JJBS

Volume 14, Number 1, March 2021
ISSN 1995-6673
Pages 1- 6

Jordan Journal of Biological Sciences

Development of biodegradable poly (vinyl alcohol) /chitosan cross linked membranes for antibacterial wound dressing applications

Mohamed E. Hassan[1*], Hassan A. Shehata[2], Alaa Fahmy[2], Mohand Badr[2], Tamer M. Tamer[3], Ahmed M. Omer[3*]

[1]Center of Excellence, Encapsulation & Nano biotechnology Group, Chemistry of Natural and Microbial Products Department, National Research Centre, El Behouth Street, Cairo 12622, Egypt,[2]Department of Chemistry, Faculty of Science, Al-Azhar University, Cairo, Egypt,[3]Polymeric Materials Research Department, Advanced Technology & New Materials Research Institute (ATNMRI), City of Scientific Research and Technological Applications (SRTA-City), Alexandria, Egypt.

Received February 6, 2020; Revised April 11, 2020; Accepted May 5, 2020

Abstract

In this study, poly vinyl alcohol/chitosan (PVA/Cs) membranes were developed via surface crosslinking technique for advanced wound dressing applications. Fourier Transform Infrared Spectrophotometer (FT-IR) and Scanning Electron Microscope (SEM) analysis tools were conducted to illustrate the chemical structures and the morphological changes of the developed membranes. Moreover, the mechanical analysis also investigated using tensile testing machine. The obtained results showed that PVA/Cs cross linked membrane recorded maximum force of 46.2 N compared with 26.09 N for Cs, indicating that the crosslinking process improved the developed membrane. Besides, the hydrophilic character of the developed membranes was examined using water uptake studies and decreased from 187% and 142% recorded by native Cs and PVA, respectively to 115% for the cross linked membrane. Two different types of bacteria were used for studying the anti-bacterial activities of the developed cross linked membranes, namely gram-positive bacteria *(Staphylococcus aureus)* and gram-negative bacteria *(Escherichia coli)*. The anti-bacterial activity of the developed cross linked membranes was augmented compared with native PVA and Cs membranes as the maximum inhibition (%) value increased from10 and 12% to 22 and 30% after crosslinking. Besides, the cross linked membranes exhibited better bio-degradability; moreover, the mechanical strength of the cross linked membranes showed good mechanical stability. The findings suggest that the cross linked PVA/Cs membranes could be efficiently applied as anti-bacterial and bio-degradable dressers for accelerating the wound healing process.

Keywords: Poly (vinyl alcohol); Chitosan; Anti-bacterial activity; Wound dressings

1. Introduction

Retrieval of wound is a specific biological process linked with the phenomena of growth and tissue regeneration (Bowler et al., 2001; Sen 2009). Recently, modern wound dressing has been applied to care for wounds instead of the traditional wound dressing, since it offers a moist environment at the wound region and hastens the migration process of epithelial cells to replace the dead cells and reconstitute the injured tissue (Alvarez et al., 1983; Boateng et al., 2008). Various requirements should be present in the modern wound dressing such as the ability for inhibition of the bacterial attack, reducing the inflammation of wound, easy sterilization/application, bio-compatibility, water/oxygen permeability and biodegradability (Guo and Luisa 2010; Jones et al., 2002). Polymers are widely used in biomedical industries including drug delivery systems, tissue engineering and wound dressing applications (Van de Velde and Paul 2002; Mona et al., 2016). Among these polymers, polyvinyl alcohol (PVA) is an amorphous vinyl polymer and is considered one of the oldest synthetic hydrophilic polymers (Hassan and Nikolaos 2000). PVA has been receiving much interest for several applications such as food industry, resins, water treatment and cosmetics (Kamal et al. 2014; Moulay 2015). Owing to its unique advantages such as hydrophilicity, bio-safety to the human cells, film forming ability, chemical resistance, mechanical resistance, good biocompatibility and biodegradability, PVA has been also applied for advanced bio-medical applications including wound dressing, surgical sutures, drug vehicles, contact lenses and artificial organs (Baker et al. 2012; Kamoun et al., 2017; Marin et al., 2014). Numerous physic-chemical modifications such as blending, chemical crosslinking, grafting and composite formation have been adopted for PVA in order to improve its clinical features. PVA has been modified with other

* Corresponding author e-mail: mohassan81@gmail.com.

** Abbreviations: FT-IR: Fourier Transform Infrared Spetrophotometer; SEM: Scanning Electron Microscope; PVA/Cs: poly vinyl alcohol/chitosan; PMN: polymorphonuclear; DD: degree of deacetylation; LB medium: Luria-Bertani medium; DNS: dinitrosalicylic acid.

natural polymers such as alginate, dextran and chitosan biopolymers to widen its bio-medical application range (Fathi et al., 2011; Kanatt et al., 2012; Levi et al., 2011).

Chitosan (Cs) is a nitrogen contained polysaccharide, and could be easily obtained via a simple deacetylation of chitin which is the main constituent of the exoskeleton of crustacean shells such as shrimps, lobsters, and crabs (Zargar et al., 2015; Samia et al., 2020). The structure of chitosan comprises randomly distributed (1-4)-linked 2-amino-2-deoxy-β -d-glucopyranose units (Sahoo et al., 2009; Ismail et al., 2019). Moreover, chitosan has reactive amine and hydroxyl groups along its backbone, soluble in aqueous acidic conditions and possesses a high viscosity which enables it to form intermolecular hydrogen bonds (Rinaudo 2006). Furthermore, chitosan has appealing biological properties including bio-degradability, mucoadhesive character, non-toxicity, excellent bio-compatibility and bio-activity (Moutinho et al., 2019; Pokhrel and Paras 2019). Therefore, chitosan has been employed in a wide range of advanced wound dressing, tissue engineering, drug delivery systems and health-care food supplements (Rami et al., 2014; Sun et al., 2019). The bio-activity of chitosan involves blood anti-coagulants, anti-inflammatory, anti-oxidant and antibacterial activities; hence it is able to form a strong protective film and can inhibit the attack of several types of microorganisms (Elchinger et al., 2015; Tamer et al., 2018). Several studies reported that chitosan has the ability to accelerate the wound healing process through stimulation of the migration of mononuclear cells and polymorphonuclear (PMN), which enhances the re-epithelization and skin tissue regeneration (Tamer et al., 2018). Besides, several modification techniques such as blending (Murakami et al., 2010), combination with bioactive agents (Sudheesh Kumar et al., 2012), Schiff base formation (Tamer et al., 2016) and chemical crosslinking (Kenawy et al., 2019) have been conducted for chitosan in order to promote its antibacterial activity against several types of bacteria, which in turn accelerates the process of wound healing.

Herein, we aimed to develop anti-bacterial and bio-degradable cross linked membranes based on PVA and chitosan bio-polymer for wound dressing applications. FT-IR and SEM analysis tools were conducted to investigate the chemical structure and the changes in the surface morphologies of the developed membranes. Moreover, the mechanical stability and hydrophilicity were also explored. The anti-bacterial activities of the cross linked membrane against two different types of bacteria were also evaluated.

2. Materials and methods

2.1. Materials

Chitosan (DD % = 93 %) was supplied from polymer department, ATNMRI, SRTA City, Alexandria; (Egypt). Poly (vinyl alcohol); Mwt 72000; hydrolyzed) was obtained from Biochemica, Germany. Sodium hydroxide (98 %), acetone (99%), ethanol (99%) and acetic acid (98%) were brought from El-Nasr Company (Alexandria), Glutaraldehyde (25%) was obtained from Sigma-Aldrich

2.2. Experimental

2.2.1. Preparation of chitosan membrane

Chitosan solution was prepared by dissolving chitosan in acetic acid solution 2% (w/v) and distilled water for 24 h at room temperature under a gentle stirring with a final concentration of 2 % (w/v). Thereafter, chitosan solution was casted in a clean petri-dish at room temperature for 48 h to ensure complete solvent evaporation.

2.2.2. Preparation of PVA membrane

PVA solution was obtained by dissolving PVA powder in hot distilled water under a gentle stirring at 80 ^{o}C for 3 h to obtain a homogenous solution with a final concentration 5% (w/v). On a clean petri dish, PVA solution was casted at room temperature for 48 h to ensure complete solvent evaporation. The membrane was separated, rinsed with 50 mL of acetone and allowed to dry for 24 h at room temperature.

2.2.3. Preparation of PVA/Cs membrane

A fixed volume of the previously prepared PVA and Cs solutions were mixed under a constant stirring for 1 h at room temperature to obtain a homogenous blend solution with a final ratio of PVA/Cs (1:1). On a clean petri dish, the PVA/Cs solution was casted at room temperature for 48 h to ensure complete solvent evaporation. The membrane was separated and rinsed with 50 mL of acetone.

2.2.4. Surface crosslinking process

Finally, prepared Cs, PVA, and PVA/Cs membranes were surface cross linked by soaking in 0.01% of glutaraldehyde/acetone solution at room temperature under a gentle stirring overnight. Then, the crosslinked membrane was washed several times with acetone to eliminate any unreacted glutaraldehyde and followed with air-drying overnight at room temperature.

2.2.5. Physicochemical characterization

The chemical structures of the developed cross linked membranes were investigated using Fourier Transform Infrared Spectrophotometer (Shimadzu FT-IR - 8400 S, Japan), while the surface morphological changes were examined by Scanning Electron Microscope (SEM; Model Jsm 6360 LA, Joel, Japan). Furthermore, the mechanical properties were studied using a universal testing machine (AG-1S, Shimadzu, Japan). Besides, water uptake (%) estimation was performed by soaking an accurate amount of dried samples in distilled water (pH 7) for 6 h at room temperature. The swollen samples were gently separated from the swelling medium and carefully bolted between two filter papers to eliminate the excess of adherent surface water and followed by weighing in an electronic balance. The percent of water uptake was calculated according to the following equation:

$$\text{Water uptake (\%)} = [(M_1 - M_0) / M_0] \times 100 \quad (1)$$

Where, M_0 and M_1 are weights of dried and swollen sample, respectively (Mohy Eldin et al. 2015a, Mohy Eldin et al. 2015b)

2.2.6. Anti-bacterial activity assay

Anti-microbial activity of the prepared membranes was tested depending on the previously reported procedure (Hassan et al., 2018). In brief, bacteria (*Staphylococcus*

aureus and *Escherichia coli*) were incubated in LB medium containing 0.5 % yeast extract, 1 % peptone and 1 % NaCl). The inoculation was at 37 °C for 24 h under shaking conditions. The obtained bacterial suspension was diluted with the previous peptone medium. Formerly, 0.1 mL of diluted bacteria suspension was cultured in 10 mL of liquid peptone medium. 10 mg of the tested membrane sample was dissolved in the previous medium and followed with sterilization at 121°C for 30 min. The inoculated medium remained shaking at 37 °C for 24 h. The bacterial growth inhibition (%) was assayed via measuring the absorbance of the culture medium at 620 nm using visible spectroscopy. The inhibition (%) was estimated by the following equation:

Where, A_b and A_a are the absorbance of bacterial culture in absence and in presence of tested sample, respectively.

2.2.7. Bio-degradability test

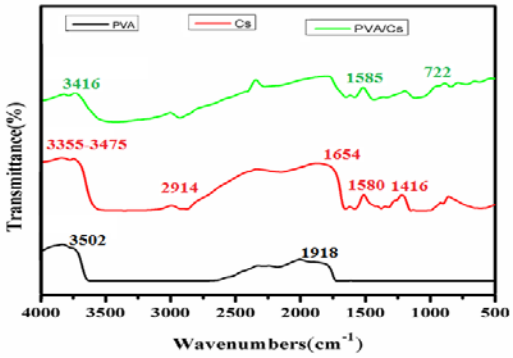
Consistent with the reported method (Miller 1959), bio-degradability of the developed cross linked membranes was measured using dinitrosalicylic acid (DNS) reagent. A definite amount of samples (0.1g) was soaked for 24 h in 2 mL of phosphate buffer (pH 7.0) and 0.5 mL of lysozyme solution at 37 °C. Next, 1.5 mL of DNS reagent was added to stop the activity of lysozyme and followed with boiling in for 15 min, and finally left to cool. The generated color from the reaction of liberated reduced sugars and DNS reagent was analyzed by measuring the optical density (OD) at 540 nm using a visible spectroscopy (Hassan et al., 2019).

3. Results

3.1. FT-IR analysis

FT-IR spectra of Cs, PVA, and PVA/Cs cross linked membranes were presented in Fig.1. From the spectrum of PVA, the characteristic band at 3502 cm^{-1} associated with the presence O-H groups, while the broad C-H stretching band appeared at 1918 cm^{-1}. The spectrum of the native chitosan showed a broad band around 3355-3475 cm^{-1} which corresponded to the stretching vibration of NH_2 and O-H groups (Abeer et al., 2017). The absorption band at 2914 cm^{-1} corresponded to both methyl and methylene groups. Moreover, the peak at 1654 cm^{-1}confirmed the presence of C=O and NH-C-O groups (Mostafa et al., 2019). The band at 1580 cm^{-1} was a result of C=C stretching in the aromatic ring. In addition, the band at 1416 cm^{-1} could be related to deformation vibration of C-N. Besides, the IR spectrum of PVA/Cs cross linked membrane showed absorption band at 3416 cm^{-1} as a result of the stretching vibration of NH_2 in secondary amides and OH groups on Cs combined with the present OH groups of PVA. Band at 1585 cm^{-1} corresponded to vibration of –NH- of Cs, and C-O stretching and C-H bending were observed at 722 cm^{-1} (Wahba and Hassan 2017). The results showed also that the crosslinking process clearly decreased the intensity of bands arising from the NH bending (amide II) at 1585 cm^{-1} of chitosan.

Figure1. FT-IR spectra PVA, Cs, and PVA/Cs cross linked



membranes.

3.2. SEM analysis

SEM images of native Cs, PVA and the cross linked PVA/Cs membranes were clarified in Fig. 2. As it can be seen, Cs showed a smooth surface with some wrinkles, while PVA exhibited more rough and un-homogenous surface. On the other hand, PVA/Cs membrane displayed a uniform surface without pores. All cross linked membranes exhibited a uniform surface without pores, which indicates the uniform distribution of PVA and PVA molecules throughout the films.

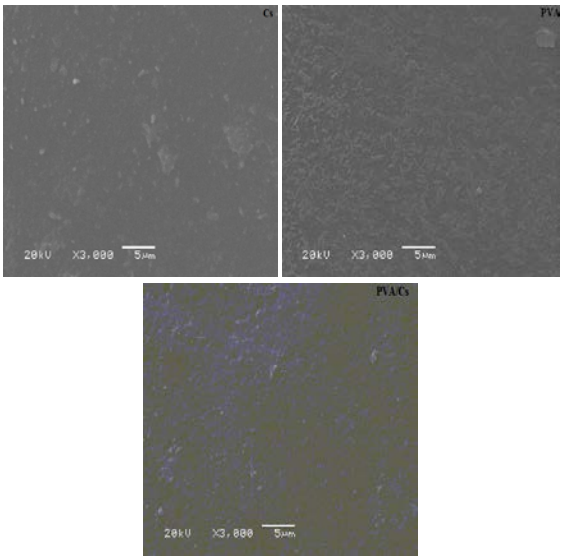


Figure2. SEM images of Cs, PVA and PVA / Cs cross linked membranes

3.3. Mechanical properties

Table 1 showed an increase in the tensile strength values for PVA membrane and associated with the strong interactions between the present ionic function groups. Moreover, the mechanical properties of Cs membrane were clearly improved after crosslinking with PVA. Where, PVA/Cs cross linked membrane recorded maximum force of 46.2 N compared with 26.09 N for neat Cs, indicating that the crosslinking process improved the developed membrane owing to the generated covalent bonds between PVA and Cs in addition to the present hydrogen bonds.

Table 1. Mechanical parameters of PVA, Cs, and PVA/Cs crosslinked membranes.

Sample	Max Force (N)	Max Dis. (mm)	Max stress (N/mm^2)	Max strain (%)	Energy (J)	Energy Max (J)
PVA	70.5	73.69	38.81	263.2	2.80	2.01
Cs	26.09	2.78	21.47	11.60	0.0548	0.0520
PVA/Cs (1:1)	46.2	41.70	34.45	129.3	1.05	1.01

3.4. Water uptake evaluation

Fig. 3 demonstrates the water uptake values of Cs, PVA and PVA/Cs cross linked membranes. The results showed that maximum water uptake values were 187% and 142% recorded by native Cs and PVA respectively, which can be attributed to the hydrophilic nature of both Cs and PVA. On the other hand, a significant decrease in water uptake value (115%) was observed in case of cross linked PVA/Cs membrane owing to the consumption of some hydrophilic groups through the crosslinking process in the membrane matrix.

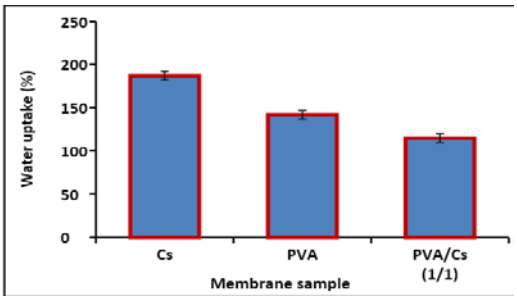


Figure 3. Water uptake values for PVA, Cs and PVA/Cs crosslinked membranes.

3.5. Anti-bacterial activity evaluation

Fig. 4 investigated the anti-bacterial activity of the developed cross linked membranes against *Staphylococcus aureus* as gram-positive bacteria and *Escherichia coli* as gram-negative bacteria. It was clear from the results that the anti-bacterial activity of the developed cross linked membranes were improved compared with native Cs and PVA membranes, whereas maximum inhibition (%) value increased from 10 and 12% for PVA membrane to 22 and 30 % after crosslinking with Cs.

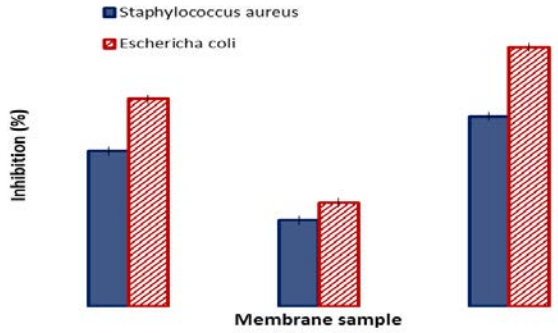


Figure 4: Anti-bacterial activity values for PVA, Cs and PVA/Cs cross linked membranes.

3.6. Bio-degradability evaluation

Fig. 5 clarified that all prepared membranes were bio-degraded in presence of lysozime. It is well known that the enzyme biodegrades polysaccharide via hydrolyzing the glycosidic bonds that existing in their structures.

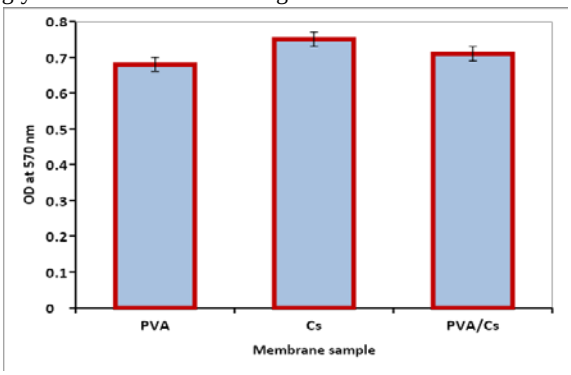


Figure 5. Bio-degradability of PVA, Cs and PVA/Cs cross linked membranes.

4. Discussion

Developing membrane to be used in wound healing process is an essential work, especially if this membrane formed from natural, biodegradable biopolymers such as polyvinyl chloride and chitosan (Balasubramaniet al., 2001).

Polyvinyl chloride/chitosan membrane shows better mechanical properties; besides, it has more function groups than the two constituents separately as shown in figure 1 (Omer et al., 2016; Hassan et al., 2019). Bio-degradability of the developed membranes could be related to the bio-degradation nature of native PVA and Cs, since both of them have hydrophilic groups such as OH groups (in case of PVA and Cs) and NH_2 group (in case of Cs) (Ueno and Toru 2001). These groups could impart the hydrophilicity of the developed membranes and offer several potentials for lysozime enzyme adsorption (Shewan and Jason 2013). Bacterial infection delays the process of wound healing and could lead to death (Dutta et al., 2009). Therefore, it is essential to evaluate the capability of the developed membranes for inhibiting the growth of different types of bacteria. The results showed that the inhibition (%) against gram-negative bacteria was higher than those against gram-positive bacteria. The interaction of positively charged amine groups present on the surface of cross linked membranes with the negatively charged cell surface that could block the feeding channels responsible for exchanging electrolytes and nutrients and, as a result, an inhibition of the normal bacteria metabolism took place and was followed with death of the bacterial cells. FT-IR, SEM and water uptake analysis concluded that the developed membrane has become more suitable for use in wound dressing with its new functional groups that make it more flexible and more antibacterial.

5. Conclusion

PVA/Cs cross linked membranes were prepared as anti-bacterial wound dressing membranes. FT-IR and SEM analysis tools were applied to investigate the chemical structure and surface morphologies of the developed membranes. Moreover, water uptake and mechanical properties were also evaluated. The developed membranes displayed a decent inhibition (%) against both gram positive and gram-negative bacteria. Results also showed

that PVA/Cs cross linked membranes were bio-degradable and could be applied for modern wound dressing applications.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Abeer AA, Faten AM, Mohamed EH, Eman RH, Mona AE (2017) Covalent immobilization of *Alternaria tenuissima* KM651985 laccase and some applied aspects. *Biocatalysis and Agricultural Biotechnology* 9: 74–81.

Alvarez OM, Patricia MM, William HE (1983) The Effect of Occlusive Dressings on Collagen Synthesis and Re-Epithelialization in Superficial Wounds. *Journal of Surgical Research* 35(2):142–48.

Baker MI, Steven PW, Zvi S, Barbara DB (2012) A Review of Polyvinyl Alcohol and Its Uses in Cartilage and Orthopedic Applications. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 100(5):1451–57.

Balasubramani M, Ravi KT, Mary B (2001) Skin Substitutes: A Review. *Burns: Journal of the International Society for Burn Injuries* 27(5):534.

Boateng JS, Kerr HM, Howard NES, Gillian ME (2008) Wound Healing Dressings and Drug Delivery Systems: A Review. *Journal of Pharmaceutical Sciences* 97(8):2892–2923.

Bowler PG, Duerden BI, David GA (2001) Wound Microbiology and Associated Approaches to Wound Management. *Clinical Microbiology Reviews* 14(2):244–69.

Dutta PK, Shipra T, Mehrotra GK, Joydeep D (2009) Perspectives for Chitosan Based Antimicrobial Films in Food Applications. *Food Chemistry* 114(4):1173–82.

Elchinger P, Cédric D, Sophie F, Olivier R, Stéphanie B, Thierry B, Claude T, Philippe M (2015) Immobilization of Proteases on Chitosan for the Development of Films with Anti-Biofilm Properties. *International Journal of Biological Macromolecules* 72:1063–68.

Fathi E, Nahid A, Mohamad I, Zeinab A (2011) Physically Crosslinked Polyvinyl Alcohol–Dextran Blend Xerogels: Morphology and Thermal Behavior. *Carbohydrate Polymers* 84(1):145–52.

Guo S, Luisa AD (2010) Factors Affecting Wound Healing. *Journal of Dental Research* 89(3):219–29.

Hassan CM, Nikolaos AP (2000) Structure and Applications of Poly (Vinyl Alcohol) Hydrogels Produced by Conventional Crosslinking or by Freezing/Thawing Methods. Pp. 37–65 in *Biopolymers· PVA Hydrogels, Anionic Polymerisation Nanocomposites*. Springer.

Hassan MA, Omer AM, Abbas E, Based WMA, Tamer TM (2018) Preparation, Physicochemical Characterization and Antimicrobial Activities of Novel Two Phenolic Chitosan Schiff Base Derivatives. *Scientific Reports* 8(1):11416.

Hassan ME, Jun B, Dou DQ (2019) Biopolymers; Definition, Classification and Applications. *Egyptian Journal of Chemistry* 62(9):1725-1737.

Hassan ME, Yang Q and Xiao Z (2019) Covalent immobilization of glucoamylase enzyme onto chemically activated surface of κ-carrageenan. *Bulletin of the National Research Centre* 43:102.

Ismail SA, Hassan ME, Hashem AM (2019) Single step hydrolysis of chitin using thermophilic immobilized exochitinase on carrageenan-guar gum gel beads. Biocatalysis and Agricultural Biotechnology 21: 101281

Jones I, Lachlan C, Robin M (2002) A Guide to Biological Skin Substitutes. *British Journal of Plastic Surgery* 55(3):185–93.

Kamal H, Hegazy E, Mohamed MM, Sabaa MW, El-Dessouky MM (2014) Adsorption Properties of PVA/PAA/Clay Composite Hydrogel Synthesized by Gamma Radiation and Its Application in Removal of Crystal Violet Dye from Its Aqueous Solution. *Journal of Nuclear Technology in Applied Science* 2(5):523–37.

Kamoun EA, Kenawy E, Xin C (2017) A Review on Polymeric Hydrogel Membranes for Wound Dressing Applications: PVA-Based Hydrogel Dressings. *Journal of Advanced Research* 8(3):217–33.

Kanatt SR, Rao MS, Chawla SP, Arun S (2012) Active Chitosan–Polyvinyl Alcohol Films with Natural Extracts. *Food Hydrocolloids* 29(2):290–97.

Kenawy E, Omer AM, Tamer TM, Elmeligy MA, Mohy Eldin MS (2019) Fabrication of Biodegradable Gelatin/Chitosan/Cinnamaldehyde Crosslinked Membranes for Antibacterial Wound Dressing Applications. *International Journal of Biological Macromolecules* 139:440–48.

Levi S, Vladislav R, Verica M, Vesna R, Branko B, Teresa F, Katarzyna EK, Viktor N (2011) Limonene Encapsulation in Alginate/Poly (Vinyl Alcohol). *Procedia Food Science* 1:1816–20.

Marin E, John R, Yhors C (2014) A Review of Polyvinyl Alcohol Derivatives: Promising Materials for Pharmaceutical and Biomedical Applications. *African Journal of Pharmacy and Pharmacology* 8(24):674–84.

Miller GL (1959) Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry* 31(3):426–28.

Mohy Eldin MS, Omer AM, Wassel MA, Tamer TM, Abd-Elmonem MS, Ibrahim SA (2015a) Novel Smart pH Sensitive Chitosan Grafted Alginate Hydrogel Microcapsules for Oral Protein Delivery: I. Preparation and Characterization. *International Journal of Pharmacy and Pharmaceutical Sciences* 7(10):320–26.

Mohy Eldin MS, Omer AM, Wassel MA, Tamer TM, Abd-Elmonem MS, Ibrahim SA(2015b) Novel smart pH sensitive chitosan grafted alginate hydrogel microcapsules for oral protein delivery: II. Evaluation of the swelling behavior. *International Journal of Pharmacy and Pharmaceutical Sciences* 7(10) 331-337.

Mona AE, Amira AG, Mohamed MIH, Mohamed EH, Naziha MH, Amal MH (2016) Enzymatic synthesis using immobilized *Enterococcus faecalis* Esawy dextransucrase and some applied studies. *Int J Biol Macromol* 92:56–62

Mostafa FA, El Aty AAA, Hassan ME, Awad GEA (2019) Immobilization of xylanase on modified grafted alginate polyethyleneimine bead based on impact of sodium cation effect. *International Journal of Biological Macromolecules* 140: 1284-1295.

Moulay S (2015) Poly (Vinyl Alcohol) Functionalizations and Applications. *Polymer-Plastics Technology and Engineering* 54(12):1289–1319.

Moutinho I, Inês CO, Mariana CS, Vasconcelos M, Ana P (2019) Different Chitosan-Based Biomaterials and Their Biomedical Applications. *Eur J Med Res Clin Trials* 1: 1-12.

Murakami K, Hiroshi A, Shingo N, Shin-ichiro N, Megumi T, Motoaki H, Satoko K, Hidemi H, Yoshihiro T, Tomoharu K (2010) Hydrogel Blends of Chitin/Chitosan, Fucoidan and Alginate as Healing-Impaired Wound Dressings. *Biomaterials* 31(1):83–90.

Omer AM, Tamer TM, Hassan MA, Rychter P, Mohy Eldin MS, Koseva N (2016) Development of Amphoteric Alginate/Aminated Chitosan Coated Microbeads for Oral Protein Delivery. *International Journal of Biological Macromolecules* 92:362–70.

Pokhrel S, Paras NY (2019) Functionalization of Chitosan Polymer and Their Applications. *Journal of Macromolecular Science, Part A* 56(5):450–75.

Rami L, Sebastien M, Samantha D, Jean-Christophe F, Robin S, Silke S, Eric L, Joëlle A, Alexandra M, Laurent D (2014) Physicochemical Modulation of Chitosan-based Hydrogels Induces Different Biological Responses: Interest for Tissue Engineering. *Journal of Biomedical Materials Research Part A* 102(10):3666–76.

Rinaudo M (2006) Chitin and Chitosan: Properties and Applications. *Progress in Polymer Science* 31(7):603–32.

Sahoo D, Sarmila S, Priyanka M, Sasmal S, Nayak PL (2009) Chitosan: A New Versatile Bio-Polymer for Various Applications. *Designed Monomers and Polymers* 12(5):377–404.

Sen CK (2009) Wound Healing Essentials: Let There Be Oxygen. *Wound Repair and Regeneration* 17(1):1–18.

Shewan HM, Jason RS (2013) Review of Techniques to Manufacture Micro-Hydrogel Particles for the Food Industry and Their Applications. *Journal of Food Engineering* 119(4):781–92.

Samia AA, Mohamed AAA, Gamal MES, Atef MI, Aliaa RES, Sherien MMA, Mohamed EH (2020) Catalytic, kinetic and thermal properties of free and immobilized Bacillus subtilis -MK1 α-amylase on Chitosan-magnetic nanoparticles. *Biotechnology Reports* 26: e00443

Sudheesh Kumar PT, Vinoth-Kumar L, Anilkumar TV, Ramya C, Reshmi P, Unnikrishnan AG, Shantikumar VN, Jayakumar R (2012) Flexible and Microporous Chitosan Hydrogel/Nano ZnO Composite Bandages for Wound Dressing: In Vitro and in Vivo Evaluation. *ACS Applied Materials & Interfaces* 4(5):2618–29.

Sun X, Chao L, Omer AM, Li-Ye Y, Xiao-kun O (2019) Dual-Layered PH-Sensitive Alginate/Chitosan/Kappa-Carrageenan Microbeads for Colon-Targeted Release of 5-Fluorouracil. *International Journal of Biological Macromolecules* 132:487–94.

Tamer TM, Valachová K, Collins MN, Hassan MA, Omer AM, Mohy Eldin MS, Švík K, Jurˇcík R, Ondruška L, Biró C, Albadarin AB, Šoltés L (2018) MitoQ Loaded Chitosan-Hyaluronan Composite Membranes for Wound Healing. *Materials* 11, 569

Tamer TM, Hassan MA, Omer AM, Based WMA, Hassan ME, El-Shafeey M, Mohy Eldin MS (2016) Synthesis, Characterization and Antimicrobial Evaluation of Two Aromatic Chitosan Schiff Base Derivatives. *Process Biochemistry* 51(10):1721–30.

Tamer TM, Valachová V, Hassan MA, Omer AM, El-Shafeey M, Mohy Eldin MS, Šoltés L (2018) Chitosan/Hyaluronan/Edaravone Membranes for Anti-Inflammatory Wound Dressing: In Vitro and in Vivo Evaluation Studies. *Materials Science and Engineering: C* 90:227–35.

Ueno H, Takashi M, Toru F (2001) Topical Formulations and Wound Healing Applications of Chitosan. *Advanced Drug Delivery Reviews* 52(2):105–15.

Van de Velde K, Paul K (2002) Biopolymers: Overview of Several Properties and Consequences on Their Applications. *Polymer Testing* 21(4):433–42.

Wahba MI, Hassan ME (2017) Agar-Carrageenan Hydrogel Blend as a Carrier for the Covalent Immobilization of ®-D-Galactosidase. *Macromol Res* 25(9):913–923

Zargar V, Morteza A, Amir D (2015) A Review on Chitin and Chitosan Polymers: Structure, Chemistry, Solubility, Derivatives, and Applications. *ChemBioEng Reviews* 2(3):204–26.