the gold layer are also visible. In comparison with the previous pictures

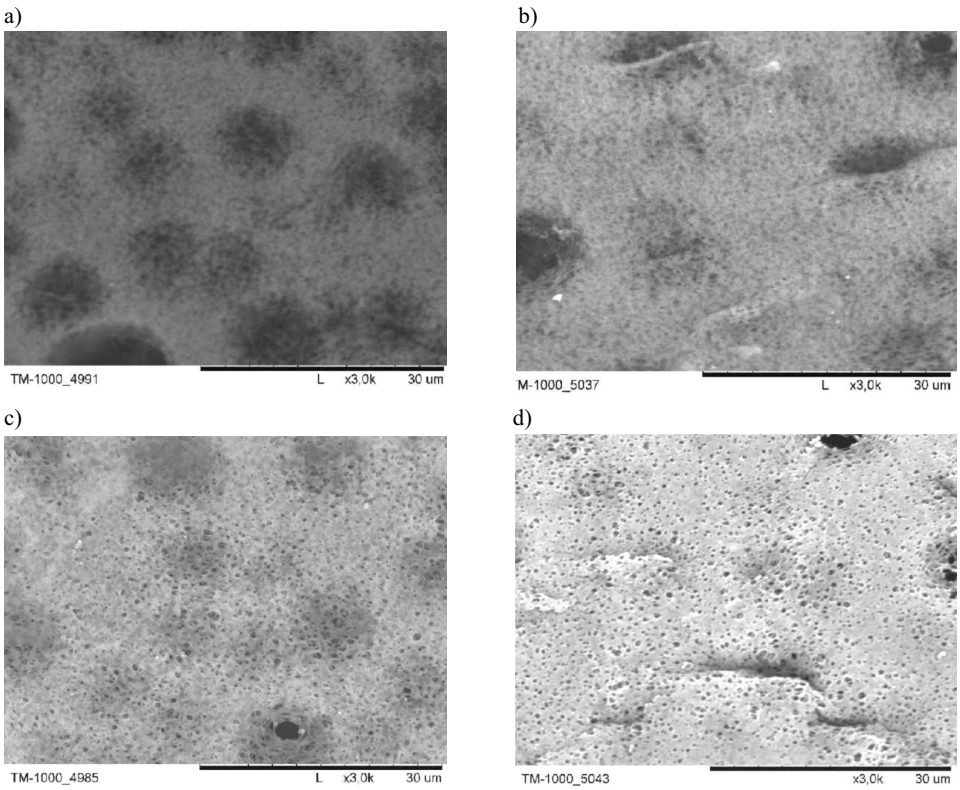

Fig. 4. Comparison of the view of the hollow fiber polysulfone membrane of magnification ×3000. a) membrane not covered with a conductor; b) membrane covered with a 5–6 nm layer of Au; c) membrane covered with a 12–14 nm layer of Au; d) membrane covered with a 25 nm layer of Au

it can be suspected that under these cracks macropores are present. However, without knowledge of the previous pictures such a suggestion would not be possible.

The next set of images shows the outer surface of the polyethersulfone membrane at a ×5000 magnification (Fig. 5).

The pictures were taken using the same procedure as for the previous membranes. Although the cuticle layer in the case of this membrane was somewhat thinner and more delicate, when looking at the subsequent pictures nearly the same conclusions can be drawn as when looking at the previous set. An increase in the thickness of the sputtered conductor allows obtaining more and more clear and focused magnifications. But at the same time one should bear in mind that sputtering with a conductor may result in "pouring over" the surface when a too thick layer of the conductor is applied, leading to erroneous conclusions. An example of applying a too thick sputtered layer on the flat membranes is shown in the set of pictures included in Fig. 6. The 30 nm layer of gold completely "poured over" the membrane surface. In this case we can only suspect existence of the pores in the membrane.

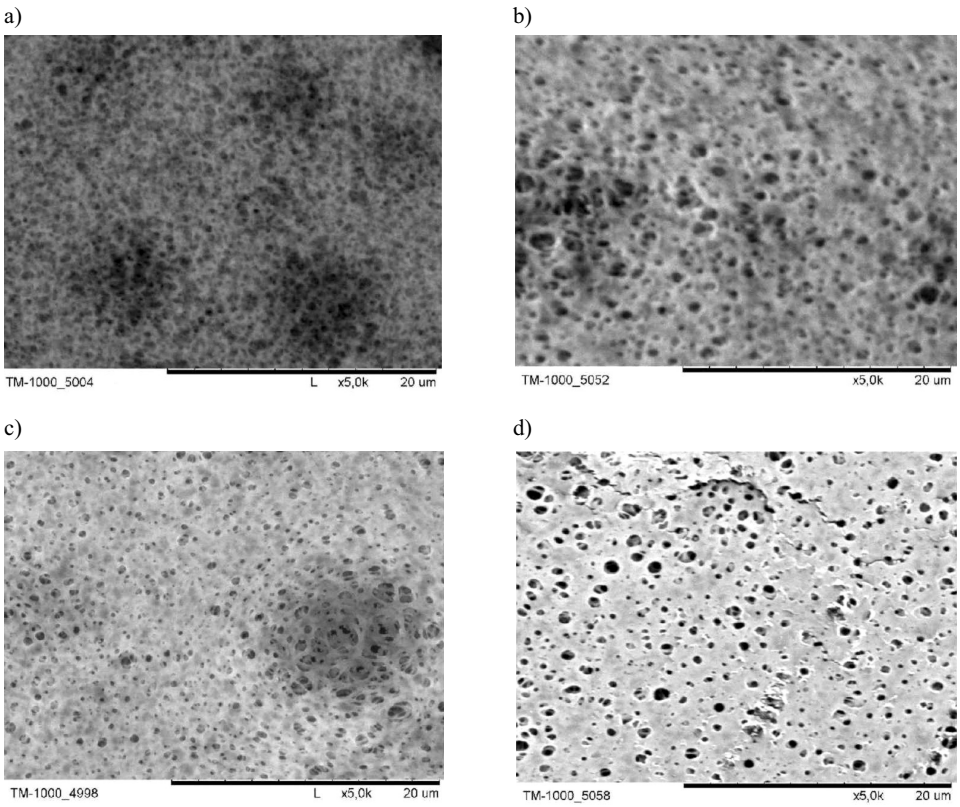

Fig. 5. Comparison of the view of the hollow fiber polyethersulfone membrane of magnification ×5000. a) the membrane not covered with a conductor; b) the membrane covered with a 6–8nm layer of Au; c) the membrane covered with a 15–18 nm layer of Au; d) the membrane covered with a 30 nm layer of Au

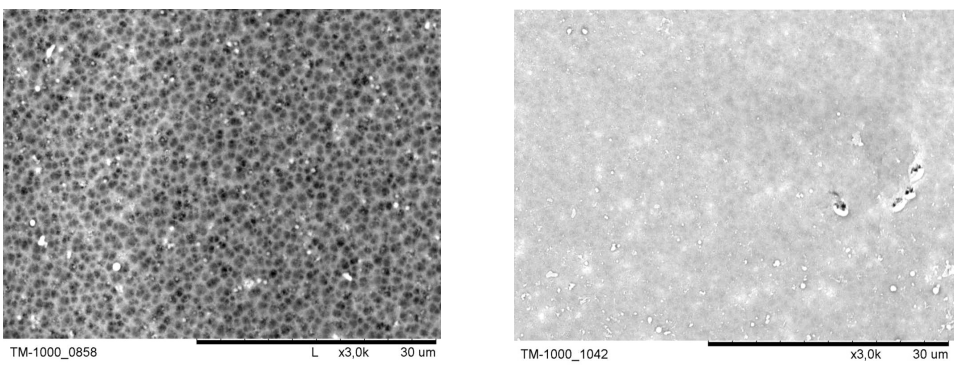

Fig. 6. View of the flat membrane with polysulfone cuticle layer designed for cell cultivation. Magnification ×5000. The left side of the membrane is unsputtered and the right side is sputtered with a gold layer of 30 nm thickness

A similar effect was observed when studying the surface of microcapsule membranes obtained from polyethersulfones (Fig. 7). When comparing the picture of the non-sputtered surface with that of the sputtered one it can be clearly seen how sputtering changes the appearance of the surface. Looking at the same microcapsule before and after sputtering there is an impression that the inner surface of the capsule is completely different, which is an obvious error suggested by the deposited layer of the conductor.

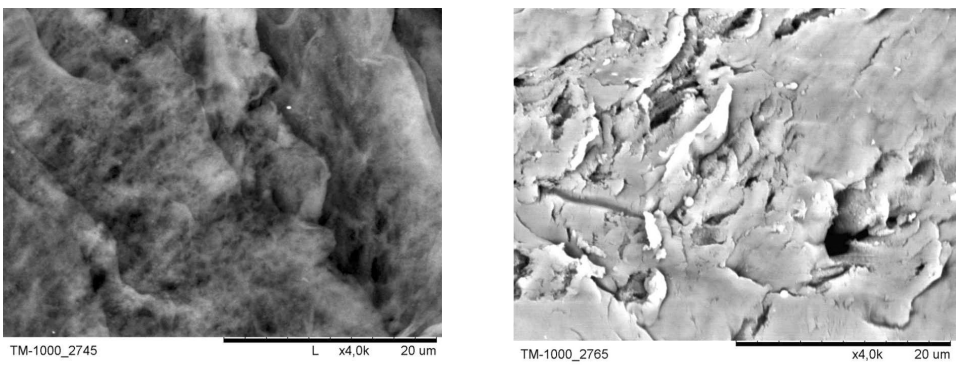

Fig. 7. View of the inner polyethersulfone microcapsule surface. Magnification ×4000. The left side presents an unsputtered surface, the right one presents a surface sputtered with a layer of gold of 25 nm thickness

However, as practice shows, very often sputtering with a conductor allows observation, which without a conductor would be difficult or even impossible. An example of an advantage provided by sputtering is a comparison of images of the wall ravine of the same microcapsule, which is observed in Fig. 8 taken without sputtering and after sputtering with a layer of gold of 25 nm thickness.

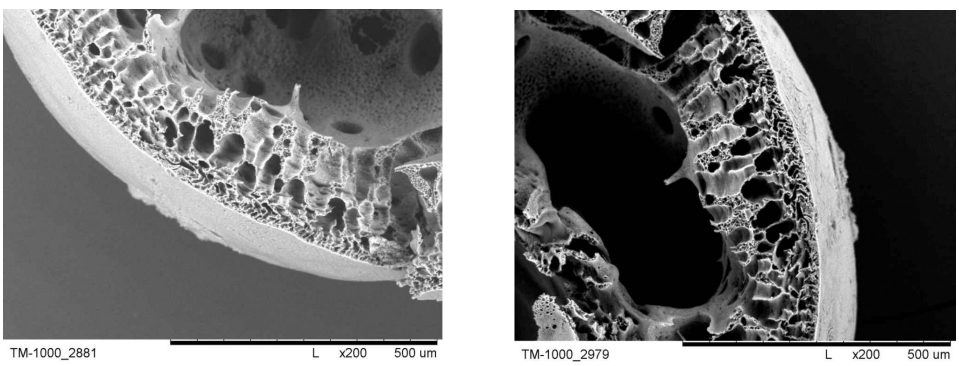

Fig. 8. View of the polyethersulfone microcapsule wall ravine. Magnification ×200. The left side presents the unsputtered surface, the right one presents the surface sputtered with a layer of gold of 25 nm thickness

In this case the details of the membrane wall structure become much clearer after sputtering. The picture is in focus what makes it is easier to evaluate the microcapsule wall structure. Therefore, for obtaining a complete picture of the membrane structure, it is favourable to take pictures of the same capsule both with and without sputtering with a layer of conductor.

3.2. The Influence of Deposition Time on Appearance of the Membrane Sample

In order to study weather the effect of "pouring over" the porous structure of the membrane can be reduced by shortening exposition to argon plasma, we have performed additional experiments.

The deposition times (applying deposition current of 20 mA) for gold layers of 5–6 nm were equal to 55 s, for that of 12–14 nm – 120 s and for that of 25 nm – 260 s. We have decided to shorten the deposition time by increasing the deposition current to 50 mA. This caused shortening of the time of 15 nm deposition to 60 s. This gave a different effect to that expected. The membrane after deposition, was completely riddled with holes, as it is shown in Fig. 9. The delicate polymer layer covering the macropores (visible on the left) was completely destroyed after sputtering (on right). This showed that an increase in current density in the case of such a delicate skin layer structures is unfavourable and may lead to still greater errors.

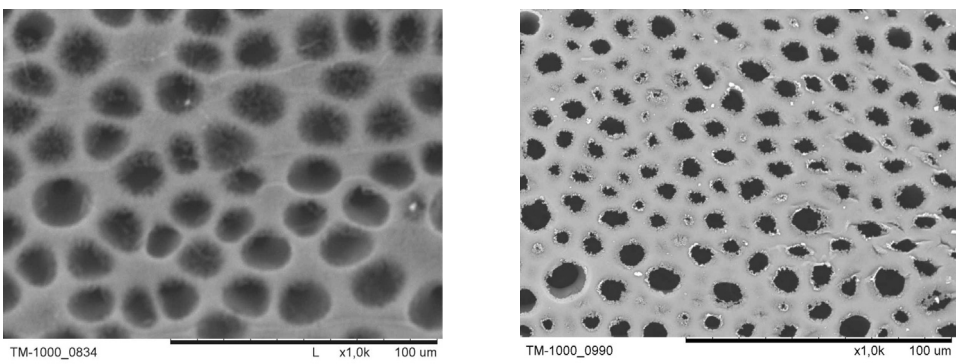

Fig. 9. Comparison of the view of the hollow fiber polysulfone membrane of magnification ×1000, left – the membrane not covered with a conductor; right – the membrane sputtered by 15 nm Au layer

3.3. The Way of Carrying out Magnifications' of the Membranes Made of Low Softening Point Polymers

Another kind of problems appears when it is necessary to carry out magnifications of the layers of membranes made of polymers of low softening points. Here it is often impossible to achieve greater magnifications, since the electron beam melts

the examined surface. Examples of how important is interference of the electron beam action are shown in Figs 10 and 11. These pictures present semi-permeable membranes, made of glicolide/ε-caprolactone/L-lactide and intended for cultivation of keratynocites. On the left side of Fig. 10 is shown a 1000-fold magnification, and on the right side a 2000-fold magnification of the same place of the membrane. On the left side of the photomicrograph the porous, unsputtered membrane is clearly visible, whereas on the right the membrane surface starts to melt under the action of the electron beam.

Another example of the harmful interactions between the electron beam and examined material is shown in Fig. 11. The left part of the image presents the dense cuticle layer of the membrane, which central part has been destroyed by electrons. It occurred during carrying out the second image (magnification ×2500) shown on the right side of Fig. 11. As it is seen, there are great differences in the surface structure

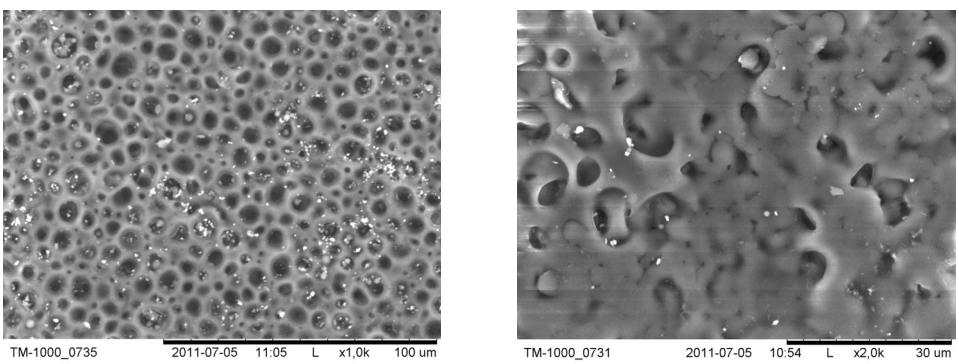

Fig. 10. Glicolid/ε-caprolactone/L-lactyd tertpolymere porous, unsputtered membrane. Left picture magnification ×1000, right picture magnification × 2500

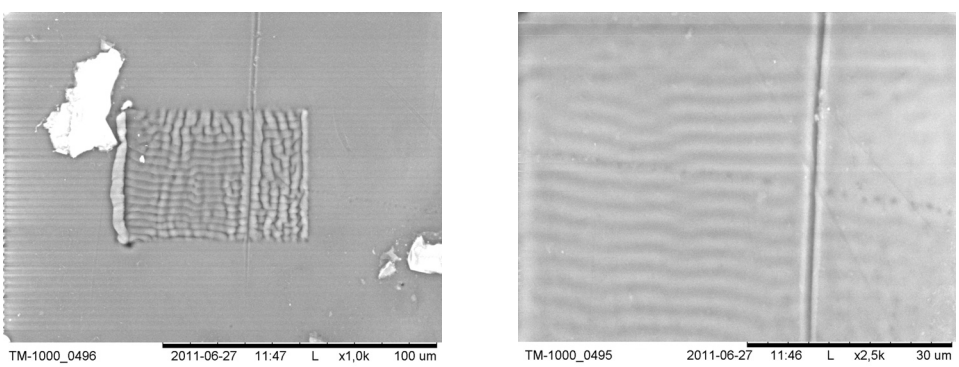

Fig. 11. Glicolid/ε-caprolactone/L-lactyd tertpolymere membrane with the close skin layer, melted by the electron beam. Magnification of the left image ×1000. Right side: the close-up of the central part of the membrane destroyed by the electron beam (magnification ×2500)

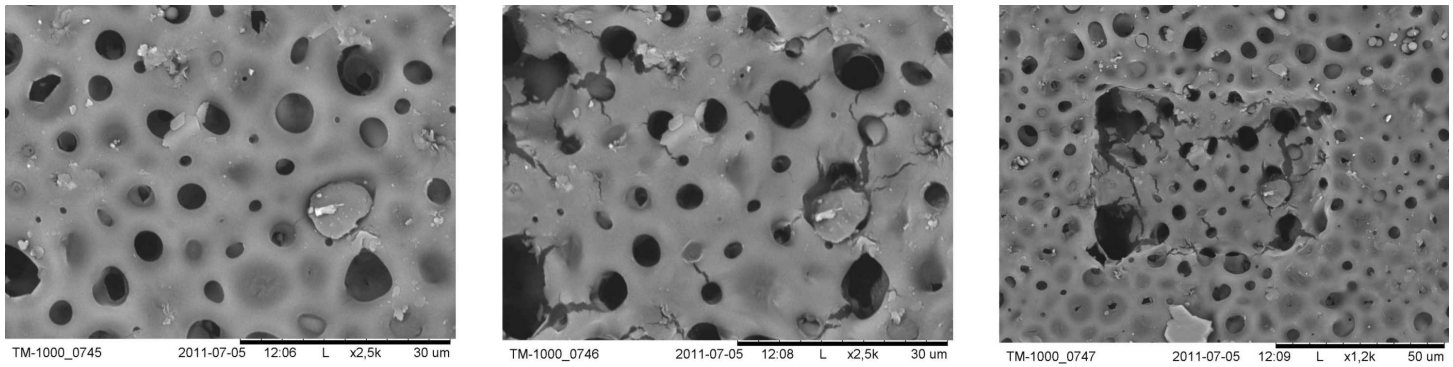

Fig. 12. Glicolid/ε-caprolactone/L-lactyd tertpolymer porous membrane sputtered by 15 nm of Au. Left picture magnification ×2500, central picture magnification×2500, right picture magnification × 1200

of the membrane. Actually, it appeared that carrying out magnifications greater than 1500 caused considerable changes in the observed surface. Therefore, to obtain reliable results magnification should be limited to about ×1000.

To improve the quality of the images of the tertpolymer membrane we decided to covered the sample with 15 nm layer of gold. Figure12 presents three images of the same place of the membrane at ×2500 (left and middle one) and ×1200 (right one) magnifications. The photomicrograph on the left side was recorded after about 15 seconds after selecting the pictured fragment. The obtained picture correlates well with that shown in Fig. 10 (magnification x1000).

The middle photomicrograph of Fig. 12 was taken from exactly the same place about 90 seconds later. Cracks in the conductor's layer are clearly visible. The picture on the right side was taken after the next 15 seconds, but at smaller magnification. It is clearly visible that the trace resulting from the electron beam interaction caused the collapse of both the membrane and the conductor layer. These observations show that in the case of membranes made of polymers with low melting points there is a higher possibility of achieving greater magnifications after deposition with a conductor than without deposition, but they have to be carried out very quickly and there is practically no chance to obtain very large magnifications without a risk of destroying the microscope screen. So, in the case of these kind of materials deposition with a thin layer of a conductor gave a fairly good result, but only when magnification was performed rapidly.

4. Conclusion

Utilization of SEM technique for the study of semi-permeable membranes is an efficient and very convenient method. However, in the case when the studied objects are bad conductors or isolators, it is difficult to obtain magnifications of good quality, showing the real structure of the examined sample. In such cases, apparatuses having the possibility of carrying out magnifications at biological mood and in which charge discharge from the sample studied occurs by means of gas (air) ionization, should be utilized first of all. Omitting of this stage of studies might be a serious mistake. Only when the carrying out of magnifications of unsputtered objects fails, then sputtering with a conductor (metal) can be taken into account. One should have in mind that the pictures obtained may be distorted by the presence of the sputtered layer. In such a situation, part of information can be recovered by sputtering the surface studied with an as thin as possible layer of a conductor. In every case it is necessary to experimentally select the deposited conducting layer thickness. The results of our studies clearly show the necessity of minimizing thickness of the layer of the conductor deposited on semi-permeable membranes. Deposition of the conductor layers thicker than absolutely necessary may lead to considerable errors in the evaluation of the membrane subtle structure. The usefulness of sputtering depends also on the

kind of observed surface. In the case of uneven surfaces, of definitely differed sputtering depth, the picture quality improves (better focusing and contrast). However, in the case of flat, little differing surfaces, or when very subtle structures are observed, sputtering often hinders the evaluation of their porosity and observation of details. Thus in every case it is worthwhile to compare pictures obtained without and with sputtering. Thanks to this interpretation errors are avoided and no essential details in the studied material structure are omitted.

Other danger is present when the membranes have been made from polymers with low melting points. In this case there is a possibility to achieve images of greater magnifications after deposition with a conductor than without deposition, but they have to be carried out very quickly and there is practically no chance to obtain very large magnifications without a risk of destroying the microscope screen.

Due to the fact that in sputtering we deal with layers of single nanometers thickness, it is necessary to apply a sputter of good quality and very carefully obey the procedures and sputtering times. Also, information on the layer thickness and what conductor was used for covering the sample presented in the picture should not be omitted.

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