

## Preparation, Characterization and *In vitro* Evaluation of Poly (L-lactide) and Sodium Calcium Silicate Ceramic Composites

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**T**HE MAIN objectives of the present study were to fabricate sodium calcium silicate ceramic /poly(L-lactide) composite membranes for bone engineering applications, by using liquid-liquid phase separation. The membranes were characterized by SEM, FT-IR, TGA and TF- XRD. Examination of the SEM microphotographs revealed that the pore diameter of the composites decreased as the ceramic content increased. For the composites containing ceramic content of 20 wt% (CP20) and 30 wt% (CP30), the maximum pore diameter was 70  $\mu\text{m}$  and 55  $\mu\text{m}$ , respectively.

*In vitro* bioactivity evaluation showed that the composites were able to induce the formation of hydroxyapatite layer on their surfaces, demonstrating their potential application in bone engineering.

**Keywords:** Poly (L-lactide), Sodium calcium silicate ceramic, Sol-gel.

Among the biodegradable polymers, poly L lactide (PLA) have been receiving a special interest as biomedical materials, PLA has widespread applications in sutures, drug delivery devices, prosthetics, scaffolds, vascular grafts, bone screws, pins, a bone reinforcement material and plates for temporary internal fixation<sup>(1)</sup>.

PLA can be considered an eco-friendly biomaterial (consume carbon dioxide in its production), it is biodegradable, recyclable, biocompatible with excellent mechanical properties. However; it has many drawbacks such as it is hydrophobic, with slower degradable rate, acidic in its degradation products, in addition to its, inability to induce a bone like apatite<sup>(2)</sup>. Therefore, bioactive material such as sodium calcium silicate ceramic<sup>(3)</sup> is added to the prepared composites.

### Material and Methods

#### Materials

Tetraethyl orthosilicate (TEOS), calcium nitrate tetrahydrate,  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , were all  $\geq 98\%$  pure and purchased from Fluka (Buchs, Switzerland). Sodium nitrate

(NaNO<sub>3</sub>) was purchased from Sigma Aldrich. Ammonia solution, 33%, and Nitric acid, 68%, were obtained from Merck, USA. Poly (L-lactide) (M. Wt. 152,000) was obtained from Fluka, USA. Chloroform was obtained from Acros (Acros Organics, Belgium). Both nitric acid and ammonia solutions were diluted to 2 M using distilled water.

*Preparation of bioactive sodium calcium silicate ceramic (Na<sub>4</sub>Ca<sub>4</sub>Si<sub>6</sub>O<sub>18</sub>) by sol-gel method*

Na<sub>4</sub>Ca<sub>4</sub>Si<sub>6</sub>O<sub>18</sub> was prepared using the sol-gel technique and the preparation procedure was similar to the preparation of bioactive glass 58S<sup>(3)</sup>. The previous method was modified through a quick alkali mediated sol gel technique to obtain nano ceramic particles.

*Preparation of biocomposites based on poly L-lactide and contain sodium calcium silicate ceramic particles as a filler*

The composite membranes were prepared through the liquid-liquid phase separation as illustrated elsewhere<sup>(4,5)</sup>. Briefly, poly (L-lactide) was dissolved in chloroform to form a polymer solution with a concentration of 10% (w/v). Bioactive ceramic powder was added and the mixture stirred for 3 hr to ensure the complete dissolution of the polymer and the formation of a homogenous solution. Then the solution poured into a closed petri dish and frozen at -20 °C for 2 days after that the dish was opened and still in freezing for 1 day to allow chloroform to evaporate. This lead to the formation of the porous composites.

The prepared composites containing 20 and 30 wt.% of sodium calcium silicate ceramic. The composites were denoted as CP20 and CP30 respectively.

The ceramic content in each membrane was calculated according to Eq. 1.

$$\text{Ceramic content (wt. \%)} = \left\{ \frac{W_c}{W_c + W_p} \right\} * 100 \quad (1)$$

where  $W_c$  was weight of the ceramic and  $W_p$  was weight of the polymer.

*Characterization techniques*

The morphology and the porous structure of the composite membrane, as well as their elemental composition, were analyzed with Scanning Electron Microscopy coupled with Energy-Dispersive Spectroscopy, SEM/EDXA (JEOL JXA-840A, Electron probe micro-analyzer, Japan) at 30 kV. The scaffolds were cut with a razor blade and coated with carbon. The SEM analysis was carried out for the sample with different magnifications.

Thermogravimetric analysis (TGA) of the prepared composite was obtained. Scans were performed in an air atmosphere in a temperature range 50–500°C for the scaffolds at a rate of 10°C/min using aluminum oxide powder as a reference. The phase analysis of samples was examined by an X-ray diffractometer model BRUKER axs, D8ADVANCE (Germany) employing Ni-filtered Cu Ka irradiation at 40 kV and 25mA.

The Fourier-Transform Infrared spectra, (FT-IR) of the prepared glass and the scaffolds were obtained using the FT/IR-6100 type A machine (The Netherlands) in the range of 400–4000  $\text{cm}^{-1}$ . Disks were prepared by mixing powders of membrane with KBr. Pure KBr powder was used as a background.

#### *Assessment of in vitro bioactivity*

The assessment of the *in vitro* bioactivity was carried out by soaking the composites in simulated body fluid (SBF) in sterilized polyethylene containers maintained at 37°C. The SBF had an ion concentration nearly equal to that of the human blood plasma. The SBF is prepared by dissolving reagent-grade NaCl,  $\text{NaHCO}_3$ , KCl,  $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ ,  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{CaCl}_2$ , and  $\text{Na}_2\text{SO}_4$  in deionized water. The solution buffered to pH 7.4 with Tris- hydroxymethyl aminomethane ( $(\text{CH}_2\text{OH})_3\text{CNH}_3$  and hydrochloric acid<sup>(6)</sup>). The samples is removed from the SBF, rinsed gently with absolute ethanol and then with deionized water, and left to dry. The formation and growth of apatite layer on the ceramic and composite surfaces were verified by Scanning Electron Microscope coupled with Energy-Dispersive Spectroscopy, SEM/EDXA (JEOL JXA-840A, Electron probe micro-analyzer, Japan) , Thin-Film X-ray Diffraction (TF-XRD) (Panalytical, X'Pert Pro, The Netherlands), and Fourier-Transform Infrared spectra, (FT-IR) (6100 type A machine) in the range of 400–4000  $\text{cm}^{-1}$ .

## Results and Discussion

#### *Results of characterizations*

##### *Thermal analysis*

The thermogravimetric analysis (TGA) of the composites were indicated in Fig. 1. The TGA results of CP20 and CP30 showed that the thermal destruction of the polymer started at 100 °C and ended at 395 °C recording a total weight loss of 80.816% and 66.53% , respectively due to release of organic materials.

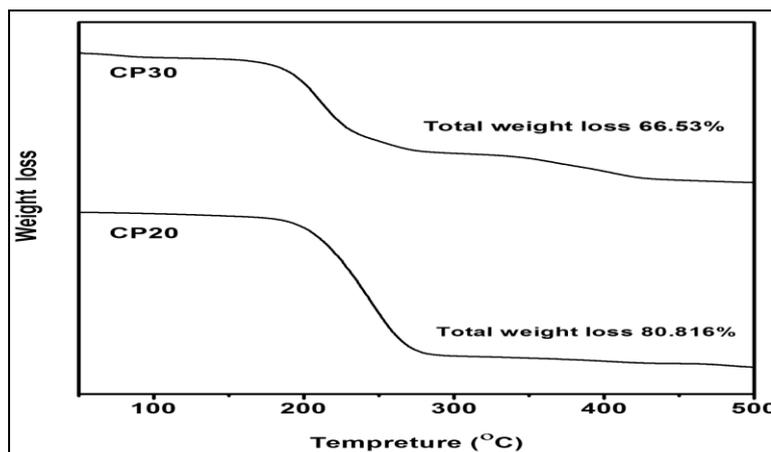


Fig. 1. Thermogravimetric analysis (TGA) of CP20 and CP30 composites.

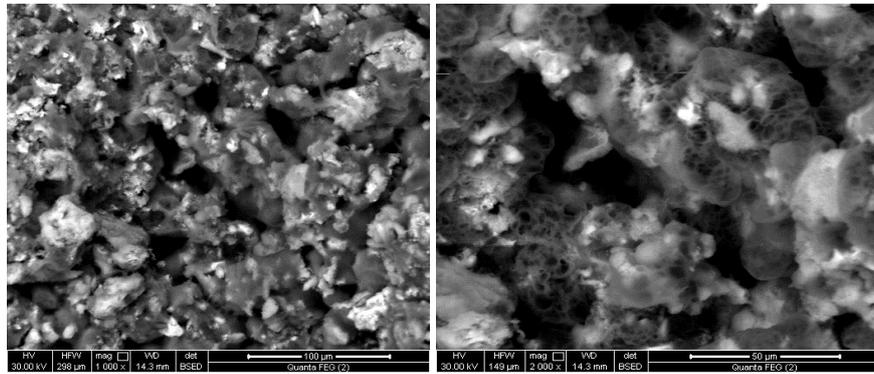
The ceramic content in the fabricated composites could be calculated from the TGA data and from Equation 1 for comparison.

The ceramic content from Eq. 1 was 20% and 30% for CP20 and CP30, respectively while that from TGA was 19.184% and 33.47% for the same composites, respectively.

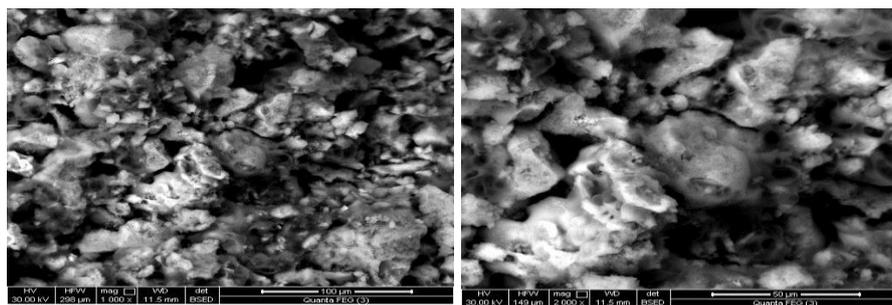
The comparison showed a difference between the amount of the ceramic content calculated from TGA data and those calculated by Eq. 1. Such difference might be due to some partial sedimentation of the ceramic particles during fabrication of composites<sup>(7)</sup>.

#### SEM examination

Figure 2 and Fig. 3 represent the SEM micrographs of a cross-section of CP20 and CP30 before immersion in SBF, respectively.



**Fig. 2.** SEM micrograph showing a cross-section of the composite membrane CP20. The figure shows a pore size varying from a few microns to about 70 $\mu$ m.



**Fig. 3.** SEM micrograph showing a cross-section of the composite membrane CP30. The figure shows a pore size varying from a few microns to about 55  $\mu$ m.

The composite membranes CP20 showed a pore size varying from a few microns to about 70  $\mu\text{m}$  (Fig. 2). By increasing the ceramic filler the pore size of the composites decreased, ranging from few microns to about 55  $\mu\text{m}$  for CP30.

In this study, the polymer solution is frozen at  $-20^{\circ}\text{C}$ , which is higher than the freezing point of the chloroform ( $-64^{\circ}\text{C}$ ), thus, inducing the Liquid-liquid phase separation by freezing the polymer. The SEM micrographs demonstrated that the porosity of the polymer matrix could be affected by the incorporation of bioactive ceramic. When an inorganic filler, such as ceramic added to the polymer/solvent solution, the growth of polymer crystals is impeded during the phase separation mechanism by the randomly distributed solid inorganic phase, hence the porosity of the scaffold decreases and the pores became irregular in shape (Fig. 2 and 3). Many other studies<sup>(8-11)</sup> showed the same observations.

#### *X-ray diffraction analysis*

The TF-XRD patterns of the composites CP20 and CP30 before immersion in the SBF were illustrated in Fig. 4.

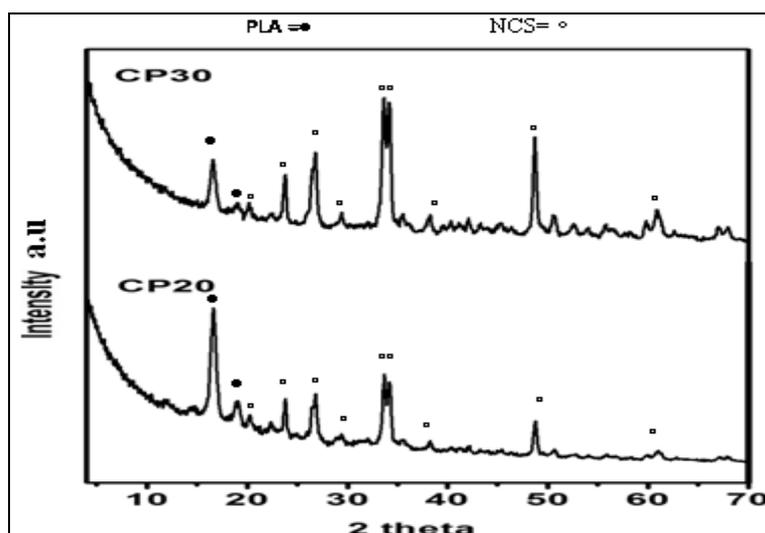


Fig. 4. Thin film X-ray of CP20, and CP30 before immersion in SBF.

For the composite membranes, two XRD peaks of the crystalline poly(L-lactide) found at  $2\theta$  values of 16.63 and 18.86 corresponding to d-spacing of 5.34  $\text{\AA}$  and 4.67  $\text{\AA}$ , respectively<sup>(12)</sup>. The figure also indicates that the intensity of the two peaks has gradually decreased as the ceramic filler content increased in the composite membranes. This could be attributed to the interaction between the polymer and ceramic fillers. Therefore, the two characteristic peaks of the poly(L-lactide) were masked by the ceramic fillers.

Also all peaks of sodium calcium silicate ceramic were noticed and it was identical to peaks of the standard PDF No. 75-1687 quite well. The intensity of ceramic peaks was increased as the ceramic filler content increased.

#### FT-IR analysis

FTIR spectra of the composite membranes CP20 and CP30 before SBF soaking are indicated in Fig. 5.

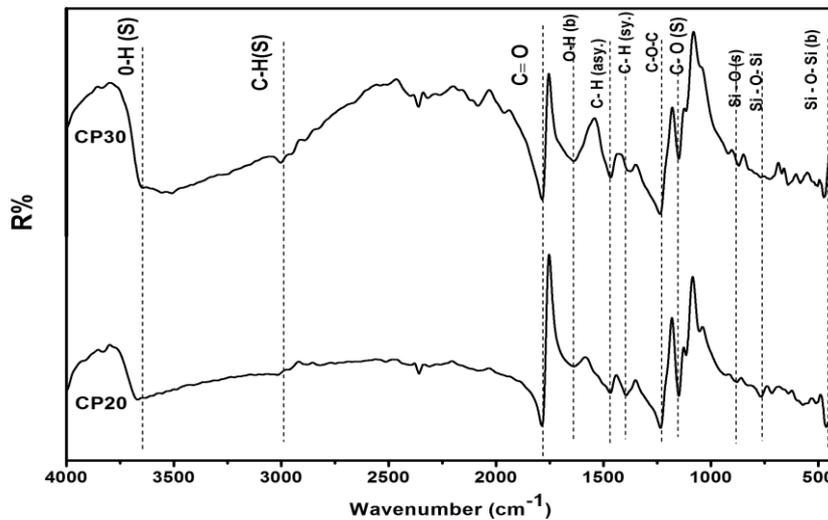


Fig. 5. FTIR spectra of the composites CP20 and CP30 before soaking in SBF. (R%)=Reflectance%.

FTIR spectra of the composite membranes illustrate all the characteristic absorption peaks of poly(L-lactide), and sol-gel sodium calcium silicate ceramic.

The main characteristic bands of poly(L-lactide) as reported elsewhere<sup>(13, 14)</sup>, these bands are ascribed to: carbonyl modes [C=O] at  $1788\text{ cm}^{-1}$ , asymmetric  $\text{CH}_3$  bending mode at  $1470\text{ cm}^{-1}$ , symmetric  $\text{CH}_3$  stretch at  $1397\text{ cm}^{-1}$ , [C-O] stretching mode at  $1150\text{ cm}^{-1}$  and other methyl bands at  $3018\text{ cm}^{-1}$ .

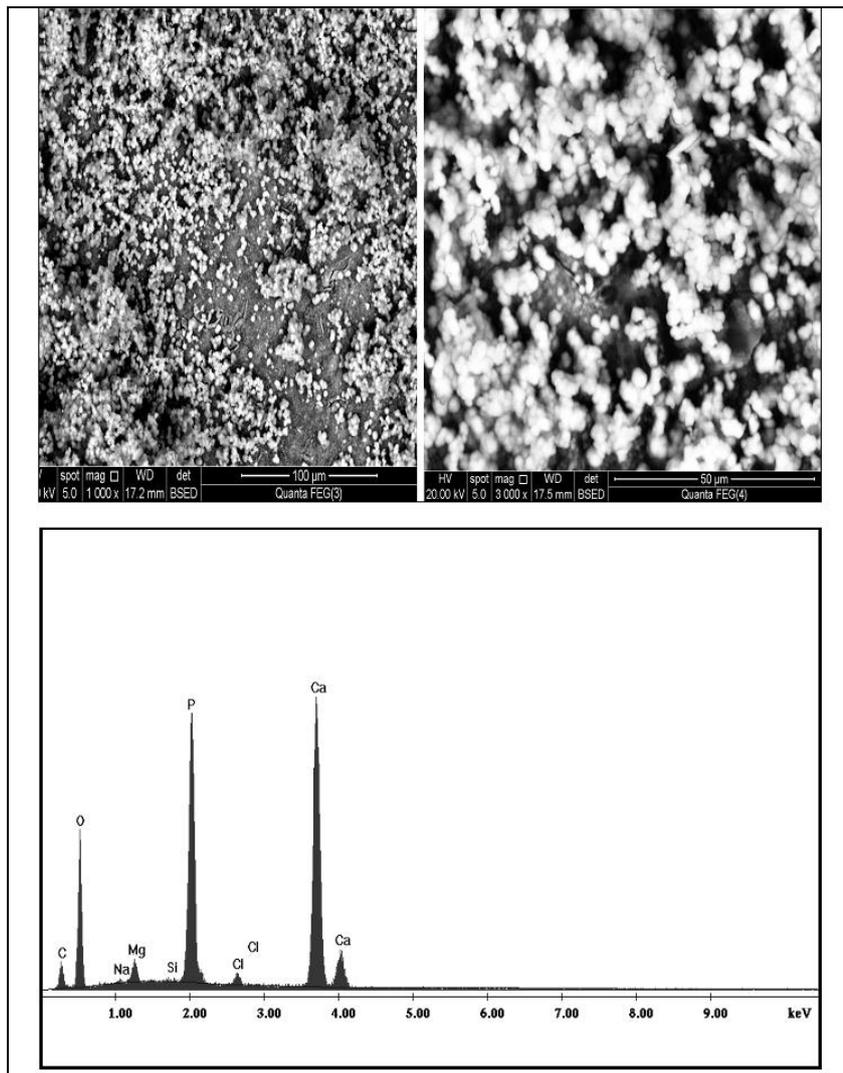
The sol-gel sodium calcium silicate ceramic characteristic bands was reported by El Batal *et al.*<sup>(15)</sup>, these bands are ascribed to: Si-O-Si (b) in the range of  $400\text{--}500\text{ cm}^{-1}$ , Si-O-Si (tetrahedral) at  $733\text{ cm}^{-1}$ , Si-O (stretch) at  $922\text{ cm}^{-1}$ , Si-O-Si (stretch) at  $1042\text{ cm}^{-1}$  and additional band at wavenumbers  $627\text{ cm}^{-1}$  which is due to presence of sodium calcium silicate crystalline phase<sup>(16)</sup>.

A slight shift was noticed for the main peaks of poly (L-lactide) to lower frequency which may be attributed to the interaction between the components of composites.

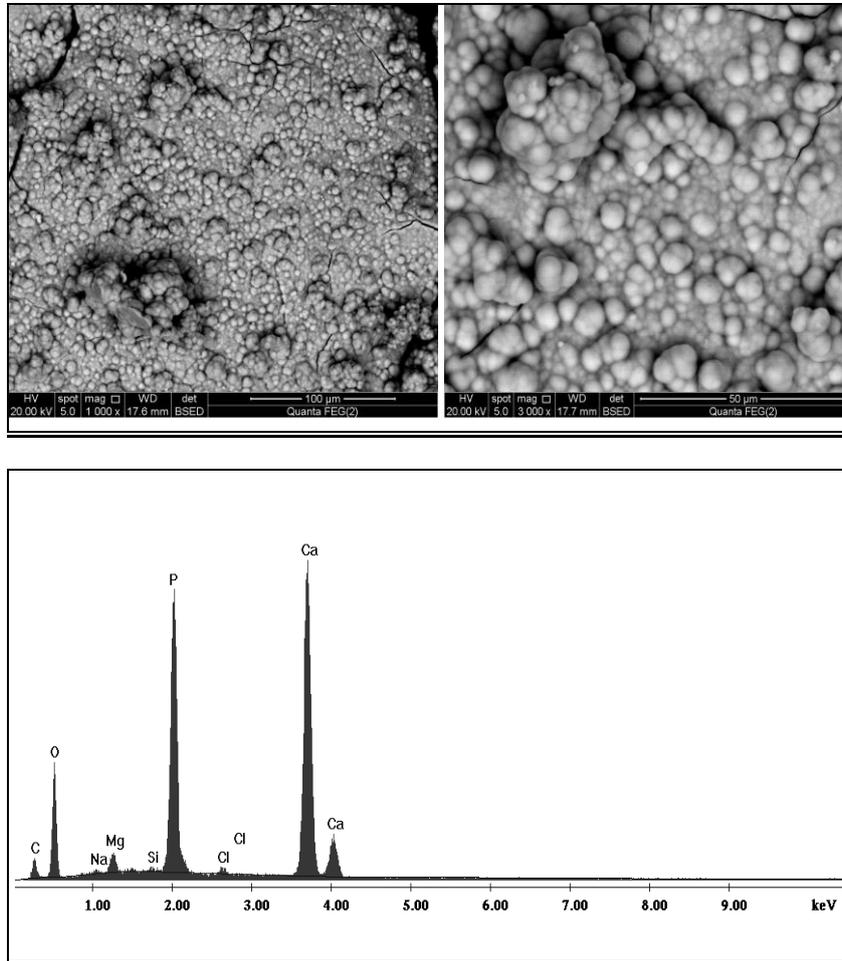
*In vitro* bioactivity evaluation

Scanning electron microscope coupled with energy dispersive X-ray analysis (SEM/EDX)

SEM micrographs of the surface of composite membranes CP20, and CP30 after immersion in the SBF for 15 days are shown in Fig. 6 and 7. The EDX analysis of the sample surfaces is also indicated.



**Fig. 6. SEM micrographs and EDX analysis for CP20 composite for 15 days of immersion in the SBF.**



**Fig. 7.** SEM micrographs and EDX analysis of CP30 composites after immersion in the SBF for 15 days.

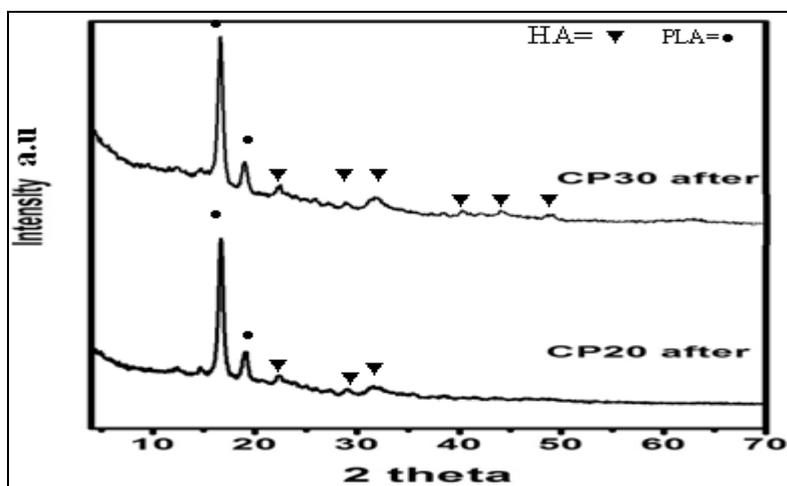
The Figures illustrate that a layer of spherical particles fully covered the surfaces of samples CP20 and CP30. The EDX analysis in these figures suggested that these spherical particles could be calcium-deficient and non-stoichiometric apatite with Ca/P ratio of 1.37 and 1.43 for CP20 and CP30, respectively. This ratio is close to that of natural apatite in bone. Comparing the EDX analyses for both CP20 and CP30, showed that the apatite layer formed on the surface of CP30 was more mineralized than that formed on the surface of CP20. Other studies have reported that the induced apatite layer on the surfaces of different bioactive materials during their incubation in SBF was also calcium-deficient<sup>(12, 17)</sup>.

The formation of the hydroxyapatite layer on the surface of composite membranes, immersed in SBF, could be explained by the hydrolysis of ester bonds of the polymer and the formation of carboxylate groups (COOH). These reactive groups have the ability to attract silica ions released from the scaffolds due to the dissolution of the ceramic particles.

These ions could, in turn, act as nucleation sites for calcium and phosphorus ions, leading to the formation of hydroxyapatite layer on the surfaces of the composites<sup>(11,12)</sup>.

*TF-XRD analysis*

The TF-XRD patterns of the surface of composite membranes (CP20 and CP30) after immersion in the SBF for 15 days is illustrated in Fig.8.



**Fig. 8.** Thin film X-ray analysis of CP20 and CP30 composites after immersion in SBF for 15 days.

The Fig. shows two sharp XRD peaks of the crystalline poly(L-lactide) found at 2θ values of 16.63 and 18.86 corresponding to d-spacing of 5.34 Å and 4.67 Å, respectively.

The figure also clearly indicate the formation of the apatite layer, on the surfaces of the samples, after 15days of immersion in the SBF.

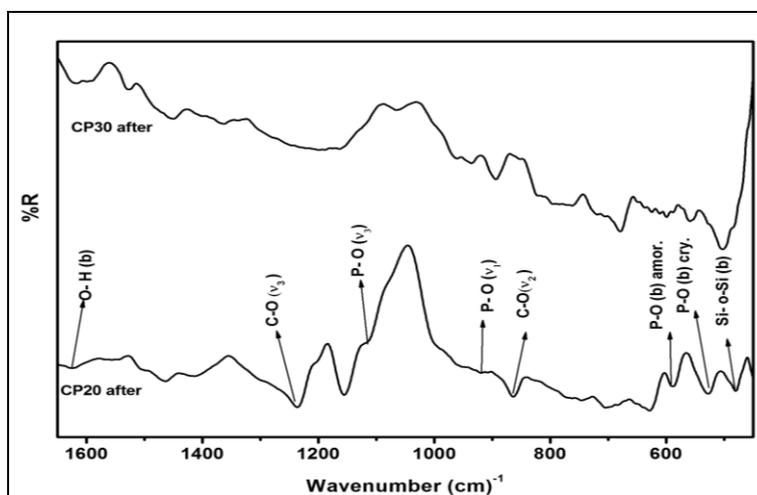
The typical diffraction pattern of the crystalline apatite could be observed, which was associated with an evident peaks at d-spacing values of 2.81 Å , 2.72 Å and 2.78 Å [matched with the corresponding ICSD card no (82-1934)]. Those diffraction peaks became sharper and their intensity increased as the ceramic filler increase, indicating a higher apatite crystallinity. In addition, the appearance of other less intense peaks at d-spacing values of 2.44 Å, 2.28 Å,

1.94 Å, 1.84 Å and 1.72 Å [matched with the corresponding ICSD card no (82-1934)] is also noticed for the composite CP30. Those results further confirmed the apatite formation and crystallization for this composite.

The diffraction peak of the formed apatite at d-spacing value of 8.27 Å was not appeared for the samples, indicating the incomplete crystallization of the apatite layer. It may be observed when immersion time in SBF increased.

#### *FTIR analysis after soaking in SBF*

Figure 9 indicates FTIR spectra of composite membranes CP20 and CP30 after 15 days of SBF immersion.



**Fig. 9.** FTIR spectra of CP20 and CP30 composites after soaking in SBF for 15 days. (R%)= Reflectance%

This Figure shows all the characteristic absorption peaks of hydroxyapatite as illustrated elsewhere<sup>(18-20)</sup>. After SBF treatment new peaks are developed for (P-O Bend- Crystalline) in the range of (500- 560  $\text{cm}^{-1}$ ), (P-O Bend- Amorphous) in the range of ( 560 – 600  $\text{cm}^{-1}$ ), [p-o ( $\nu_1$ )] at 960  $\text{cm}^{-1}$  and [p-o( $\nu_3$ )] in the range of (1020-1120  $\text{cm}^{-1}$ ), also carbonate modes are appeared as [C-O ( $\nu_3$ )] in the range of (1350-1550  $\text{cm}^{-1}$ ), [C - O ( $\nu_2$ )] stretching in the range of (890 - 800  $\text{cm}^{-1}$ ) in addition to O-H (b) in the range of (1630-1650  $\text{cm}^{-1}$ ).

#### **Conclusion**

- The liquid-liquid phase separation used successfully in the fabrication of sodium calcium silicate ceramic/poly(L-lactide) composites. The composites show porous structure.
- Increasing the ceramic concentration from 20 wt.% to 30 wt.% , resulted in a decrease in pore diameter from 70  $\mu\text{m}$  for CP20 to 55  $\mu\text{m}$  for CP30.

- The addition of sodium calcium silicate ceramic to polymer is beneficial in the formation of hydroxyapatite layer. Therefore, it may improve polymer bone bonding ability *in vivo*.

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## تحضير وتوصيف وتقييم النشاط الحيوى خارج الجسم لمركبات حيوية بديلة مكونة من البولى لاكتيد وسيليكات الصوديوم والكالسيوم

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الهدف الرئيسى للدراسة الحالية هو تحضير مركبات مكونة من البولى لاكتيد  
وسيليكات الصوديوم والكالسيوم لاستخدامها فى تطبيقات العظام البديلة، بطريقة  
فصل سائل عن اخر.

تم توصيف المركبات تحت الدراسة باستخدام التقنيات المختلفة مثل حيود  
الأشعة السينية (XRD)، والمجهر الالكترونى الماسح (SEM)، والتحليل التناقل  
الحرارى (TGA)، بالإضافة إلى دراسة طيف الأشعة تحت الحمراء مع تحليل  
فورييه (FTIR). أوضحت هذه الدراسة أنه بزيادة نسبة السيراميك المضاف  
تتناقص قطر مسام العينات، حيث تناقص قطر المسام من ٧٠ إلى ٥٥ ميكرون  
بتناقص نسبة السيراميك من ٢٠ إلى ٣٠ % على الترتيب.

أوضحت كذلك نتائج تقييم النشاط الحيوى خلال العينات تحت الدراسة، أن  
المركبات المحضرة لها القدرة على تكوين طبقة الهيدروكسى اباتيت (بديلة العظام)  
على سطحها، مما يدل على امكانية تطبيقها فى هندسة الأنسجة.