

**BRNO UNIVERSITY OF TECHNOLOGY  
FACULTY OF CHEMISTRY**

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**THE SYNTHESIS AND CHARACTERISATION OF  
COMPOSITE MATERIALS FOR POTENTIAL APPLICATION  
IN MEDICINE**

SYNTÉZA A VLASTNOSTI BIOKOMPOZITNÍCH MATERIÁLŮ S POTENCIÁLNÍM VYUŽITÍM V MEDICÍNĚ

**SYLLABUS OF DOCTORAL THESIS**

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**KLÍČOVÁ SLOVA:**

biokompozity, polyvinylalkohol, hydroxyapatit, mikrostruktura, SBF bioaktivita

**KEYWORDS:**

biocomposites, polyvinyl alcohol, hydroxyapatite, microstructure, SBF bioactivity

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## ABSTRACT

The work is focused on the synthesis and the study of biocomposite materials for potential medical application. The theoretical part is a literature overview focused on different types of biomaterials especially on polyvinyl alcohol - hydroxyapatite (PVA/HA) composites.

A set of polyvinyl alcohol (PVA) membranes with various weight percent - 0%, 10%, 20%, 30%, 40% and 50% of hydroxyapatite (HA) were prepared. Hydroxyapatite was prepared by precipitation procedure using starting materials of diammonium hydrogen phosphate and calcium nitrate tetrahydrate in water alkaline environment and then mixed with solution of polyvinyl alcohol, which was prepared by dissolving it in water at 85°C. The different mixtures were casted in a mould and evaporated for 7 days at temperature 30 °C to obtain 0,5 mm thin membranes. The ATR-FTIR spectroscopy was used to identify the different functional groups in composite membranes, XRD testing was carried out to identify crystallized hydroxyapatite. The tensile testing and TGA measurement were realised to find the effect of HA amount on the mechanical properties and thermal stability of the membranes. The *in vitro* bioactivity tests in Simulated Blood Fluid (SBF) were performed for 2 hours, 7 and 28 days. SEM was used to characterise surface microstructure of biocomposite membranes before and after immersion in SBF. The surface of the tested membranes were analysed to investigate the formation of apatite, the characteristic of bioactivity. It was observed a formation of clusters within membranes with increasing amount of HA particles due to hydrogen bond and also the agglomeration and crystal growth of HA particles during drying of membranes. The bioactivity was found increasing with time immersion of biocomposite materials.

# CONTENTS

<b>1</b>	<b>INTRODUCTION</b>	<b>5</b>
<b>2</b>	<b>THEORETICAL PART</b>	<b>7</b>
2.1	Hydroxyapatite	7
2.2	Poly(vinyl alcohol)	7
2.2.1	Poly(vinyl alcohol) in medical application	8
2.3	Poly(vinyl alcohol) – Hydroxyapatite composite	8
2.3.1	PVA/HA composite application	8
<b>3</b>	<b>EXPERIMENTAL PART</b>	<b>10</b>
3.1	Samples preparation	10
3.1.1	Chemicals used	10
3.1.2	Preparation of hydroxyapatite	10
3.1.3	Preparation of poly(vinyl alcohol)	10
3.1.4	Composite membranes preparation	11
3.1.5	Simulated body fluid preparation	11
3.2	Testing methods	12
3.2.1	FTIR-ATR	12
3.2.2	Morphological characterization with scanning electron microscopy	12
3.2.3	XRD diffraction analysis	12
3.2.4	Tensile properties	12
3.2.5	Thermogravimetric Analysis (TGA)	12
3.2.6	Bioactivity testing	13
<b>4</b>	<b>RESULTS AND DISCUSSION</b>	<b>14</b>
4.1	FTIR-ATR	14
4.2	Morphological characterisation with SEM	15
4.3	Tensile properties	19
4.4	Thermogravimetric analysis	22
4.4.1	PVA thermogravimetry analysis	22
4.4.2	Hydroxyapatite thermogravimetry analysis	23
4.4.3	PVA/HA composite thermogravimetry analysis	24
4.5	Bioactivity testing	26
<b>5</b>	<b>CONCLUSION</b>	<b>31</b>
<b>6</b>	<b>REFERENCES</b>	<b>33</b>
<b>7</b>	<b>LIST OF SYMBOLS</b>	<b>36</b>
<b>8</b>	<b>LIST OF PUBLICATIONS</b>	<b>37</b>

# 1 INTRODUCTION

Biological materials are used as replacement, increasingly non-biological materials as metals, metal alloys, ceramics, polymers and composites are applied. The required feature of materials used as implants is non-toxicity for living cells, non-inflammatory, non-teratogenicity, non-mutagenicity and non-cancerous. In the present times ceramic surface bioactive materials are used as they successfully imitate the advantages of biological materials [1].

The definition of biomaterials is: "A biomaterial is a substance that has been engineered to take a form which, alone or as part of a complex system, is used to direct, by control of interactions with components of living systems, the course of any therapeutic or diagnostic procedure, in human or veterinary medicine"[2].

In any case, biomaterials are intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body and are now used in a number of different applications throughout the body [3]. The major difference between biomaterials and other classes of materials is the ability of biomaterials to remain in a biological environment without damaging the surroundings and without being damaged themselves in the process. Thus, biomaterials are solely associated with the health care domain and they must have an interface with tissues or tissue components. One should stress that any artificial materials that are simply in contact with skin, such as hearing aids and wearable artificial limbs, are not included in the definition of biomaterials since the skin acts as a protective barrier between the body and the external world. The biomaterials discipline is founded in the knowledge of the synergistic interaction of material science, biological science, chemical science, medical science and mechanical science and requires input and comprehension from all these areas so that implanted biomaterials perform adequately in a living body and interrupt normal body functions as little as possible [4].

Engineering materials could be classified as metals, polymers ceramics and composites. In the same way, biomaterials are divided into biometals, biopolymers, bioceramics and biocomposites [4, 5].

When a synthetic material is placed within the human body, tissue reacts towards the implant in a variety of ways depending on the material type. The mechanism of tissue interaction (if any) depends on the tissue response to the implant surface. In general, there are three terms in which a biomaterial may be described in or classified into representing the tissues responses. These are bioinert, bioresorbable and bioactive [6].

Nowadays, it is possible to manufacture implants for any part of our body, except for the brain. Obviously, different types of materials are in use depending on the tissue to be replaced. If we focus on functional artificial biomaterials, the choice has to be made among metals, polymers and ceramics. Each group exhibits some a priori advantages and drawbacks. Ceramics, for instance, are the most biocompatible materials and can be obtained with bioinert, bioactive or bioresorbable properties, but their main drawbacks are their hardness and fragility.

Metals exhibit problems of corrosion and toxicity, but their mechanical behaviour is optimum. Polymers offer many possibilities depending on their chemical composition and structure (biodegradability degree, hydrophilic/hydrophobic ratio, toughness/flexibility, etc.), but very few have shown good bioactive properties to ensure the implant osteo-integration.

It is quite usual to use the three types of materials in the same implant. This is the case of a total hip joint prosthesis which presents a metal beam, partially coated with a bioactive ceramic, while the head is made of an inert ceramic and the socket is made of polymer [7].

The biomaterials for the biomedical applications have evolved through three different generations, namely first generation (bioinert materials), second generation (bioactive and biodegradable materials) and third generation (materials designed to stimulate specific responses at the molecular level) [8].

## 2 THEORETICAL PART

### 2.1 Hydroxyapatite

Hydroxyapatite is described as one of the most bioactive ceramics widely used as powder or in particulate forms in various bone repairs and as coating for metallic prostheses to improve their biological properties. It has excellent biocompatibility, bioactivity and osteo-conduction properties. HA is thermodynamically the most stable calcium phosphate ceramic compound nearest to the pH, temperature and composition of the physiological fluid [9].

Contrarily HA is fragile and has low tensile strength, due to those it can not be use to replace bone in load bearing sites.

The bone consists of organic components (mostly collagen fibres) and inorganic mineralphase, known as biological hydroxyapatite which represents 65-70 wt% of natural bone [10]. Collagen fibres keep bones resilient, while mineral component is responsible for the bone stiffness. The most compatible with bone mineral phase is non-stoichiometric calcium phosphate of “apatiticstructure” It differs from stoichiometric hydroxyapatite of formula  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  with molar ratio of  $\text{Ca/P} = 1.67$  [11]. Bone contains carbonated HA crystals in the form of plates and needles with length about 40–60 nm, width 20 nm and thickness 1.5–5 nm [12].

Biological apatites are non-stoichiometric, mostly Ca-deficient with respect to phosphate ( $\text{Ca/P} < 1.67$ ) unless carbonate ion is incorporated, then  $\text{Ca/P}$  is higher than 1.67. The difference between stoichiometric hydroxyapatite and bone mineral is founded in the impurity content, which is associated mostly with ions substitution for calcium sites in the bone hydroxyapatite structure [11].

This ratio can be an important factor in cell adhesion, proliferation and in bone remodelling and formation. Calcium-deficient HA is of greater biological interest because the mineral portion of the hard tissue is primarily carbonate substituted calcium-deficient HA, with a  $\text{Ca/P}$  ratio of about 1.5, which is chemically and compositionally similar to tricalciumphosphate but structurally similar to stoichiometric hydroxyapatite.

HA is a crystal of hexagonal structure with the space group P63/m. The lower crystallographic symmetry induces mechanical anisotropy along each axis. The a, b plane is bio-active, the c plane is bioinert. That's why a specific crystal orientation is required HA should be used as a biomaterial [13].

### 2.2 Poly(vinyl alcohol)

Polyvinyl alcohol was discovered in 1915 by F. Klatte. The stoichiometric saponification of polyvinyl acetate with caustic soda to yield polyvinyl alcohol was first described in 1924 by Polyvinylalcohol

W. O. Herrmann and W. Haehnel. The monomer „vinyl alcohol“ is theoretically the enol form of acetaldehyde but can not exist as a monomer in practice. [14].

Polyvinylalcohol is a water soluble and biodegradable synthetic polymer. For food use it is an odourless and tasteless, translucent, white or cream coloured granular powder. PVA is slightly soluble in ethanol, but insoluble in other organic solvent [15].

PVA is an excellent adhesive with superior bonding strength, film forming and emulsifying properties. The film of PVA exhibits outstanding resistance to oil, grease and solvents. In

addition to its film forming property, it has excellent adhesion to both hydrophilic and hydrophobic materials. PVA has been extensively used in textile industry for warp sizing and resin finishing, in paper industry for surface sizing and pigment coating, in the production of PVAc emulsion as protective colloid, in the suspension polymerization of PVC as a dispersion agent; as binder for ceramics, magnet, foundry cores and pigments [16]. Polyvinyl alcohol is used in cosmetics as a binder, film former and viscosity-increasing agent. It is used at various concentrations up to 25% of the formulation in 1994 according to the FDA. PVA is also used by the plastic industry for various applications and in pharmaceutical products as a viscosity increasing agent as well as a lubricant in ophthalmic solutions. It is also used for several clinical applications and has been investigated as a potential carrier molecule for internal tumour investigations [17].

### **2.2.1 Poly(vinyl alcohol) in medical application**

Polyvinyl alcohol hydrogel has been recognized as a substitute material for the artificial articular cartilage due to its hydrophilicity and microporous structure, high elasticity, mechanical properties of absorption and exudation of body fluid. These properties of PVA hydrogel are closer to human body cartilage tissue than those of other artificial materials. On the other hand, PVA hydrogel presents poor strength and bioactivities. The shortcomings are overcome by adding hydroxyapatite as the reinforced phase to PVA composite hydrogel. PVA/HA composite hydrogel has been extensively studied as a cartilage substitute material [4, 18, 19].

## **2.3 Poly(vinyl alcohol) – Hydroxyapatite composite**

Biocomposite materials based on polymer matrix reinforced with (nano)hydroxyapatite (gel) have been developed as an alternative biomaterial to titanium alloy in the replacement of diseased or damaged cartilage [20 - 22].

The use of PVA became very promising in the last years for its excellent biocompatibility and biotribological properties [23 - 26]. Also, the characteristic that make it a potential biomaterial to replace the natural articular cartilage is its high porous structure with high content of free water, which is similar to that found in this soft tissue.

The clinical application of the pure polymer brings some problems. The first problem is the durability of PVA. Attempts to improve hydrogel properties for load-bearing biomedical applications have included the introduction of composite materials such as rubber or glass, the use of cross-linking agents, and the use of freeze thawing procedures to induce partial crystallinity. The other major problem in clinical application is the articular cartilage fixation method. Since PVA-H itself does not adhere to tissue, longterm fixation of PVA implant by sutures is difficult.

Therefore, a complex type artificial articular cartilage consists of PVA and a tissue-inducing material. Hydroxyapatite ceramic has already been used in the clinic for filling of bone defects due to its good biocompatibility and bioactivity. It can form bone-bonding with living tissue through osteoconduction mechanism [25].

### **2.3.1 PVA/HA composite application**

Poly vinyl alcohol- hydrogel (PVA-H) is widely used in biomedical fields, such as soft tissue replacement, drug delivery, and hemodialysis membranes for its excellent biocompatibility [27, 28]. The mechanical properties of PVA-H/HA composites have also been studied for use

as an artificial blood vessel the most important properties of biomaterials used for artificial joints [29].

Porous composites may be used as drug delivery system because of ability to take in the drug molecules [30, 31]. An artificial cornea consisted of a porous nano-HA/PVA hydrogel skirt and a transparent center of PVA hydrogel has been prepared as well. The results displayed a good biocompatibility and interlocking between artificial cornea and host tissues [32, 33].

### 3 EXPERIMENTAL PART

#### 3.1 Samples preparation

##### 3.1.1 Chemicals used

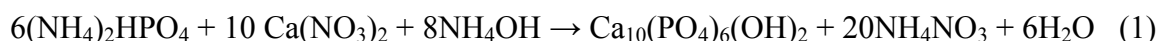
Polyvinyl alcohol Mowiol 10-98 with Mw 61 000 from Clariant  
Diammonium hydrogen phosphate  $(\text{NH}_4)_2\text{HPO}_4$  from Sigma-Aldrich  
Calcium nitrate tetrahydrate  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  from Sigma-Aldrich  
Ammonium hydroxide  $\text{NH}_4\text{OH}$  from Sigma-Aldrich  
Distilled water for all the procedures was used.  
SBF – Simulated Body Fluid

##### 3.1.2 Preparation of hydroxyapatite

In this work hydroxyapatite was prepared by the reaction using diammonium hydrogen phosphate and calcium nitrate tetrahydrate in distilled water in presence of additional  $\text{NH}_4\text{OH}$ , which acts to conduct reaction to form nanoparticles (Equation 1). The suspension obtained can not easily sediment and the white yoghurt form is like gel. The pH was kept at high value around 13 - 14, this was controlled by ammonium hydroxide. Formed HA solution was rinsed to separate  $\text{OH}^-$  ions until the pH of suspension was neutral. That was checked by simple pH paper indicator.

The weighed portion of the precursors was calculated to maintain required molar ratio Ca:P at 1, 67. It is necessary for biological response and producing a pure HA without minor fraction. Slight imbalances in the ratio of Ca/P can lead to the appearance of extraneous phases. If the Ca/P ratio is lower than 1.67, then alpha or beta tricalcium phosphate may be present after processing. If the Ca/P is higher than 1.67, calcium oxide (CaO) may be present with the HA phase [34].

Via this precipitation reaction hydroxyapatite with spherical particles was synthesised:



The sol-gel routine was the other way, which was HA prepared. The used solvent was ethanol. Diammonium hydrogen phosphate and calcium nitrate tetrahydrate were dissolved in ethanol, then mixed together. The temperature was kept at 80 °C and the pH was maintained at about 9 - 10 with ammonium hydroxide. The mixture was stirred for several hours until the high viscosity gel was obtained. This gel was dried and the newly formed HA was mixed with PVA solution. The mixture was found to be difficult to homogenise with PVA solution and the membranes created with this HA were not used for further testing, because the HA phase was separated during drying and it was not possible to disperse in the membrane body.

##### 3.1.3 Preparation of poly(vinyl alcohol)

Clariant poly(vinyl alcohol) was dissolved in distilled water during constant stirring at the temperature 85 °C for 4 hours until all the solid polymer was dissolved. The amount of polymer used was 21 g dissolved in 400 ml of distilled water.

### 3.1.4 Composite membranes preparation

Set of polyvinyl alcohol and hydroxyapatite composite membranes were made with various HA weight percent - 0%, 10%, 20%, 30%, 40% and 50%. Polyvinyl alcohol solution with temperature around 85 °C was stirred while adding calculated and weighed quantity of hydroxyapatite gel. This blend was mixed for 15 minutes to homogenise. Then 40 ml of the mixture was dosed into a mould with pipette. The mould has dimension 8 x 8 cm. This way prepared mixtures were dried at 30 °C for 7 days. After drying process, membranes of thickness near to 0,5 mm were removed and were ready for further testing.

**Table 1:** Designation of prepared composites

Composite components [in wt%]	Designation
0 HA + 100 PVA	0HA
10 HA + 90 PVA	10HA
20 HA + 80 PVA	20HA
30 HA + 70 PVA	30HA
40 HA + 60PVA	40HA
50 HA + 50 PVA	50HA
100HA + 0 PVA	100HA

### 3.1.5 Simulated body fluid preparation

In in vitro studies, the pH of the SBF solution is often buffered to 7.25-7.4 at 37°C using tris(hydroxymethyl) aminomethane and hydrochloric acid. SBF is a highly saturated solution, thus precipitation of calcium phosphate can easily take place during preparation, storage and use. Simulated body fluid is an acellular, aqueous medium that has inorganic ion concentrations similar to those of human extracellular fluid, in order to reproduce formation of apatite on bioactive materials *in vitro*. This fluid can be used for not only evaluation of bioactivity of artificial materials *in vitro*, but also coating of apatite on various materials under biomimetic conditions. The composition of prepared SBF was in mmol/l: 142.0 Na<sup>+</sup>, 5.0 K<sup>+</sup>, 1.5 Mg<sup>2+</sup>, 2.5 Ca<sup>2+</sup>, 148.8 Cl<sup>-</sup>, 0.5 SO<sub>4</sub><sup>2-</sup>, 1.0 HPO<sub>4</sub><sup>2-</sup> and 4.2 HCO<sub>3</sub><sup>-</sup>).

SBF was prepared by dissolving the components 8.035 g NaCl, 0.355 g NaHCO<sub>3</sub>, 0.225 g KCl, 0.231 g K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O, 0.311 g MgCl<sub>2</sub>·6H<sub>2</sub>O, 0.292 g CaCl<sub>2</sub>·6H<sub>2</sub>O, 0.072 g Na<sub>2</sub>SO<sub>4</sub> per litre of ultrapure water in a beaker according to the protocol given by Kokubo [35].

The medium was buffered to pH 7.3-7.4 at 36.5 ±0.5 °C with 6.118 g tris(hydroxymethyl) aminomethane ((HOCH<sub>2</sub>)<sub>3</sub>CNH<sub>2</sub>) and hydrochloric acid (HCl) to achieve the buffering capacity of blood. In preparation of SBF, each chemical was added after assuring the previous chemical dissolved fully to avoid any unexpected chemical reactions among them. Each small undesired variance in the preparation steps and the storage temperatures, may drastically affect the phase purity and high temperature stability of the produced HA on the surface, as well as the kinetics of the precipitation processes.

Sodium azide (NaN<sub>3</sub>) was added to inhibit bacterial growth. The microbiological assay of SBF medium indicated the necessity of NaN<sub>3</sub> addition to inhibit the growth of bacteria. Gil [36] stated that the use of SBF without of NaN<sub>3</sub> addition led to enormous increase of cells (10<sup>4</sup>-10<sup>5</sup> /ml) in the form of coliform bacterium, bacterium bacillus and Pseudomonas after cultivation in culture media at 37 °C. These anaerobic bacteria consume phosphorus and thus

reduce its concentration in the medium. Furthermore, it was observed that these bacteria may have a negative impact on human health.

## 3.2 Testing methods

### 3.2.1 FTIR-ATR

The functional groups in PVA, HA and composite samples were identified and the nature of bonding between the particles and the polymer matrix were analyzed by FTIR-ATR spectroscopy analysis. The used apparatus was Nicolet i50 FTIR spectrometer with single bounce diamond crystal ATR accessory within the scanning range 4 000–400  $\text{cm}^{-1}$ .

### 3.2.2 Morphological characterization with scanning electron microscopy

The surface morphology was examined through scanning electron microscope (JEOL JSM-7600F).

### 3.2.3 XRD diffraction analysis

For the XRD analysis was testing device Empyrean made by Panalytical used, measurement conditions were set as follows:

• Scan Axis	Gonio
• Start Position [ $^{\circ}$ 2Th.]	5.0066
• End Position [ $^{\circ}$ 2Th.]	89.9876
• Step Size [ $^{\circ}$ 2Th.]	0.0130
• Scan Step Time [s]	55.08
• Anode Material	Cu
• K-Alpha1 [ $\text{\AA}$ ]	1.54060
• K-Alpha2 [ $\text{\AA}$ ]	1.54443
• Generator Settings	30 mA, 40 kV

### 3.2.4 Tensile properties

In this study were tested 0HA, 10HA, 20HA, 30HA, 40HA and 50HA membranes.

Samples were cut out into shape of dog-bone according to ISO system standard ISO 527. The edges of the specimens were smooth, there was not requirement of any further treatment. Each sample used was previously inspected and those containing any defect such as air bubbles, holes. Width was constantly 4mm and thickness were gauged on 3 different points and averaged. The specimens were fixed into the grips of the testing machine. The distance between sample holders was 40 mm. Each specimen was tested for 5 times and finally characteristic curves were chosen.

For the investigation it was used universal materials testing machine ZWICK Z010/TH2A made by Zwick-Roell company (Germany). The tensile testing machine pulls the sample from both ends and measures the force required to pull the specimen apart and how is the strain before breaking. The speed of the measurements was constantly set at 20 mm/min.

### 3.2.5 Thermogravimetric Analysis (TGA)

Thermogravimetry analysis was carried out on composite samples and single components (PVA, HA) non-isothermally using a 20 $^{\circ}\text{C min}^{-1}$  heating rate up to 900 $^{\circ}\text{C}$ .

The TGA Q500 TA Instruments research-grade thermogravimetric analyzer was used. The measurement went under N<sub>2</sub> condition and results were analysed with Universal V4.7A TA Instruments analyzer.

The main goal of this measurement was to assess the effect of content of hydroxyapatite in PVA matrix on thermal stability. A derivative weight loss curve was used to determine the temperature at which weight loss was most apparent.

### **3.2.6 Bioactivity testing**

The bioactivity of the membranes with various content of HA in PVA matrix were tested with *in vitro* SBF method. The SBF was prepared in laboratory. Cleaned samples of size 1x1 cm were immersed and stored in the incubation apparatus (Binder BD 115) for 2 hours, 7 and 28 days at the temperature of 37.0 °C. The microstructure of membranes surface were tested before and after soaking in SBF by SEM. The samples were weighed before and after immersion to determine the extent of biodegradation or bioactivity.

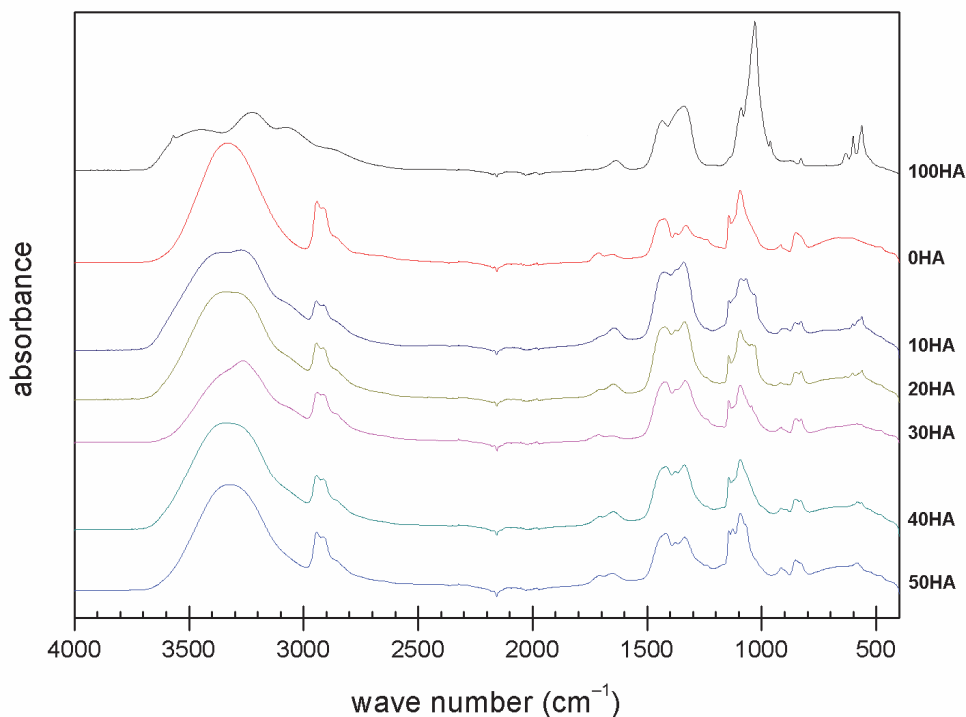
## 4 RESULTS AND DISCUSSION

### 4.1 FTIR-ATR

The FTIR spectra of different biocomposite membranes and the pure HA powder are depicted in Fig.1. The spectra are aligned from pure HA(100HA) powder, pure PVA (0HA) to 50HA composite.

The spectrum of the pure HA powder: Bands at 630 and 3570  $\text{cm}^{-1}$  indicate structural O-H groups in the HAp crystals. Bands located at about 1000 - 1100 and 560 - 570  $\text{cm}^{-1}$  are attributed to the  $\nu_3$  and  $\nu_4$  P-O vibration modes of regular tetrahedral  $\text{PO}_4^{3-}$  groups.

The observed bands at 604  $\text{cm}^{-1}$  correspond to O-P-O bending and  $\nu_1$  symmetric P stretching modes. The  $\nu_1$  symmetric stretching mode of phosphate group is observed at 957  $\text{cm}^{-1}$ . Band at 1641  $\text{cm}^{-1}$  indicates the presence of  $\text{H}_2\text{O}$  in HAp crystals [37].



**Figure 1:** Spectra of HA powder(100HA), pure PVA(0HA) and PVA/HA composites with various amount of hydroxyapatite (10HA, 20HA, 30HA, 40HA, 50HA)

The PVA spectrum indicates a wide and intense band due to the presence of hydroxyl groups (O-H) at 3441  $\text{cm}^{-1}$ . The bands corresponding to the (-CH<sub>2</sub>-) asymmetric and the symmetric stretching at around 2900  $\text{cm}^{-1}$ . The band at 1400  $\text{cm}^{-1}$  can be attributed to O-H and C-H bending. The absorption peaks at 1110  $\text{cm}^{-1}$  are related to C-O stretching.

The band at 900  $\text{cm}^{-1}$  results from an angular deformation outside the plan of O-H bond. The absorption bands at 1625  $\text{cm}^{-1}$  are due to symmetric stretching of carboxylate anion (-COO-) [38].

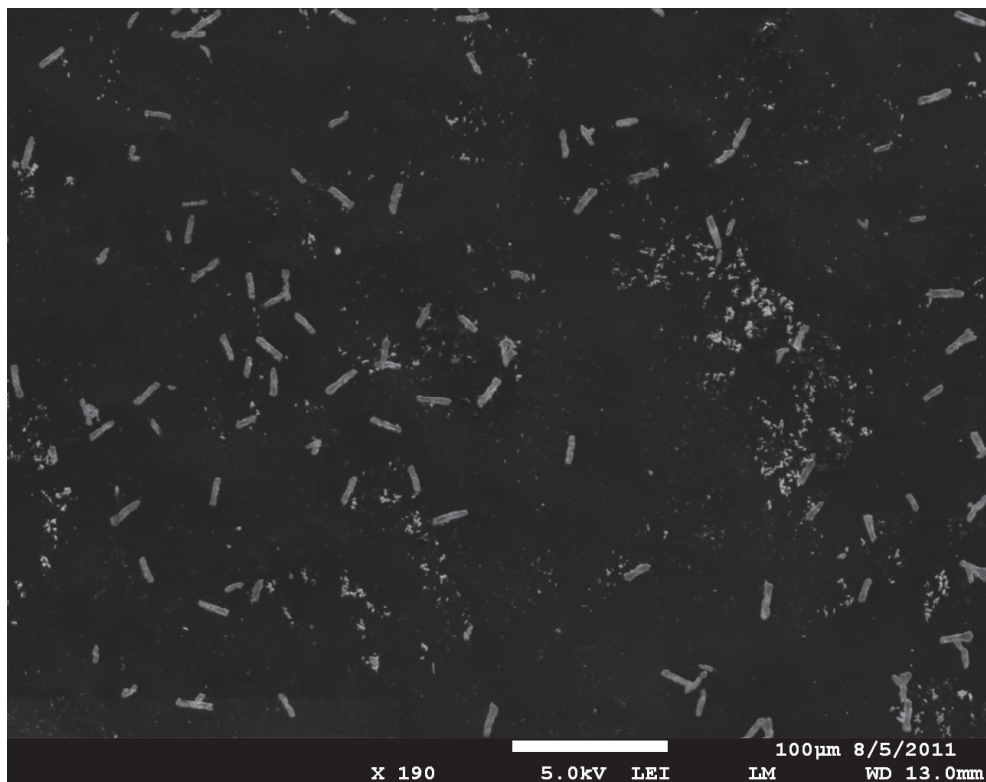
The PVA spectrum and composites spectrum show an increasing intensity of absorbance varying with the amount of HA in the samples in the adsorption range from  $3000\text{ cm}^{-1}$  to  $3500\text{ cm}^{-1}$ . Broad and strong band at  $3360\text{ cm}^{-1}$  which characterizes O-H stretching frequency is well detected due the increasing presence of HA in composite materials.

The intensity of spectrum at around  $1330\text{ cm}^{-1}$  increases with increasing content of HA in samples and is assigned to the phosphate group  $\text{PO}_4^{3-}$ .

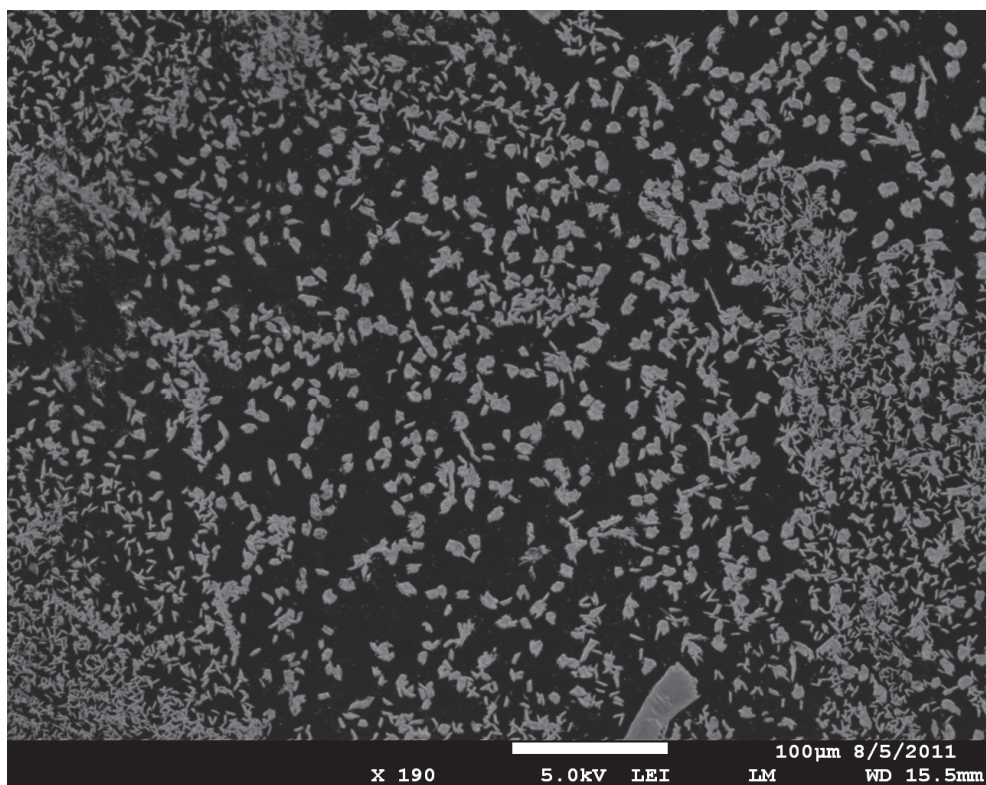
There were detected changes in spektra after composite formation, the variation and shift in the band around  $3300 - 3400\text{ cm}^{-1}$ . The band observed between  $2942-2944\text{ cm}^{-1}$  corresponds to C-H stretching band of PVA. A new peak of C-H stretching band is observed at  $2944\text{ cm}^{-1}$ , when the PVA is added. These changes mark the chemical bond interactions between HA and PVA in the composite.

## 4.2 Morphological characterisation with SEM

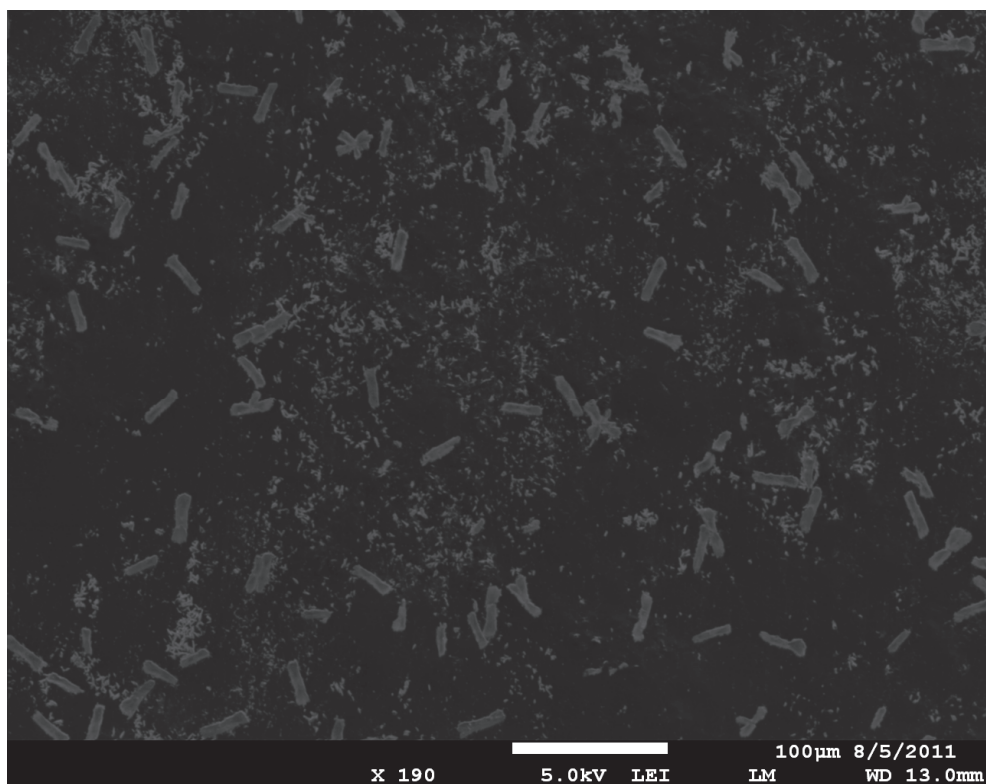
The microstructure of PVA/HA composites with different weight percentages of HA in polyvinyl alcohol matrix, pure HA and pure PVA was analysed using scanning electron microscope (SEM). The SEM pictures are displayed in Fig. 2 - 6. The PVA matrix is continuous without bubbles. Hydroxapatite in composite is present in rod-like particles, which create various structures - agglomerates. All the results show emerging of HA agglomerates which in the case of 40HA and 50HA hydroxyapatite crystals grow into surfaces and cause the roughness of the samples. The pure PVA membrane was clear (not present). The 10HA contains small and rare occuring plates-like agglomerates, which are consist of HA needles. A few solitary HA needles can be found too. The 20HA contains differently shaped agglomerates from 10HA. Short agglomerated clusters of HA needles were found in 20HA samples as well as plenty of HA needles distributed itself in the PVA matrix. The 30HA contains plenty of very similar structures as the 10HA, but in multiple amount. The long shaped clusters were observed and alone HA needles as well. The 40HA specimen shows that the matrix is saturated with the HA filler. The HA is dispersed all over the testing area and obviously the HA grows into the surface of the membrane and causes its roughness. The 50HA specimen was difficult to observe, because electron beam did interact with the sample during scanning, that's why the surface was gilded. The 50HA shows huge agglomerates, which grow through the surface and made the specimen very rough. The detailed view of HA spherical particles used to form the PVA/HA composites is displayed in Fig. 7. The particles were of micro and nano size.



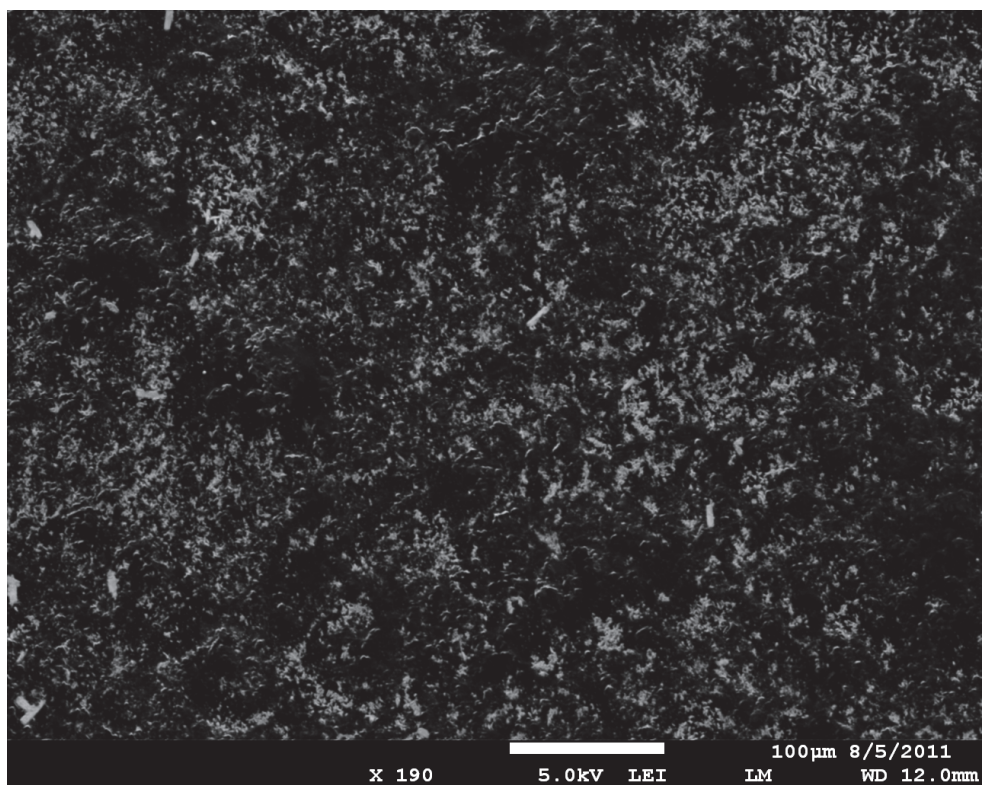
**Figure 2:** SEM image of 10HA at 150x magnification



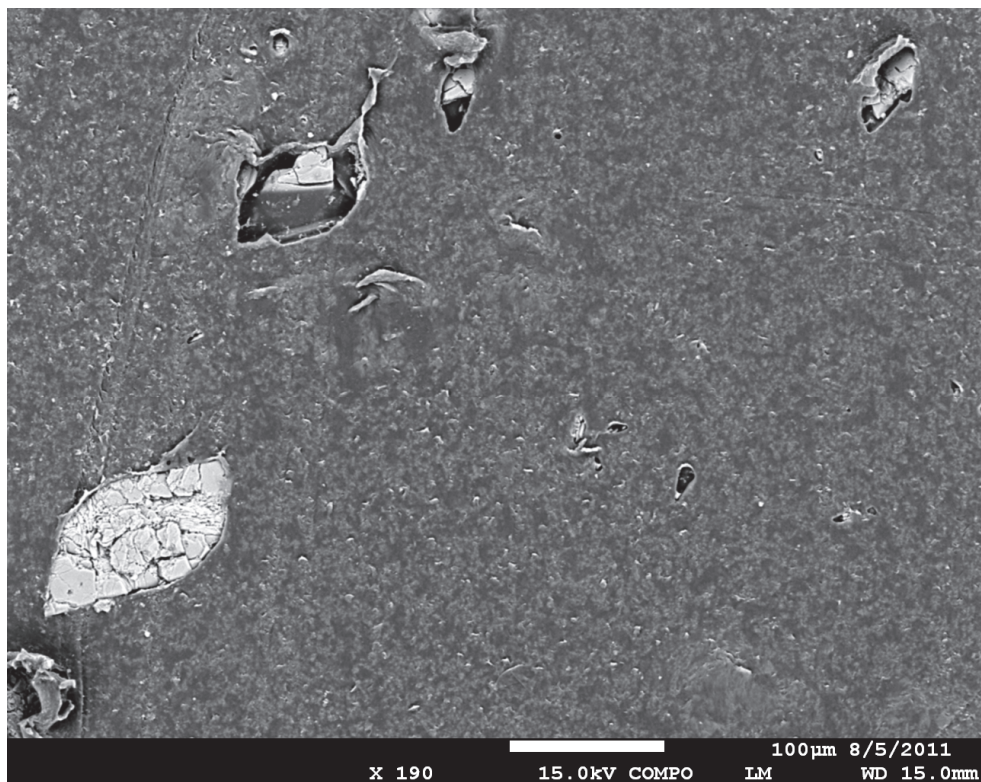
**Figure 3:** SEM image of 20HA at 150x magnification



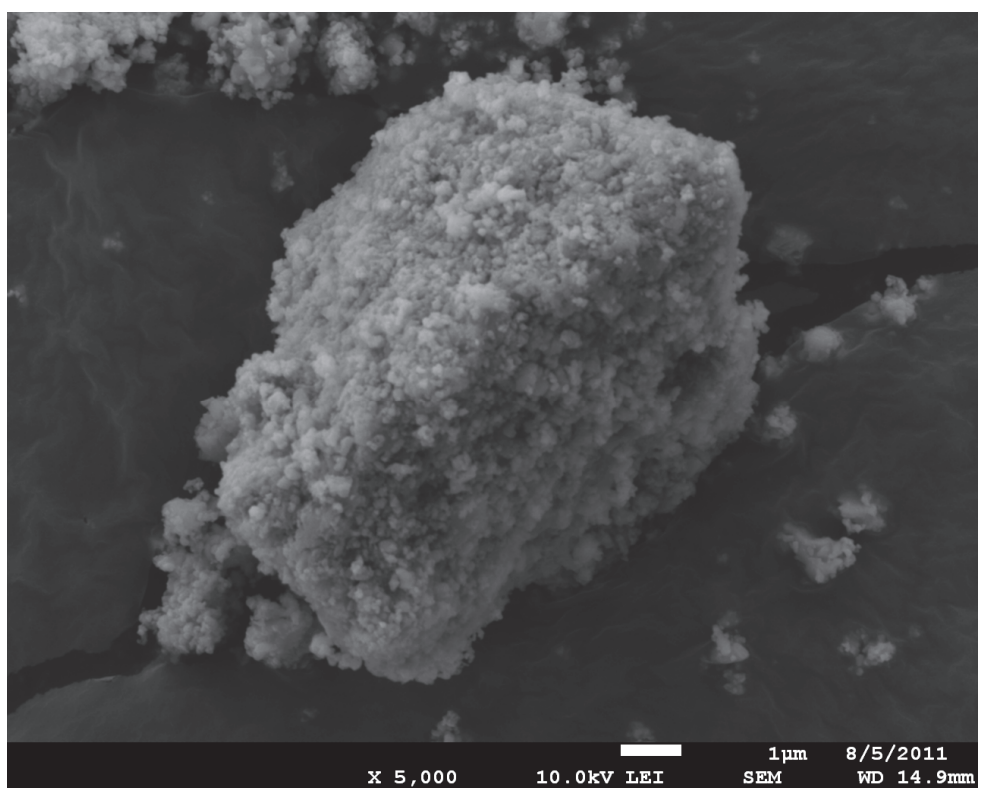
**Figure 4:** SEM image of 30HA at 150x magnification



**Figure 5:** SEM image of 40HA at 150x magnification



**Figure 6:** SEM image of 50HA at 150x magnification



**Figure 7:** Shows detailed view of HA particles at 5000 magnification

The morphology of HA particles in the composite membranes was changed, not only, due to the filler concentration, but also due to the temperature (30 °C) and time of membranes preparation (7 days). These conditions have greatly influenced the HA crystal growth.

### 4.3 Tensile properties

The mechanical behavior of the composite with various weight ratios of HA particles in terms stress-strain dependence is displayed in Fig. 8. The results show, that HA addition influences the mechanical properties of the composites. The resulted values were averaged (see Table 2) from 5 accomplished measurements.

Pure PVA shows a curve with yield point, which indicates transition between elastic and plastic deformation, in the case of composites, this point is not observed clearly.

Curves of composite have three zones - the first is characterised by an increase in the load accompanied by extension of the film, followed by a zone in which the extension occurs with slight increases in the load and the point of rupture of the membranes. The samples formed necking and white zone before rupture. The failure appears to be ductile for all the tested samples.

The significant values of the measurement are noted in Table 2. Maximum tensile stress was the highest in the case of 10HA, i.e. 55 MPa. Compared to the pure PVA, the 10HA and 20HA had higher maximum stress. The increase in the case of 10HA was 17% and 20HA was 8%. The sample 30HA had maximum tensile stress lower than that of the PVA, it was 42 MPa and the decrease was 11%, samples 40HA and 50HA had distinctly lower values of maximum tensile stress than pure polymer, namely 32 MPa and 27 MPa for 50HA, which made the decrease up to 32% and 42% comparing to the neat polymer.

The next value is the Young's modulus, as the content of HA increased further, the Young's modulus of the composites presented decreasing trend. The pure PVA has the value at 662 MPa, very close to this value was 10HA composite with 644 MPa. Similar value of Young's modulus was observed for 20HA and 40HA composites at 383 MPa and 382 MPa and the lowest was 50HA composite with 247 MPa.

Tensile stress at break have increased in comparison with the pure PVA in the case of 10HA and 20HA composites, this followed the trend of the maximum stress values. The increase in the case of 10HA was 29% and 14% for 20 HA. When the HA content in the composite reached 30%, the stress at break decreased. For 30HA, the decrease was 5% and then steeply 39% decrease for 40HA and 51% for 50HA.

Elongation at break in % compared with the pure PVA decreased for all the composites, the decrease was non-linear. The highest value of percentage of elongation was observed for neat polymer, this was 226%, the composite elongation was lower, the lowest for 20HA, then 10HA and then in ordered from lowest to highest 50HA → 30HA → 40HA.

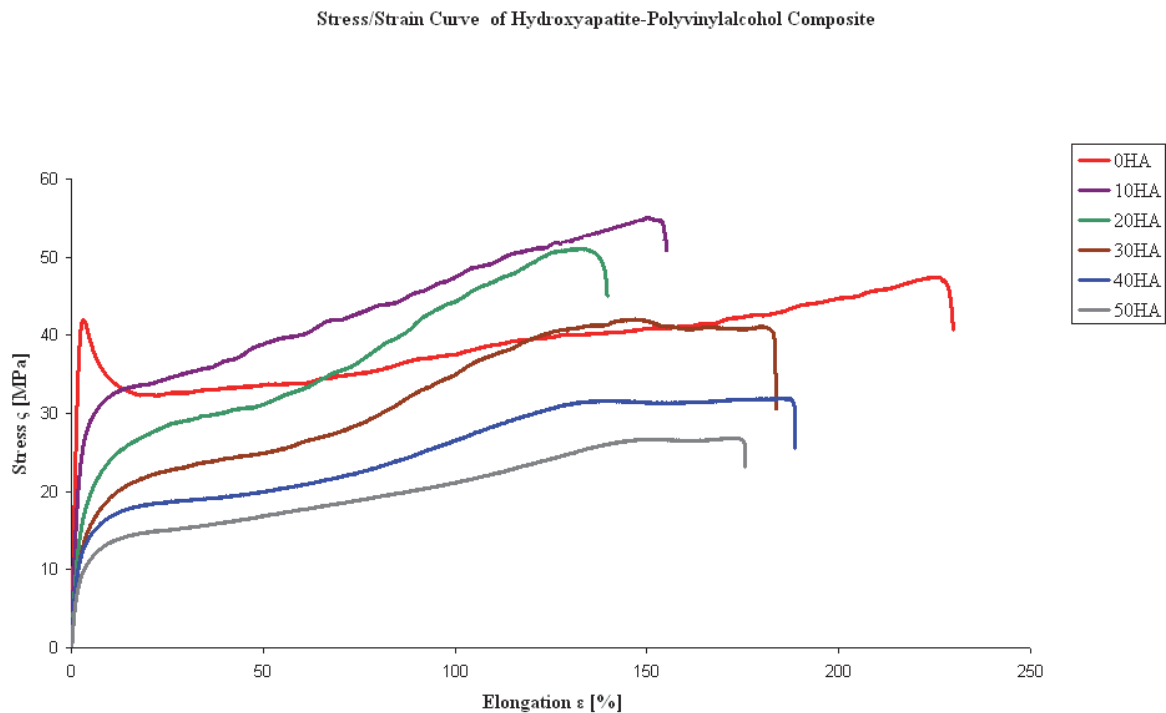
Zheng *et al.*[39] presented results where the tensile strength and modulus of PVA/HA composite hydrogel increased with HA content. The viscosity of composite hydrogel was enhanced and its stress relaxation performance was more similar to natural cartilage. Li *et al.*[40] showed that the tensile strength of PVA/HA composite hydrogel first increased and then decreased with the increase in HA content. It was found, that the tensile strength achieved the maximum value when HA concentration was 7.5%. As HA content further increased the tensile strength decreased.

In this survey the values of tensile strength are improved for 10HA and 20HA in comparison with the pure PVA (0HA). The increasing amount of HA deteriorates the tensile strength, this may be caused by generating of HA agglomerates.

The SEM Figure 9 shows rough surface which belongs to direction of the loading during testing for the 30HA specimen. Figure 10 shows the edge (fracture surface) after tear apart.

**Table 2:** Tensile properties of prepared membranes

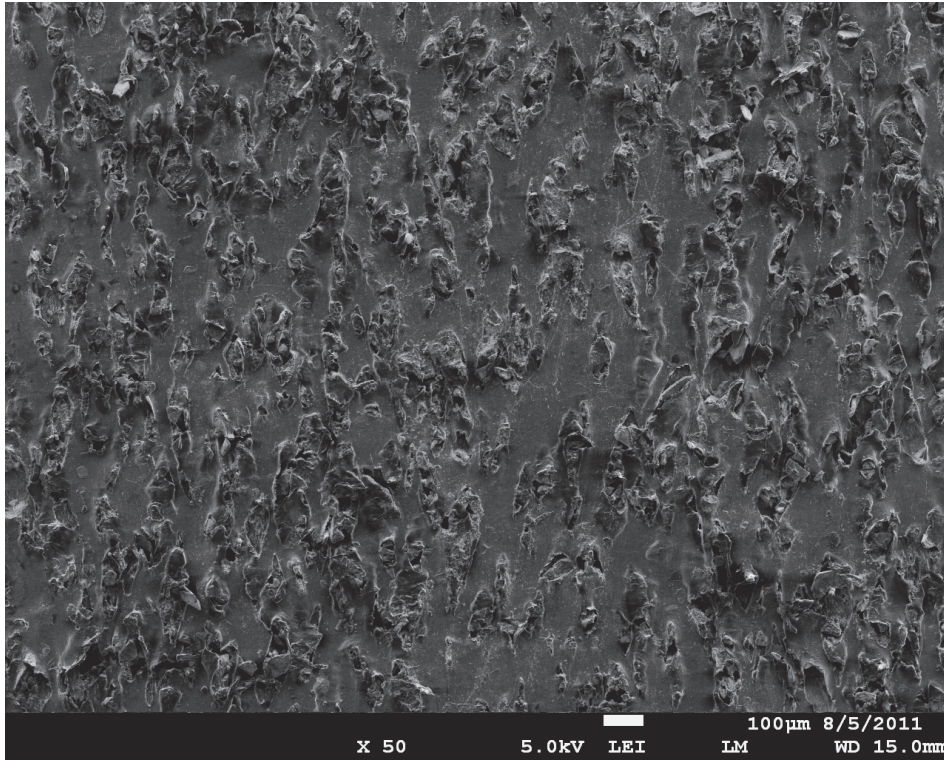
Sample	Maximum tensile stress (MPa)	Young's modulus (MPa)	Tensile stress at break (MPa)	Elongation at break (%)
0HA	47	662	41	226
10HA	55	644	53	156
20HA	51	383	47	140
30HA	42	362	39	181
40HA	32	382	25	185
50HA	27	247	20	177



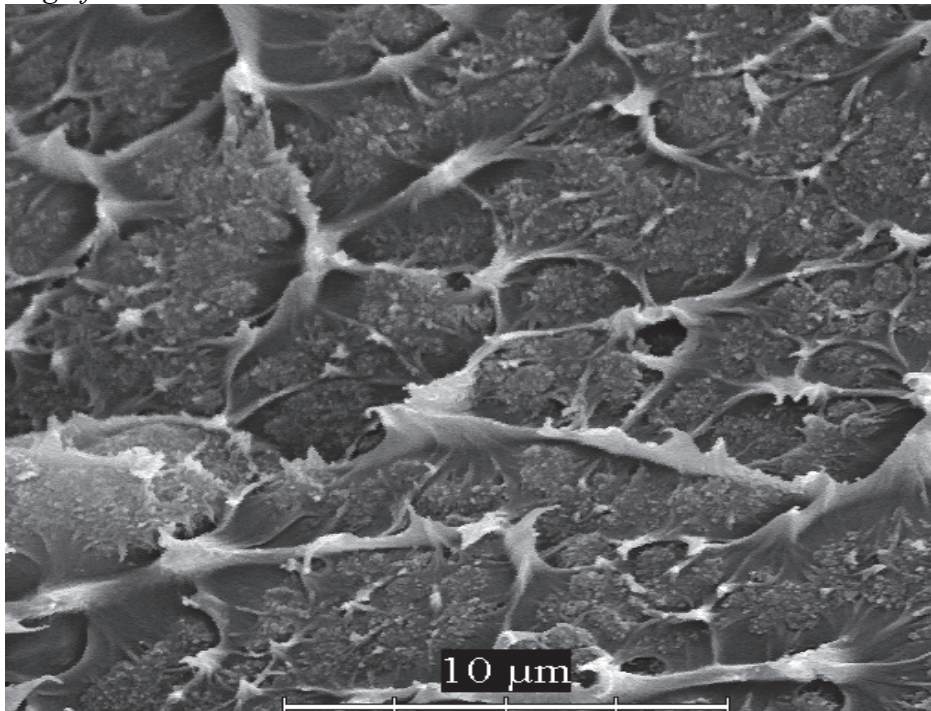
**Figure 8:** Stress/strain characteristics of the PVA/HA composite

The mechanical properties of the polymer are expected to be improved effectively by adding inorganic nanoparticles into polymer matrix due to their high mechanical strength and surface energy [30]. Ideally the interfacial bonding can effectively transfer the load from the matrix to the reinforcement and weak interfacial bonding can deflect an advancing crack thus providing enhanced fracture toughness and avoiding catastrophic failure [13].

Contrarily, the nano-HA particles can easily agglomerate because of its high surface active energy, while the content of nano-HA exceeding a certain percent. The local agglomeration of nano-HA particles can not act as a reinforcement phase but can become the original defective region, which deteriorates the tensile strength of the composites [41].



**Figure 9:** SEM image of the tensile impact fracture surfaces of the sample 30HA at 100 magnification



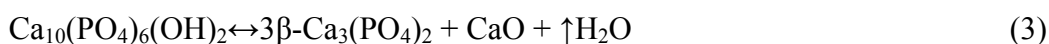
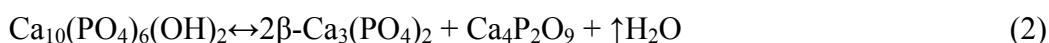
**Figure 10:** Detail of the impact edge with visible HA and PVA phase after tensile testing of 30HA

## 4.4 Thermogravimetric analysis

Peng [42] has prepared and investigated thermal behaviors of PVA/SiO<sub>2</sub> nanocomposite. The thermal decomposition of PVA carried out in two steps. The degradation temperature of the first step is not high enough to break all the backbone chains of these polyenes into low-molecular-weight polyenes. Most of these polyene structures will act as intermediate products and they will be further degraded into other products with lower molecular weights at the next degradation step, which is chain-scission reactions. The degradation products of the second degradation step are acetaldehyde, acetic acid, polyenes, benzenoid derivatives and a small amount of furan.

HA begins to decompose at about 800°C to form oxyhydroxyapatite, or Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2-2x</sub>O<sub>x</sub>, where =vacancy. This process is gradual and takes place over a range of temperatures. Furthermore, HA also decomposes to form other calcium phosphates at elevated temperatures. Two mechanisms have been proposed for the decomposition as follows:

Further heating results in the transformation of β-tricalcium phosphate (β-Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>), forming α-tricalcium phosphate. This usually requires exposure to temperatures in excess of 1350°C for this phase transformation to take place. Two mechanisms have been proposed for the decomposition as follows:

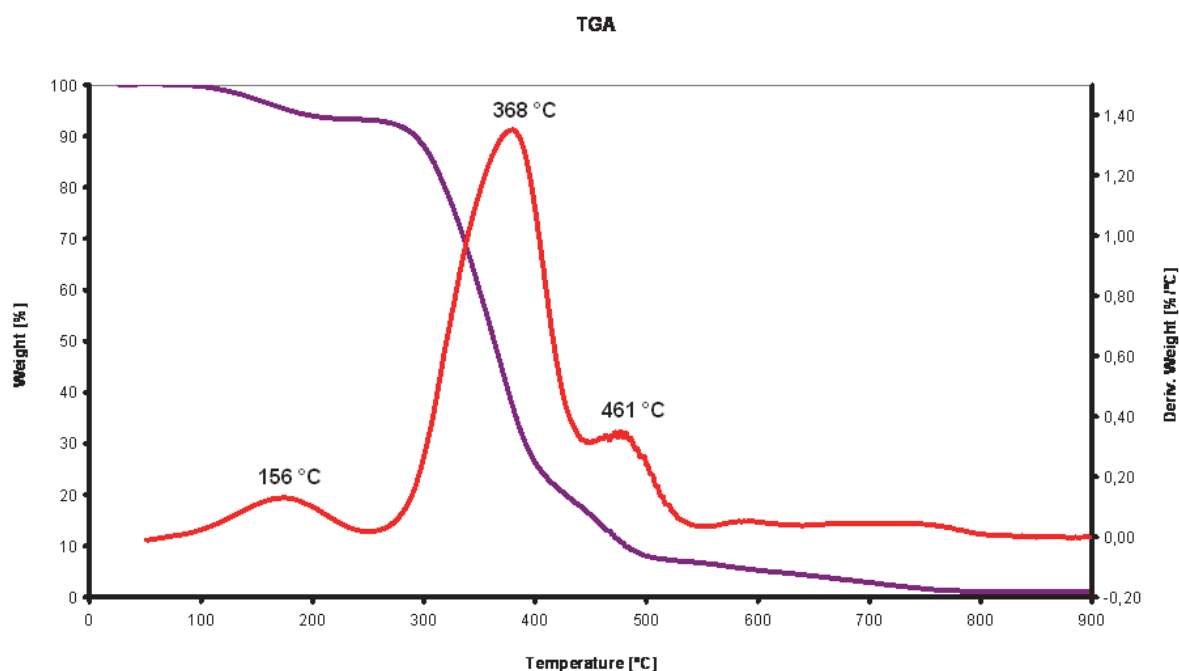


Of these, the former is the more accepted mechanism. Regardless of which mechanism takes place, both result in the formation of soluble or resorbable calcium phosphates, which dissolve when exposed to physiological environments [43].

### 4.4.1 PVA thermogravimetry analysis

The course of thermogravimetric curve of pure PVA(0HA) shows 3 main degradation steps, which are clearly expressed with DTG – derivated TG curve (Fig. 11).

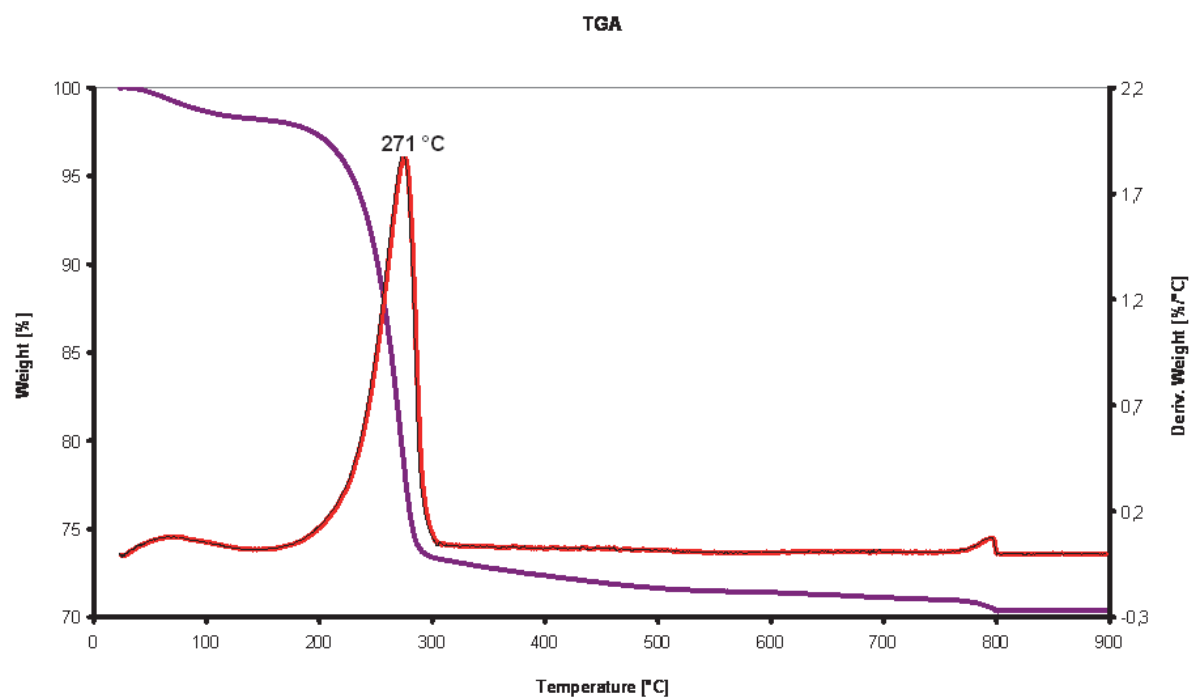
The initial weight loss for the pure PVA occurred at a temperature range of 37 to 200 °C with a peak at 156 °C in the DTG curve ( $T_{\text{max},1} = 156$  °C), due to the evaporation of physically weak and chemically strong bound H<sub>2</sub>O from the polymer matrix. The weight loss was about 7,5 wt%. The next major weight losses have been observed in the range of 250 to 415 °C ( $T_{\text{max},2} = 368$  °C). This was due to the degradation of side chain (O–H) of PVA and the weight loss at this stage was about 68 wt%. The last peak of the third stage temperature range between 450 and 500 °C ( $T_{\text{max},3} = 461$  °C). This may correspond to the cleavage of C–C backbone of PVA polymer or commonly called carbonation with a weight loss of 16 wt%.



**Figure 11:** TGA curve of pure PVA (0HA)

#### 4.4.2 Hydroxyapatite thermogravimetry analysis

The decomposition of the pure HA powder was monitored up to 900 °C (Fig. 12). There was revealed one significant peak at DTG curve around 272 °C, which belongs to massive loss of physically bond water from hydroxyapatite structure. This mass loss was near 25 wt% in range 200 – 300 °C.



**Figure 12:** TGA curve of pure hydroxyapatite powder (100HA)

#### 4.4.3 PVA/HA composite thermogravimetry analysis

The main loss of weight in composite shows peaks in DTG curves and declination of the TGA curve. The thermograms of all measured PVA/HA composites show common features. The main weight loss carry out in three ranges. The first one takes place by 250 °C, the second range is approximately between 250 and 450 °C and the last one 450 °C and higher.

**Table 3:** Weight loss of PVA/HA composites with variable HA content in PVA matrix sorted into three temperature ranges

Sample	1st Range		2nd Range		3rd Range	
	T [°C]	Weight loss [%]	T [°C]	Weight loss [%]	T [°C]	Weight loss [%]
<b>10HA</b>	37 - 230	14.9	250 – 450	49.6	450– 540	18.9
<b>20HA</b>	37 - 240	17.0	260 – 430	43.1	430– 530	15.8
<b>30HA</b>	37 – 250	19.9	270 – 420	41.8	430– 500	10.7
<b>40HA</b>	37 - 210	22.9	250 – 400	35.0	500- 630	15.0
<b>50HA</b>	37 - 200	31.9	250 - 400	25.5	450- 600	21.0

First temperature range reveals, that with increasing hydroxyapatite in PVA matrix increases weight loss. This phenomenon might be explained as weight releasing in form of water from hydroxyapatite and breaking of weak bonds between matrix and the filler.

The second range, approx. between 250 and 450 °C shows opposite tendency comparing to previous range, so with increasing amount of HA in PVA matrix, the weight loss decreases. It was also found out, that the interval of decomposition is shortened, so the broadest is in 10HA. The temperature range is in line from the lowest presence of HA to the highest wide 200, 160, 160, 150 and 150 °C.

The weight loss in this range is influenced by both – the filler and the matrix decomposition, as the hydroxyapatite has its main weight loss in this survey between 200 – 300 °C, and it is 25%wt. and the PVA which show in range 250 – 450 °C massive weight loss about 68%. This loss is associated with the degradation of PVA polymer membrane.

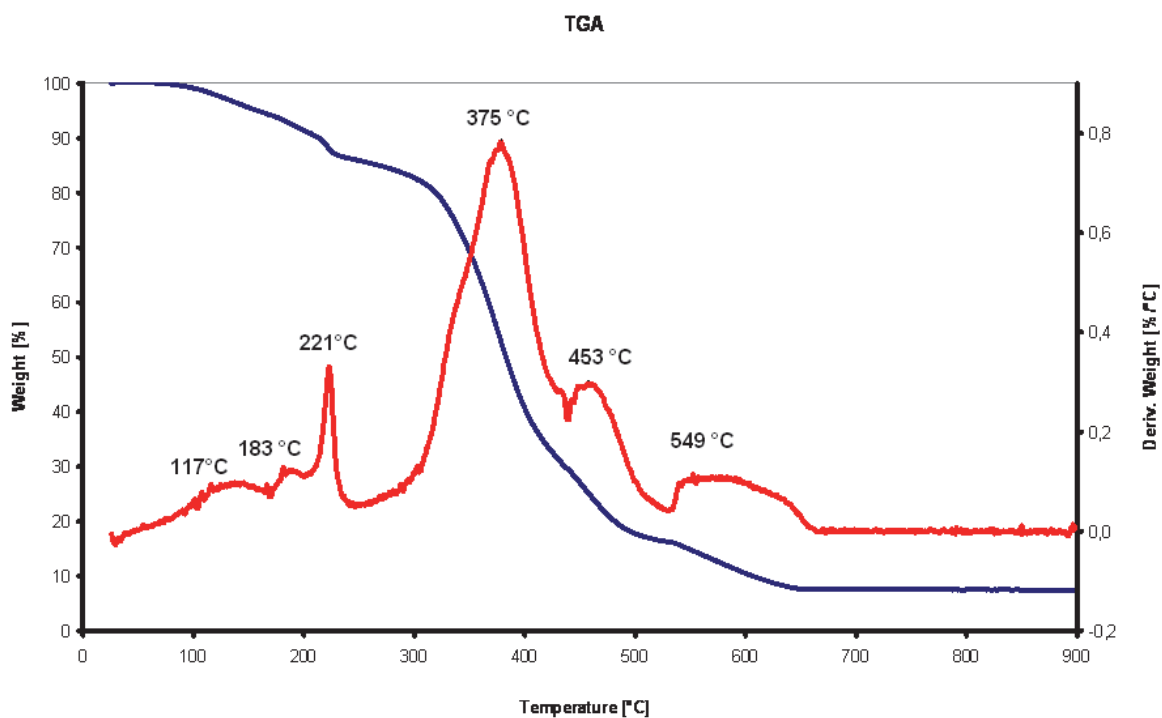
The third decomposition region is in range approx. 450 °C and higher, which is due to the cleavage backbone of PVA polymer membrane (or so-called carbonation).

The 20HA shows peculiar run of the curve at around 240 °C, this was recorded the bouncing measuring pan during testing which was caused by foaming of the PVA in the sample at this temperature.

The PVA/HA composite shows improved resistance in comparison with the pure PVA. Degradation temperatures of the composite are noticeably higher which is due to interfacial bonding between HA particles and the PVA polymer matrix.

The total weight loss was determined at all of the measured samples at 900 °C and as expected, the highest residue was noticed at pure HA, the lowest at pure PVA and according to amount of HA in PVA matrix. The results of the measurement are noticed in Tab. 4.

TGA curves of 10HA composites is displayed in Fig 13.

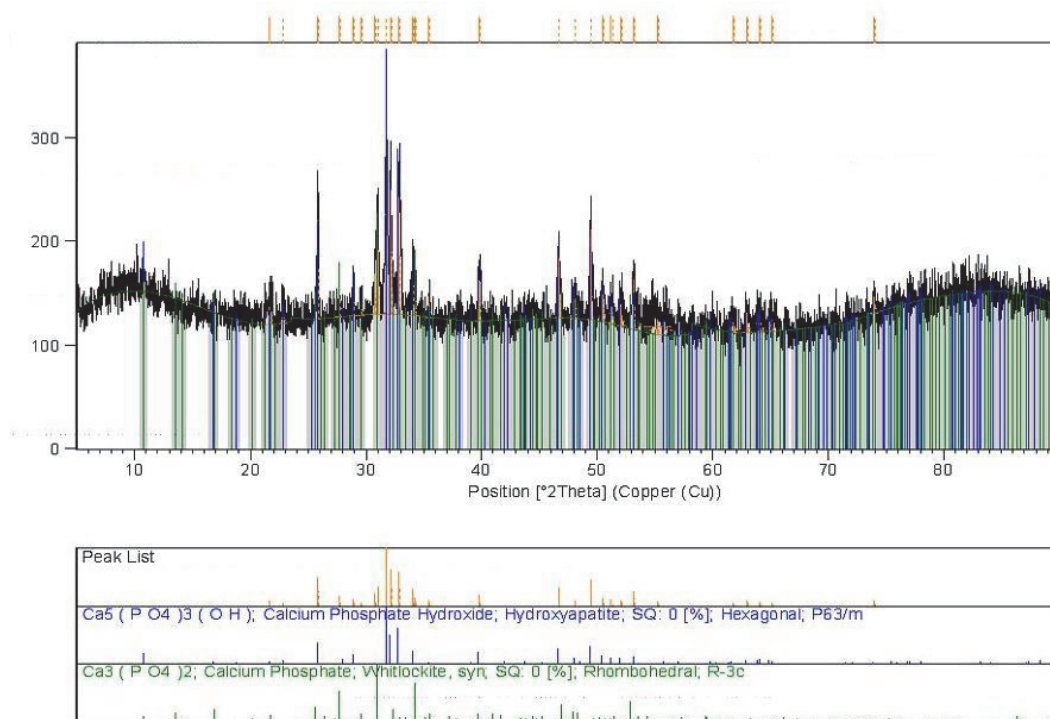


**Figure 13:** TGA curve of composite 10HA

**Table 4:** Comparison of total weight loss at 900°C

Sample	Total weight loss at 900°C in %
0HA	98.99
10 HA	92.61
20 HA	90.82
30 HA	85.40
40 HA	80.39
50 HA	79.50
100 HA	33.04

The residuum of the TGA measured samples was a white powder, which was further analysed by XRD measurement, which showed supposed results - equations (section 4.4). After TGA analysis, there was found rest of not fully decomposed hydroxyapatite and calcium phosphate  $\text{Ca}_3(\text{PO}_4)_2$ . Polyvinyl alcohol was not more found in sample, but whitlockite as product of partial decomposition of HA (Fig. 14).



**Figure 14:** XRD spectrum of 20HA composite after TGA analysis

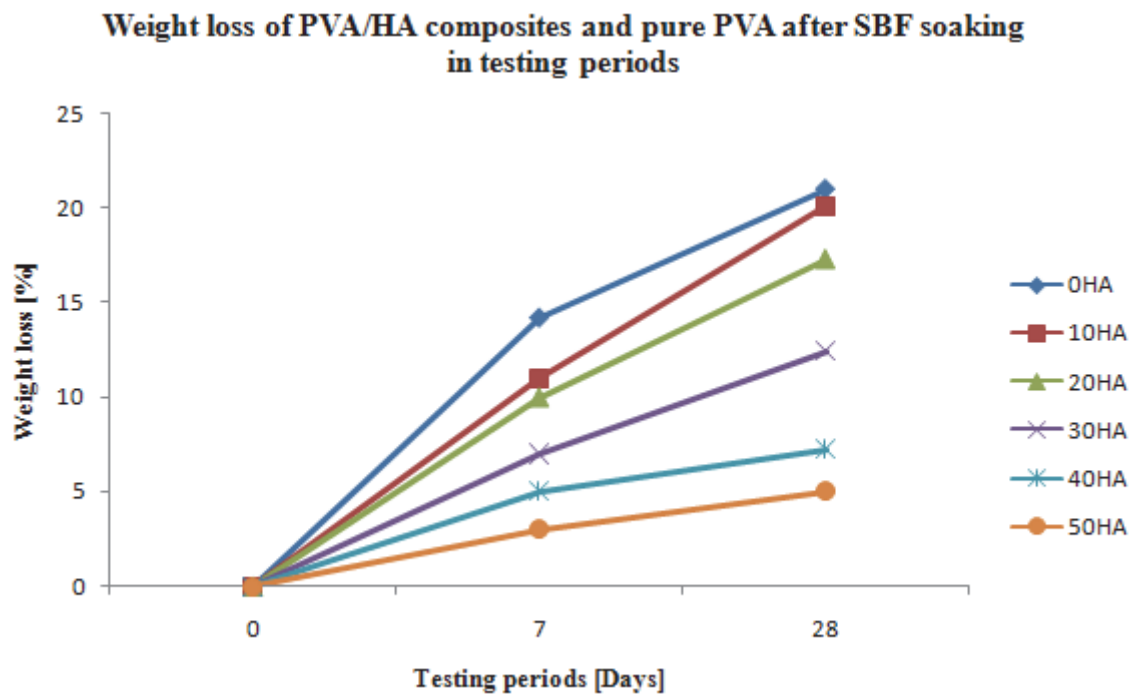
#### 4.5 Bioactivity testing

Samples removed from the microincubator were rinsed with distilled water and dried at laboratory temperature. All the samples were weighed before and after the SBF immersion to compare weight loss respectively estimate the biodegradation of the composite. The weight loss was calculated according to percentage of initial weight. These results are depicted in Tab. 5. There are not included samples soaking for two hours as the weigh loss was negligible. It is obvious, that HA content strongly influences the degradation rate of the composite. The weigh loss was as expected highest in the case of pure PVA and stepped up with the increasing time soaked in SBF. Conversely the lowest weight loss was registered in the case of 50HA, this is due to high content of HA in PVA matrix and because of new HA formation. Nie [44] tested foamed BCP/PVA bodies and the results described the same degradation effect, but the degradation rate was higher because of higher contact area.

Graphical representation of weight change see in Fig. 15. The limitation of PVA are obvious, including fast hydrolysis [45] and its bioinert nature [46] that hinder protein and cell adhesion. This requires the incorporation of component, which reduces the rate and time of biodegradation, this demand fulfills hydroxyapatite.

**Table 5:** Weigh loss of PVA/HA biocomposites in testing time

	Weight loss after 7 days [%]	Weight loss after 28 days
<b>0HA</b>	14.2	21.0
<b>10HA</b>	11.1	20.1
<b>20HA</b>	10.1	17.3
<b>30HA</b>	7.0	12.4
<b>40HA</b>	4.9	7.2
<b>50HA</b>	3.0	5.0

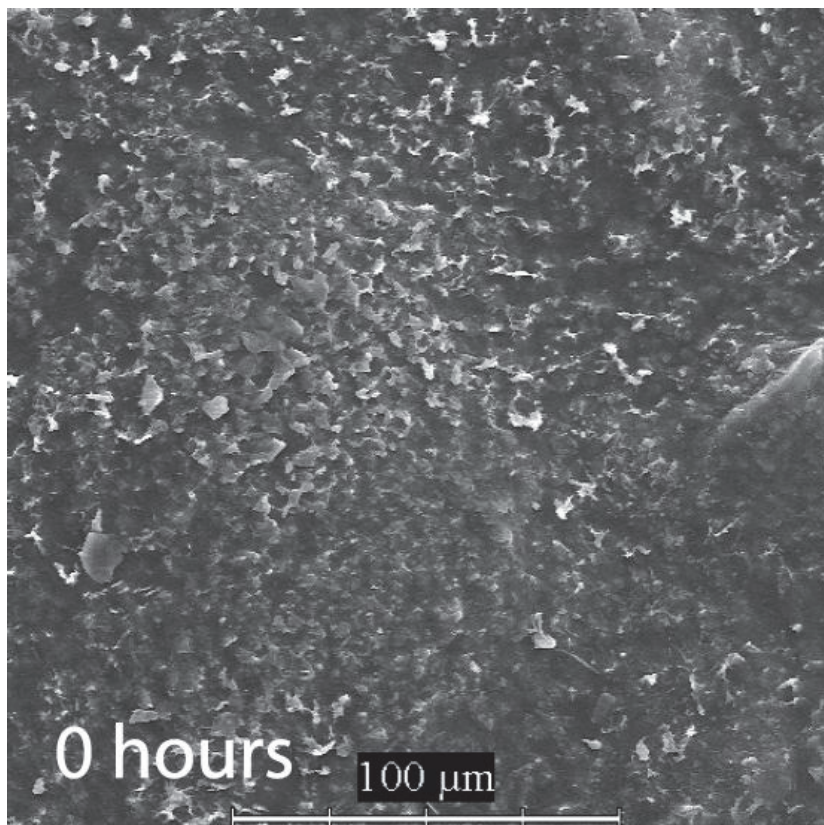


**Figure 15:** Increasing weight loss of composites and the pure PVA in time

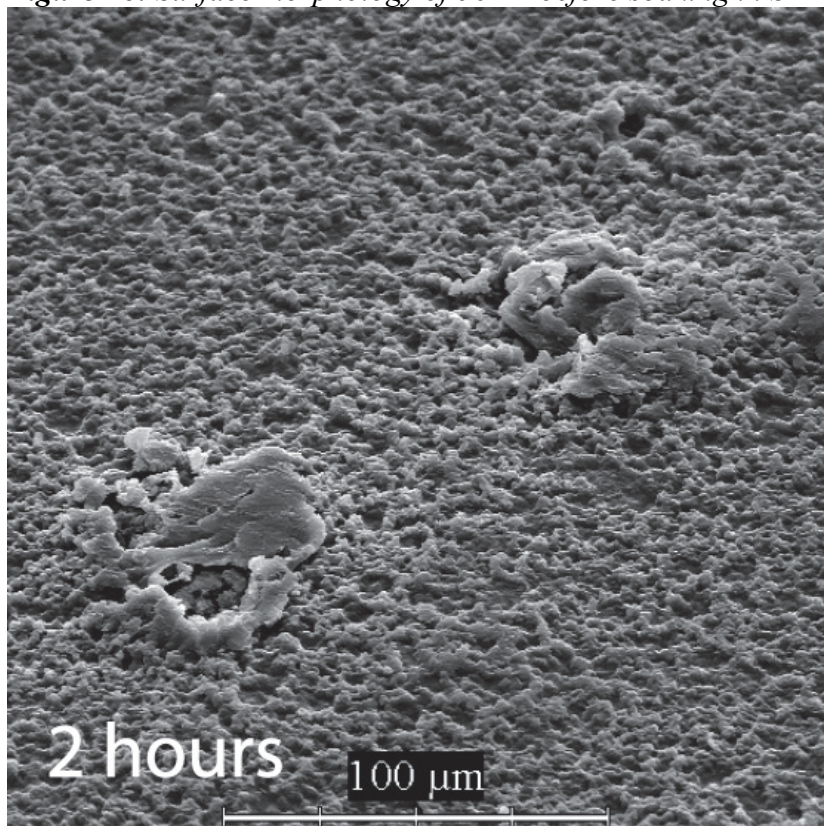
The SEM pictures Fig. 16 - 19 display HA formation on the surface of 50HA composite during testing periods.

HA crystals were observed on all composite membranes just after 2 hours of soaking in SBF. Most of the membranes surface was entirely covered with crystals after 7 days of soaking in SBF. The number of nucleation locations of apatite crystals was adequate to the experimental time and the apatite layer formed more rapidly on composite with higher content of HA.

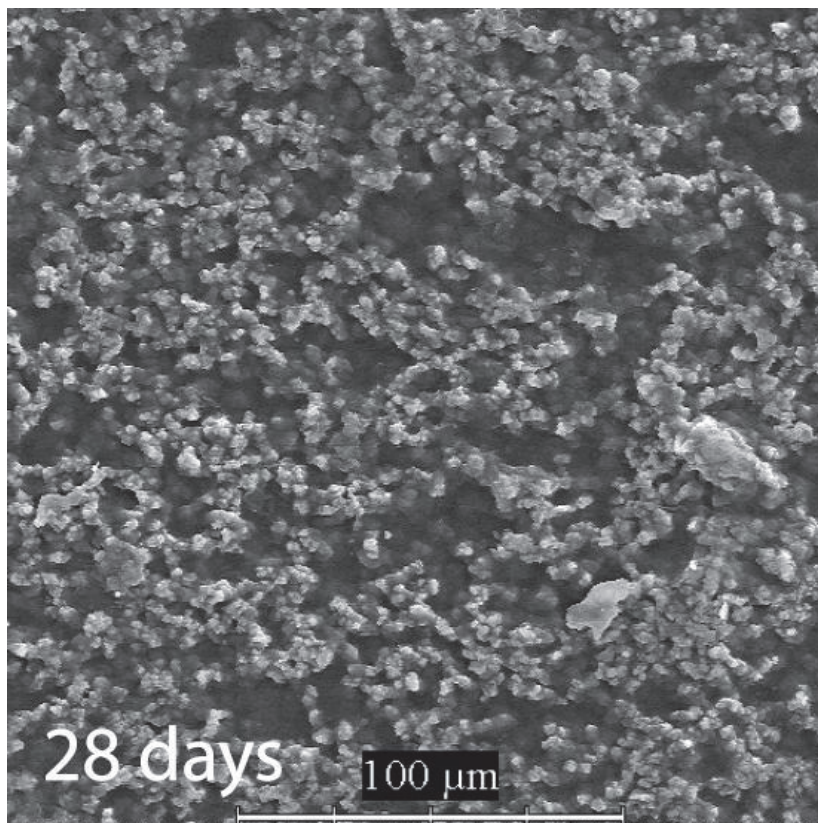
The *in vitro* testing proved high bioactive properties of the PVA/HA composites. The possibility to manage the rate of bioactivity makes PVA/HA composite suitable to fulfill diverse requirements and can be useful as tailor-made materials for medical application.



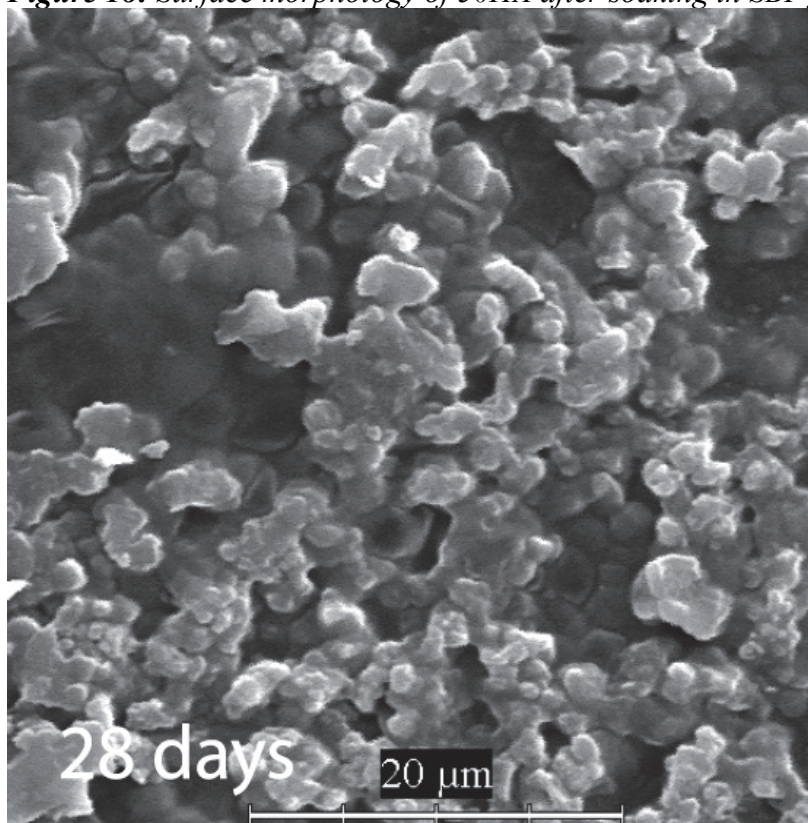
**Figure 16:** Surface morphology of 50HA before soaking in SBF



**Figure 17:** Surface morphology of 50HA after soaking in SBF for 2 hours

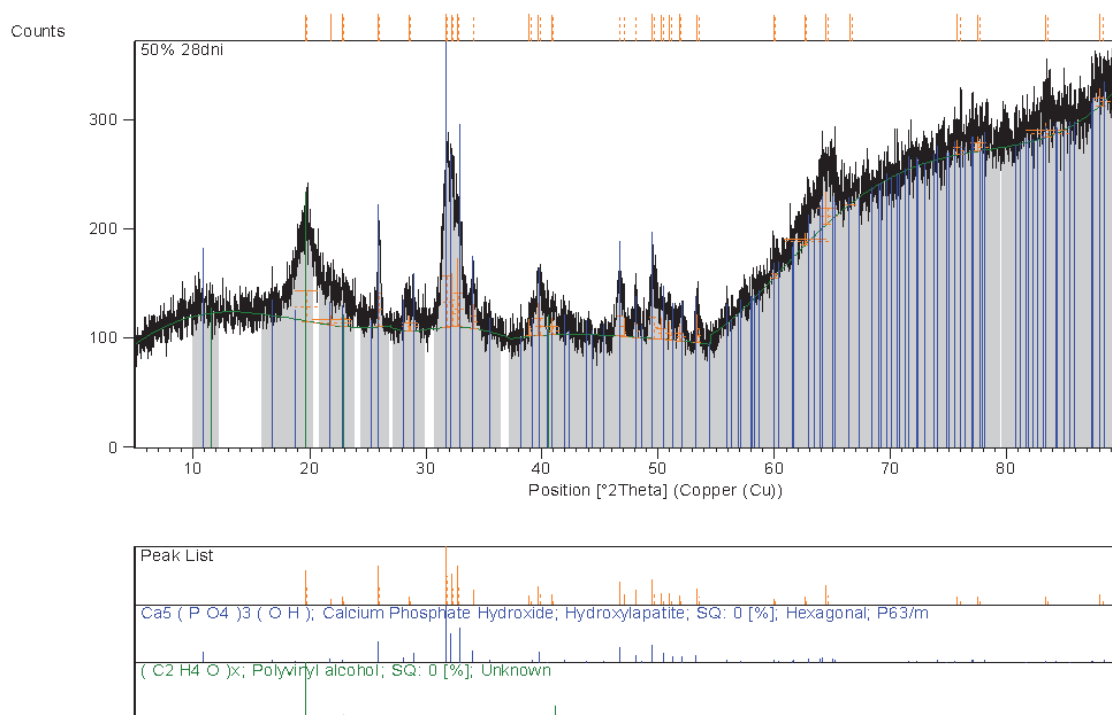


**Figure 18:** Surface morphology of 50HA after soaking in SBF for 28 days



**Figure 19:** Detail of new formed hydroxyapatite on the surface

The newly formed layer was analysed by XRD analysis, which proved HA on the surface - Fig. 20. The diffractograms should be not considered as quantitative analysis, because of the high amount amorphous phase in the membranes, this misrepresents the results.



**Figure 20:** Diffractogram of 50HA composite surface after 28 days of soaking in SBF

## 5 CONCLUSION

The aim of the work was to synthesize biocomposite poly(vinyl alcohol) membranes reinforced with particles of hydroxyapatite and to characterise the properties of the composites. The main part of the work was focused on study of the *in vitro* bioactivity of synthesized membrane biocomposites.

As the first step, hydroxyapatite was prepared by action of diammonium hydrogen phosphate  $(\text{NH}_4)_2\text{HPO}_4$  on calcium nitrate tetrahydrate  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  in the presence of ammonium hydroxide  $\text{NH}_4\text{OH}$  in water environment to form yogurt-like suspension. After washing the ammonium ions out, the solution was further used to prepare PVA/HA composite membranes with various weight HA content. The prepared composites were characterised by various methods and the bioactivity of the material was also tested.

ATR-FTIR spectroscopy was used to identify the different functional groups in composite membranes and composite components (HA filler, PVA matrix) themselves. The bonding between HA and PVA appeared in spectra after composite formation.

The SEM was used to determine surface morphology of synthesized composite membranes and the influence of HA amount on morphology. The dried HA was observed and it was round particles before processed into composite. The HA changed its structure after composite formation, there were detected needles shaped structures, which created clusters of various shapes. The agglomerates amounts increased with increasing wt% amount of HA. The change in morphology was caused not only by amount of HA in PVA matrix, but also because of the procedure, which was used to synthesize the membranes. They were dried for 7 days at 30°C. These condition caused HA crystal growth and the agglomeration trend as well.

The tensile testing was used to compare pure PVA and composites mechanical properties. There was a noticeable influence of HA filler. The values of tensile strength are improved for 10HA and 20HA in comparison with the pure PVA. The increasing amount of HA deteriorated the tensile strength, this was probably caused by HA agglomerates.

The bioactivity testing results indicated that the synthesized HA/PVA composite membranes showed the high bioactivity *in vitro* in the SBF solution. At all the tested membranes new apatite layer was formed, this was detected with SEM measurements. The tested samples showed weight loss after SBF soaking which decreases with increasing content of HA in the PVA matrix, this was caused by biodegradation ability of PVA. The bioactivity of membrane containing 50 % of HA had the highest ability to form the apatite and the layer increased with increasing soaking time. This ability makes the composite a suitable candidate for applications as artificial cartilage.

The main contributions of this work is that PVA/HA biocomposites were characterised with wide scope of methods. In the case of FTIR, the bonding between components was detected, the thermal stability of the composites was improved in comparison to the pure PVA and the mechanical properties were strongly influenced with bioceramic filler. The distribution and the arrangement of the HA crystals were observed with SEM. Finally the *in vitro* method was

used for the study of the membranes, this test allows prediction of the approximate behaviour of such materials *in vivo*. The bioactivity was proved due to formation of new HA layer on the membranes surface.

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## 7 LIST OF SYMBOLS

ATR-FTIR	Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy
HA	Hydroxyapatite
PVA	Polyvinyl alcohol
SEM	Scanning electron microscopy
SBF	Simulated Body Fluid
TGA	Thermogravimetric analysis
XRD	X-ray diffraction

## 8 LIST OF PUBLICATIONS

### Publications

BALGOVÁ, Z.; PALOU, M.; WASSERBAUER, J.; KOZÁNKOVÁ, J.; LUTIŠANOVÁ, G. Preparation, characterization and in vitro bioactivity of polyvinyl alcohol- hydroxyapatite biphasique membranes. *Acta Chemica Slovaca*, 2013, roč. 6, č. 1, s. 8-14. ISSN: 1337- 978X.

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### Conference proceedings

OPRAVIL, T.; HAVLICA, J.; BRANDŠTETR, J.; PTÁČEK, P.; ŠOUKAL, F.; BALGOVÁ, Z. Preparation and properties of binders based on roman cement. In *11th INTERNATIONAL CONFERENCE Ecology and new building materials and product*. 2007.s. 255-259. ISBN: 978-80-239-9347- 9.

BALGOVÁ, Z.; PALOU, M. *Synthesis of polyvinyl alcohol- hydroxyapatite membranes*. Chemické listy. Brno: Česká společnost chemická, 2011. s. 943-943. ISSN: 0009- 2770.

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