Journal of Polymers and the Environment (2022) 30:4675–4689
https://doi.org/10.1007/s10924-022-02538-7

ORIGINAL PAPER

PVA/CMC/Attapulgite Clay Composite Hydrogel Membranes for Biomedical Applications: Factors Affecting Hydrogel Membranes Crosslinking and Bio-evaluation Tests

Eman Farid[1] · Elbadawy A. Kamoun[2,3] · Tarek H. Taha[4] · Ali El-Dissouky[1] · Tarek E. Khalil[1]

Accepted: 19 July 2022 / Published online: 6 August 2022

Abstract

Herein, polyvinyl alcohol-carboxymethyl cellulose (PVA-CMC) composite hydrogel membranes were prepared using solution-casting method, where citric acid (CA) was added as crosslinker in different ratios of (7, 10 and12 wt%). Attapulgite clay extracted from Northwestern Desert of Borg El-Arab, Egypt; was incorporated as nanofiller (1, 2, 4, and 5 wt%) into membranes for improving their mechanical/ thermal stability. Results revealed that, physicochemical properties of membranes e.g. swelling%, tensile strength and morphology of membranes affected significantly by different clay concentrations and citric acid crosslinker. Also, attapulgite clay with concentration 1 (wt%) enhanced mechanical strength of composite membranes, compared to other clay concentrations. Furthermore, protein adsorption %, hydrolytic degradation, hemolysis (%) and antimicrobial activity significantly affected by clay contents and CA concentrations. Four bacterial pathogens e.g. *Candida albicans*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Bacillus cereus* were used for testing antimicrobial activity of prepared membranes. Results referred to increasing of clay contents led to a high hemolysis %; however, increasing CA concentration significantly reduced hemolysis %. Meanwhile, membranes with low clay contents offered the most effective resistance against tested microbes. These findings are referring to the ability of using PVA-CMC-attapulgite composite membranes crosslinked by CA as good candidate of biomaterials for dermal wound dressings.

Keywords PVA-CMC · Attapulgite clay · Hydrogel membranes · Antimicrobial activity

Introduction

Wound healing is one of the most important and complex processes occurs to human involving interaction between body cells and its extracellular cover to protect skin hurt zone. Recently, wound dressings examples like hydrogels have specific properties required for wound closure such as keeping humidity and cooling sensation for the wound, preventing bacterial infections, permitting gas exchange and biocompatible to be easily removed from wounds [1] hydrogels are highly hydrated three-dimensional crosslinked polymeric network with porous molecular structure that can absorb wound exudates and retains moisture making them suitable candidate for wound dressing applications [2]. Last decade, many wound dressings have been prepared using natural and synthetic polymers.

Polyvinyl alcohol (PVA) is hydrophilic synthetic polymer that easily soluble in water, slightly soluble in ethanol, but insoluble in other organic solvents, it was prepared by the polymerization of vinyl acetate to form Poly (vinyl acetate)

✉ Elbadawy A. Kamoun
badawykamoun@yahoo.com; e-b.kamoun@tu-bs.de

1 Chemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt

2 Polymeric Materials Research Department, Advanced Technology and New Materials Research Institute (ATNMRI), City of Scientific Research and Technological Applications, New Borg Al-Arab City, Alexandria 21934, Egypt

3 Nanotechnology Research Center (NTRC), The British University in Egypt, El-Shreouk City, Cairo, Egypt

4 Environmental Biotechnology Research Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), City of Scientific Research and Technological Applications (SRTA-City), New Borg Al-Arab City, Alexandria 21934, Egypt

followed by partial hydrolysis [3]. PVA hydrogel is considered an effective agent in biomedical and pharmaceutical applications due to its excellent chemical and physical properties such as biocompatibility, non-toxicity, on-carcinogenic, high flexibility, high tensile strength, film forming, emulsifying, and adhesive properties. However, PVA hydrogel possesses high water holding capacity or biological fluids due to its rubbery or elastic structure. As result of these advantages PVA hydrogel can stimulate natural tissue and can be accepted in the body which make it suitable for wound dressing application [4].

Carboxymethyl cellulose (CMC) is a natural polymer derived from cellulose, it is biodegradable, has high swelling ability, and hydrophilic carboxylate groups in its backbone. So, it is suitable for forming hydrogel but with low mechanical strength and stability [5]. Thus, blending with other high mechanical polymers., PVA to obtain composite hydrogel is inexpensive and advantageous way to prepare new structural material combined properties of both polymers [6].

Citric acid is used as popular chemical crosslinker because it is cheap, nontoxic, and antimicrobial ability. Citric acid crosslinked PVA-CMC hydrogel films were prepared by esterification reaction in which citric acid forms a cyclic anhydride at high temperature followed by esterification of the hydroxyl group of the adjacent polymer chains leads to the formation of crosslinks [7]. Previously, blending of PVA-CMC polymers lead to semi-transparent, flexible, and highly efficient hydrogel with excellent physical and mechanical properties suitable for wound dressing as can absorb wound exudates due to their highly swelling capacity [8, 9]. Herein, an extended research work of using attapulgite clay as antimicrobial clay was recommended for incorporation with PVA/CMC composite hydrogel membranes as promising biomaterials for wound dressing applications. It is well known that pristine hydrogel with high strength and elasticity will be better as dressing material. However, reinforcing agents e.g., naturally favored for incorporation into membrane composition [10]. Attapulgite clay mineral was identified and basically extracted from the calcareous soil of North Western desert, Borg El-Arab, Egypt. Attapulgite is known as ribbon of 2:1 phyllosilicates and, unlike other clay minerals, which have a fibrous morphology structure. The composition of attapulgite was reported through our previous published study [11], who has discussed the theoretical formula of attapulgite as $[Si_8Mg_5O_{20}(OH)_2](H_2O)_{44}H_2O]$. Attapulgite clay has rectangular channels containing cations, zeolitic water, and water molecules bound to coordinative unsaturated metal ion centers [11]. Raw attapulgite clay is high hydrophilic, and it has permanent negative charges. Hence, the intercalation of cationic surfactants is needed to reverse its surface charge [11]. The intrinsic structure of attapulgite makes it accessible for fabricating many practical materials as good alternatives for expensive and environmentally hazardous nano-materials. Thus, Attapulgite was first purified and incorporated into hydrogel membranes for avoiding the quick deterioration of hydrogel membranes due to its high swelling capacity to wound exudates. Furthermore, clay was incorporated into hydrogel membranes to adjust the swelling degree, prolonging the degradation rate and for enhancing the mechanical and thermal stability of hydrogel membranes. However, previous studies were reported on PVA/clay composite membranes, hence the Egyptian mineral clay of deserts needs further investigations to gain more information about its utilization for biomedical application. Also, previous studies have discussed the silica–based clay e.g. montmorillonite, bentonite and Laponite composed with polymers for biomedical applications [10, 11].

This work aims to prepare of effective CA crosslinked PVA-CMC-attapulgite composite hydrogel membranes for wound dressings. Both the effect of CA concentration and clay contents were studied intensively and their influences on physicochemical properties of PVA-CMC-attapulgite composite hydrogel members. The prepared composite membranes were instrumentally characterized by FTIR and SEM, while the bio-assessment test e.g. hemolysis % and antimicrobial activity were carried out *in vitro.*

Materials and Methods

Materials

Polyvinyl alcohol, PVA (typically average M_{wt} ~ 72,000 g/mol; 98.9% hydrolyzed) and high viscosity carboxymethyl cellulose, CMC sodium salt (degree of substitution 0.6–0.0.95) were purchased from Biochemika, Germany. Citric acid anhydrous (CA) (purity > 96%) was purchased from Sigma-Aldrich, Germany. Attapulgite clay was obtained from Northwestern desert of Borg El-Arab city, Alexandria, Egypt. Attapulgite clay was purified from sands and rocks before use. Distilled water was used during this research. All other chemicals were used without further purification. Attapulgite clay was collected, extracted, separated, purified and activated as previously reported in our published work [11], where this part was further explained in (supplementary Data). The average particle size of used attapulgite clay ranged between 500 and 1.0 µm.

Four bacterial pathogens; *Candida albicans* ATCC 700, *Escherichia coli* NCTC10418, *Klebsiella pneumoniae* ATCC13883, and *Bacillus cereus* ATCC6633 were kindly provided by National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt. Luria-Bertani (LB) Broth powder was obtained from Bio Basic, Canada.

Preparation of PVA-CMC-Attapulgite Hydrogel Membranes

PVA-CMC hydrogel membranes were prepared by solution-casting method according to previous reported study by Hussein et al. [12], with some modifications. Briefly, aqueous solution of 10 (%, w/v) PVA and 1 (%, w/v) CMC were carefully dissolved in 15 mL distilled water with stirring at 60 °C for 4 h. After getting a clear polymeric PVA-CMC mixture solution, it was cooled to the room temperature, then10 (%, w/v) of citric acid was added as crosslinker at room temperature with gentle stirring for 15 min to prevent quick premature crosslinking. Purified attapulgite clay was added as nano-filler followed by ultrasonic in water bath for grantee the clay well dispersion in polymeric solution, and then 1 mL of glycerol was added as plasticizer and 0.25 g of polyethylene glycol (PEG) was added as pore forming agent. The mixture solution was left under stirring at room temperature for two hours to remove the entrapped air bubbles. Finally, the solution was casted in (20 mm × 100 mm) petri dish and dried in oven overnight at 60 °C to evaporate excess water, and then dried films were transferred into 80°C for two hours to complete the esterification reaction. Different concentrations of PVA (5,7 and10%, w/v), CMC (0.25, 0.5, 1.0 and 2.0% w/v), citric acid (7, 10 and 12%, w/v) and attapulgite clay contents (1, 2, 4 and 5%, w/v) were incorporated and tested for optimizing of composite hydrogel membranes.

Instrumental Characterization

FT-IR

Vacuumed and dried samples of PVA, CMC, and PVA–CMC hydrogel were analyzed by FTIR on an EQUINOX 55 instrument (BRUKER, Germany). Translucent KBr-disks were prepared by grinding the dried sample materials together with infrared grade KBr and then pressing. The FTIR spectra were obtained by recording 64 scans between 4000 and 400 cm^{-1} with a resolution of 2 cm^{-1}. All samples were freeze-dried using liquid nitrogen, crushed to a fine powder (KBr: sample = 140 mg: 2 mg), and pressed by applying a force 105 N into a transparent disk (maximum disk weight = 145 mg) with a diameter of 13 mm. All samples were measured in transmission mode.

SEM

The surface and internal structure of the hydrogel membrane samples were investigated by Analytical-SEM (JEOL, JSM-6360LA, Japan) with 15 kV voltage for secondary electron imaging. The hydrogel membranes were 11425, USA dehydrated by freeze-dryer and coated with Au using an ion sputter coater in (model: 11430, USA, combined with vacuum base unit or SPi module.

TGA

The thermal decomposition of vacuumed-dried PVA–CMC membranes was measured using TGA thermogram. The thermo-gravimetric analysis (TGA) was performed on a 204 Phoenix TGA instrument (NETZSCH, Germany) from 50-600°C at a heating rate of 10 °C/min. TGA thermographs calculated the onset temperature T_{onset} which known as the temperature at the intersection of the baseline mass and the tangent attracted to the mass curve at the point of inflection or point of maximum mass loss percentage.

Mechanical Properties

To measure maximum tensile strength and degree of elongation to break PVA-CMC composite hydrogel membranes were drawn on a tensile testing machine (model: AG-I/50-10KN, Japan). PVA–CMC membranes were cut into a rectangular or plate shape (5 cm long, 1 cm width). The analysis was carried out at a stretching rate of (20 mm/min) with preload (0.5 N) for each sample [13].

Physicochemical Characterization

Swelling Ratio (%)

Dried PVA-CMC membrane samples were cut into (2 × 2 cm) pieces and its dry weight was determined (W_d) and then was soaked in distilled water, the weight of swelled samples (W_s)was determined at different specific time intervals till saturation or equilibrium. Water uptake was measured according to the following formula:

$$\text{Swelling ratio or Water uptake (SR)} = \left[\left(W_s - W_d\right)/W_d\right] \times 100. \quad (1)$$

where, W_d is weight of dry sample and W_s is weight of swollen sample at specific time intervals [14].

Protein Adsorption

Bovine serum albumin (BSA) was used as a medication or protein model in this study. Weighed BSA (25 mg/mL) depending on the weight of samples tested) it was fine dissolved at pH 7.4 at 37 °C in phosphate buffered saline (PBS) solution. PVA-CMC hydrogels with specific weights were immersed in BSA-PBS solution overnight at 37 °C. Overall sum of BSA adsorbed on the hydrogel surface was calculated by removing the hydrogel bits from BSA solution and then calculate the BSA concentration before/after the

immersion of hydrogel in BSA solution. The adsorbed BSA on hydrogels surface was measured spectrophotometer at 280 nm [15].

Hydrolytic Degradation

Hydrolytic degradation or weight loss (%) of PVA-CMC-CA hydrogel membranes, as a function of citric acid different concentrations, prepared membranes were immersed in phosphate buffered saline (PBS) solution (pH 7.4, at 37 °C for 1–3 weeks). The degradation or weight loss (%) of hydrogels was measured after equilibrium swelling weight of swollen hydrogels. At specific time intervals, samples were removed from PBS solution and were softly wiped with filter papers. The weight loss (%) of hydrogels was detected as a function of time of incubation using the following equation.

$$\text{Weight loss } (\%) = [(W_0 - W_t)/W_0] \times 100. \quad (2)$$

where, W_0 is the hydrogel's initial weight, Wt is the removed PBS hydrogel and weighed at specific incubation time intervals [16].

Bio-assessment Tests

Disc Diffusion Assay

Antimicrobial activity of prepared composite hydrogel membranes was tested using disc-diffusion assay [17]. The surfaces of LB agar plates were inoculated with the microbial pathogens using sterile cotton swabs after their dilution to 0.5 McFarland standard. A single disc (0.7 mm diameter) of each membrane was aseptically added to the plates' surface using tip-ignited forceps. All the plates were then incubated at 30 °C for 24 h. After incubation, the plates were checked for the formation of clear zones which were measured and recorded [18].

Hemolysis Assay

Hemolysis test is an investigating assay that is widely used to determine the biocompatibility of a certain compound or a polymeric material. It depends on testing the ability of that compound to destroy the human RBCs and releasing their hemoglobin content. It started by the collection of a healthy blood sample in EDTA containing tube to avoid its clotting. A net volume of 700 µl of Ca^{+2}-Mg^{+2} free Dulbecco's phosphate-buffered saline (DPBS) buffer was mixed with 10 µl of the blood sample with gentle mixing. After that, 100 mg of each tested sample were separately added. Both of Triton X-100 and DMSO (0.5%) were used as the positive and negative controls, respectively. The tested and controls tubes were incubated for 3.5 h at 37 °C with gentle interval mixing followed by centrifugation at 10.000 rpm for 15 min. Equal volumes of the supernatants and the reagent (cyanmethemoglobin) were mixed, followed by spectrophotometric measuring of each mixture at 540 nm against the blank (Ca^{+2}-Mg^{+2} free DPBS buffer plus the sample without blood [19].

Results and Discussion

Instrumental Characterization

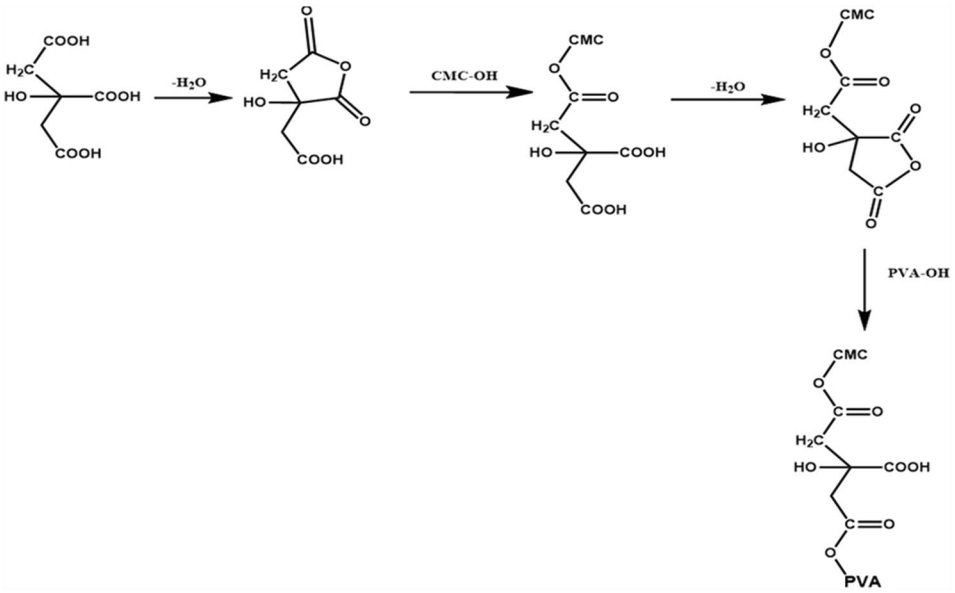
Polyvinyl alcohol-carboxymethyl cellulose blended hydrogel membranes were prepared using citric acid as chemical crosslinker through esterification reaction as displayed in Scheme 1., where the feature peaks verifying the chemical crosslinking is proven by FTIR analysis. While physical interaction between PVA and CMC as is explained in Scheme1 is also verified by FTIR analysis.

FTIR Analysis

FTIR spectra of pure PVA, pure CMC, PVA-CMC non-crosslinked membrane and citric acid crosslinked hydrogel membrane are represented in Fig. 1a. The peaks associated with PVA shows C-H alkyl-asymmetric stretching vibration at ν 2850 cm^{-1}. A notable stretching band for $–CH_2$ appears at ν 1560–1450 cm^{-1} was found. Also, C–O stretching band appeared approximately at ν 1100 cm^{-1} [20].

For CMC, aliphatic C–H band appears approximately at ν 2900 cm^{-1}, a new peak represents asymmetric stretching vibration of carbonyl group of COO– at ν 1600-1640 cm^{-1} and symmetric stretches at approximately 1400 cm^{-1}, also a band at 1070 cm^{-1} is assigned to C–O–C stretching, were clearly detected [21].

FT-IR spectrum of PVA/CMC membranes crosslinked by 10 (wt%) of CA shows abroad band appeared at ν 3200–3600 cm^{-1}, which represents O–H stretching vibration [22] also –OH peak intensity decreased with the crosslinking reaction. This is due to the esterification reaction with citric acid and formation of ester bonds. In addition, new peaks are observed at ν 1750 and 1300 cm^{-1}, which represent the formation of C=O and C–O stretching of ester bonds, respectively; because of the crosslinking reaction [23]. However, IR spectra were difficult to be evaluated due of the peaks overlapping occurred with carboxylic group bands of CMC, and addition of citric acid protonation reaction of carboxymethyl groups of CMC [24]. Also, the spectrum of pristine attapulgite clay and interaction between attapulgite clay and PVA polymeric matrix is relatively clear and was



Scheme 1 Reaction mechanism representing crosslinking reaction of PVA/CMC via esterification reaction between PVA and citric acid first followed by physical crosslinking between PVA and CMC

lately explained and discussed well in our previous published work [11].

Figure 1b represents the effect of citric acid concentration, when CA concentration increases from 7, 10, to 12 (wt%) in membrane composition, the intensity of carbonyl band increases. This is owing to a result of increase the number of ester crosslinks and also free –COOH groups are associated to the increase of CA concentration, where it can't be significantly determined [25]. Interestingly, esterification mechanism leads to the formation of crosslinks between PVA and CMC, whereas a cyclic anhydride intermediate is formed by post heat-treatment to complete the crosslinking cycle. This intermediate is responsible for the formation of crosslinks with CMC by esterification of –OH group of CMC resulting in another carboxylic acid occurring intramolecular cyclic anhydride with neighboring carboxylate group [26].

SEM Investigation

The surface morphology of crosslinked PVA-CMC composite hydrogel membranes with different CA concentrations (7, 10 and 12 wt%) were investigated using SEM, as represented in Fig. 2. SEM micrographs showed that, citric acid content is clearly responsible for changing the surface morphology of tested membrane, since the amount of PVA and CMC were uniform in all films. At membrane of (7 wt%) CA concentration, few pores with smooth, regular and homogenous surface is observed due to low crosslinking degree in the obtained composite membranes. By increasing the CA concentration up to (10 wt%), the film exhibits more porous network that enables the diffusion of water through the pores. These results might be attributed to the presence of three (-COOH) groups for each CA molecule. Thus, it was found with increasing CA percentage, increases the number of –COOH groups available for crosslinking with –OH group of PVA leading to increasing of the crosslinking density and hence, promoting the porosity. This process decreases the water absorption of PVA composite hydrogels. Further, increase of CA ratio up to (12 wt%), hyper-crosslinking was triggered due to further increasing available free –COOH groups of CA for crosslinking process, causing much pores forming and limited water uptake of hydrogel membranes occurred. These results are supporting swelling findings which were discussed below and consistent with previous findings of Nataraj et al. [27].

Figure 3 displays the surface morphology alternations due to incorporation of attapulgite clay in different contents (1, 2, 4 and 5 wt%) into PVA-CMC composite hydrogel membranes. As clearly seen, the low attapulgite contents (i.e. 1 and 2 wt%) showed compacted, smooth and uniform shape structure. However, high incorporated clay contents (4 and 5 wt%) showed clay aggregation onto the membrane surface. The high clay incorporation behavior is similar and consistent with previous results obtained by Hussein et al. [16], regarding the aggregated high concentrations of CNCs onto PVA/HA hydrogel membranes.

Mechanical Properties

To assess the mechanical properties of crosslinked PVA/CMC membranes with different CA concentrations (7, 10 and 12 wt%), the maximum tensile strength and elongation to break were tested and obtained data was listed in Table 1. Unexpectedly, maximum tensile strength and elongation to break decreases with increasing the citric acid concentration

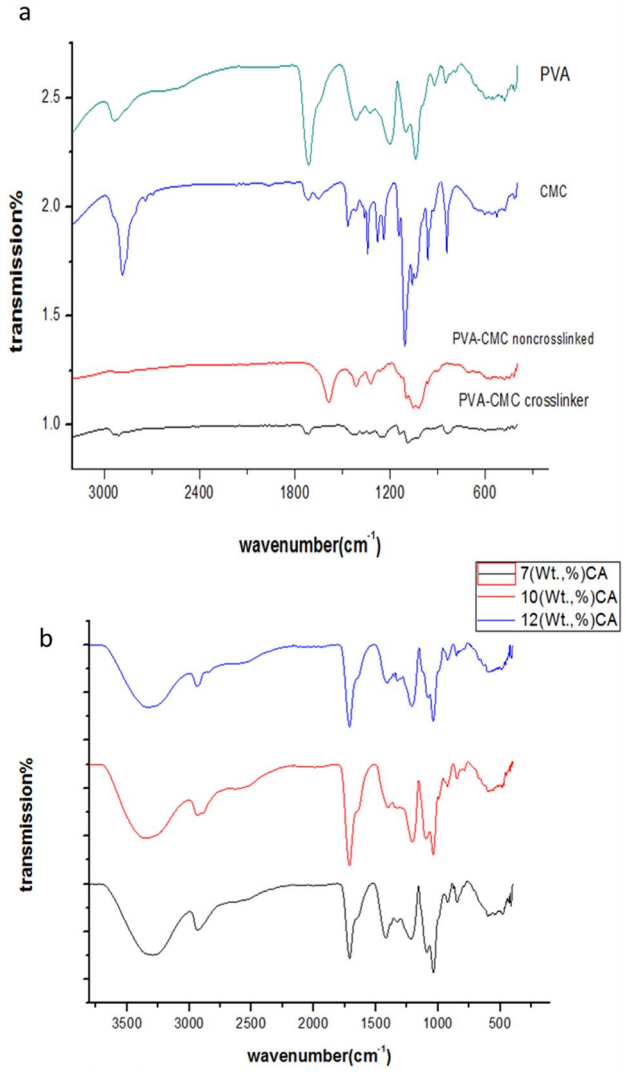


Fig. 1 FTIR spectra of pure PVA, pure CMC, uncrosslinked PVA/CMC membranes and crosslinked PVA/CMC hydrogel membranes (**a**) and crosslinked PVA/CMC hydrogel membranes using different citric acid concentration at 7, 10, and 12 wt% (**b**)

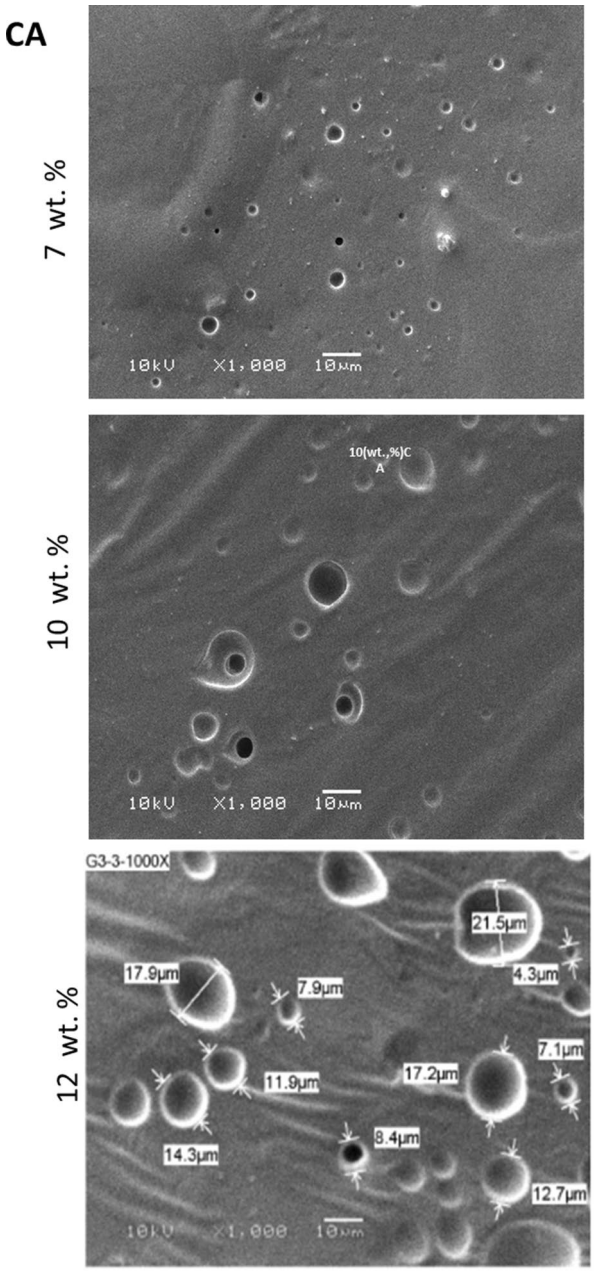


Fig. 2 SEM micrographs of crosslinked PVA/CMC hydrogel membranes, using different citric acid concentration at 7, 10, and 12 wt%, (original magnifications at ×1000, scale 10 µm, and applied voltage 10 kV)

and they show the same mechanical pattern behavior. These results refer to addition of citric acid to PVA-CMC hydrogel membranes might destabilize and accelerate the elongation-to-break of membranes resulting insignificant decreasing in the maximum tensile strength. These results are almost matched with results obtained by Jiang et al. [28], who demonstrated that increasing CA concentration up to 9 wt% boosted the CA molecules available for crosslinking leading to an increase in mechanical strength. However, further increase of CA concentration triggered the hyper-crosslinking reaction resulting in sudden mechanical deterioration of CA crosslinked zein NFs [29].

Different attapulgite clay contents (1, 2, 4, and 5 wt%) were incorporated into PVA-CMC composite hydrogel membranes and their mechanical stability were tested and displayed in Table 2. It was found that, addition of clay up to 1(wt%) significantly increased the entire parameters of mechanical properties of PVA/CMC/clay membrane (Table 2). It was observed that, further increase of attapulgite clay ≥ 1.0 (wt%) might result in an agglomeration of clay occurring the weak interaction between PVA-CMC chains, followed by reducing the crosslinking degree of PVA/CMC due to spacing of polymeric chains accompanied with a sudden mechanical deterioration of hybrid hydrogel membranes

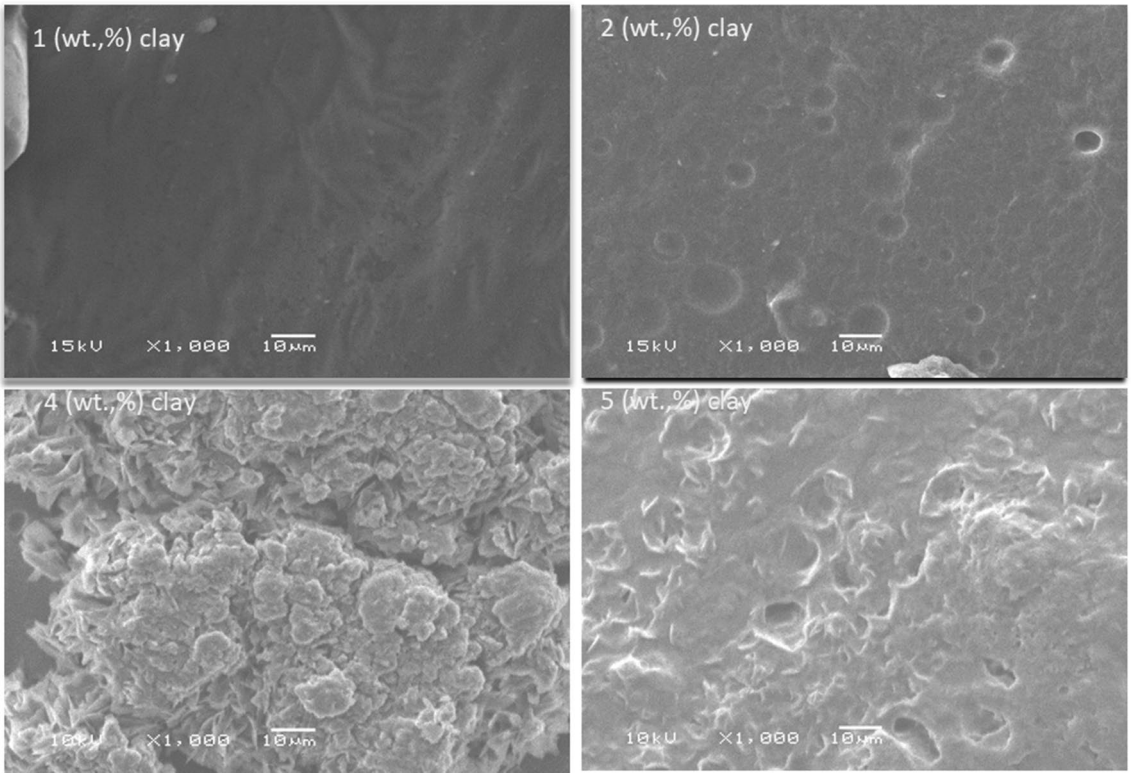


Fig. 3 SEM micrographs of crosslinked PVA/CMC/attapulgite clay composite hydrogel membranes, using different clay concentration at (1, 2, 4, and 5 wt%), (original magnifications at ×1000, scale 10 µm, and applied voltage 10 kV)

Table 1 Effect of citric acid concentration on the mechanical properties of crosslinked PVA-CMC hydrogel membrane

CA concentration (wt%)	Elongation to break (%)	Max. stress (N/mm^2)	Max. force (N)
7	372.2	4.9	15.8
10	340.6	4.85	15.5
12	270	3.7	11.7

Table 2 Effect of attapulgite clay contents on the mechanical properties of PVA/CMC/Clay composite hydrogel membrane

Clay content (wt%)	Elongation to break (%)	Max. stress (N/mm^2)	Max. force (N)
0	123.4	2.7	8.9
1	249.3	5.8	15.2
2	140.9	6.4	10.2
4	188	10.5	10.5
5	128.6	10.2	10.2

[11, 29]. Hence, 1 wt% clay was considered as optimal concentration in terms of cost and high mechanical stability of composite membranes. Similar results were previously reported by Du et al.[30]. They have tested mechanical properties of high Laponite clay contents in Laponite-poly (acrylic acid) nanocomposite membranes.

Thermal Properties (TGA Measurements)

Thermal stability of PVA/CMC hydrogel membranes crosslinked with different CA concentration (7, 10, and 12 wt%) were conducted by TGA measurement (Fig. 4). It was shown that, (9.5, 8.1 and 2.4% of weight loss (%) of PVA/CMC membranes crosslinked with CA (7, 10 and 12 wt%), respectively occurred up to 66–107 °C, owing to evaporation of free water, bounded water and stored humidity. The highest crosslinked membranes with 12 (wt%) of CA was stable until 180 °C, then a steep and sharp weight loss (%) occurred until 459 °C, as known T_{onset} or the second decomposition stage. This biggest weight loss (%) around 79% was allied with organic matters decomposition and destruction of PVA/CMC chain linkage. Meanwhile, in this second thermal decomposition; some volatile compounds e.g. CO_2 were thermally-decomposed. Finally, the pyrolysis or the third complete volatilization stage is detected from 459 to 550 °C with total weight loss (%) of 82% was detected (Fig. 4). Overall, increasing of used CA for crosslinking PVA/CMC membranes sharply improved crosslinking

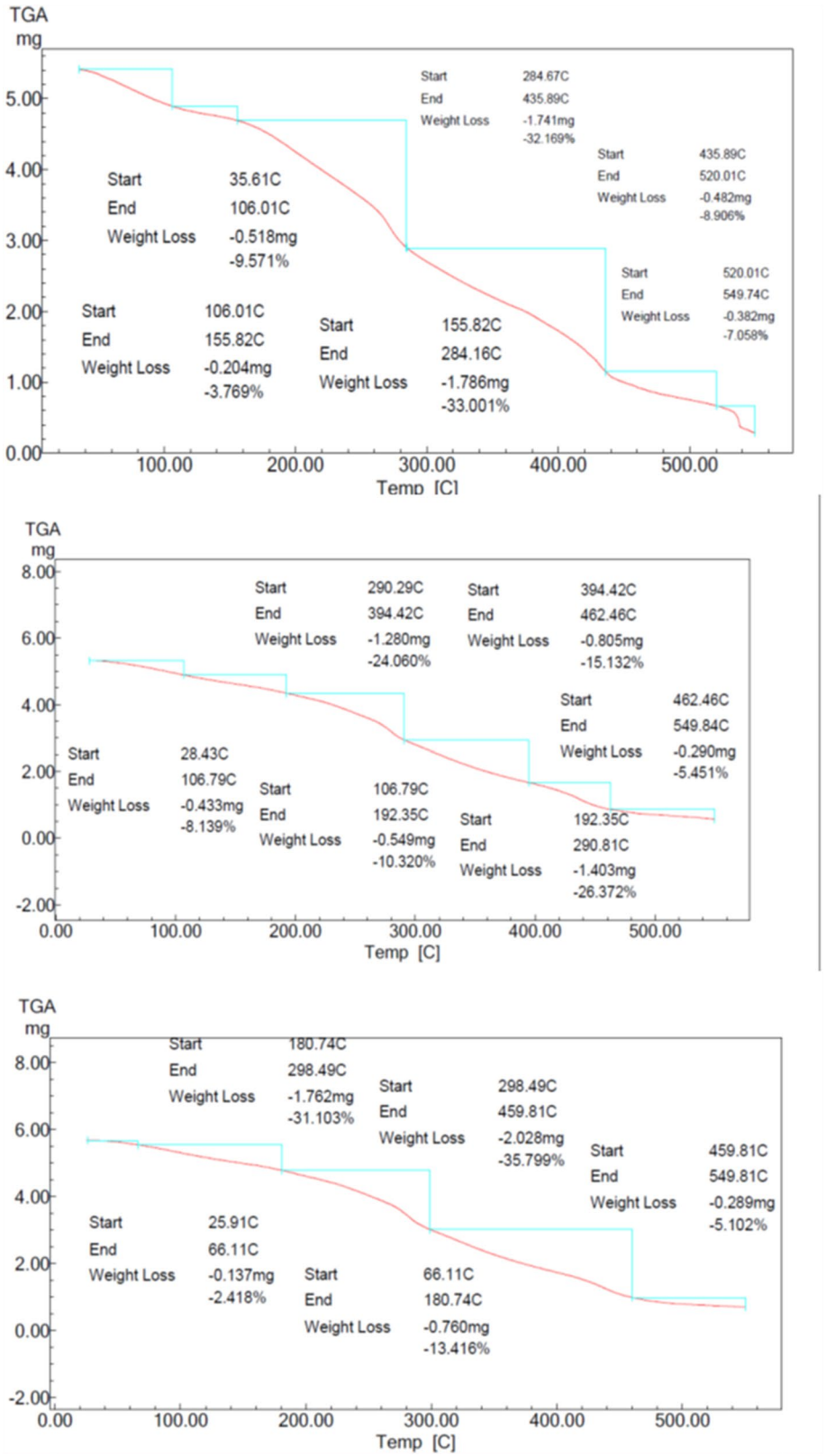


Fig. 4 TGA results of crosslinked PVA/CMC hydrogel membranes, using different citric acid concentration at 7, 10, and 12 wt%

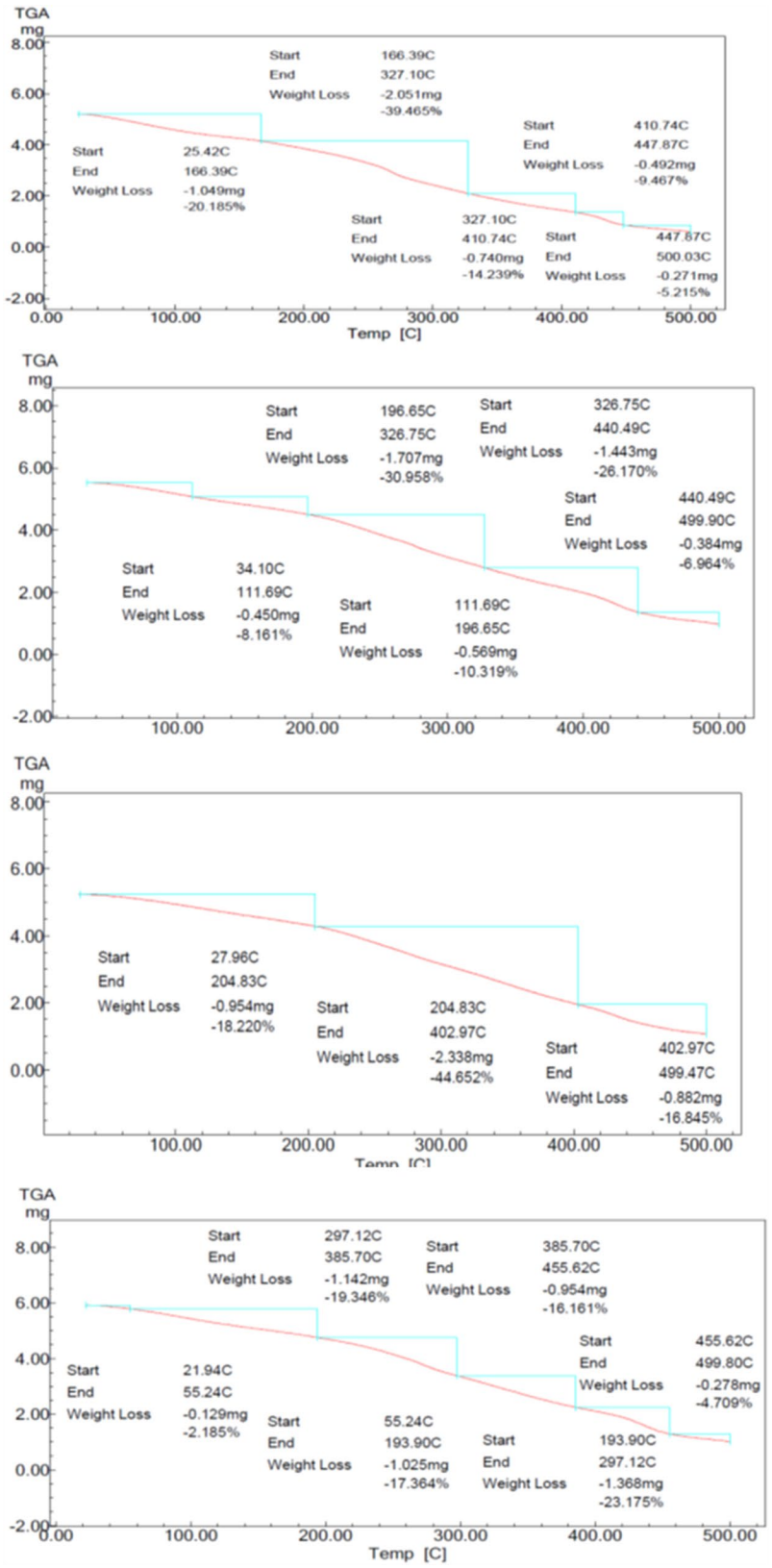


Fig. 5 TGA results of crosslinked PVA/CMC/attapulgite clay composite hydrogel membranes, using different attapulgite clay contents in membranes at 1, 2, 4 and 5 wt%

degree, accompanied with enhancing the thermal stability of membranes.

As shown in Fig. 5, the weight loss of PVA/CMC/clay composite membranes sharply reduced from 20 to 2% with increasing the incorporated attapulgite clay contents from 1 to 5 (wt%) owing to evaporation of free water, bounded water and stored humidity. While T_{onset} of the beginning of second decomposition stage increased from 166 to 193°C, when the incorporated clay content was increased from 1 to 5 (wt%), respectively. Notably, the total weight loss % after pyrolysis thermal decomposition stage (i.e. third decomposition stage) decreased from 92 to 81% with increasing the incorporated clay contents in membranes from 1 to 5 (wt%). These findings refer to the addition of attapulgite clay into PVA-CMC membranes remarkably the entire thermal stability behavior of composite membrane, in addition prolonged the time of decomposition and the total weight loss decreased due to existing of inorganic matters. These results are consistent with obtained results of Du et al. [30], where who demonstrated that thermal stability of poly (acrylic acid) nanocomposite membranes were sharply enhanced with increasing Laponite clay contents in membranes.

Physicochemical Characterization

Swelling Ratio % of Hydrogel Membranes

It was found, citric acid concentration affects significantly on the swelling ratio or water uptake of prepared PVA/CMC composite hydrogel membranes, as shown in Fig. 6a. It was proven previously that, swellability of hydrogel membranes increases with increasing the concentration of citric acid, due to increase the hydrophilicity which associated with increasing the carboxyl content in membranes [31]. Notably, the swelling ration of membranes decreased with increase the used concentration of CA, due to increase the crosslinking degree of membranes. These findings could be explained that fact that, increasing clay content might fill the hydrogel pores and forming compacted and condensed interior structure which hinder the water exchange and adsorption, resulting a significant reducing of hybrid hydrogel swellability. On the other side, increase the CA caused further crosslinking for both PVA and CMC until certain concentration (10 wt% CA). However, membranes crosslinked with ≥ 12 wt% of CA show an opposite swelling effect, where the swelling increased significantly owing to imaginably termination reaction occurred. Thus, 10 wt% of CA was chosen as an optimum concentration for further experiments. Our resuts also matched with swelling results obtained by Demitri et al. [32], who reported that the optimal degree of swelling for practical applications was achieved using low CA concentrations.

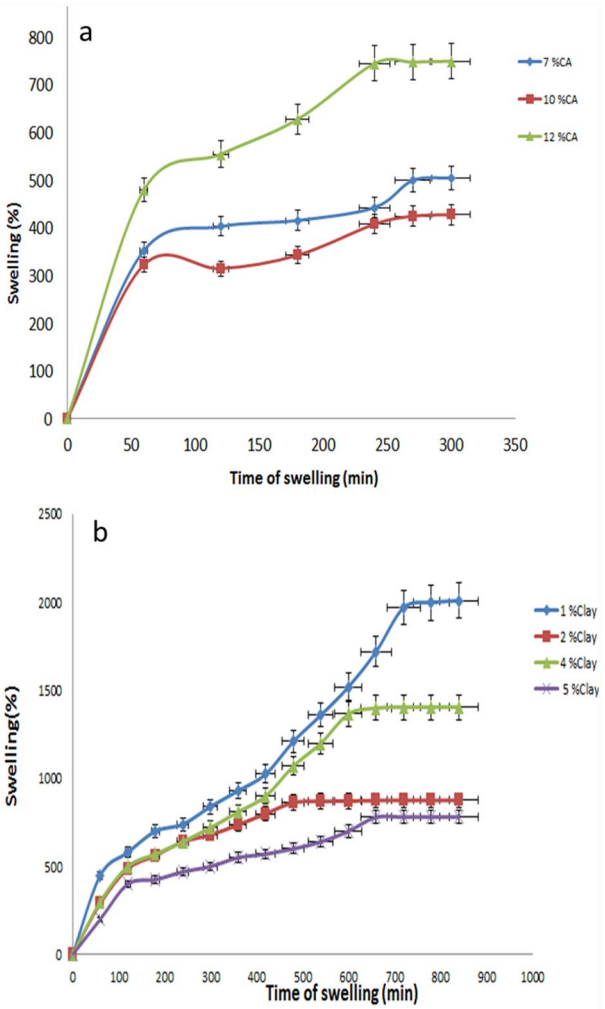


Fig. 6 Swelling ratio % of crosslinked PVA/CMC hydrogel membranes, using different citric acid concentration at 7, 10, and 12 wt% (**a**) and using different incorporated attapulgite clay contents (1, 2, 4, and 5 wt%) (**b**)

As presented in Fig. 6b, it was observed that with increasing attapulgite clay contents, the swelling ability of hydrogel membranes decreases. This might be ascribed to the blocking available pores of membranes and formation of a very compacted interior-structure of hydrogel network causing swelling reduction. In the same context, Mahdavinia et al. [33] reported that the incorporation of Laponite clay reduced dramatically the swelling ability. Because of, addition of clay acts as additional physical crosslinker preventing from water absorption. Despite decreasing water uptake, but the results are still with acceptable for water uptake capacity to absorb wound exudates and keeping high water resistance, in case employing the prepared membranes as topical wound dressings.

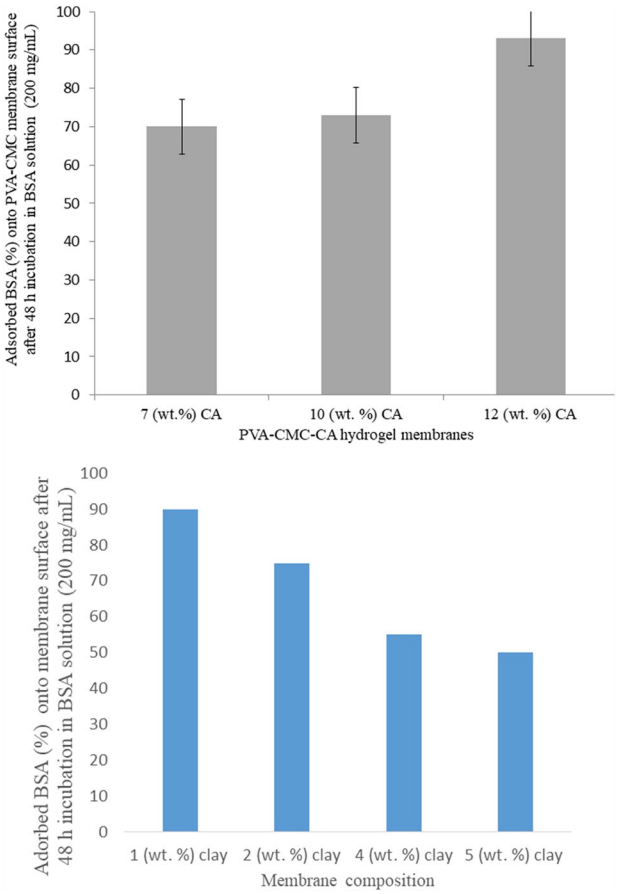


Fig. 7 Protein adsorption (%) onto the surface of crosslinked PVA/CMC hydrogel membranes, using different citric acid concentration at 7, 10, and 12 wt% (up) and using different incorporated attapulgite clay contents (1, 2, 4, and 5 wt%) (down)

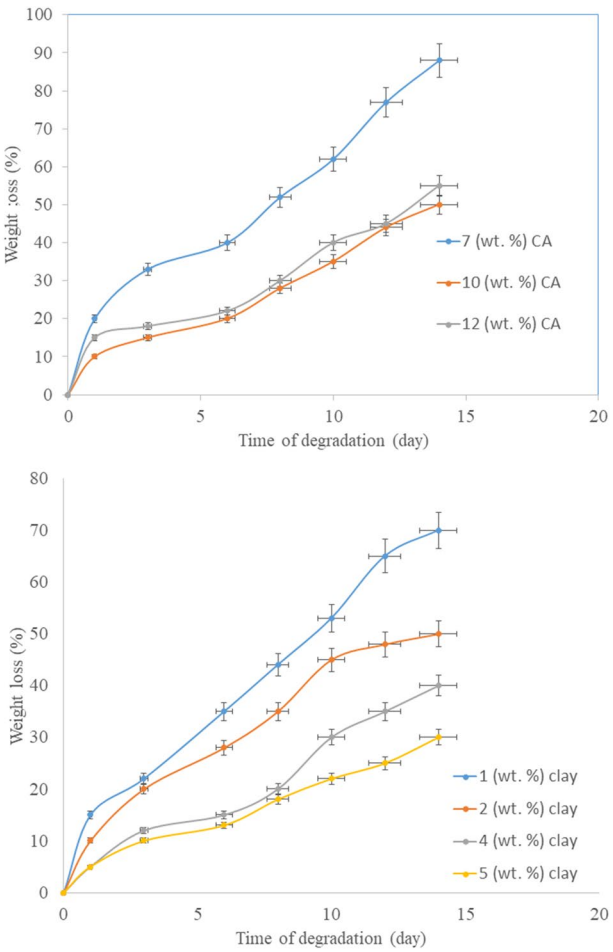


Fig. 8 Hydrolytic degradation or weight loss (%) of crosslinked PVA/CMC hydrogel membranes, using different citric acid concentration at 7, 10, and 12 wt% (up) and using different incorporated attapulgite clay contents (1, 2, 4, and 5 wt%) (down)

Protein Adsorption (%)

The assessing of adsorbed BSA onto the surface of composite hydrogel membranes is a real significant factor for the ability of physiological and cells attachment manner, which also verify the biocompatibility of tested biomaterials. The influence of both CA crosslinker concentrations and contents of incorporated clay on the amounts of adsorbed BSA onto the surface of membranes after incubation for 48 h, were assessed and shown in Fig. 7. As seen, the adsorbed BSA onto the membrane surfaces generally increased significantly with the low CA concentration and low contents of incorporated attapulgite clay (7 wt% CA and 1 wt% clay, respectively). It is due to the fact that, the adsorbed BSA onto membrane surface increases significantly with the highest swollen composite hydrogel membranes, or with other mean the lowest crosslinked membranes. Notable, the presented findings here are fully consistent with the presented results of swelling measurements in Fig. 6. Also, the speculation of reduction of adsorbed protein was found with the highest crosslinked membranes and lowest values of swollen membranes, which is fully consistent with our previous and reported findings [15, 18, 19, 31].

Hydrolytic Degradation

The hydrolytic degradation or weight loss (%) of crosslinked PVA/CMC hydrogel membranes were assessed as function of different CA concentrations (7, 10, and 12 wt%) and different attapulgite clay contents (1, 2, 4 and 5 wt%), is shown in Fig. 8. It was obviously shown that, both the high CA concentration and attapulgite clay content, might hinder and resist the degradation behavior of hydrogel membranes, compared to the low CA concentration and clay content, respectively. Notably, CA concentration ≥ 10 (wt%) and incorporated clay ≥ 4 (wt%) have progressively kept the mechanical stability of crosslinked membranes, where membranes lost almost 40% and 25–30% of their

weights after 15 days, respectively. This implies that, both using CA as crosslinker and incorporation of attapulgite clay have improved dramatically mechanical stability and hindered the hydrolytic degradation behavior of crosslinked PVA/CMC/attapulgite composite membranes. The current degradation results are entirely consistent with our previous reported results [15, 18, 19].

Table 3. The inhibition zones of crosslinked PVA/CMC andPVA/CMC/attapulgite clay composite hydrogel membranes against human pathogensusing disc diffusion assay; antimicrobial activity of membranes was tested asfunction of different CA concentrations and different clay contents

Conc./Content (wt%)	Inhibition zones (mm)			
	Candida albicans	*Escherichia coli*	*Klebsiella pneumoniae*	*Bacillus cereus*
CA				
7	18	20	20	17
10	20	12	19	15
12	25	16	22	11
Clay				
0	25	15	24	17
1	21	13	15	11
2	23	13	21	13
4	17	13	22	12
5	13	20	15	10

Bio-assessment Tests

Antimicrobial Activity

The antimicrobial activity of crosslinked PVA/CMC and PVA/CMC/clay hydrogel membranes was investigated using disc diffusion assay against different human pathogens, as function of different CA and clay contents; was presented in Fig. 7; Table 3.

Crosslinked PVA/CMC hydrogel membranes show remarked resistances against *C. albicans* with all used CA concentrations (Fig. 9; Table 3). It was observed that, increasing the concentration of citric acid was matched with the increase of the measured inhibition zones (Tale 3). When the CA concentration was raised from 7 to 12 (wt%), shows an increasing in the inhibition zones from 18 to 25 mm. On the other hand, prepared membranes show the lowest resistance against *B. cereus* microbe (Table 3). However, increasing CA concentrations from 7 to 12 (wt%) results in decreasing in the measured inhibition zones from 17 to 11 mm, respectively. While prepared hydrogel membranes exhibit a moderately resistance toward *E. coli* and *Klebsiella pneumonia*. A maximum inhibition zone was recorded of 22 mm with 12 (wt%) CA against *K. pneumoniae* and 12 mm as the lowest measured inhibition zone with 10 (wt%) CA was applied against *E. coli*. These results are consistent with obtained results by Mahdavinia et al. [33] and Siregar et al. [34], who reported that CA crosslinked PVA-CMC films have powerful antimicrobial properties. These results could

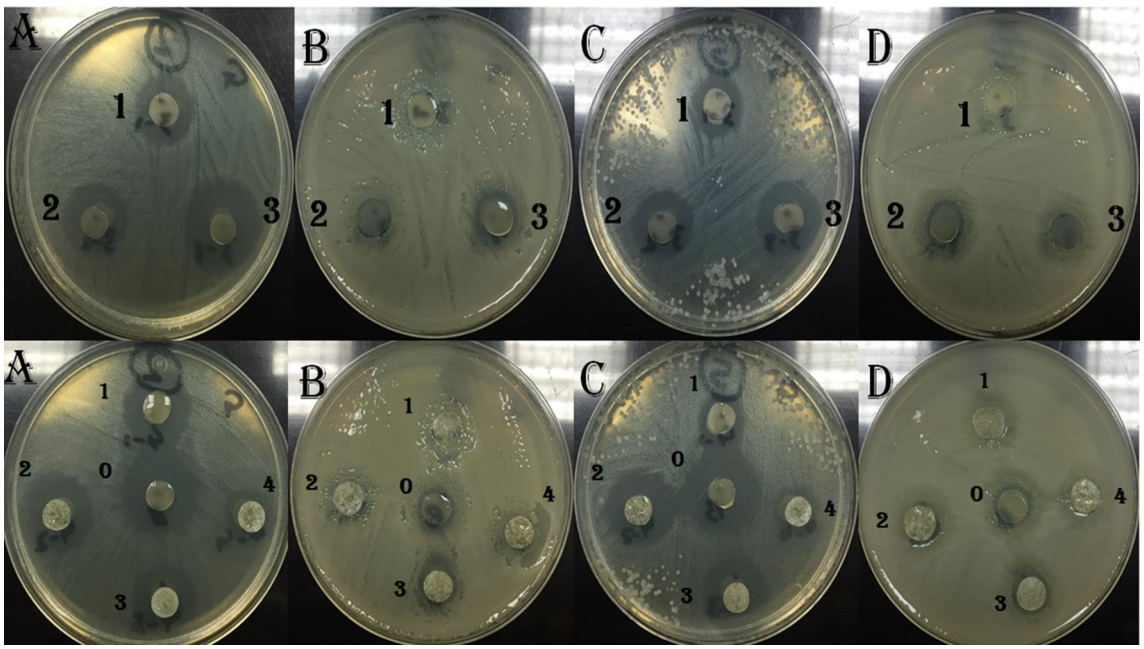


Fig. 9 Antimicrobial activity images representing the inhibition zones of crosslinked PVA/CMC hydrogel membranes, using different citric acid concentration at 7, 10, and 12 wt% (up) and using different incorporated attapulgite clay contents (1, 2, 4, and 5 wt%) (down)

be attributed to citric acid possesses non dissociated form (–COOH) that can permeate the bacterial cell membrane, and allowing to donate hydrogen ions to the system. To maintain the intracellular pH, H^+ ions are released resulting in weak pH, these acidic conditions lead to deformation and damage to cells also damage enzymatic activity, protein structure and DNA of the microorganism [35].

Also, crosslinked PVA/CMC/clay composite hydrogel membranes show remarked resistances against all tested microbes (Table 3). *C. albicans* was the most affected microbe by hydrogel membranes, while *B. cereus* was the lowest affected one. It was observed that, the membranes that lack clay was generally most effective against the tested microbes than clay-containing membranes. The clay-lacking membrane succeeded to affect the pathogens *C. albicans*, *K. pneumonia* and *B. cereus* and show inhibition zones measuring of 25, 24, and 17 mm, respectively. However, the pathogen of *E. coli* is highly affected by 5% incorporated clay that resulted in 20 mm inhibition zone, compared to 15 mm inhibition zone of clay-lacking membrane was applied. Thus, it could be concluded that each prepared membrane resulted in the formation of clear zones that are considered pathogen dependent. These results are consistent with results obtained by El-Bassyoni et al.[11], who demonstrated that PVA-HES-attapulgite composite hydrogel membranes tested for wound dressing showed significant inhibition zones with antimicrobial potency. It was varied according to the tested microbe and these results might be attributed to the presence of some metals in attapulgite clay composition which have antimicrobial character.

Hemolysis Test

The effect of citric acid concentration on the biocompatibility of prepared membranes was investigated according to the hemolysis percentage of a tested healthy blood sample. As shown in Table 4 (up), the percentage of citric acid is significantly affecting the blood hemolysis percentage. It was found that the lowest used concentration of citric acid (7 wt%) results in the highest hemolysis of tested membrane at (66%). Increasing citric acid concentration to 10 (wt%) shows a slightly reduction of hemolysis at (64%). However, the lowest hemolysis percentage is recorded as 35.5%, when the citric acid concentration is elevated to 12 (wt%). It could be concluded that the descending order of hemolysis would be summarized as: CA concentrations of 7 > 10 > 12 (wt%). Our results are matched with the biocompatible nature of citric acid as previously reported by Salihu et al. [36]. Also, Mali et al.[37] demonstrated that, hemolysis study of CMC composite hydrogel films crosslinked with citric acid indicated their hemocompatibility making them effective for drug delivery application. These results are attributed to the presence of three carboxylic groups in CA structure, so crosslinking of polymers with CA provides some pendant free carboxylic groups which is responsible for enhancing biocompatibility [38].

Table 4 Hemolysis percentage of crosslinked PVA/CMC and PVA/CMC/attapulgite clay composite hydrogel membranes, biocompatibility of membranes was tested as function of different CA concentrations and different clay contents

Tested parameters	Concentration/content (wt%)	OD $_{540\ nm}$	Hemolysis (%)
CA	7	0.924	66
	10	0.901	64.3
	12	0.496	35.4
Clay content	0	0.931	66.5
	1	1.038	74
	2	0.732	52.3
	4	1.060	75.7
	5	1.136	81
	Positive control	1.4	100

Different clay contents were tested for their biocompatibility depending on their behavior towards a healthy blood sample. As depicted in Table 4 (down), the percentage of blood hemolysis was varied according to the tested clay concentration. It was shown that, the lowest clay content in membranes of (1 wt%) results in a hemolytic percentage at 74%. While, increasing clay concentration to 4 and 5 (wt%) results in a gradual increase of hemolysis percentages of 75.7 and 81%, respectively. However, 2 (wt%) of clay provides the lowest blood hemolysis of 52.3%. According to these results, the ascending order of the clay concentration that resulted in high percentages of blood hemolysis could be summarized as: clay contents in membranes (2 < 1 < 4 < 5 (wt%)). Our results are consistent with results of Golafshan et al. [29], who demonstrated that the hemolysis ratio of PVA-alginate-Laponite clay hydrogel was promoted by increasing Laponite clay content for wound healing application. Also, it was reported that the clay incorporation into PVA hydrogels improves the hemolysis percentage and coagulation activity of blood making the hydrogel potential candidate for wound healing application [38, 39]. Moreover, gelatin-PVA-hydroxyapatite composite hydrogel membranes have high hemocompatibility making them suitable for biomedical applications [40].

Conclusions

In conclusion, PVA-CMC-CA composite hydrogel membranes were successfully prepared and tested for wound dressing's application, due to their unique features such as porosity, biocompatibility, non-cytotoxicity, and high

thermal and mechanical stability. The prepared hydrogel membranes were crosslinked *via* esterification mechanism using citric acid as a safe chemical crosslinker. Membranes were mechanically supported by incorporating of attapulgite mineral clay as additional physical crosslinker. PVA/CMC/attapulgite clay composite hydrogel membranes were characterized by FTIR, SEM, swelling tests, mechanical/thermal stability, as well as antimicrobial activity. The obtained results showed that, the properties of composite hydrogel membranes have significantly affected by the used CA concentration and incorporated attapulgite clay contents. It was concluded that, high CA concentration and high attapulgite clay contents in membranes kept the mechanical/thermal stability of crosslinked membranes, and resisted the degradation growth of crosslinked membranes; compared to low concentration and content of CA and clay, respectively. While, optimized CA concentration and attapulgite clay content in PVA/CMC membranes showed remarked antimicrobial activity, adequate swelling behavior, and high mechanical and thermal stability. Biocompatibility of PVA/CMC-attapulgite clay films was determined using hemolysis test which indicated that, CA crosslinked PVA-CMC-attapulgite clay hydrogels are safe for employing as a potential wound dressings and promising biomaterials for versatile biomedical purposes.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10924-022-02538-7.

Funding Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB). The authors have not disclosed any funding.

Declarations

Conflict of interest The authors report no financial or nonfinancial conflict of interest.

Ethical Approval All experiments were performed in accordance with the Guidelines of World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects, and approved by the ethics committee at Alexandria University and The British University in Egypt (BUE).

References

1. Namazi H, Rakhshaei R, Hamishehkar H, Kafil HS (2016) Antibiotic loaded carboxymethylcellulose/MCM-41 nanocomposite hydrogel films as potential wound dressing. Int J Biolog Macromol 85:327–334
2. Li Y, Zhu C, Fan D, Fu R, Ma P, Duan Z, Li X, Lei H, Chi L (2020) Construction of porous sponge-like PVA-CMC-PEG hydrogels with pH-sensitivity via phase separation for wound dressing. Int J Polym Mater Polym Biomater 69:505–515
3. Gajra B, Pandya SS, Vidyasagar G, Rabari H, Dedania RR, Rao S (2012) Poly vinyl alcohol hydrogel and its pharmaceutical and biomedical applications: a review. Int J Pharm Res 4:2026
4. Kenawy E-R, Kamoun EA, Mohy Eldin MS, El-Meligy MA (2014) Physically crosslinked poly (vinyl alcohol)-hydroxyethyl starch blend hydrogel membranes: synthesis and characterization for biomedical applications. Arab J Chem 7:372–380
5. Mali K, Dhawale S, Dias R, Dhane N, Ghorpade V (2018) Citric acid crosslinked carboxymethyl cellulose-based composite hydrogel films for drug delivery. Indian J Pharm Sci 80:657–667
6. Ibrahim MM, Koschella A, Kadry G, Heinze T (2013) Evaluation of cellulose and carboxymethyl cellulose/poly (vinyl alcohol) membranes. Carbohyd Polym 95:414–420
7. Ghorpade VS, Dias RJ, Mali KK, Mulla SI (2019) Citric acid crosslinked carboxymethylcellulose-polyvinyl alcohol hydrogel films for extended release of water soluble basic drugs. J Drug Deliv Sci Technol 52:421–430
8. El-Gamal S, El Sayed AM, Abdel-Hady E (2018) Effect of cobalt oxide nanoparticles on the nano-scale free volume and optical properties of biodegradable CMC/PVA films. J Polym Environment 26:2536–2545
9. Djumaev A, Tashmukhamedova S (2020) Physical and chemical properties of PVA-CMC based hydrogel carrier loaded with herbal hemostatic agent for application as wound dressings. Natl J Physiol Pharm Pharmacol 10:905–909
10. Kokabi M, Sirousazar M, Hassan ZM (2007) PVA–clay nanocomposite hydrogels for wound dressing. Eur Polym J 43:773–781
11. Elbassyoni S, Kamoun EA, Taha TH, Rashed MA, ElNozahi FA (2020) Effect of Egyptian attapulgite clay on the properties of PVA-HES–clay nanocomposite hydrogel membranes for wound dressing applications. Arab J Sci Eng 45:4737–4749
12. Hussein Y, Loutfy SA, Kamoun EA, El-Moslamy SH, Radwan EM, Elbehairi SEI (2021) Enhanced anti-cancer activity by localized delivery of curcumin form PVA/CNCs hydrogel membranes: preparation and in vitro bioevaluation. Int J Biolog Macromol 170:107–122
13. Kamoun EA, Kenawy E-RS, Tamer TM, El-Meligy MA, Mohy Eldin MS (2015) Poly (vinyl alcohol)-alginate physically crosslinked hydrogel membranes for wound dressing applications: characterization and bio-evaluation. Arab J Chem 8:38–47
14. Xu ZY, Li J-Y (2018) Enhanced swelling, mechanical and thermal properties of cellulose nanofibrils (CNF)/poly (vinyl alcohol)(PVA) hydrogels with controlled porous structure. J Nanosci Nanatechnol 18:668–675
15. Kamoun EA, Omer AM, Abu-Serie MM, Khattab SN, Ahmed HM, Elbardan AA (2018) Photopolymerized PVA-g-GMA hydrogels for biomedical applications: factors affecting hydrogel formation and bioevaluation tests. Arab J Sci Eng 43:3565–3575
16. Hussein Y, Kamoun EA, Loutfy SA, Amen R, Taha TH, Mansour AS, Abdel-Salam AI, Amer M (2021) Plant nanocellulose and its composite hydrogel membranes-based polyvinyl alcohol/hyaluronic acid for biomedical applications: extraction, characterization, and in vitro bioevaluation. J Appl Pharma Sci 11(1):49–60

17. Ahmed A, Niazi MBK, Jahan Z, Ahmad T, Hussain A, Pervaiz E, Janjua HA, Hussain Z (2020) In-vitro and in-vivo study of superabsorbent PVA/Starch/g-C3N4/Ag@ TiO2 NPs hydrogel membranes for wound dressing. Eur Polym J 130:109650
18. Fahmy A, Kamoun EA, El-Eisawy R, El-Fakharany EM, Taha TH, El-Damhougy BK, Abdelhai F (2015) Poly (vinyl alcohol)-hyaluronic acid membranes for wound dressing applications: synthesis and in vitro bio-evaluations. J Brazil Chem Soc 26:466–1474
19. Hussein Y, El-Fakharany EM, Kamoun EA, Loutfy SA, Amin R, Taha TH, Salim SA, Amer M (2020) Electrospun PVA/hyaluronic acid/L-arginine nanofibers for wound healing applications: nanofibers optimization and in vitro bioevaluation. Int J Biol Macromol 164:667–676
20. Reis EFD, Campos FS, Lage AP, Leite RC, Heneine LG, Vasconcelos WL, Lobato ZIP, Mansur HS (2006) Synthesis and characterization of poly (vinyl alcohol) hydrogels and hybrids for rMPB70 protein adsorption. Mater Res 9:185–191
21. Capanema NS, Mansur AA, de Jesus AC, Carvalho SM, de Oliveira LC, Mansur HS (2018) Superabsorbent crosslinked carboxymethyl cellulose-PEG hydrogels for potential wound dressing applications. Int J Biolog Macromol 106:1218–1234
22. Ghorpade VS, Yadav AV, Dias RJ, Mali KK, Pargaonkar SS, Shinde PV, Dhane NS (2018) Citric acid crosslinked carboxymethylcellulose-poly (ethylene glycol) hydrogel films for delivery of poorly soluble drugs. Int J Biolog Macromol 118:783–791
23. Shi R, Bi J, Zhang Z, Zhu A, Chen D, Zhou X, Zhang L, Tian W (2008) The effect of citric acid on the structural properties and cytotoxicity of the polyvinyl alcohol/starch films when molding at high temperature. Carbohydr Polym 74:763–770
24. Azeredo HM, Kontou-Vrettou C, Moates GK, Wellner N, Cross K, Pereira PH, Waldron KW (2015) Wheat straw hemicellulose films as affected by citric acid. Food Hydrocoll 50:1–6
25. Ghorpade VS, Yadav AV, Dias RJ (2016) Citric acid crosslinked cyclodextrin/hydroxypropylmethylcellulose hydrogel films for hydrophobic drug delivery. Int J Biological Macromol 93:75–86
26. Saputra AH, Apriliana NH (2018) Polyvinyl alcohol (PVA) partially hydrolyzed addition in synthesis of natural hydrogel carboxymethyl cellulose (CMC) based from water hyacinth. MATEC Web Confs EDP Sci 156:01007
27. Nataraj D, Reddy R, Reddy N (2020) Crosslinking electrospun poly (vinyl) alcohol fibers with citric acid to impart aqueous stability for medical applications. Eur Polym J 124:109484
28. Jiang Q, Reddy N, Yang Y (2010) Cytocompatible cross-linking of electrospun zein fibers for the development of water-stable tissue engineering scaffolds. Acta Biomater 6:4042–4051
29. Golafshan N, Rezahasani R, Esfahani MT, Kharaziha M, Khorasani S (2017) Nanohybrid hydrogels of laponite: PVA-Alginate as a potential wound healing material. Carbohydr Polym 176:392–401
30. Du J, Zhu J, Wu R, Xu S, Tan Y, Wang J (2015) A facile approach to prepare strong poly (acrylic acid)/LAPONITE® ionic nanocomposite hydrogels at high clay concentrations. RSC Adv 5:60152–60160
31. El-Lakany SA, Abd-Elhamid AI, Kamoun EA, El-Fakharany EM, Samy WM, Elgindy NA (2019) α-Bisabolol-loaded cross-linked zein nanofibrous 3d-scaffolds for accelerating wound healing and tissue regeneration in rats. Inter J Nanomed 14:8251
32. Demitri C, Del Sole R, Scalera F, Sannino A, Vasapollo G, Maffezzoli A, Ambrosio L, Nicolais L (2008) Novel superabsorbent cellulose-based hydrogels crosslinked with citric acid. J Appl Polym Sci 110:2453–2460
33. Mahdavinia GR, Mousanezhad S, Hosseinzadeh H, Darvishi F, Sabzi M (2016) Magnetic hydrogel beads based on PVA/sodium alginate/laponite RD and studying their BSA adsorption. Carbohydr Polym 147:379–391
34. Siregar F, Nasution D, Muis Y, Kaban D (2019) Preparation and characterization of antibacterial film based on carboxylmethylcellulose from Gebang leaf (Coryphautan), polyvynil alcohol and citric acid. RASAYAN J Chem 12:554–564
35. Mani-López E, García H, López-Malo A (2012) Organic acids as antimicrobials to control Salmonella in meat and poultry products. Food Res Int 45:713–721
36. Salihu R, Abd Razak SI, Zawawi NA, Kadir MRA, Ismail NI, Jusoh N, Mohamad MR, Nayan NHM (2021) Citric acid: A green cross-linker of biomaterials for biomedical applications. Eur Polym J 146:110271
37. Mali KK, Dhawale SC, Dias RJ (2017) Synthesis and characterization of hydrogel films of carboxymethyl tamarind gum using citric acid. Inter J Biological Macromol 105:463–470
38. Sabzi M, Afshari MJ, Babaahmadi M, Shafagh N (2020) pH-dependent swelling and antibiotic release from citric acid crosslinked poly (vinyl alcohol)(PVA)/nano silver hydrogels. Colloids Surf B 188:110757
39. Morariu S, Bercea M, Gradinaru LM, Rosca I, Avadanei M (2020) Versatile poly (vinyl alcohol)/clay physical hydrogels with tailorable structure as potential candidates for wound healing applications. Mater Sci Eng C 109:110395
40. Basak P, Pahari P, Das P, Das N, Samanta SK, Roy S (2018) Synthesis and characterisation of gelatin-PVA/hydroxyapetite (HAP) composite for medical applications. IOP Conf Ser: Mater Sci Eng 410:012021