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EVALUATION OF THE MORPHOLOGY AND STRUCTURE OF E GLASS FIBER-REINFORCED COMPOSITES FOR CRANIO-FACIAL BONE RECONSTRUCTION

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ABSTRACT. New E-glass fiber reinforced composites developed to serve cranio-facial bone reconstruction were investigated using scanning electron microscopy, atomic force microscopy and X ray diffraction to evaluate their morphology and structure. The morphological analysis of the external surface of experimental composite materials has shown that the reinforcing material was well incorporated in the polymer matrix and no monofilaments of fiber glass could be observed on the material surface. Structure analysis of the experimental composites revealed the optimal interfacial adhesion obtained using A 174 silane. Woven E glass fibers were more favourable than unidirectional fibers for manufacturing cranio-facial implants. The flexural strength of the experimental composite materials was directly influenced by the degree of reinforcement. Taking into account the obtained results, the perspective of continuing the studies appears for improving the mechanical properties so as to be as close as possible to the ones of the human bone.

Keywords: *biocomposites, reconstructive surgery, surface, interface, bending test, scanning electron microscopy, atomic force microscopy, elemental distribution*

INTRODUCTION

In spite of the huge progress made in reconstructive surgery in recent decades, restoring cranio-facial bone structures, pathologically affected, keeps being an endless challenge. The consecutive morphological and functional deficits, significantly impairs life quality because they involve a highly exposed anatomic segment.

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Reconstruction of craniofacial bone defects, especially dysfunctions and dysmorphysm that they induce, is a burning issue in the medical field. Materials used to serve this purpose are of great diversity, but they still have some shortcomings that limit their application and sometimes cause clinical problems [1, 2].

Recently, the biomaterial research is focused also on biodegradable and biocompatible composites. The main concerns related to clinical application of composite implants are the negative biological reactions that these materials may produce. The local reactions that composites may induce are due not only to the elution of leachable components from composites, but also to the mechanical irritation they cause [3, 4]. Also, mechanical properties of composites meant for bone reconstruction should be tailored in order to avoid stress-shielding and bone over-loading, which have negative effects on surrounding bone and ultimately lead to bone resorption, loosening and failure of the implant [5, 6, 7].

This study aims to analyse the morphology and the structure of experimental E-glass fiber reinforced composites that will serve the fabrication of custom-made implants for craniofacial bone reconstruction.

The surface morphological analysis had as an objective to determine the implants quality in terms of surface roughness and the influence of the gentamicin coating on this aspect through atomic force microscopy and scanning electronic microscopy examinations.

Likewise, the structural analysis of the experimental composite materials has pursued to investigate the following aspects: if the structure of the composite materials is uniform or if any material defect is found; if the suitable compatibility is made between the organic phase and the inorganic phase of the composite materials; which is the influence of the degree of reinforcement and of the glass fibers geometry on the mechanical properties of the composite materials. In order to investigate these aspects, the analysis of the experimental composite materials was made through scanning electronic microscopy, both before and after subjecting the specimen tubes to the bending test. Taking into account that cranial implants are fixed rigidly marginally, out of all mechanical trials the bending test was chosen because it best stimulates the conditions in which such an implant might be subjected to an accidental force.

RESULTS AND DISCUSSION

Morphological analysis of the surface

The morphological analysis of the external surface of experimental composite materials (MCE) using SEM (fig. 1) has shown that the reinforcing material was well incorporated in the matrix. Monofilaments of fiber glass could not be observed on the material surface. The quality of the obtained surface was

good, the roughness being acceptable for the surface of an implant. In fact, this roughness copies the quality of the matrix surface which was used to prepare the respective samples. This aspect is critical for composites meant for medical implant fabrication, since the inflammatory reaction of the surrounding tissues may be caused not only by the leachable compounds, but also by the surface of the implant itself. The presence of the gentamicin coating (fig. 1 D) did not notably influence the roughness of the obtained material. Moreover, the presence of gentamicin on the surface of the implant impaired bacterial adhesion and growth [8].

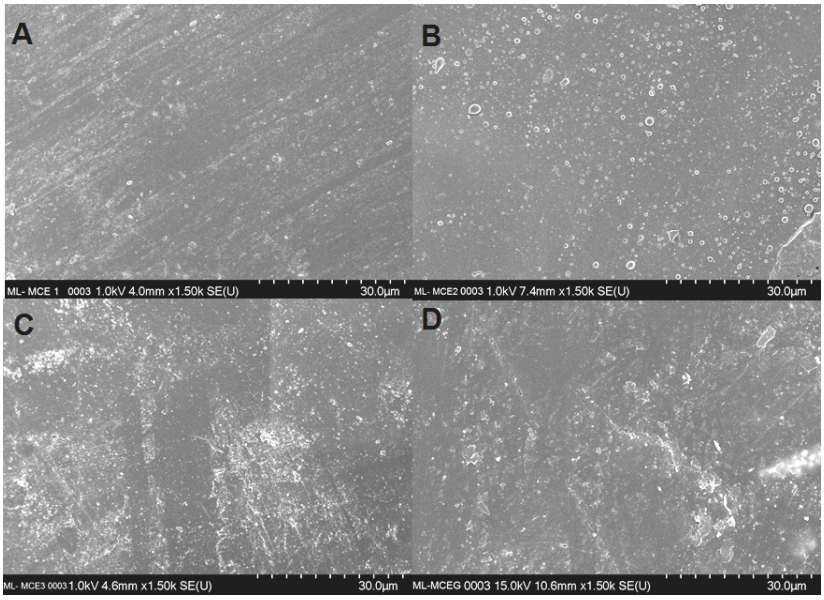


Figure 1. Morphological analysis of the external surface of the experimental composite materials MCE 1 (A), MCE 2 (B), MCE 3 (C), MCE 1-G (D).

The 3D topography of the surface of the four materials analysed using atomic force microscopy emphasized their minimal roughness, with level differences of maximum 30 nm for MCE 1- MCE 3. In the situation where the gentamicin layer was added, the level differences were smaller, to a maximum of 8 nm. This can be explained by the fact that the gentamicin molecules have smaller sizes thus creating a higher uniformity degree. In the figure 2 are exemplified the AFM images obtained for MCE 1 and MCE 1G.

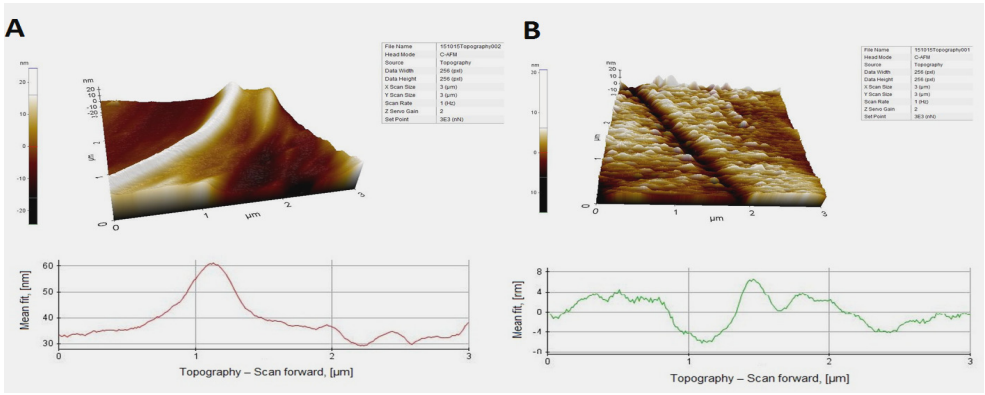


Figure 2. 3D Topography and the variation of topographic heights of MCE 1 (A) and MCE1- G (B) on the analyzed surface

Structure analysis using X-Ray diffraction

The diffractogram of the resin sample (fig. 3 A) was specific to amorphous carbon based materials meaning disordered polymer chains. Also the diffractogram of glass fibers was rendered (fig. 3B), which was specific to amorphous vitreous materials. No matter the fiber glass used in the composition of composites, the degree of reinforcement or the geometry of reinforcement, their diffractograms present a totally amorphous character (fig. 3 C).

In the diffractogram of the composite coated with gentamicin MCE 1-G one can identify the peaks characteristic to the crystalline phase of the gentamicin (18 peaks) (fig. 3D), besides the amorphous halo given by the composite made of resin and glass fibers.

The structural analysis of the experimental composite materials before and after subjecting them to the bending test.

The structural analysis of the MCE1- MCE3 experimental composite materials has revealed that the organic matrix integrates the reinforcing material, with no material defects detected.

In figure 4 was exemplified MCE 1 for which the bidirectional fibers could be observed, before the composite was subjected to mechanical stress for its characterization. The singular points represent the warp of the fabric and the fibers which appear to be continuous represent the weft of the fabric. There were no material defects highlighted, the matrix integrating the enforcing material throughout the entire structure of the material.

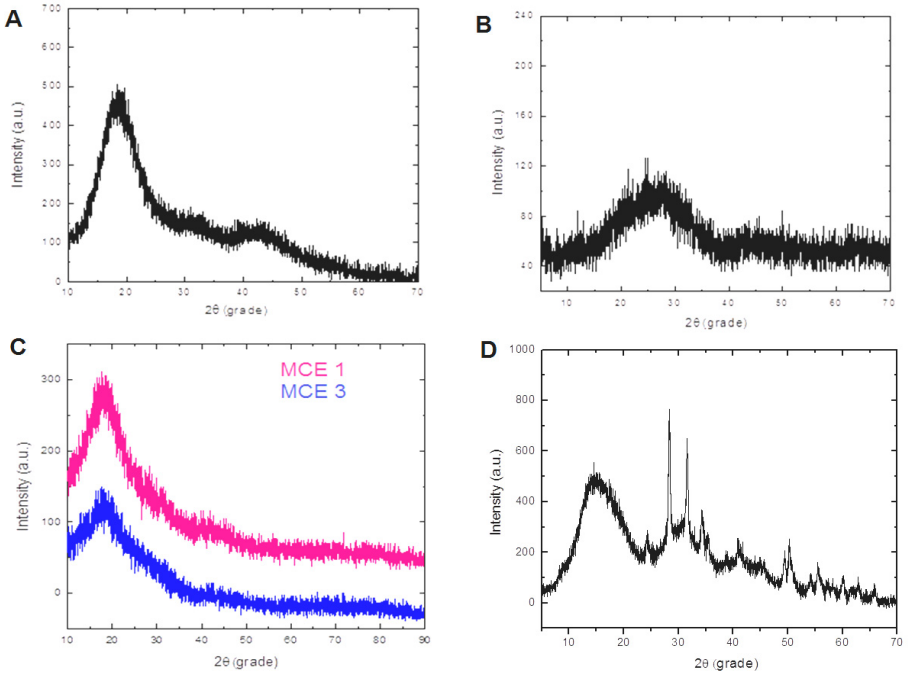


Figure 3. Diffractograms of resin (A), E-glass fibers (B), MCE1 AND MCE 3 (C), MCE 1-G (D)

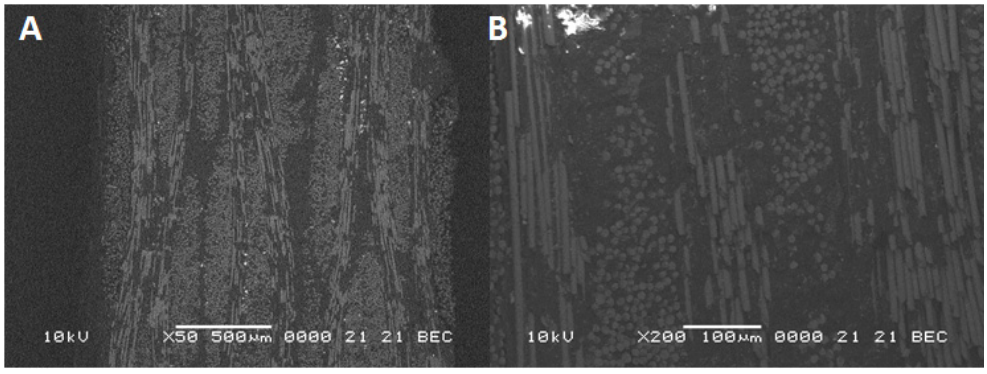


Figure 4. SEM Images for sample MCE 1 before the bending test
A- Image recorded lengthwise (x50)
B- Image recorded transversally (x200)

The bending test to which the samples made of experimental composite materials were subjected to has indicated the values for flexural strength and respectively for Young's modulus comprised in table 1.

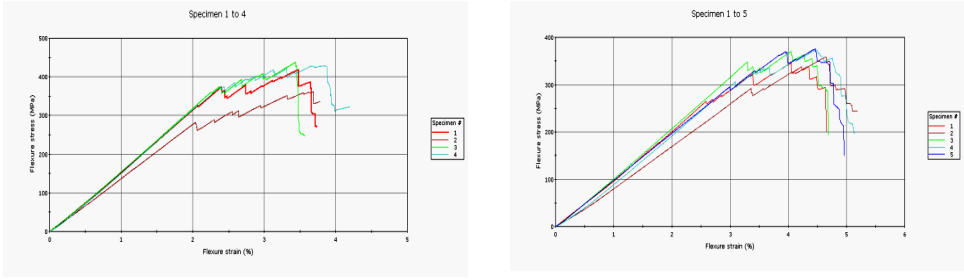
Table 1. Mechanical properties of the experimental composite materials

Codes of experimental composite materials	Glass fibers	Reinforcement /degree of reinforcement %	Flexural strength (Mpa)	Young's modulus (Gpa)
MCE 1	Woven	65	394.45	16.295
MCE 2	Woven	60	319.35	14.849
MCE 3	Unidirectional	65	717.50	21.706

After subjecting the samples from composite materials to the bending test, other important structural aspects have been identified by analyzing the obtained values and through further examination with scanning electronic microscopy. Therefore, during the bending process, the first to cede was the polymer matrix while the reinforcement fought against fissure propagation, thus taking place a stress transfer from the matrix towards the fibers.

It was also observed that for the samples MCE 1 the reinforcing fibers had broken gradually. Also, according to the diagram presented in figure 5, MCE 1 has flexural strength lowering thresholds near the maximum breaking values. While crossing the thresholds, the sample continues to stretch and then it breaks completely. From a value point of view, the flexural strength for MCE 1 and MCE 2 was measured at approximately 350 MPa and of over 700 MPa for the MCE 3 samples.

For the samples type MCE 2 one can observe a specific longer stretch, because the quantity of resin is larger, thus assuring a greater elongation (4% as opposed to 3%).



MCE 1 MCE 2

Figure 5. The flexural strength diagrams for MCE 1 as compared to MCE 2

The phenomena taking place in the MCE 1 structure during the bending test are exemplified in figure 6. The blackened zone which appears after the bending stress represents the delamination of the layers of the reinforcing material. After the start of this phenomenon, the actual breaking of the monofilaments may occur.

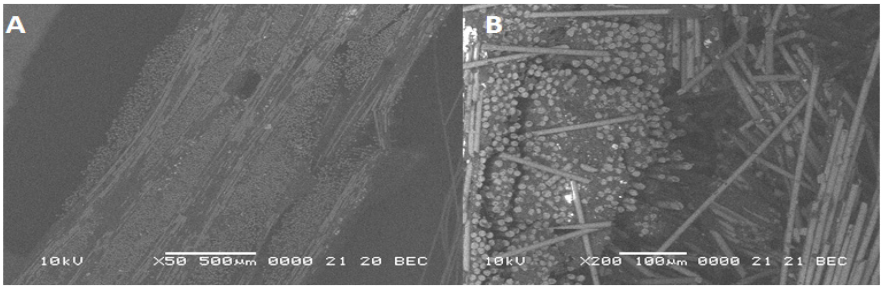


Figure 6. SEM Images for MCE 1 after the bending test
A - Imagine recorded lengthwise (x50),
B - Imagine recorded transversally (x200)

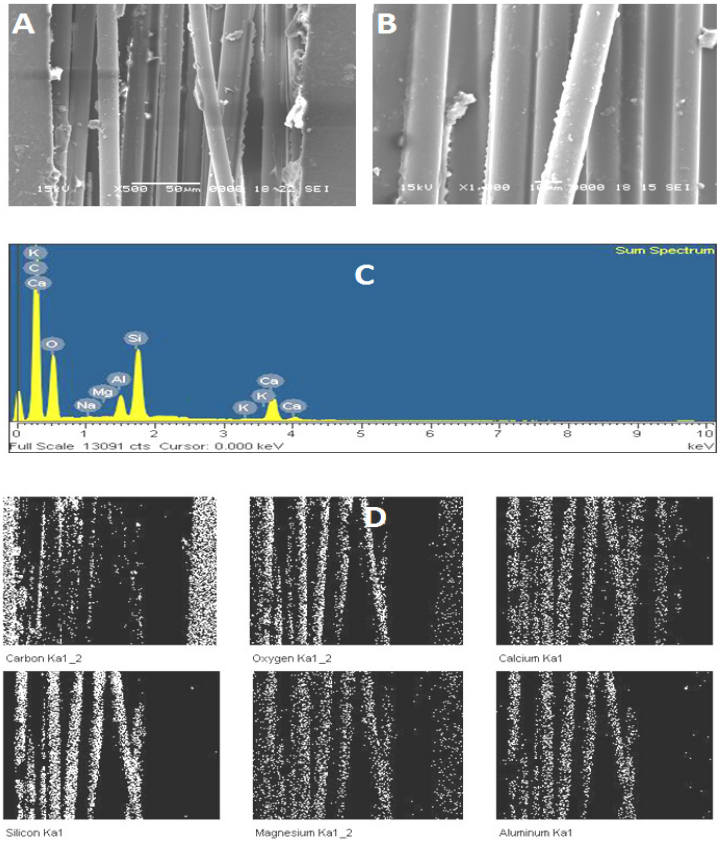


Figure 7. SEM and EDX images for MCE 3 after the bending test
A - SEM Image for MCE 3 after the bending test (x1000)
B - SEM Image for MCE 3 after the bending test (x 5000)
C - EDX spectrum corresponding to the area from point A
D - The distribution of elements on the surface of the sample from point A

For a much better visualization of the polymer matrix which remains attached to the glass fibers superior enhancements were used and the samples were covered in Au, thus avoiding electrostatic charge. Figure 7 presents electronic microscopy aspects for sample MCE 3, after the bending test. The glass fibers had polymer portions on their external surface. This indicated a very good compatibility between the fibers and the matrix, the treatment used being adequate for obtaining the suitable interface. The monofilaments are grouped, having a uniform allocation in the structure of the composite material. They are arranged longitudinally in regard to the direction of the mechanical stress. During mechanical stress, they have acted together and remained grouped even after breaking.

With the help of the X-ray microanalysis a quantification was made of the chemical elements detected on the surface from figure 7A, thus creating a compositional analysis of the sample after the mechanical testing. The elemental chemical compositions on the analyzed microarea are presented in figure 7C.

The distribution of the main chemical elements (carbon, calcium, oxygen, aluminum, magnesium and silicon) on the surface of the sample from figure 7B is characterized by a relative uniformity. Although the calcium, oxygen, aluminum, magnesium and silicon are elements of the glass fibers, the presence of the carbon is given by the existence of polymer resin traces on the surface of the reinforcement. The relatively uniform distribution of the carbon atoms strengthens the affirmation stating that the compatibility organic phase – inorganic phase was made accordingly.

The flexural strength of the experimental composite materials was directly influenced by the degree of reinforcement, as it is also shown by other literature studies [9]. However, it must be taken into account that in the case of high degrees of charge the glass fibers can remains “too poor”, which leads to structural defects. Moreover, considering the field of application for the manufactured materials there is no need for the mechanical resistances / Young’s modulus to have great values. For a degree of reinforcement of 60%, the flexural strength still exceeds the one of human bone structures [10].

The mechanical properties of composite materials were also influenced by the geometry of the reinforcement [11]. The fabrics used in this study for the samples MCE 1 and MCE 2 have had the weft and warp in equal proportions, arranged perpendicularly to each other. Such a fabric is favorable for manufacturing the landmarks with a complex geometry, to which the stresses are not only unidirectional or only bidirectional, but the stresses may also be complex. In the case of the material with unidirectional fibers, MCE 3, all the monofilaments are arranged on the direction of the stress if it is a unidirectional stress (traction, bend or compression). This makes the values of

the specific resistance of the material to be double compared to a similar material but reinforced with fabric, in which case only half the quantity of integrated fibers takes the respective stress.

CONCLUSIONS

The morphological analysis of the external surface of experimental composite materials has shown that the reinforcing material was well incorporated in the polymer matrix and no monofilaments of fiber glass could be observed on the material surface. The usage of the A 174 silane has allowed the chemical binding of the two phases of the experimental composite materials. Thus was created a stable interface, which notably increases the mechanical resistance. Taking into account the obtained results, the perspective of continuing the studies appears for tailoring the mechanical properties so as to be as close as possible to the ones of the human bone, starting from the already proven aspects: the reinforcement shoud use woven fibers and the degree of reinforcement must not exceed 60%.

EXPERIMENTAL SECTION

In order to produce the experimental composite materials (MCE) included in the study, following materials were used: bisphenol A glycidylmethacrylate (Bis-GMA), urethane dimethacylate (UDMA), triethylene glycol dimethacrylate (TEGDMA), benzoyl peroxide (POB), 2,2-dihydroxyethyl-p-toluidine (DHEPT), butylatedhydroxy toluene (BHT), 3-methacryloyloxypropyl-1-trimethoxy-silane (A-174 silane, Sigma Aldrich Chemical Co., Taufkirchen, Germany), bi-woven E-glass fibers (Owens Corning, Brussels, Belgium), Gentamycin sulphate (BioChemica, Activity 641 I.U./mg; Lot:3Q008363).

Two resins (base resin and catalyst resin) were prepared to produce each MCE. The composition of resins is UDMA 60 wt%, Bis-GMA 10 wt%, TEGDMA 30 wt%. POB (1 wt%) was used as the initiator in the catalyst resins and DHEPT (1 wt%) was used as the accelerator in the base resin. BHT was used as an antioxidant and dissolved in each resin in an amount of 0.65 wt%. To ensure good interfacial adhesion and stress transfer across the interface, E glass fibers were treated with a coupling agent [12, 13]. 1 wt % A-174 (γ-methacryloxypropyl-1-trimethoxysilane) was added to the amount of E-glass fibers. The silane solution was prepared by dissolving A-174 silane in ethanol–water 90/10 vol.% acidified to pH 3.8. PH level was maintained using glacial acetic acid. The glass fibers were immersed in this solution for 1 h. After that, the silane layer was dried at 110 °C for 2 h [14].

Each MCE was obtained using the laminate lay-up process. The bottom layer in the mold was the base resin, over which the E- glass fibers were applied. On the top surface of this last material a layer of catalyst resin was added. Then the glass fibers and the resins were applied alternatively using the following pattern: base resin, woven glass fibers, catalyst resin, woven glass fibers, base resin, etc.

For MCE 1 the degree of reinforcement was of 65% and it was made with woven fiber glass; for MCE 2 the degree of reinforcement was of 60% and it was made with woven glass fibers; for MCE 3 the degree of reinforcement was of 65% and it was made with unidirectional glass fibers extracted from the same fabric used for manufacturing MCE 1 and MCE 2. A part of the specimens manufactured from MCE 1 were covered with a gentamicin layer following the previously published protocol [8], and were coded MCE 1-G.

The MCE hardened through the chemically initiated polymerization of the resins at room temperature, followed by a thermic treatment (post-polymerization) for 2 hours at 100 ^{0}C in a specialized oven. The polymerization started the moment a base resin is mixed with a catalyst resin and continued during the application of the successive layers of resins and woven E-glass fibers.

Scanning electronic microscopy and atomic force microscopy techniques were used for the morphological analysis of the surface. The amorphous character of the composite materials was highlighted through X-ray diffractometry. For the structural analysis of the experimental composite materials, scanning electronic microscopy techniques were used both before and after the mechanical tests.

Hereinafter are presented the details regarding the devices and techniques used, and also the protocol followed for the bending test.

The examination through electronic microscopy was made with a SEM scanning microscope JEOL – JSM 5600 LV, equipped with an EDX spectrometer (Energy dispersive X-ray spectrometer) (Oxford Instruments Soft Inca 200). The EDX spectrometer was used to quantify the chemical elements detected on the specimens' surfaces. In order to avoid electrostatic charge, the test tubes of experimental biocomposite materials which are not electrically conductive have been metallized with gold using a Denton Vacuum apparatus (Desk V) (They have a thickness of layer of the order of 25 Å).

The *XE 70* ***atomic force microscope*** was used to determine the roughness state of MCE. This type of microscope is used to determine different mechanical, structural and morphological characteristics, but also to obtain

the topography of some surfaces with a resolution of 2 nm. The tests have been at a 25°C temperature and a relative humidity of 56%. The testing strength was of 25 μN. Using the same microscope, 3D images were made of the tested samples. The resulted images were processed by using the XEI Image Processing Tool for SPM Data software.

For the analysis of specimens through ***X-ray diffraction*** a Shimadzu XRD-6000 diffractometer was used, with Cu Ka radiation (λ = 1.5418 Å) and a Ni filter. The specters have been registered between 10° and 70°, and 20° and 110° in the continuous scanning mode 2θ, with 2°/minute. The tension and current were of 40 kV, respectively 30 mA.

The analyses have been made within the laboratory of Micro- and Nano- Systems (MiNaS) from the Department of Mechanical Systems Engineering, the Technical University from Cluj-Napoca.

The bending test

The *flexural strength and modulus of elasticity* were determined using MCE specimens of rectangular form (length 25.0 mm, height 2.0 mm and width 2.0 mm), according to ISO 4049/2000 three-point bending test. The measurements of the flexural properties were made using a Lloyd LR5K Plus mechanical testing apparatus connected with a computer. The crosshead speed of the testing machine was 1.0 mm/min.

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