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Preparation and characterization of biodegradable nanocomposites derived from carboxymethyl cellulose and hydroxyapatite

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ABSTRACT

Development of a cost-effective technology for extraction and quantification of carcinogenic and toxic organic materials which are widely used in the industry are critical to humans. Membrane technology received much attention and has already been widely used in this area. In this work, we offer a newly developed bio-based nanocomposite membrane for removal of bisphenol-A (BPA) from water. Three natural components hydroxyapatite (HAp), carboxymethyl cellulose and lysine as a diluent were used for making the bio-based membrane. The membrane was fabricated by two different methods, the conventional casting method and the double decomposition method. Analysis and testing results showed that, membrane produced by the casting method is rough, stiff and partially soluble in water. However, the film made by the double decomposition method is smooth, flexible, and has low water solubility. The physicochemical characteristics of the prepared membranes were determined by Fourier transform infrared spectroscopy (FT-IR) and thermal gravimetric analysis (TGA/DTA). The morphology and components interface were observed by X-ray wide angle (WAXD), scanning electron microscopy (SEM) and electron microscopy (SEM/FEG). The spectral and crystallographic data showed the presence of an effective interaction between hydroxyapatite and CMC plasticized with lysine. Results also show that, the particles size of the composite decrease as the content of CMC increases, with an increase of a 2% by weight of CMC the size increases by 18 ± 3 nm. Produced membrane composite could be classified as tri-functional material: it could be useful for extracting toxic material bisphenol A (BPA) from baby food containers; has antimicrobial and antifungal properties; and a valuable candidate for use in bone tissue engineering.

1. Introduction

During the last decade, there was an increase interest in a man-made biodegradable composite to fill the high demand for functional bone grafts. So far, calcium phosphates hydroxyapatite (HAp) is the most useful material for this purpose due to its biocompatibilities with the mineral phase of the bone tissues (Skrtic, Antonucci, & Eanes, 2003). However, the brittleness and the low degradation rate of this material have limited its use in the field of bone tissue engineering. Recently several studies have been conducted to improve the physicochemical and mechanical properties of HAp by converting it to a composite. In one study, a composite with improved characteristics was made from HAp and chitosan (Cs) (Bala'zsi, Bishop, & Yang, 2009; Chen, Chen, & Lai, 2012; Danilchenko, Kalinkevich, & Pogorelov, 2011). Lately, it was found that, this type of composites (HAp/Cs) has poor interface bonding between HAp and Cs which lead to a composite with poor plasticity and mechanical property. In another study, a tri-component composite based on HAp/CMC (carboxymethyl cellulose)/Cs was prepared and evaluated in the field of bone tissue engineering. The presence of CMC improved the interface between HAp and Cs, as

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a result of that, a composite with enhanced mechanical, chemical and biological properties was obtained (Alge, Goebel et al., 2012).

In this work, we seek to avoid the use of conventional materials and replace them with new bioactive materials to prepare biopolymer-based nanocomposites that could be suitable inducing bone growth and for removing toxic chemicals from wastewater. The materials chosen for this purpose were carboxymethyl cellulose, HAp and lysine.

Biodegradable nanocomposite films are generally made from nano-materials uniformly dispersed in a biopolymer matrix such as protein, lipid and polysaccharides (Kushwaha, Avadhani, & Singh, 2014; Kushwaha, Ver Avadhani, Tomer, & Singh, 2014). These bio-nanocomposite materials are considered a prospective replacement to the synthetic polymers. They are widely used in medicine, drug delivery, tissue culture, implants, alleviation of environmental pollution, food preservation, and antimicrobial food packaging.

As stated before, the compositions of hydroxyapatite are similar to that of the mineral phase of bone, it also has other useful properties (Azzaoui, Lamhamdi, et al., 2013; Azzaoui, Berrabah, et al., 2013; Lamhamdi, Azzaoui, Mejdoubi, Garoiz, et al., 2014; Lamhamdi, Azzaoui, Mejdoubi, Hammouti, et al., 2014; Azzaoui et al., 2014).

Carboxymethyl cellulose (CMC) is an anionic, water-soluble cellulose derivative obtained by introducing $-CH_2COOH$ groups into cellulose molecular chain. It has received much attention due to its unique properties such as water solubility, transparency, hydrophilicity, non-toxicity, biocompatibility, biodegradability and good film forming ability. It has been widely used in critical medical applications such as drug delivery and in other applications such as textile printing, paper industry, detergents, food, and oil good drilling (Yang & Zhu, 2007).

Lysine was chosen as a plasticizing agent in this work. Lysine is an essential amino acid that helps the growth of bones in the formation of collagen and antibodies. It's been used as a plasticizer for CMC to improve its flexibility and plasticity.

In this work, a composite based on hydroxyapatite (HAp), carboxymethyl cellulose and lysine was prepared and evaluated by various spectroscopic and analytical techniques. The HAp/CMC/lysine type of composites have valuable properties such as biocompatibility, bioactivity, osteo-conductivity, and is made from environmentally friendly materials. The target nanocomposites membrane was prepared by two different methods for a comparison purpose: The casting and the double decomposition methods. The casting method is known to produce a composite membrane that is fragile and easy to break. While the other process is known to produce membrane that is transparent, soft and flexible.

The extraction efficiency of the developed membranes for bisphenol A (BPA) was examined. It was used as a based stationary phase (TFME) for extracting BPA from baby food matrix. Quantification of the extracted BPA by the developed membrane was carried out by a unique chromatographic method that was developed at our laboratory. The developed chromatographic method is convenient and easy to use for routine practice. Bisphenol A is an organic compound that is toxic and carcinogenic. It is widely used in the industry, the main applications for it is as a precursor for making epoxy resins and in manufacturing of plastic bottles.

2. Experimental

2.1. Material and methods

Hydroxyapatite (HAp) and carboxymethyl cellulose (CMC) were synthesized at our laboratory using reported procedures (El Barkany, El Idrissi, Ouslimane, & Amhamdi, 2009; Qi, Lina, Ming, Xiaojun, & Gongzhen, 2005). Calcium nitrate $Ca(NO_3)_2{\cdot}4H_2O$ (99%), ammonium hydrogen phosphate $(NH_4)_2{\cdot}HPO_4$ (99%), dimethylformamide (DMF, polyethylene glycol (PEG-MW1000) were purchased from Sigma Aldrich and used as received without any further purification. High purity distilled water was used in all runs.

2.1.1. Preparation of standards and samples solutions

A standard solution of BPA (100 ppm, S0) was prepared by dissolving 10.0 mg of BPA in a 100 g water at pH = 3.0. Diluted solutions S1 and S2 of BPA were prepared from S0 solution with 10 and 1 pm, respectively. Membranes of HAp/CMCp/lysine composites before use were washed with deionized water, absolute ethanol and dried at 100 °C for 10 min. The extraction and desorption procedures were performed as follow: two of the prepared membranes were each immersed in 50 ml of each solutions S1 and S2, the membranes and the solution were stirred from 5 min. The membranes were then withdrawn from the solutions and dried at 40 °C for 40 min. Then, they were placed in centrifuge tubes (1.5 ml) containing 1.0 mL of dry tetrahydrofuran and reacted with the derivatizing reagent TMCS-BSTFA 1% (200 μl) under ultrasound at 70 °C for 30 min. The reaction product (10.0 μL) was injected directly into the GC/MS chromatograph. The analysis was carried out in triplicate (Azzaoui et al., 2015).

2.2. Characterization techniques

2.2.1. FT-IR measurements

Samples of the prepared composites were characterized by Fourier transform infrared spectroscopy (FT-IR) using a Schimadzu 300 series FT-IR (Shimadzu Scientific Instruments). Spectra were acquired over the range 400–4000 cm^{-1} for samples in pellet form prepared by mixing 1.0 mg of powder samples with 200.0 mg KBr spectroscopic grade.

2.2.2. Morphological studies

Emission-scanning electron microscopy (SEM/FEG) was used to study the composites morphology and composites components interface. It was carried out using a SU 8020, 3.0 KV SE (U). Before the analysis specimens were frozen with liquid nitrogen, fractured, mounted, coated with gold/palladium and observed using an applied tension of 10 kV.

2.2.3. Thermogravimetric analysis

Standard thermogravimetric analysis was performed on the composites using the TGA Q500 and Q50 TA instrument at a temperature range from 20–900 °C and a heating rate of 10 °C/min. The Differential Scanning Calorimeter (DSC) Q2000 V24.4 Build 116 from TA instrument was used for DS analysis. A 10.0 mg sample was sealed in aluminum DSC pan and placed in the DSC cell. The apparatus Gas chromatography/mass spectrometry (GC/MS) used in this work was GC/MS-QP2010 (Shimadzu, Japan) equipped with a solution 2.5 software from a Shimadzu.

2.2.4. Gas chromatography–mass spectrometry (GC/MS) analysis

GC/MS analysis was performed on a Shimadzu GCMS-QP2010 (Shimadzu, Germany) with GC-MS solution 2.5 software, which features a newly designed data processing platform that achieves maximum scan speeds of 20,000 μ/s and also features an advanced scanning speed protocol (ASSPTM), which realizes higher sensitivity at high scan speed.

A TFME-GC/MS [(Thin film micro extraction)-GC-MS] method was used in the analysis of bisphenol-A extracted from various samples of food matrices. The method was developed at our laboratory and could be crucial for controlling the concentration of BPA and in the identification of its degradation products. Thin film micro extraction (TFME) combined with GC/MS method is simple, fast,

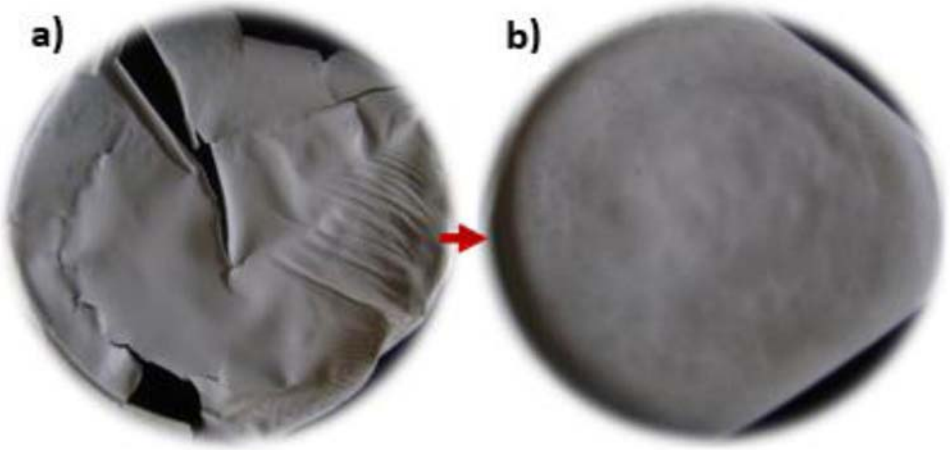

Fig. 1. Photographs of membranes CMC_p/HAp/lysine obtained in the absence (a) and presence (b) of lysine.

reliable and solvents are not needed to analyze extraction efficiency of CMCp/HAp/lysine composite for BPA in water. Membrane SPME, also known as thin-film micro-extraction (TFME) is a relatively new avenue in micro-extraction techniques that has been successfully used in GC/MS based applications.

Commercially available membranes developed for use in standard SPME fibers all have similar components, including polydimethylsiloxane (PDMS), polydivinylbenzene (DVB), and carboxen (Jonathan, Ezel, & Janusz, 2015).

Extraction of BPA by florisil was used as a control in this study.

2.2.5. Antibacterial and antifungal test

The antimicrobial activities of the HAp/CMCp/lysine membranes were evaluated using the disc diffusion method and the susceptibility test of NCCLS (National Committee for Clinical Laboratory Standards). Both tests are recommended by the WHO and the French standard NF-U-47-107 AFNOR 2004.

The microorganisms used in this study were two gram positives *Bacillus subtilis and Micrococcus luteus* and a gram negative *E. coli.* The fungus used in this study was Candida (*Candida albicans*) (see Annex S1).

The disc diffusion method was carried out according to a standard method reported by Bauer, Kirby, Serris, and Turck (1966). A bacteria culture standard with 0.5 McFarland was used to lawn Muller Hinton agar plates evenly using a sterile swab. The plates were left at room temperature 15 min, then used for the sensitivity test. Each test plate comprises of six discs, one positive control (a standard the commercial antibiotic disc tetracycline 1.0 mg/ml), one negative control (DMSO), and four discs with membranes with various compositions. The six discs were placed about equidistance from each other. The plates were then incubated depending on the species of bacteria used in the test at 37 °C for *Micrococcus luteus* and *E. coil* and at 33 °C for *Bacillus subtilis* for 18 to 24 h. The plate with fungi Candida albicans containing PDA was incubated at 37 °C for 48 h. Cycloheximide was used as antifungal control. After incubation, the plates were examined for inhibition zone. The inhibition zone was then measured using calipers and recorded. The tests were done in triplicates to ensure reliability.

2.3. Preparation of calcium phosphate/carboxymethyl cellulose/lysine composite

This section consists of three parts: The first part covers the lamination of pure carboxymethyl cellulose (CMC). The second part shows the synthesis of HAp and third part is devoted to the synthesis of calcium phosphate/carboxymethyl cellulose/*lysine* composites (Hasan & Nurhan, 2004).

2.3.1. Preparation of plasticized CMC

A sample of CMC powder (10.0 g) was mixed with a suitable amount of lysine in 50 mL of water. The mixture was stirred magnetically until a clear solution was obtained. The product was precipitated from the clear solution by the addition of a 100 mL ethanol. The dissolution/precipitation procedure was repeated three times to remove all impurities. Produced plasticized carboxymethyl cellulose (CMC_P) was left to dry at 50 °C for 12 h. A summary of the process of making CMC_{Pis} shown in Annex S2 and S3.

2.3.2. Synthesis of HAp powder

The HAp powder was prepared by a wet chemical method using $Ca(NO_3)_2{,}4H_2O$ and $(NH_4)_2HPO_4$ as Ca and P precursors, respectively. A solution of $(NH_4)_2HPO_4$ (11.93 g) in 50 mL water was added dropwise to an aqueous solution of $Ca(NO_3)_2{\cdot}4H_2O$ (4.09 g/50 mL) with stirring. In all runs the pH of $Ca(NO_3)_2{,}4H_2O$ solution was adjusted to 10.5 by ammonium hydroxide and the solution temperature was maintained at 85.0 °C during the addition. A know amount of lysine was added to the mixture then it was cooled down to room temperature. Precipitated particles of HAp were aged for 24 h at room temperature and then calcined at 900 °C.

2.3.3. Synthesis of CMC_p/HAp/lysine membrane

2.3.3.1. Casting method. Solution A: In a three-necked round bottom flask (100 mL) a known weight of CMC_p was dissolved in a 50 mL of distilled water by stirring at 50 °C for 30 min as shown in Annex S3.

Solution B: A known weight of hydroxyapatite powder treated with lysine was dispersed in 5 mL DMF, and added to solution A, followed with a 1 mL of glycerol. Produced mixture was stirred at 40 °C for 24. Several solutions with various compositions were prepared as above, a summary of the amounts of reagents used to make the solutions are shown in Annex S4. The obtained clear solution was casted into a petri dish, the temperature of the solution in the petri dish was raised to 50 °C at a rate of 2 °C/min and maintaining at 50 °C for 2 h, after cooling back to room temperature a thin clear film was obtained. The film was collected, washed with absolute ethanol to remove any possible impurities or unreacted materials and air dried at 37 °C. A photo image of the obtained membrane is shown in Fig. 1.

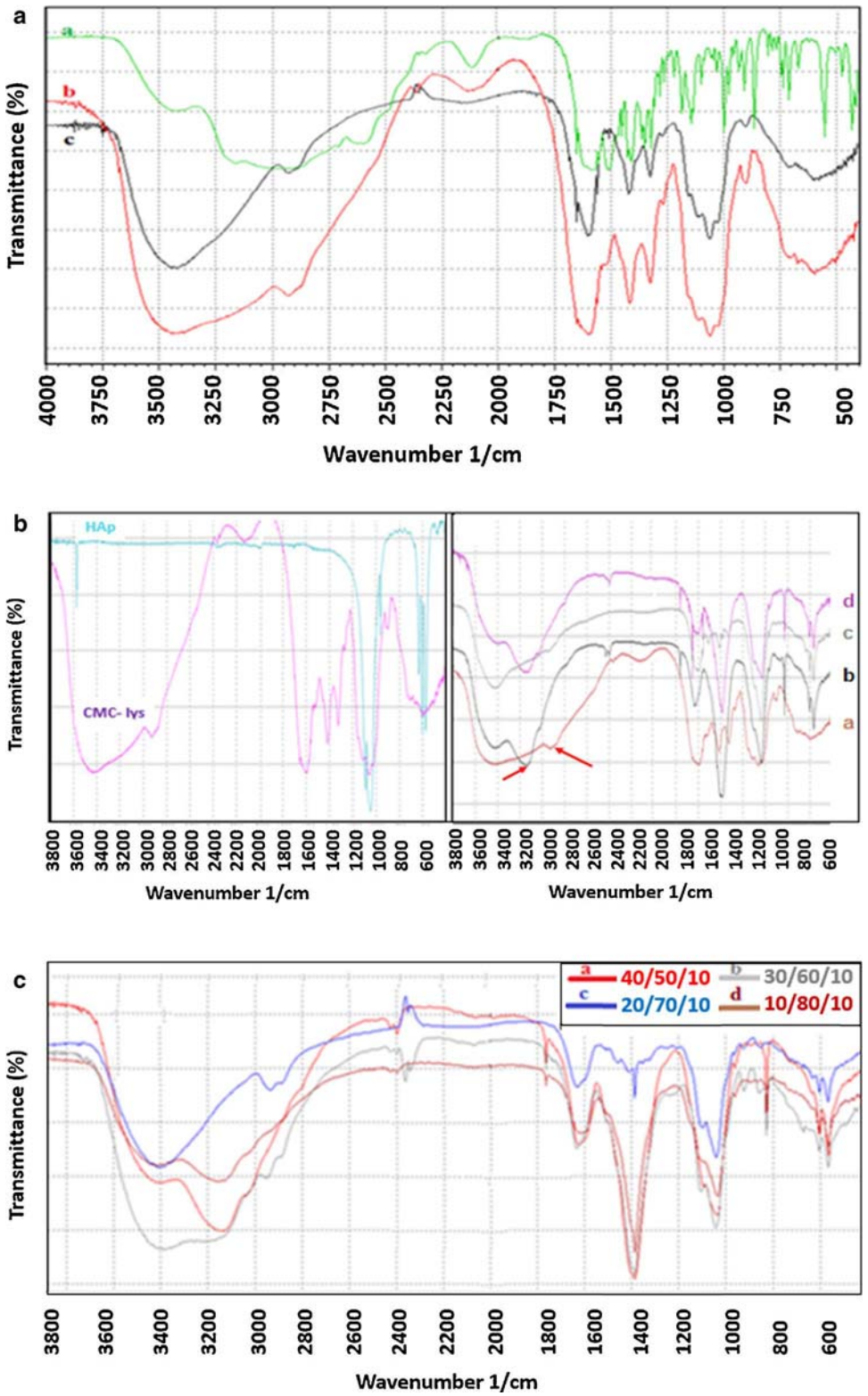

Fig. 2. (a) FT-IR of *a* = L-lysine, *b* = CMC_P and *c* = CMC. (b) FT-IR spectra of HAp, CMC-lysine three composites of CMC/HAp/lysine with various mass ratios: *a* = 100/00/00, *b* = 40/50/10, *c* = 30/60/10 and *d* = 20/70/10. (c) FT-IR spectra of four composites of CMC_P/HAp/lysine.

Fig. 1shows images of the membranes in the absence and presence of lysine. The membrane obtained in the absence of lysine (left) is less ductile and more brittle. In contrast, the membrane obtained in the presence of lysine (right) is flexible and smooth.

2.3.3.2. Double decomposition method. Several CMC_P/HAp/lysine composites were prepared using various ratios of the three components as shown in Annex S5. Aqueous solutions of the three components were prepared at room temperature as follow: A solution of $Ca(NO_3)_2{\cdot}4H_2O$ in water (60 mL) with a known con-

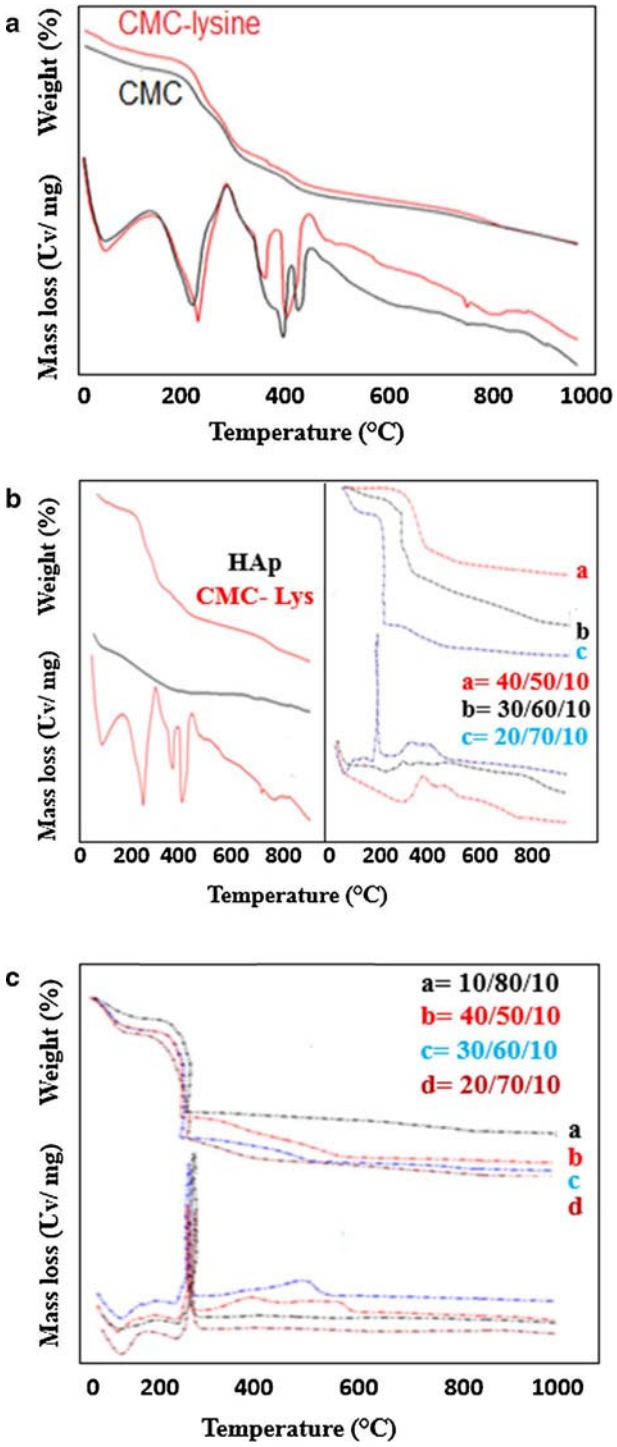

Fig. 3. (a) TGA and DTA curves of CMC and CMC plasticized by lysine. (b) ATGA/DTA curves of CMC-lysine, HAp and CMC_p/HAp lysine composites with various weight ratios. (c) TGA and DTA spectra of four composites CMC_p/HAp/lysine with various mass ratios.

centration was prepared. To it was added a solution of know concentration of CMCp in a 30 mL water. To the produced mixture was added a third solution containing known amount of $(NH_4)_2HPO_4$ dissolved in a 100 mL water over a period of 24 h. In all prepared composites, the amount of reagents used affords a Ca/P molar ratio of about 1.67. The pH of the solutions reached about 10.5 during the mixing. The resultant solution was poured into a petri dish and heated at 50 °C for 1.5 h to produce a film. The film was collected washed with absolute ethanol to remove any

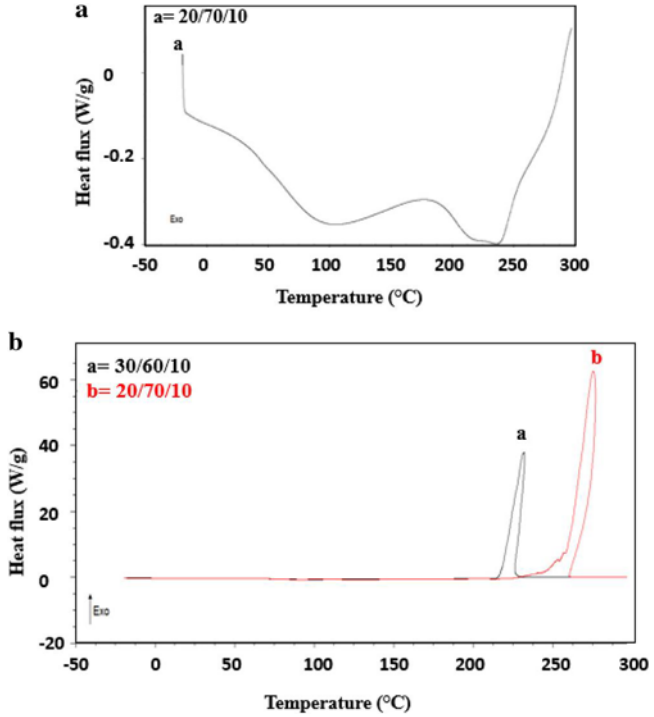

Fig. 4. (a) DSC thermogram of the composite CMC_p/HAp/lysine. (b) DSC thermograms DSC recorded during the first heating cycle of the composites.

possible impurities and air dried at 37 °C. The prepared films are summarized in Annex S6.

3. Results and discussion

3.1. FT-IR analysis

3.1.1. FT-IR spectra of lysine, CMC and CMC_P

The FT-IR spectra of lysine, CMC and CMCp are shown in Fig. 2a. The IR spectrum of CMC shows a broad band centered at 3450 cm^{-1} corresponds to the stretching vibrations of the O–H bond of carboxyl and hydroxyl groups. The band at 3450 cm^{-1} may include OH groups from absorbed water (Biswal & Singh, 2004) and ethanol. A band also appears at 2928 cm^{-1} which could be related to a C–H stretching bond (Lii, Tomasik, Zaleska, & Liaw, 2002). The FT-IR spectrum of CMC also shows two bands at 1600 and 1415 cm^{-1}, the bands are attributed to the symmetric and anti-symmetric vibration COO^- (carboxylate group), respectively (Adinugraha & Marseno, 2005). The bands at about 1420 and 1320 cm^{-1} are due to the stretching elongation of CH_2 bonds and OH bending vibration. A band also shows at 1060 cm^{-1} which correspond to CH–O–CH_2 stretching (Biswal & Singh, 2004).

The main characteristics of the FT-IR of CMC_p are a broad band at 3670–2600 cm^{-1}, the band could have attributed to the stretching vibration of O–H of carboxyl (CMC) and NH of NH_3^+ (lysine). A stretching intense band at 1800–1550 cm^{-1} corresponding to C=O of the carboxyl groups in lysine and CMC. Other bands appear in the FT-IR spectrum are related to CH, C–N, C–O, C–CO and other bonds in CMC and lysine. The FT-IR of CMC_P shows clearly the presence of two compounds CMC and lysine. By comparing the two IR spectra of CMC and CMCp, there is a clear shift in frequencies of certain bands like in the frequency of the O–H group (Fig. 2a), this could be attributed to the presence of an interaction between the functional groups of the two compounds in CMCp.

3.1.2. FT-IR of a membrane prepared by the casting method

The FT-IR spectra of CMC_p and membranes with various compositions are shown in Fig. 2b. The FT-IR spectrum of HAp shows clearly a sharp medium peak at 3580 cm^{-1} corresponds to the O–H Stretching, two strong bands between 1100 and 1000 cm^{-1} which could be are related to PO_4^- and three sharp bands between 620 and 580 cm^{-1} are attributed to OH and PO_4^-. Comparing the spectrum of CMC_p/HAp/lysine composite with that of CMCp, it can be seen that the addition of HAp to CMCp resulted in a red-shift in the OH and C=O bands, and the intensity of these two main peaks of CMC_p/HAp/lysine composite decreased obviously. Two new peaks were also detected at 1615 cm^{-1} (weak) and 1415 cm^{-1} (Stretching) due to the asymmetric and symmetric stretching modes of the –COO– groups. The results reveal that there is a certain degree of interaction between the HAp particles and CMCp. The interaction between the composite components is mainly related to H-bonding among OH, COOH, and NH and Metal–ligand complexation between carboxyl, amine, and hydroxyl from one side and HAp metals for the other side (Koutsopoulos, 2002; Sukhodub et al., 2004).

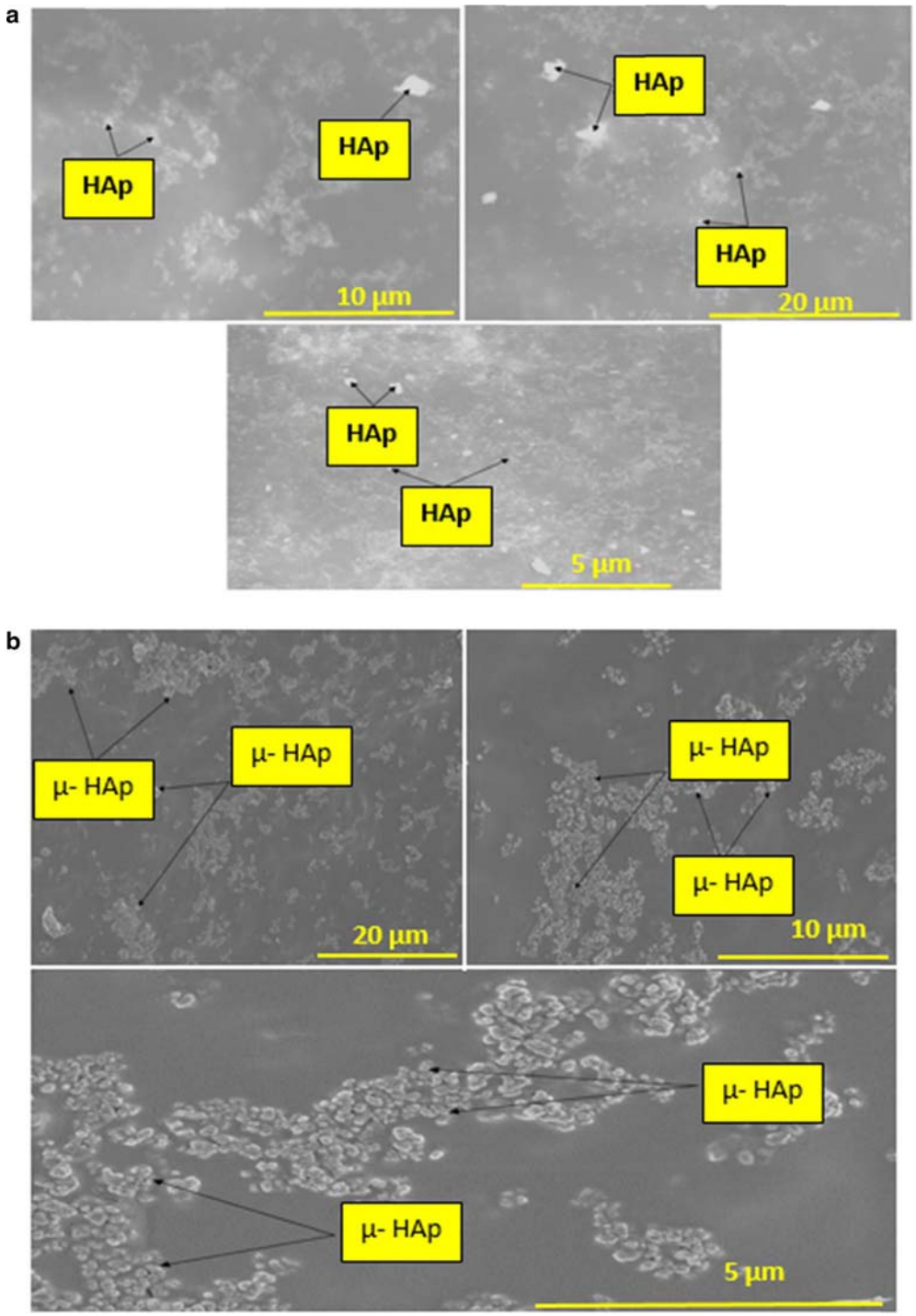

Fig. 5. (a) SEM images of CMC_p/HAp/lysine composite. (b) Analysis by SEM-FEG composites CMC_p/HAp_t/lysine. (c) SEM-FEG images of composites CMC_p/HAp/lysine.

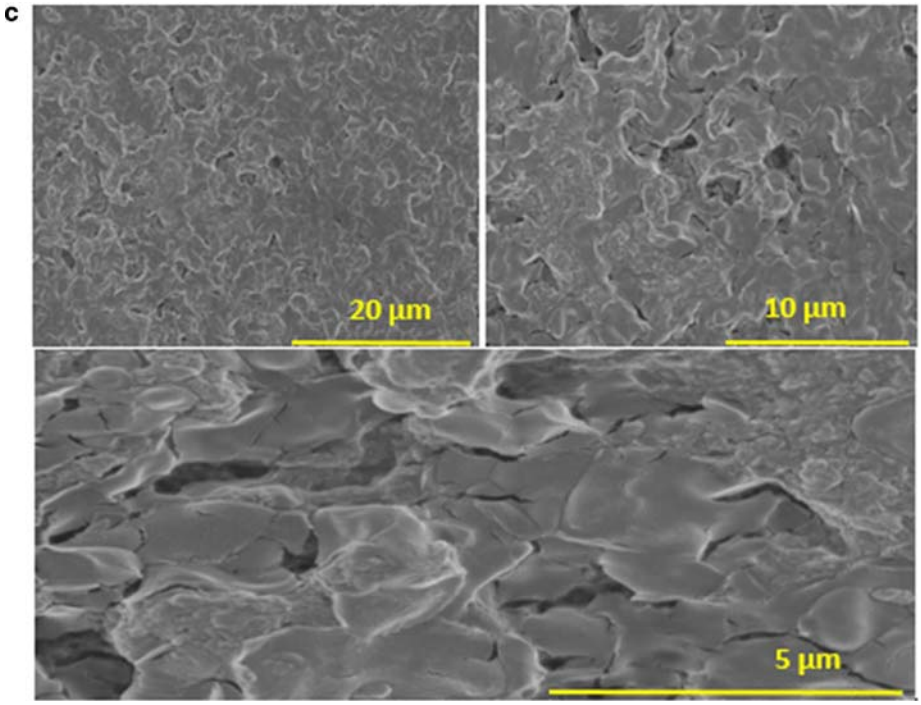

Fig. 5. (*Continued*)

3.1.3. FT-IR of a membrane prepared by the double decomposition method

The FT-IR spectra of four membranes with various mass ratios prepared by the double decomposition method are shown in Fig. 2c. The main characteristics bands in the FT-IR spectra (Fig. 2c) are related to CMC_p. The spectra show stretching vibration bands that are broad and strong in the range of 3670–2600 cm^{-1}, the bands could be attributed to the OH of carboxyl groups in CMCp. Stretching intense bands appear at 1650 cm^{-1} corresponding to C=O of the carboxyl groups in CMCp. Other bands appear in the FT-IR spectrum correspond to CH, C–O, C–CO and other bonds present in CMCp. The peaks appear in the FF-IR spectrum of CMC_p/HAp/lysine composite prepared by the double decomposition method are almost similar to those appear in the FF-IR spectrum of the one prepared by the casting method (Fig. 2b) with more noticeable shifts in the IR bands.

3.2. Thermogravimetric analysis

3.2.1. Thermgravimetric analysis of CMC, CMCp, HAp

Thermal analysis was conducted on CMC, CMC_p and HAp. Obtained TGA–DTA curves are shown in Fig. 3. No transition points were observed in the TGA of HAp (Fig. 3a) up to a 1000 °C. The TGA curve of CMCp shows five transition temperatures points at 100, 172, 245 °C, 324.11 °C and 765.4 °C. The results were confirmed by DTA shown in Fig. 3a. The first transition point occurred at a 100 °C which could be related to the complete loss of water molecules. The second point at 170 °C, could be attributed to the oxidation of the OH groups on the polymer chains as was described by Proniewicz et al. (Lojewski et al., 2005). The results also show that, the rate of weight loss in HAp increased by increasing the temperature. The highest rate of weight loss occurred between 240 °C and 278.5 °C and thereafter decreases and attained a constant rate. Almost 55% of CMCp weight was lost observed at temperature lower than 375.0 C. The polymer decomposition occurred at 250 °C and the maximum degradation occurred at 286.5 °C. The results were confirmed by the presence of an exothermic curve in DTA at 290.2 °C. The maximum loss could be related decarboxylation o COO^- and loss of CO_2 (Biswal & Singh, 2004).

It was also noted that, the CMC_p has slightly higher thermally stability than CMC. There is some blue shift in the transition points as shown in the overlaid curves. Thus, it could be concluded that the lamination of the polysaccharide backbone slightly improves its thermal stability.

3.2.2. Thermgravimetric analysis of composites prepared by the casting method

Thermal analysis was also conducted on of CMC_p/HAp/lysine composites with various mass ratios. Obtained thermal diagrams are shown in Fig. 3b. The degradation of CMCp in the composite shifted to a higher temperature as the composite contents of HAP increases. The results indicate the presence of an interaction between CMC_p and HAp that occurred via H-bonding and coordination to carboxyl group as was shown by FT-IR. The rate of weight loss increased by increasing the temperature. The highest rate of weight loss occurred at 390.0 °C for the composite with 40% HAp which could be attributed to d elimination of CO_2 molecule from the polymeric backbone. The results were confirmed by the presence of an exothermic peak in the DTA curve at 395 °C. The blue shift in the maximum temperature of the weight loss indicate that the composite is more stable than CMC_p alone.

3.2.3. Thermal analysis of composites prepared by the double decomposition method.

Thermal analysis was conducted on four CMCp/HAp/lysine composites with various weight ratios. Obtained TGA–DTA curves are demonstrated in Fig. 3c. The TGA graphs of the composites of high content of CMC_p show that, the composites go through at least five transition temperatures points at 100, 190, 245, 380.1 and 560.2 °C, the number of the transition points varies from one composite to another. The results were confirmed by DTA shown also in Fig. 3c. The degradation of organic part of the composites CMCp starts at about 191.0 °C. As shown in Fig. 3c, the weight loss decreases as the content of HAp increases, the composite that contains 40% CMC

showed the highest weight loss, which could be attributed to the decarboxylation and to the bond (O–C–O) decomposition in the polymer backbone. The composite with the highest content of HAp and lowest content of CMC showed the highest stability, since as shown in the DTA and TGA graphs it showed the lowest number of transition points. Again, the results indicate that HAp stabilizes CMC_p by both coordination to carboxyl group and H-bonding as was shown by FT-IR.

3.3. DSC analysis

The thermal images of the composites prepared by the casting method are shown in Fig. 4a. The melting temperatures and enthalpies of the reactions are summarized in Annex S7.

The thermal properties of the composites were determined by DSC analysis, results are shown in Fig. 4a. As shown in Fig. 4a, the composite endothermic peak observed at 240 °C could be due to the de-polymerization of CMC.

Thermograms obtained by DSC for composites prepared by the double decomposition method are shown in Fig. 4b. The composite melting temperatures were observed at about 220 for composite with a weight ratio of CMC_p/HAp/lysine is about 30/60/10 and 260 °C for the weight ratio of 20/70/10. An increase in the melting temperature was observed, when the percentage of hydroxyapatite increases. This result is in agreement with the results obtained by TGA/DTA.

Enthalpies values of the composite are shown in (Annex S8).

3.4. Scanning electron microscope analysis (SEM) by the casting method

Obtained SEM images of CMCp/HAp/lysine composites are shown in Fig. 5a. It is evident from images that; HAp particles are uniformly distributed in the composites matrices. The images also show the presence of few agglomerations of crystals in CMC matrix obtained by the blend method. Therefore, HAp particles can yet keep general nano effects in the nanocomposites. Although it can't be concluded from the images the type of bonding interface between the two phases. But, the uniform contact interface and the presence of few agglomerations of crystals could be an indication that, both physical chemical bonding are presents between the components composites.

FEG-SEM analysis results of composite CMC_p/HAp/lysine obtained by the double decomposition method (Fig. 5b) show the presence of nano particles with various sizes, the highest is about 600 nm. The morphology images (Fig. 5c) of the same composite show a homogenous distribution of HAp in the composite matrix

3.5. A process of composite formation by the casting method

A summary of the method followed in making a CMC_p/HAp/lysine composites is summarized in Annex S9. The method involved four steps. It starts with the dissolution of the organic matrix in an appropriate solvent, in the second step a homogenize solution of HAp in DMF is prepared, then HAp is precipitated on CMC_p to produce a film of CMC_p/HAp/lysine composite.

3.6. Process of composite formation by the double decomposition method

The process of CMC_p/HAp/lysine composite formation by the double decomposition method is shown in Annex S10. The process involved four steps. The first step involved the dissolution of the organic substrates in water, the diffusion of water through the organic and the inorganic matrices, links formed between reagents

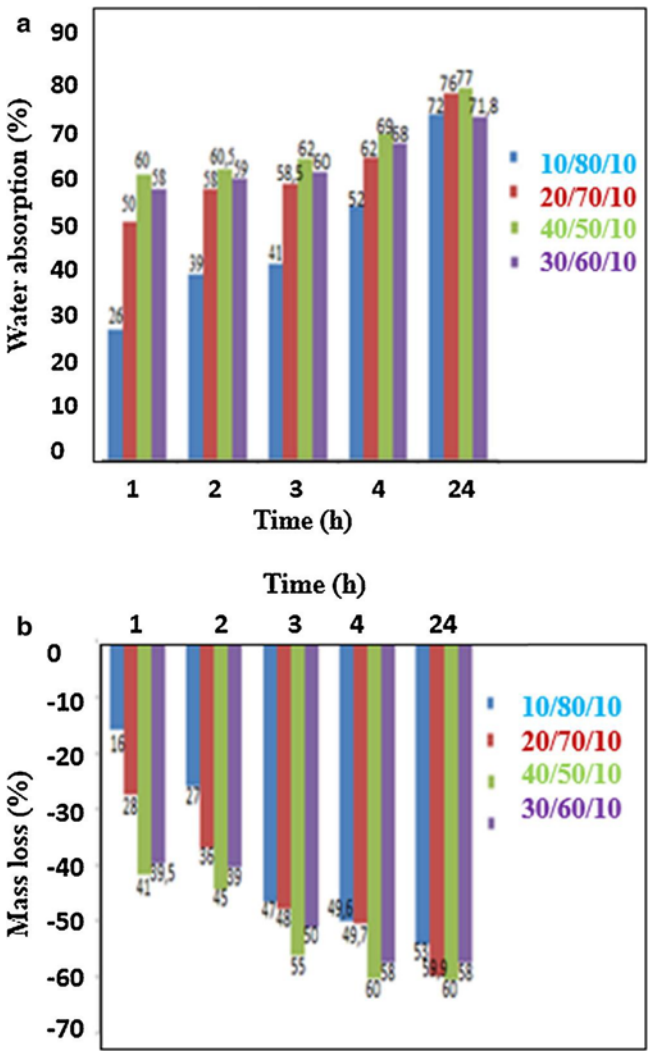

Fig. 6. (a) Swelling behavior of various composites. (b) Biodegradability of composites in PBS.

functional groups, and finally the formation of the composites of CMC_p/HAp//lysine.

3.7. Film transparency

Results show that, the optical properties of the composite CMC_p/HAp/lysine are controlled by the method of synthesis. Annex S11 shows photographs of CMC_p/HAp/lysine films prepared by the above mentioned two methods. As shown in the figure, membrane synthesized by the casting method is thick. In contrast, the membrane prepared by the double decomposition method is thin and transparent (images a and b).

Transparent membranes prepared by the double decomposition method indicate that, this method produces a better dispersion of the apatite grains and the inorganic phase in the organic matrix.

3.8. The swelling behavior of the composites membranes

The swelling behavior of several membrane of CMC_p/HAp/lysine in water was evaluated. Sample of the membranes were immersed in water for 24 h, the selling behavior was monitored during this period of time. The results are summarized in Fig. 6a.

As shown in Fig. 6a, after immersion for 24 h, a membrane with a mass ratio of 10/80/10 swelled by 72% of its weight. Membrane

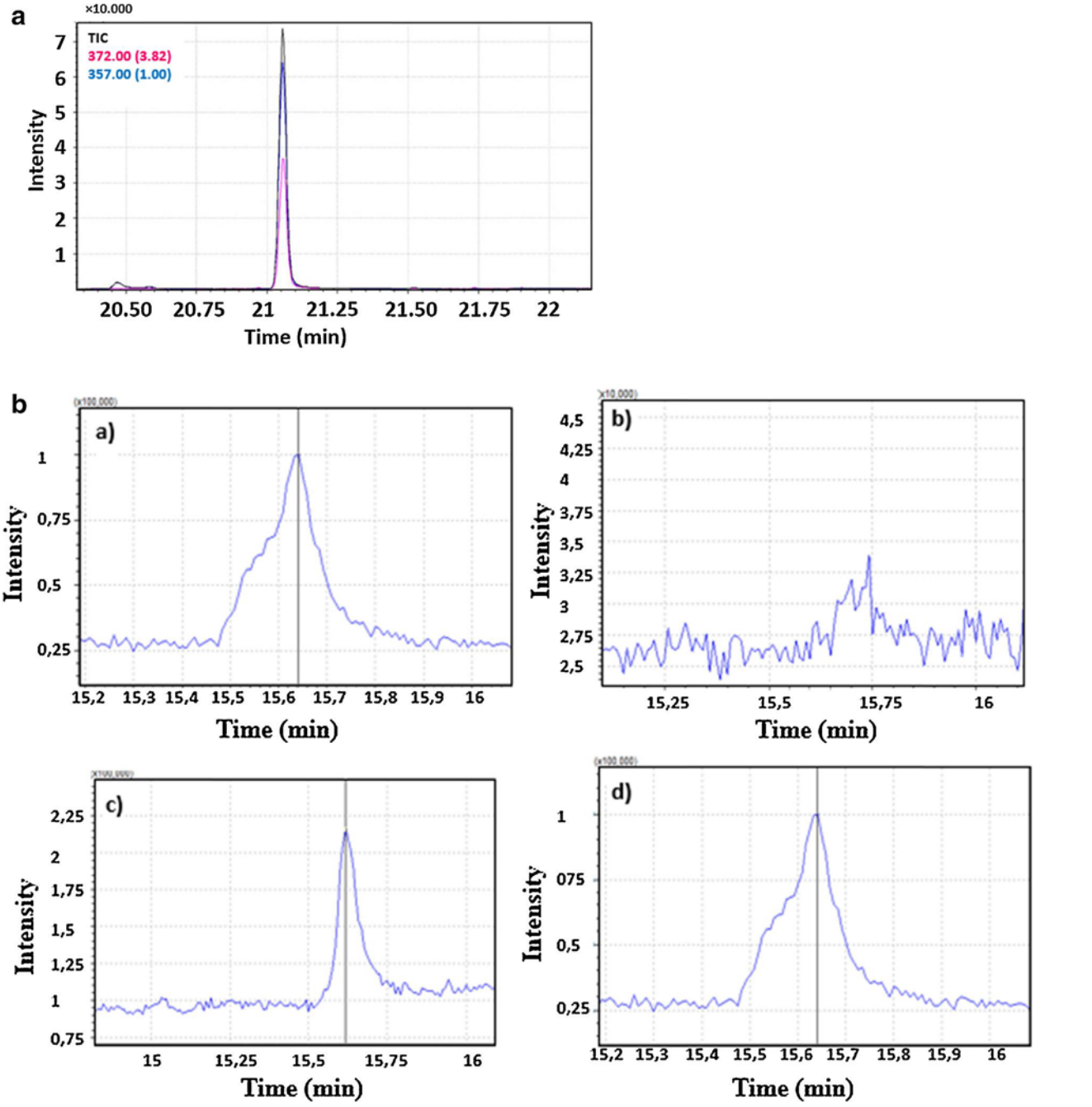

Fig. 7. (a) Chromatography of BPA extracted with commercial florisil. (b) Chromatography BPA desorbed from composite plasticized CMC_p/HAp/lysine with respectively equal molar ratios: *a* = 40/50/10, *b* = 20/70/10, *c* = 30/60/10, *d* = 10/80/10.

with 20:70:10 showed a slightly higher swelling of about 76%, and the membrane with a mass ratio of 40:50:10 present swelled by about 77%.

The results also showed that, samples with higher contents of HAp showed higher rate of absorbency during the first few hours of immersion in water. However, after 24 h, all composites swelled almost the same amount of water.

3.9. Mass loss evaluation

The mass loss of the composites CMC_p/HAp/lysine was determined by immersion of a known weight of the composite in a phosphate-buffered saline (PBS) solution with a pH of 6.4. The results showed that the mass loss increased by increasing the immersion time. After one hour of immersion, the mass loss was about 41% for the composite with the mass ratio of 40/50/10. After 4 h, the mass loss increased to about 60% (Fig. 6b). No mass loss was detected after 4 h of immersion.

The results also show that, the mass loss of the membrane with higher content of HAp was lower. Membrane with high content of HAp produced by casting method become rough and porous after immersion in PBS. An indication that, the membrane is going through some dissolution and swelling.

Membrane produced by the double decomposition method (Annex S12) was more resistant to swelling and degradation, which could be related to the crosslinking reaction occurred between the composite main components HAp and CMC_p.

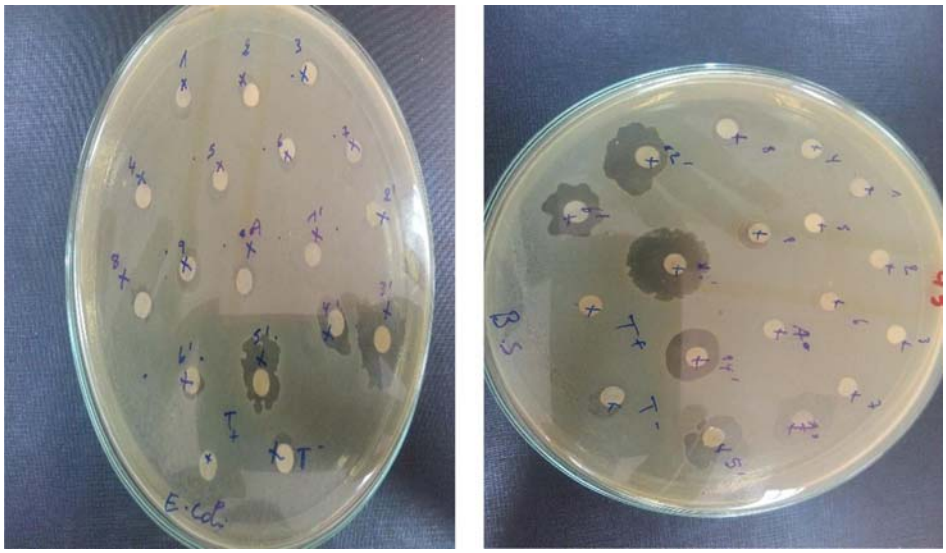

Fig. 8. Sensitivity test in agar media (*D*: diameter of inhibition in mm).

So, it can be concluded that the method of synthesis has some effect on membrane stability. Membrane prepared by double decomposition has higher stability due to crosslinking between membrane components. However, membrane produced by the casting method shows only physical interaction.

3.10. Absorption of bisphenol A (BPA) by CMC_p/HAp/lysine membrane

The membranes extraction efficiency of BPA from aqueous media were evaluated. Gas chromatography coupled with mass spectrometry for the analysis of extracted BPA. The results are presented in Fig. 7a and b. The extraction time was fixed for 15 min with stirring and sonication followed with desorption and analysis by GC/MS.

The highest BPA recovery was achieved using a CMC_p/HAp/lysine membrane with a mass ratio of 20:70:10 as shown in Fig. 7b. Extraction of BPA by florisil was used as a control in this study. The adsorption efficiency of florisil for BPA is shown in Fig. 7a. The results revealed that, the amount of BPA extracted by CMC_p/HAp/lysine membrane was lower than that extracted by florisil.

The biodegradability and the efficiency of the tri-components membrane toward the BPA makes it a useful candidate for removing toxic materials from environment.

3.11. Antibacterial and antifungal results of composites CMC_p/HAp/lysine

The sensitivity test in agar media showed that, there is some selectivity in bacteria inhibition by the membranes. For instance, membrane O8 (Annex 13) inhibited *Bacillus subtilis* with a diameter of inhibition of 9 mm, it also showed a major activity against *E. coli*. Annex S13 shows the diameter of inhibition (D) in mm for each of the membranes against three bacteria cells and one fungus stains. Products O6, O5 and O3 exhibited some activity against *E. coli* with 6 mm diameter of inhibition.

All membranes (O3, O4, O5, O6, O7 and O8) showed antifungal activity against *Candida*, products O3 and O5 showed excellent inhibition efficiency with D of 12 mm.

The positive control, showed a higher diameter of inhibition of about 25 mm against *Micrococcus luteus*, 24 against *Bacillus subtilis*, 23 against *E. coli* and 28 for Candida. The negative control showed no activity against bacterial cells or fungus (Fig. 8).

From these results, it could be concluded that all products inhibit fungi and composite with an exception of O8, it showed the highest efficiency against bacterial and fungi.

4. Conclusion

In this study, three components bio-composites were prepared using various mass ratios of CMC_p, HAp, and lysine. The composites were prepared by two different methods: the casting and the double decomposition. The casting method produced a membrane with relatively high thicknesses, opaque with rough surface and low flexibility. However, the double decomposition method produced a nano thickness membrane that is smooth, transparent and flexible.

Several spectroscopic and analytical methods were used to characterize the physical and chemical properties of CMC_p/HAp/lysine composites. The SEM analysis results showed that, the particles of hydroxyapatite are well dispersed in the CMC_p matrix. Microscopic results obtained by SEM/FEG showed that, the presence glycerol as a second plasticizer made the membrane even more homogeneous and stabilized it against breaking during its use. All composites showed some inhibition efficiency against bacteria and fungi. The composites also showed good extraction efficiency for BPA. Their extraction efficiency and their low water solubility make them useful in extracting toxic organic chemicals such as BPA from industrial waste. The composite could also find a use in bone tissue engineering.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2017.02.092.

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