PREPARAREA ŞI CARACTERIZAREA MICROSFERELOR CERAMICE POROASE PE BAZĂ DE FOSFAT TRICALCIC
FABRICATION AND CHARACTERIZATION OF POROUS TRI-CALCIUM PHOSPHATE CERAMIC MICROSPHERES

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The paper presents the preparation and characterization of alginate-tricalcium phosphate porous microspheres, in terms of size, morphology, water sorption and structure. Microspheres were prepared by the ionotropic gelation technique using $CaCl_2$ as cross-linking agent. The effect of the amount of alginate, addition of porogen material, tricalcium phosphate and sintering temperature on density, porosity and microstructure of microporous ceramic granules, and the influence of tricalcium phosphate / alginate solution report on rheological characteristics of ceramic suspensions were investigated. Archimedes method was used for investigating physical properties. Depending on their characteristics, microporous granules may find application in dental, oral/maxillofacial and other orthopaedic surgical procedures.

Lucrarea prezintă modul de preparare şi caracterizarea granulelor microporoase pe bază de alginat şi fosfat tricalcic, referitor la dimensiunea, morfologia, absorbția de apă şi structură. Microsferele au fost elaborate prin tehnica gelifierii ionotropice în prezența $CaCl_2$ ca agent de reticulare. Au fost investigate efectul cantității de alginat, adaosului de material porogen, de fosfat tricalcic şi al temperaturii de sinterizare asupra densității, porozității şi microstructurii granulelor ceramice microporoase, cât şi influența raportului fosfat tricalcic / soluție de alginat asupra caracteristicilor reologice pentru suspensiile ceramice. Caracteristicile fizice au fost evaluate prin metoda lui Arhimede. In funcție de caracteristici granulele ceramice microporoase pot fi utilizate în chirurgia maxilofacială şi alte proceduri chirurgicale din ortopedie.

Keywords: *alginate; immiscibility; ionotropic gelation, tri-calcium phosphate, rheology, beads*

1. Introduction

Bone reconstruction represents a necessity in many clinical situations in which skeletal defects are to be restored or repaired [1, 2]. Theoretically, the best material that can be used for bone reconstruction is autologous bone, considering that it does not induce foreign or immunological reactions, and does not transmit viral infections, but repair or reconstruction of skeletal defects can be carried out by removed from the iliac crest by surgery. To avoid this disadvantage autologous bone can be replaced by synthetic biomaterials [3]. An interesting research field with medical applications is represented today by *ceramics*, as they can be used to obtain useful biomaterials for the production of implants [4-6]. *Calcium phosphates* are known able to promote new bone formation and have been already used for the repair of periodontal defects, orthopaedic and maxillofacial applications [7-10]. β-tricalcium phosphate (β-TCP) is a synthetic calcium phosphate ceramic used as an alternative autologous bone graft, has good biodegradability and osteoconductivity with large applications bone tissue engineering. Beta-tricalcium phosphate (β-TCP) has the chemical formula of $Ca_3(PO_4)_2$ and the ratio of calcium to phosphate is about 1.5, which is one of the most popular bioresorbable materials for bone implants. β-TCP promotes a faster osseointegration of the calcium phosphate biomaterials and exhibits biodegrability, biocompatibility, and osteo-conductivity [11]. Moreover, TCP is degraded 10-20 times faster than HA [12]. *Substitutions* of ions such as Na^+, Zn^{2+}, Mg^{2+},Fe^{3+}, Cu^{2+}, Si^{2+}, K^+, CO^{3-}, F^-, Cl^- plays an important role in the overall performance of the bone. In this respect it is recommended that these ions be incorporated in implant because biocompatibility is closely correlated to their composition. For calcium phosphates bioresorbable characteristics can be modified by adding them in composition.

Experimental results have shown that of all these ions, and specially Mg^{2+} and Zn^{2+} has a

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significant influence in controlling degradability and in interactions with the implant [13]. Substitutions can produce changes in properties such as crystallinity, solubility and finally to the biological response. Ceramic parts fabrication is a demanding task requiring number of carefully controlled processing steps. The simplest way to obtain small particles for medical applications is to crush the bulk materials and then fractionated according to size. Great attention is focused on the use of microspheres as carriers for proteins and drugs and for bone healing/reconstruction. In a ceramic-based bone graft material, the shape of the particles has a direct influence on bone formation [14]. Several forms of particulate products are used as bone fillers including irregular multifaceted particles and rounded smooth granules with solid or porous structures. Ideal bioceramic microspheres for bone repair/healing or regeneration needs to be bioactive and biodegradable. Granules can be fabricating using a variety of technologies such as crushing of bulk material follow by pelletizing, spray drying, liquid quenching, or hydrothermal synthesis that yields granules of irregular or near-spherical shape [15-19]. In this study, we consider an alternative technology for preparation of porous tri-calcium phosphate granules by which spheroidizing of granules is carried out in immiscible liquid, under the action of surface tension forces. The formation of calcium phosphate-alginate beads by ionotropic gelation was achieved by dropping the calcium phosphate-alginate dispersion into a $CaCl_2$ bath, as cross-linking agent [20]. The ionotropic gelation technique was selected to prepare β-TCP microbeads due to its simplicity and low cost. Alginate are established among the most versatile biopolymers, used in a wide range of applications, is a nontoxic, biodegradable, naturally occurring polysaccharide obtained from marine brown algae and contains β-D-mannopyranosil uronate and α-L-gulopyranosyl uronate in regular (1-4)-linked sequences. Alginates can be ionically cross-linked, at low concentrations, by the addition of divalent cations (e.g., Ca^{2+}, Sr^{2+}, and Ba^{2+}) in aqueous solution through the ionic interaction between the cation and the carboxyl functional group [21, 22]. *Open porosity* within the microsphere can be made by adding a pore former to the material. Using this method, porous granules of spherical shape are obtained in which open pores is produced by burn out of the polymeric component. The size of spherical particles could be controlled by the control of alginate/ β-TCP ratio, distance of the dropping, by stirring rate and in general, to the rheological characteristics of the slurry [23]. The size of particles decreased with increasing stirring rate. An important step in such a formulation is the drying of calcium phosphate-alginate beads. *Drying technique* could influence several bead characteristics such as size, shape, and mechanical properties. Drying is an important factor in determining the degree to which water is removed from the ceramic matrix (microspheres). The rate (speed) is defined as "degree of extraction of water" and that will diminish as the process progresses. It will be assumed that the loss of mass of drying beads is governed by the diffusion of saturated water vapor at the surface of the bead into the surrounding air, at ambient relative humidity. As such, the water loss is given by the Fickian laws of diffusion assuming spherical symmetry [24]. Control this process allows the production of beads with desired shape and morphology, and very importantly, without contraction cracks over the entire process. Microspheres can be loaded with different agents (proteins as for instance growth factors, enzymes, antibiotics) in order to stimulate the regeneration of bone tissue or even be seeded with cells prior to implantation [25]. To be used in the medical field ceramic granules must be measured and evaluated in terms of size and size distribution, shape and surface roughness, surface area, porosity, density, brittleness and flow characteristics.

Present paper describes the preparation and characterization of microspheres intended to be used as a biomaterial for bone regeneration or as drug-delivery matrices. The method to produce porous granules is based on liquid immiscibility effect, by the ionotropic gelation technique, using $CaCl_2$ as cross-linking agent. The scope was to investigate the influence of some processing parameters on size, shape and morphology of calcium phosphate-alginate beads. The effects of slurry concentration, and porosifier content on the density, porosity and open pore content of the porous β-TCP microspheres were investigated. The small particle size and interconnected microporosity are believed to improve osteoconductive properties and promote timely resorption concomitant with the process of remodeling.

2. Materials and experimental procedures

2.1. Ceramic powders for β-TCP *porous microspheres preparation*

Ceramic powders**,** produced in our laboratory, were prepared via solid-state reaction using a methodology previously described [26, 27]. Briefly, β-TCP powders, with ionic substitution (0.5%mol. Mg^{2+} and 0.5%mol. Zn^{2+}) were prepared by sintering the stoichiometric mixture of $NH_4H_2PO_4$ and $CaCO_3$ (2:3 molar ratio), with magnesium oxide and zinc oxide (Fluka) as Mg and Zn sources, at 925° and 1100ºC. A heating rate of 5ºC·min^{-1} and a cooling rate of 10ºC·min^{-1} were used.

The two sort of the β-TCP powders, coded as ‘‘B1’’ (β-TCP powder calcined at ~ 925ºC) and

''B2'' (β-TCP powder calcined and sintered at ~ 1100ºC) were used to obtain ceramic microsphere.

2.2. Slip preparation

Slips were prepared at a solid loading of ~ 60 wt %. Deionized water was used as suspending medium for the powders. In order to enhance slip stability specific materials were used: 1.5 wt% commercial organic, polyacrylic acid sodium salt (Fluka) as deflocculant and 1% carboxymethyl Cellulose Sodium (Fluka) as binder. The homogenization time was of 3 hours, under ultrasonic agitation.

2.3. Preparation of β-TCP porous microspheres

The slips prepared before were homogenized with a biopolymer solution (sodium alginate solution), using a magnetic agitator, at room temperature. One of the most important and useful properties of alginates is the ability to form gels in the presence of divalent cations, especially calcium, resulting in formation of three-dimensional network which is usually described by 'egg-box' model.

The sodium alginate water solution (1 - 3% wt.) was prepared by dispersing the sodium alginate in de-ionised water under continuous stirring for 1 hour. The dispersion was sonicated for 30 min to remove any air bubbles that may have been formed during stirring process.

The resulted homogeneous dispersions were extruded into 500ml of calcium chloride (1.5% wt.) solution through hypodermic syringe with needle tip (20G) and stirred at 200rpm using magnetic stirrer. A gelation time of 30 min. was allowed to complete the curing reaction and produce spherical and rigid microbeads. The beads were collected by decantation, washed with distilled water and dried by water extraction with alcohol.

For experiments were made different compositions with powder ceramic/biopolymer solution ratio ranging in the 0.1 - 0.5 domain. Were developed suspensions with different rheology with an increasing ratio between the amount of ceramic powder and aqueous solution of sodium alginate, further defined as follows: B1-1, B1-2, B1-3 and respectively, B2-1, B2-2, B2-3 (the second numbers represents the mass ratio between ceramic powder and sodium alginate solution).

The microspheres produced were measured in terms of average diameter at different times of drying between 4h and 72h, and finally after sintering.

An important step is the drying of calcium phosphate-alginate beads. During drying, the beads shrank significantly and their shape changed. Thus, it was propose a simple model and equally economically (with minimum energy consumption) [28] for the water extraction (drying) using progressive dehydration in a series of graded ethanol solutions.

Open porosity within the microsphere was induced by sintering at a temperature less than that required to fully densify the material. For one type of suspension (B2), 10% commercial microcristalline cellulose (Merck), was used as porosifier, to emphasize the influence of adding a pore generator to the material open porosity. The obtained spherical particles were further heated at 1000 to 1150°C.

3. Microsferes characterization

3.1. Phase composition of the powders and microsferes

The calcined β-TCP powders (B1 - β-TCP powder calcined at ~ 925ºC and B2 - β-TCP powder calcined and sintered at ~ 1100ºC) was characterized by X-ray powder diffraction (XRD) method, using CuKα radiation, type Bruker-AXS, D8 ADVANCE high-resolution diffractometer, over the 2θ range of 20 to 60º. The identification was carried out by comparing the peak positions and intensities with those in the joint Committee on powder diffraction Standards (JCPDS, 09-0169) data files.

3.2. Rheological characteristics of slips

were determined by measuring their viscosities and flow behavior by using a rotating-spindle Brookfield Viscometer (Brookfield DV-II +Pro (Brookfield Engineering Laboratories Inc., Middleboro, USA). The viscosity can be measured based on statically and dynamic methods, working at room temperature or at higher temperatures, up to 1000ºC. The small sample adapter of the viscometer permitted to determine viscosity in small volume (2 ml) slip aliquots. The flow properties of slips were determined in accordance with the procedure specified in ASTM D 2196 test. The shear rates in these measurements were varied in the interval 2.5–100 rpm of the spindle rotation. The viscosity data were recorded at a constant shear rate of 18.6 s^{-1} corresponding to 20 rpm of spindle rotation

3.3. Characterization of porous beads

The *granules* thus prepared were measured for linear shrinkage and open-pore size distribution. The morphology and microscopic structure of granules were studied by scanning electron microscopy. Different physical properties – such as porosity and density-were measured

Linear shrinkage of the β-TCP beads was determined by measuring the microspheres dimensions, using Digimatic callipers after drying and sintering, respectively.

For *drying* experiments, it was used an extraction algorithm starting from 50% alcohol solution to pure alcohol (ethyl alcohol-96% pa) and the weight difference was measured at pre-determined intervals (between 4h and 72h).

The open-pore porosity (P_a) of the microspheres was tested by Archimedes' principle, using SORTORIUS density measurement kit. The measurements were carried out in distilled water. These were then compared with the theoretical densities (density of 3.07 g/cm^3 for β-TCP).

All the microspheres were evaluated with respect to their size and shape using optical microscope. The particle diameters of more than 20 microspheres were measured randomly by optical microscope. Morpho-structural analysis by optical microscopy was performed by visualization with Carl Zeiss Jena microscope NU2 type, with DinoLite digital attached camera and image acquisition software. Photographs of samples were made in direct reflected light to increase the magnification to 300X. The morphological properties of beads surface and the internal structure characteristics of beads (e.g. porosity, pore size, surface area) were evaluated using scanning electron microscope scopy (SEM), using scanning electron microscope type FESEM-FIB Auriga model produced by Carl Zeiss Germany.

4. Results and discussions

4.1. β-TCP powder characterization

The XRD pattern shows that the calcined powders (B1 - β-TCP powder calcined at 925ºC and B2 - β-TCP powder calcined at 1100ºC) contain only beta-tricalcium phosphate peaks. In Figure 1, the X ray diffraction pattern for β-TCP powder calcined at 1100°C (B2), is presented. Same diffraction effects are present for β-TCP powder calcined at 925°C.

The main features of the two types of ceramic powders, average particle size and surface area (determined by BET method) are presented in Table 1. Increasing the sintering temperature increases the average size of particles and therefore a reduction in surface area from 18.34 m^2/g to 5.85 m^2/g.

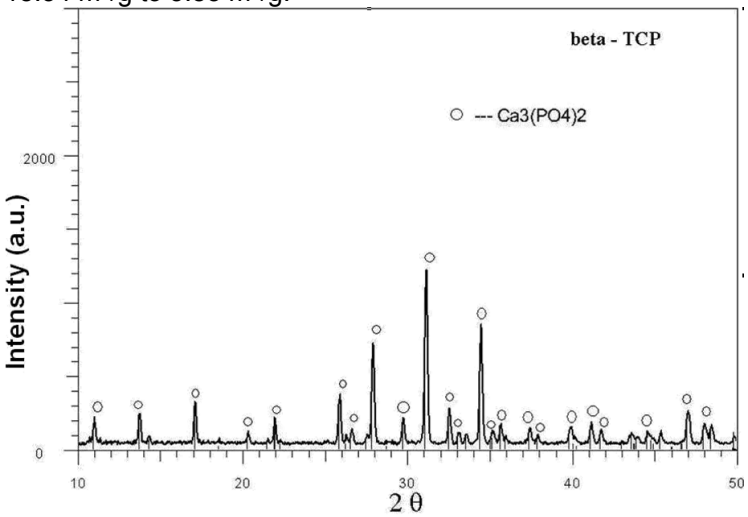

Fig.1.- XRD spectra for powder B2 of β-TCP compared with the JCPDS card 09-0169.
Difractograma pulberii B2 de β-TCP raportata la fişele JCPDS, card 09-0169.

4.2. Viscosity of the β-TCP slips

Apparent viscosities of β-TCP slips, containing 60% solid and 1% deflocculant are displayed in Figure 2. The viscosity data were recorded at a constant shear rate of 18.6 s^{-1} corresponding to 20 rpm of spindle rotation.

The viscosity curve revealed that slip could attain sufficient fluidity (the frictional forces decreases due to the presence of deflocculant) at a velocity gradient below 10 s^{-1}.

The viscosity of the ceramic suspensions is strongly dependent on the powder characteristics, such as the specific surface area, the particle shape and the extent of agglomeration. Therefore, the slips obtained from powder B2 (with low specific surface) present higher viscosity values due to the presence of larger grains and hard agglomerates.

4.3. β-TCP-alginate porous microspheres

Spherical shaped microparticles presenting a uniform size were prepared by ionotropic gelation method, and are presented in Figure 3a. When the alginate drops were in contact with calcium ions, almost instantly calcium alginate formed and it maintained the shape even at low concentration of the electrolyte solution. Its shape is spherical but sometimes skewed at the low concentration of sodium alginate, less than 1.75% wt., Figure 3b. Increasing the concentration of $CaCl_2$ to 3% wt. should increase the porosity of beads, while using 0.5% wt. of $CaCl_2$ gives weak gel due to insufficient cross-linking of alginate. For this process is proposed a theoretical model. The model proposed by German scientist Heinrich Thiele [29] consists essentially in a process of phase separation; the ionotropic gelation process being accompanied by a phenomenon of dehydration, according to equation below:

$$\text{Alginate}_{(sol.)} + \text{Electrolyte (A)} \leftrightarrow$$
$$\leftrightarrow \text{Gel} + \text{Electrolyte (B)} + H_2O$$

Table 1

Characteristics of β-TCP prepared ceramic powders
Caracteristicile pulberilor ceramice de β-TCP preparate

Sample/*Proba*	d_{50}[μm]	S_{sp} [m^2/g]
β-TCP-**B1**	1.27	18.34
β-TCP-**B2**	3.64	5.85

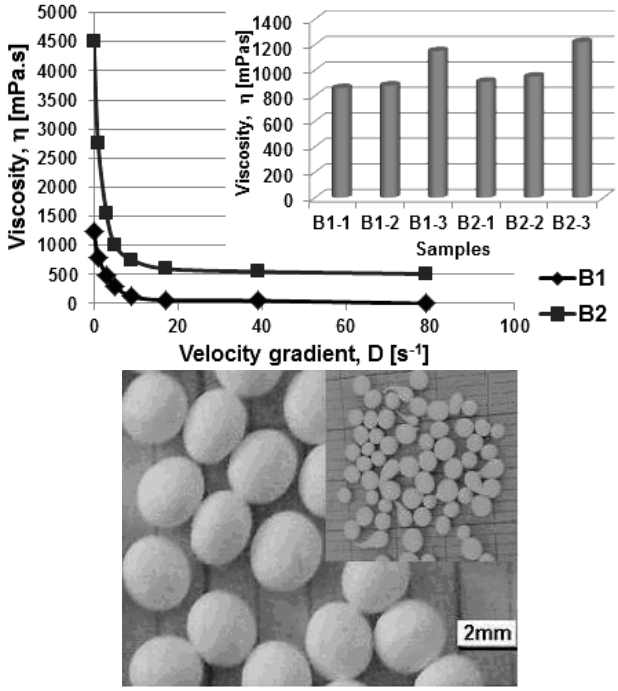

Fig. 3 - Spherical shaped microparticles prepared by ionotropic gelation method using $CaCl_2$ as cross-linking agent *Microparticule sferice obţinute prin metoda de gelifiere ionotropică, utilizând soluţie de $CaCl_2$ ca agent de întărire.*

The water formed during the process will be "trapped" in the interface between the solution of alginate and gel formed; the water elimination, according to the model proposed, will be made by penetration of Ca^{2+} ions from the electrolyte (solution of $CaCl_2$) through the newly gel membrane formed. The specimens were dehydrated through a series of aqueous solutions containing ethanol with increasing concentration up to ~ 100 %.

For experiments using a mining algorithm from 50% alcohol solution or pure alcohol (ethyl alcohol-96%p.a), the weight difference was measured at predetermined intervals (4h, 16h, 48h and 72h)**.** Main results are presented in Figure 4. It is obviously that the drying process dynamic is higher for samples obtained with finer β-TCP powders, the water elimination being easier.

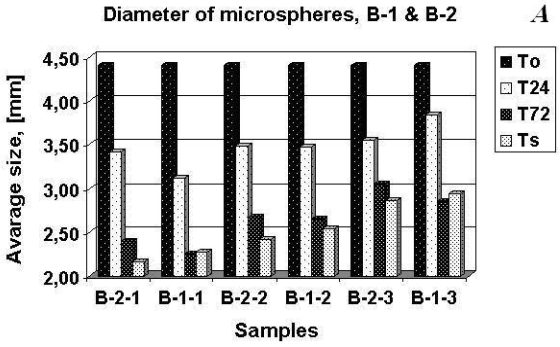

Fig.2 - Viscosity curves for the two ceramic powdes used (B1 - calcined at 925ºC and B2 - calcined at 1100ºC) (the second numbers on the sample codes represents the mass ratio between ceramic powder and sodium alginate solution) / *Curbele de viscozitate pentru cele două pulberi ceramice utilizate (B1 – calcinată la 925°C şi B2 – calcinată la 1100°C) (cel de-al doilea număr de la codul probelor reprezintă raportul gravimetric între pulberea ceramică şi soluţia de alginat de sodiu).*

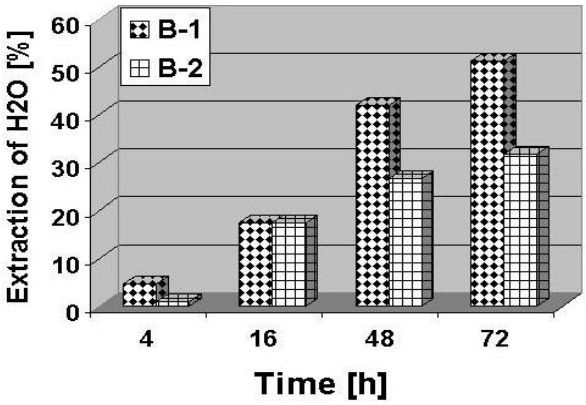

Fig. 4 - Drying process dynamic by means of water extraction in the time interval 4-72 hours, for microspheres from B1 & B2 powders / *Dinamica procesului de uscare cu extracţie de apă în intervalul de timp 4-72 ore, pentru microsferele din pulberile B1 şi B2.*

The presence of deformed beads is due to the ratio of precursor concentration to $CaCl_2$ concentration, which is increased above certain limits. This is most likely due to the fact that calcium is consumed by alginate gelation, thus the gel formation is not rapid enough to produce a stable network. The ionotropic gelation takes place within a small range of alginate concentration. The upper limit is supposed to be at 4wt. % alginate solution. The diameters of the microspheres obtained (before and after drying and sintering) using the 3%wt. polymer solution and the different ceramic-to-polymer ratios are presented in Figure 5.

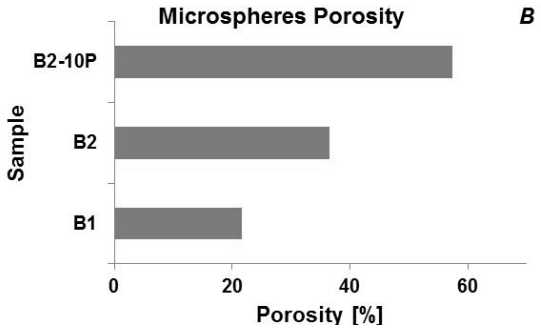

Fig. 5. - **A**. Diameters of the microspheres prepared using the 3 %wt. alginate solution, at wet state and, after drying and sintering stage / *Diametrul microsferelor tip B1 şi B2 obţinute din soluţie de alginat 3%, după elaborare, uscare şi sinterizare.* / **B**. Microspheres porosity with and without 10% wt. porogen addition / *Porozitatea microsferelor cu şi fără adaos de 10%gr. porogen.*

Upon drying, microspheres have undergone a volume contraction, which was more significant for the lower ceramic-to-polymer solution mass ratio (0.2). The bulk density of the dried body is about 32% of the theoretical density, which is prove that the microspheres are porous. By adding 10% of porogen agent, open porosity increase with more than 50%.

4.4. Morpho-structural analysis

The spherical geometry of granules, analysis by optical microscopy is reflected in Figure 6. Diameter of the bead was about 2500 μm, and micropores were observed on the surface. The analysis revealed that microspheres were homogeneous, in terms of size and shape. No evidence of cracks was found.

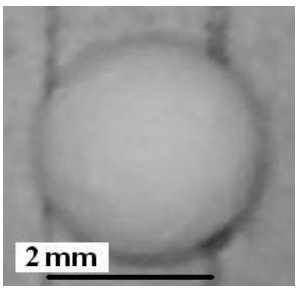

Fig. 6. - Microscopy for ceramic microspheres based on β-TCP, obtained by ionotropic gelification technique / *Microscopie optică pentru microsferele ceramice pe bază de β-TCP obținute prin gelifiere ionotropică.*

The morphology and the microstructure of the two types of beads are shown in Figures 7a-b. The surfaces of the B2 microspheres were coarser than the surface of the B1 microspheres. SEM images shows that B1 granules are constituted of agglomerated elementary particles due to diffusion bonding particles; powders exhibit grains with a significant size which are strongly bonded ones with the others. For B2 granules pores are preferentially located on the grain boundaries. The burning out of biopolymers is accompanied by the formation of fine pores (intra-pores) in granules, whereas the formation of inter-granular inter - connected pores (inter-pores) is determined by the packing of granules during the sintering stage. The proportion and particle size of the pore-forming agent influences the porosity and the pore structure.

It was demonstrated that the porosity of the obtained granules depended mainly on the amount of the added pore-creating medium and the temperature of sintering. The high porosity favours the ectopic formation of bone tissue due to the increased specific surface and higher solubility.

5. Conclusions

The microspheres were prepared by the ionotropic gelation technique using $CaCl_2$ as cross-linking agent. The size of spherical particles of β-TCP with alginate was depended on the stirring rate of cross-linking solution and the viscosity of the slurry. Dispersed suspensions with high content of β-TCP powder (up to 60 wt. %) and low viscosity were obtained. Surface area, porosity and phase composition of the β-TCP powder affected significantly the viscosity of slurries. The slurries with the highest concentration of solids were obtained using β-TCP calcined at 1100ºC. Despite rapid interaction between Ca^{2+} and alginate, a certain time period is required for adequate cross-linking of the polymer matrix to confer sufficient mechanical strength to the microspheres for ease of collection and handling. Cross-linking of β-TCP/alginate microspheres for at least 15 minutes was necessary for the formation of sufficiently strong microspheres. It was observed that reducing the needle diameter could lead to smaller beads. However, the shape of wet beads was influenced by the distance between the top of a needle, through which the dispersion was dropped, and hardening solution as well as the stirring speed of the hardening solution. Drying technique could influence several bead characteristics such as size, shape, and mechanical properties. Compared to other methods of preparation of ceramic-polymer microspheres, this process presents the advantage of being simple and of being carried out

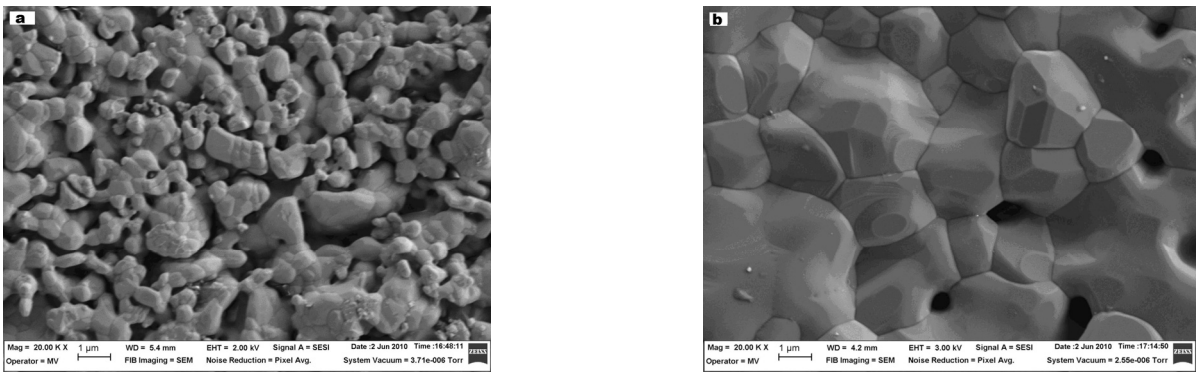

Fig. 7 - SEM photographs for a) ceramic microsphere obtained from B1 powder and b) ceramic microsphere obtained from B2 powder / *Microscopie electronică (SEM) pentru: a) microsfere ceramice obținute din pulberea B1 şi b) microsfere ceramice obținute din B2.*

at room temperature and in the absence of organic solvents. We can conclude that calcium phosphate porous microspheres prepared by ionotropic gelation technique are effective for development of drug delivery matrices or as fillers in bone surgery.

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