



Original Paper

**Journal of Innovative Engineering  
and Natural Science**

(Yenilikçi Mühendislik ve Doğa Bilimleri Dergisi)

<https://dergipark.org.tr/en/pub/jiens>

## Influence of essential oil on the properties of UV-crosslinked Polyacrylamide/sodium caseinate (PAAM/SC) hydrogels

Ahmetcan Gün<sup>a</sup>, Azra Albayrak<sup>a</sup>, Fatma Nur Parın<sup>a\*</sup>, Recep İlhan<sup>a,b</sup>, Uğur Parın<sup>c</sup>

<sup>a</sup>Polymer Materials Engineering, Bursa Technical University, Bursa, 16310, Turkey<sup>b</sup>Mechanical Engineering, Bursa Technical University, Bursa, 16310, Turkey<sup>c</sup>Department of Microbiology, Aydın Adnan Menderes University, Aydın, 09000, Turkey

### ARTICLE INFO

#### Article history:

Received 20 Sep 2024

Received in revised form 12 Dec 2024

Accepted 11 Jan 2025

Available online

#### Keywords:

Polyacrylamide/sodium caseinate hydrogels

β-CD

Orange blossom essential oil (OBEO)

Compression test

Antibacterial efficiency

### ABSTRACT

Presently, numerous studies have shown that hydrogels can help with wound healing in a variety of approaches. Oil-loaded protein-based hydrogels were fast produced via free radical photopolymerization (UV crosslinking). The water phase consisted of sodium caseinate polymer and acrylamide monomer, whereas the oil phase included orange blossom essential oil (OBEO). The bio-based surfactant β-cyclodextrin (β-CD) stabilized oil loaded-hydrogels. β-CD/OBEO complexes in specific proportions (1:1, 1:2, and 1:4) was added to water phase. ATR-FT-IR confirms the functional groups in hydrogels. The hydrogels have a swelling ratio above 280 % for 24 hours. The maximum compression strength for hydrogels with (β-CD/OBEO, 1:2) is almost 1 MPa. All oil-loaded hydrogels showed antibacterial efficiency against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*), with inhibition zones of 6-10 mm. According to the findings, the synthesized hydrogels can be used as wound dressings in wound healing applications.

## I. INTRODUCTION

The skin on the human body exerts a significant influence on many different human activities, including protection from pathogens, sensing the external environment and regulating body temperature. In addition to its elastic and soft tissue, the skin is susceptible to damage as a result of external factors because it envelops the body. Skin damage is one of the most common physical injuries in human history over many years. Designing new wound treatment materials is a very necessary issue in the findings of modern medical technology [1]. Human skin has the ability to self-repair these damages to maintain its structural integrity. Even though it has this ability, the skin must be supported for wound care (especially for large and open wounds or burns) to prevent infection and desiccation, relieve pain, protect the open area, accelerate the healing process and prevent scarring [2]. Wound dressings play an important role in the healing process. Wound dressings are developed in various materials and forms for different wound types and healing stages. Examples of these forms are gel, alginate, hydrogel, foam, hydrocolloid, film. Hydrogels have been the center of attention of researchers in recent years due to their high water content and providing a moist environment for wound tissue.

Hydrogels are cross-linked, hydrophilic polymer chains containing water without dissolving [3]. Hydrogels have been around for many years and these days, hydrogels continue to fascinate materials scientists and biomedical researchers [4]. Because of their high swelling ratio, biocompatibility, and porous shape, hydrogels with

\*Corresponding author. Tel.: +90-224-300-3611; e-mail: nur.parin@btu.edu.tr

antibacterial characteristics have been thoroughly researched [5]. Hydrogels are frequently used in agriculture to maintain soil moisture in dry climates, as well as in sanitary applications like tampons and diapers. Another crucial category of wet wound dressings that may meet most of the ideal wound dressing requirements is hydrogel. Hydrogel wound dressings have many standards for a suitable wound dressing, including supporting moist healing, rehydrating dead tissues, improving debridement healing, cleansing dry, sloughy or necrotic wounds, avoiding biological reactions, cooling the wound surface, and being non-irritant, non-adherent, and permeable to metabolites. Natural materials offer therapeutic value in enhancing stem cell activity and speeding wound healing due to their bioactive components. Hydrogels may be prepared using many methods, such as freeze-thawing, gas foaming, emulsion templating, and particle leaching. The oil-in-water emulsion template is increasing in popularity for creating composite hydrogels due to its ease of usage and versatility [6].

Sodium caseinate is a compound derived from casein, a protein present in mammals' milk. Therefore, casein-based hydrogels are biocompatible, biodegradable, renewable, readily available, affordable, and non-toxic [7]. Various forms of casein hydrogels have been utilized for the delivery and controlled release of a wide range of biomolecules [8]. To enhance a crosslink reaction, casein hydrogels must contain a crosslinking agent. Casein crosslinking agents that have been researched in previous studies include glutaraldehyde (Glu) [7].

Phytochemicals such as essential oils, tannins, flavonoids and alkaloids may exhibit broad-spectrum antimicrobial activity against bacteria, fungi and other pathogens. Some synthetic drugs may also have antimicrobial effects, but the development of antibiotic resistance is a growing concern related to synthetic antibiotics. In addition, medicinal plants often contain compounds that stimulate tissue regeneration and wound closure.

The chemical composition of essential oils and their effects on tissue have been extensively researched for decades. Anti-inflammatory, antioxidant and antimicrobial effects of the material used during tissue repair are very important. Essential oils exhibit strong properties related to these three main issues [9,10]. Orange blossom essential oil (OBEO) is known as neroli and it has therapeutic characteristics, including antibacterial activity, anti-inflammatory, and antioxidant effects [11]. These bioactivities owing to many terpenes such as linalool (44-53%),  $\alpha$ -terpineol (5-6%), geraniol (3-4%), (E)-nerolidol and (E,E)-2,6-farnesol (2-5%), limonene (8-12%), (E)- $\beta$ -ocimene (3-5%), and  $\beta$ -pinene (2-4%) from the oil [12]. Many of the studies showed that these terpene groups protect pathogens. Furthermore, these groups may be polymerized or functionalized with other monomers and chemical groups.

In the last 5 years, researchers have become increasingly interested in the effect of essential oils on hydrogels. For instance, Naik et al. (2021) studied the potential of polyacrylamide-based materials loaded with essential oils for wound healing applications [13]. Moradi et al. (2024) synthesized hydrogels loaded with antibacterial myrtle oil nanoemulsions for burn treatment, using a free radical polymerization method to graft acrylamide and acrylic acid [14]. Li et al. (2022) explored the antibacterial properties of film-shaped acrylamide-based nanocomposite hydrogels incorporating essential oils [15]. These studies collectively demonstrate the potential of acrylamide-based hydrogels to acquire diverse properties when loaded with essential oils.

This study aims to be able to produce cross-linked hydrogels in very short periods with UV, as opposed to cross-linking by thermal method. Therefore, the OBEO-loaded polyacrylamide/sodium caseinate hydrogels were synthesized via UV crosslinking method. The changes in the amount of OBEO on the morphological,

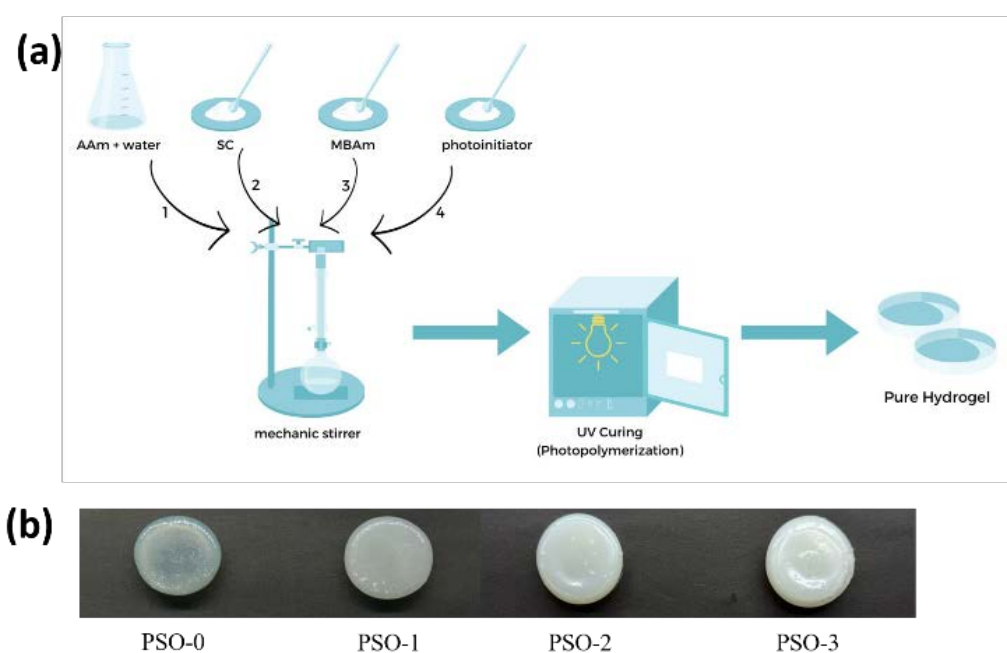
spectroscopical, swelling, and mechanical properties of the hydrogels produced have been studied. Moreover, antibacterial efficiency against gram (-) *Escherichia coli*, and gram (+) *Staphylococcus aureus* bacteria has been examined.

## II. EXPERIMENTAL METHOD

### 2.1 Materials and Preparation Techniques

Acrylamide powder (AAm) Veskim Chemical Company, Turkey, sodium caseinate (Alfasol, Turkey) and orange blossom essential essential oil (>95% purity) were purchased from KDR-Natural, Turkey.  $\beta$ -cyclodextrin ( $\beta$ -CD) was kindly donated by Wacker Chemical Company, Germany. MBAAm (99% purity, Sigma Aldrich Chemie GmbH, Steinheim, Germany) and Irgacure 2959 (99% purity, Sigma Aldrich Chemie GmbH, Steinheim, Germany) were used as received. Glycerol and citric acid were purchased from the local market. Ethanol (98% purity) was bought out from Sigma Aldrich Chemie GmbH, Steinheim, Germany). Distilled water was used in the experiments.

To obtain hydrogels, a certain amount of AAm monomer was dissolved in water using a magnetical stirrer. Then, a certain amount of sodium caseinate also was added to the solution and the added to materials were mixed by mechanical stirrer at 100 rpm for 5 minutes. After obtaining a homogeneous mixture, a certain amount of MBAAm crosslinker, and Irgacure 2959 (photoinitiator) was added to the mixture, respectively. All materials were stirred until completely dissolved (about 15 min). With this method, pure PAAm/SC hydrogel was obtained.



**Figure 1.** (a) Schematical illustration and (b) digital images of preparation of PAAm/SC hydrogels

The resulting solutions were cured for 30 minutes to ensure crosslinking. UV curing (300W, 20 cm distance) was used in this study. The cured hydrogels were washed in alcohol (10 mL) to remove impurities. The pure hydrogel sample was coded as PSO-0.

To obtain oil-loaded hydrogels, different ratios of OBEO were determined to consider  $\beta$ -CD/OBEO ratios. After preparing homogenous hydrogel solutions, OBEO has been added drop by drop to the solution mixture (Figure 1). These  $\beta$ -CD/OBEO ratios were adjusted as 1:1, 1:2, and 1:4, and sample codes were identified as PSO-1, PSO-2, and PSO-3, respectively. All synthesized hydrogel samples were kept in a desiccator until material characterization. The hydrogel composition is given in Table 1. These ratios were calculated based on the total polymer solution (5 mL).

**Table 1.** The compositions of the hydrogel

Samples	AAm (%)	Sodium caseinate (%)	MBAAm (%)	$\beta$ -CD/OBEO	Photoinitiator (w/v) (%)
PSO-0	50	10	10	-	0.03
PSO-1	50	10	10	1:1	0.03
PSO-2	50	10	10	1:2	0.03
PSO-3	50	10	10	1:4	0.03

## 2.2 Optical Microscope Analysis

The morphology of pure hydrogel and oil-loaded hydrogel samples was observed optical microscope (Leica-M125) with 10X magnification.

## 2.3 Spectroscopic Analysis

FT-IR spectrophotometer (Perkin Elmer/Spectrum Two) was used for chemical structural characterization of the obtained films. The films were scanned using diamond crystal in the wavenumber range from 4000 to 500  $\text{cm}^{-1}$  with an average 16 scans at 4  $\text{cm}^{-1}$  resolution by transmission mode.

## 2.4 Swelling Test

Swelling ratios (SR) of all samples have been calculated as follows. Firstly, a dried the hydrogels were soaked at room temperature in distilled water. The hydrogels were washed away and weighed at specific time intervals. The swelling ratio was calculated as follows [16].

$$SR (\%) = \frac{W_s - W_d}{W_d} \times 100 \quad (1)$$

## 2.5 Compression test

Compression tests were carried out by compressing the hydrogels (cylindrical, diameter  $33.81 \pm 0.71$  mm, height  $6.78 \pm 0.52$ , and fully hydrated) to 60% of their original height using a universal testing machine (Shimadzu, model AGS-X, Japan) at a load of 1 kN and a crosshead speed of 2 mm/min. During all tests force and displacement data were collected. Then using the data, the elastic modulus, and compression stresses of the hydrogels were obtained. The modulus was calculated from the linear slope of the stress–strain curves at low strains. Compression stress is the stress value at 60% strain. The stress ( $\sigma_c$ ) was calculated by  $\sigma_c = 4F/\pi d^2$ , where F was the loading force, and d was the original diameter of the hydrogel. All tests were performed for the hydrogels at ambient conditions (25°C, 60% RH), and results were given with their standard deviations.

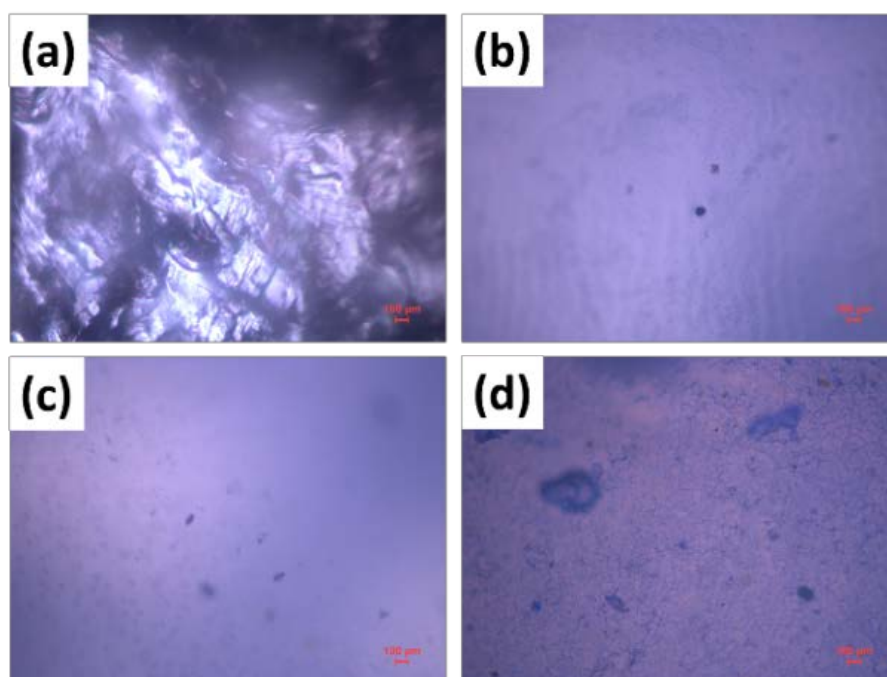
### 2.6 Antibacterial Efficiency

The antibacterial efficiencies of gels were determined by the standard strains of *Escherichia coli* ATCC® 25922, and *Staphylococcus aureus* ATCC® 25923. Trypton Soy Agar (Merck Millipore™ 105458) was used for the growth of lyophilized bacterial strains. The inoculated culture media were incubated for 24 hours (37°C) under aerobic conditions. In an isotonic saline solution, bacterial suspensions were adjusted to 0.5 McFarland ( $1 \times 10^8$  CFU/mL) turbidity. Antimicrobial efficacy was evaluated qualitatively by the disk diffusion method.

## III. RESULTS AND DISCUSSIONS

### 3.1 Optical Microscope Analysis

It is observed that the amount of oil changes the surface images of hydrogels (Figure 2). The addition of oil into hydrogel solution did not appear to alter the integrity of the hydrogels, as they maintained their form with slight fluctuations in volume. Surface morphology exceptions were observed in control hydrogels (without cells), where the pure sample contracted and wrinkled on the surface [17]. However, samples with oil have oil droplets in the sample (Figure 2b, 2c, and 2d). Further, Optical these images reveal the coarse texture of hydrogels [18]. The dispersion of oil droplets is unequal. It can be from the oil molecules' random distribution that different ester form hydrophobic interactions with the polymer molecules.



**Figure 2.** Optical microscope images of PAAm/SC hydrogels. (a) PSO-0, (b) PSO-1, (c) PSO-2, (d) PSO-3, respectively

### 3.2 Spectroscopic Analysis

The functional groups and physical interactions between polyacrylamide – sodium caseinate and OBEO were examined using FT-IR (Figure 3). The spectra show distinct absorption peaks for AAm, sodium caseinate and OBEO. Hydrogels exhibit stretching vibrations of the  $-NH_2$  group in the range  $3410-3421\text{ cm}^{-1}$ . The peaks at  $1682-$

1685  $\text{cm}^{-1}$  (stretching of  $-\text{C}=\text{O}$ ) correspond to the properties of the acrylamide unit [19]. The peaks of sodium caseinate at 1541 and 1658  $\text{cm}^{-1}$  due to amide 1 and amide 2 groups [20].

The OBEO showed a characteristic peak at 1743  $\text{cm}^{-1}$  which is identified as the stretch of the  $-\text{C}=\text{O}$  bond due to the ester group [21]. In this study, the absorption peak at 1721  $\text{cm}^{-1}$  was found ( $-\text{C}=\text{O}$ ) and the peaks at 3320  $\text{cm}^{-1}$  was related to  $-\text{OH}$  stretch. The absorption bands in 2929  $\text{cm}^{-1}$ , 2872  $\text{cm}^{-1}$  in OBEO spectrum due to the asymmetric and symmetric C-H stretching. The bands at 1038  $\text{cm}^{-1}$  and 1075  $\text{cm}^{-1}$  were attributed to C-O stretching vibration. The detected peaks are in parallel with the literature [21-23].

Changes in the FTIR spectra of hydrogels produced by incorporation of oil into the polymer solution have been observed. As the oil concentration increases, peaks caused by oil have been clearly observed in PSO-3 hydrogels at 2934  $\text{cm}^{-1}$  and 2857  $\text{cm}^{-1}$  owing to the asymmetric and symmetric C-H stretching. Further, the peaks intensity of PSO-0 compared to PSO-2 were slightly decreased at 1665  $\text{cm}^{-1}$ , 1618  $\text{cm}^{-1}$ , and 1461  $\text{cm}^{-1}$ . The peak in OBEO at 1038  $\text{cm}^{-1}$  showed its presence in the samples PSO-1 and PSO-3. A peak of 1653  $\text{cm}^{-1}$  in the PSO3 hydrogel may indicate that a new bond may be formed between the main terpenoid components (linalool, terpinen-4-ol,  $\alpha$ -terpineol) in the OBEO and acrylamide.

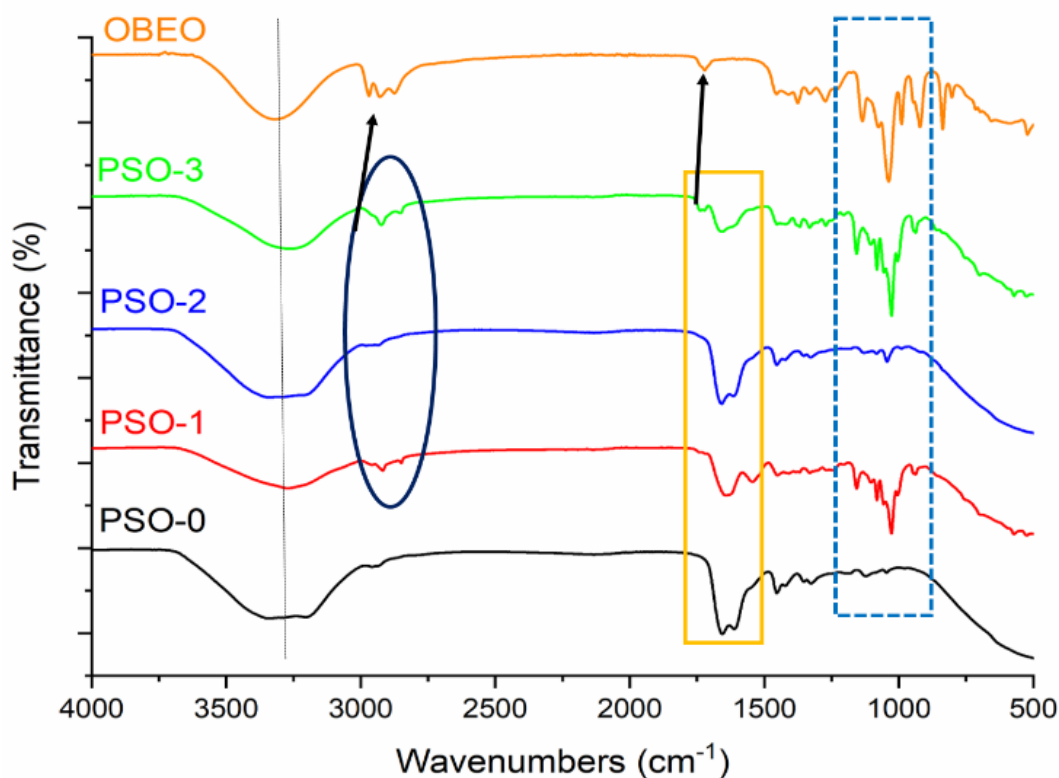


Figure 3. FT-IR spectra of the PAAm/SC hydrogels and OBEO

### 3.3 Swelling Test

Swelling is critical to investigating the properties of hydrogels. To measure the swelling ratio, a hydrogel sample's swelling capacity versus time profile is created by measuring free-absorbency capacity at regular periods [24]. The abundance of amide, carboxyl, and hydroxyl groups in sodium caseinate/acrylamide hydrogel contributes to its swelling ratio [25]. It was obvious that the oil ratio had a significant influence on the swelling behaviour of oil-

loaded hydrogels. It is expected that the swelling ratio will decrease with an increase in the amount of essential oil. However, as the amount of oil increased in the study, results showed decreased swelling ratios except for the PSO-2 hydrogel (Figure 4). Because the formation of new bonds between the OBEO and the polymer matrix (polyacrylamide and sodium caseinate), as in the mechanical test results. The swelling ratios at the end of 24 hours were found to be 310.7%, 304.5%, 286.5%, and 310.8% in the PSO-0, PSO-1, PSO-2, and PSO-3, respectively. However, any extreme difference was observed between the swelling properties of hydrogels and an increase in the amount of oil.

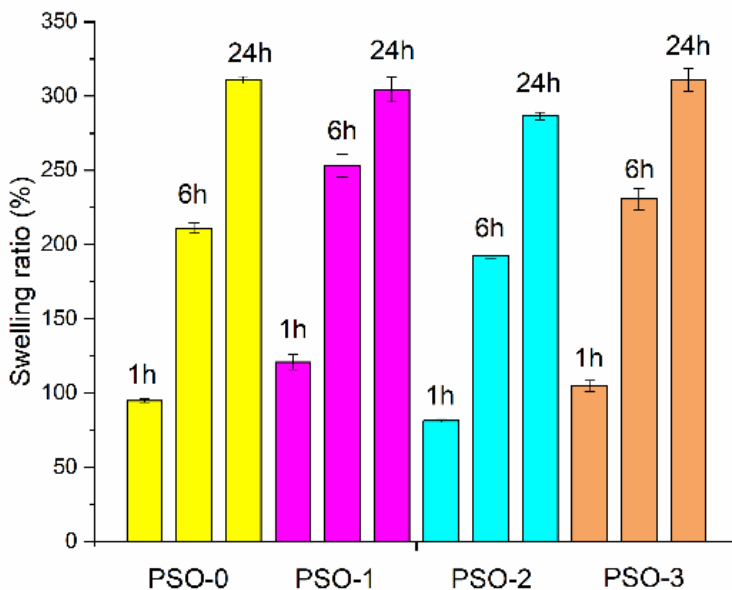


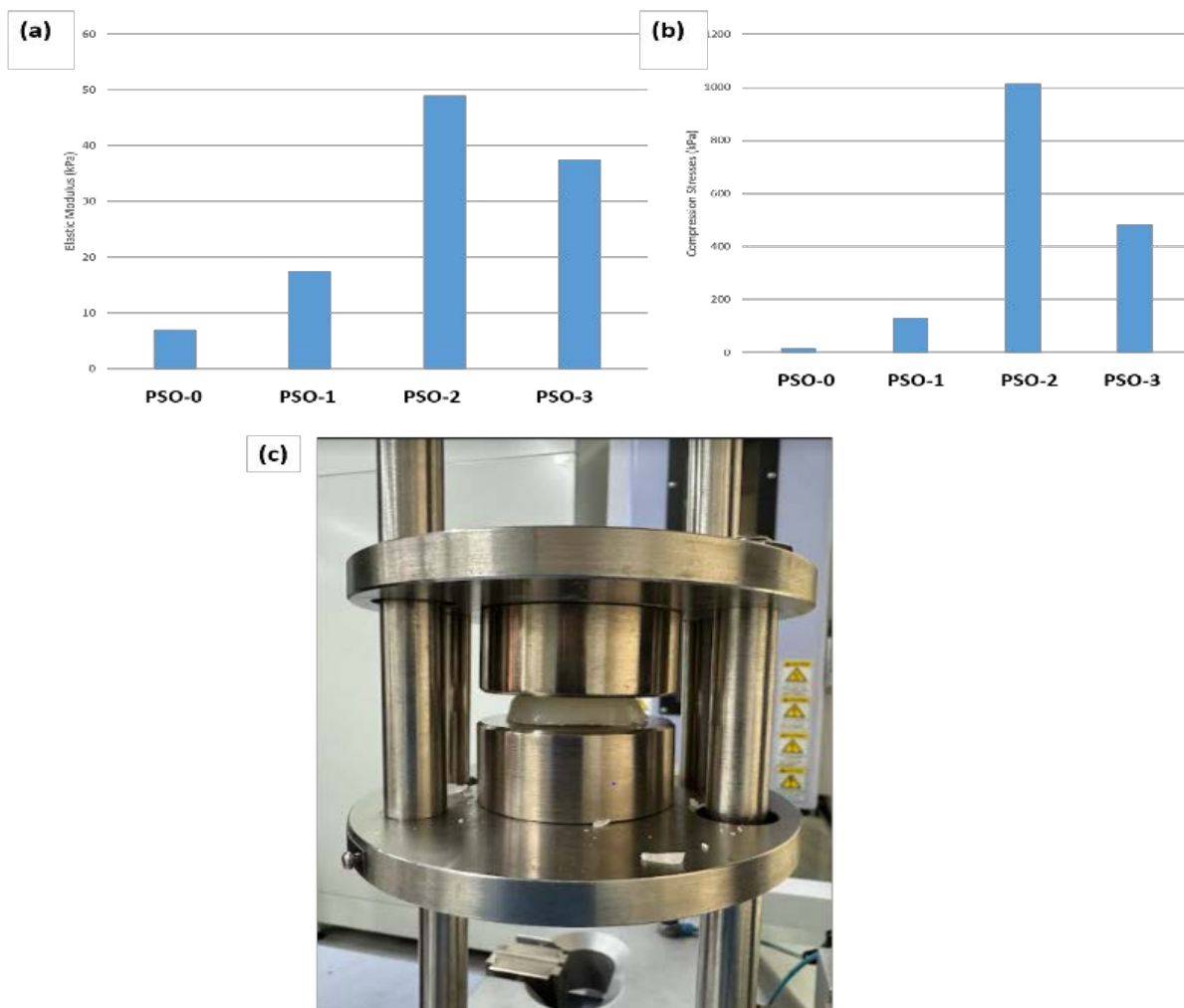
Figure 4. Swelling ratios of PAAm/SC hydrogels

### 3.4 Compression test

Hydrogels are frequently preferred materials for biomedical applications. They are of great interest in areas such as drug delivery, ligament repair, wound dressing and tissue engineering. In such applications, hydrogels are expected to exhibit good mechanical strength. Because hydrogels are subjected to certain loads such as compression load in the in-body environment. Therefore, in this study, hydrogels were subjected to compression tests and their properties were determined. In the tests, the hydrogels were compressed at a strain value of 60% of their height. As a result of the test, force and strain values were obtained and the modulus of elasticity and compressive stress values of the hydrogels were calculated. The mechanical test results of the hydrogels are given in Figure 5.

Figure 5a shows the elastic modulus values of the sample according to the changing material. When Figure 5a is examined, the elastic moduli for PSO-0, PSO-1, PSO-2, and PSO-3 hydrogels are 7, 17, 49, and 37 kPa, respectively. Generally, it can be said that an increase in the elastic modulus value was observed as the oil ratio increased, except for PSO-3 hydrogel. This decrement is due to the incompatibility of  $\beta$ -CD used as a compatibilizing agent with the oil ratio. As a result, it was determined that PSO-2 hydrogel had the optimum oil ratio. The hydrogel at this ratio has the best elastic modulus and exhibits better mechanical properties compared to other oil ratios.

The graph given in Figure 5b shows the compressive stress values depending on the changing material. When Figure 5b is examined, it is seen that the progression of the stress values obtained is the same as the modulus values. The compressive stress values of the materials with PSO-0, PSO-1, and PSO-2 hydrogels were 17 kPa, 131 kPa, and 1013 kPa, respectively. The compressive stress value of PSO-3 was 482 kPa. When the mechanical properties were analyzed in general, it was observed that the mechanical properties increased with increasing oil content. Accordingly, the optimum oil ratio was determined for PSO-2 hydrogels and the best mechanical strength values were observed in this sample. It is thought that orange blossom essential oil is also cross-linked during curing and the mechanical properties increase as a result of the increase in cross-link density.



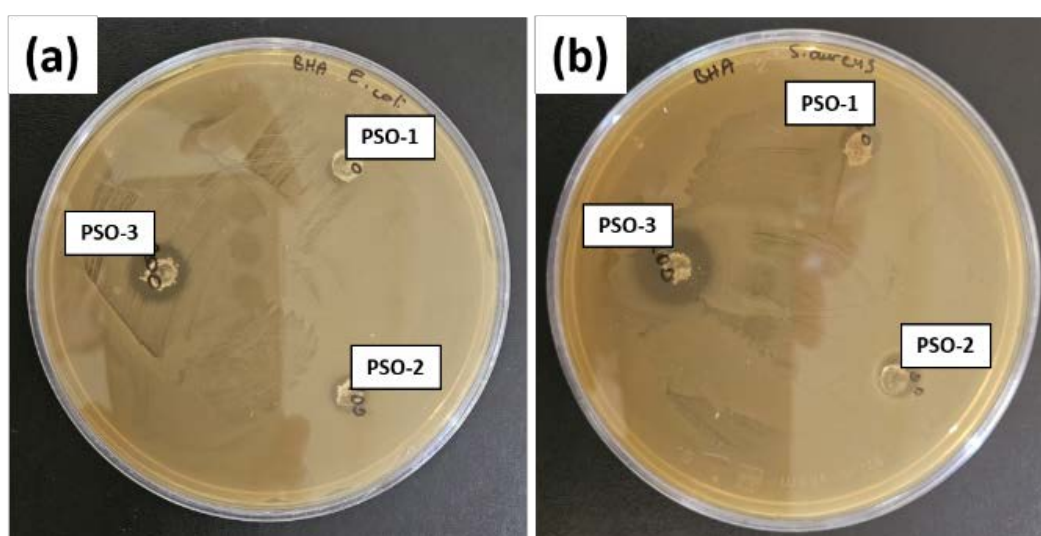
**Figure 5.** (a) Representative elastic modulus of the hydrogels. (b) Representative compressive stress of the hydrogels. (c) Demonstration of a PSO-0 hydrogel in the mechanical test

### 3.5 Antibacterial Efficiency

Bacterial infections cause a major threat to human health. Strong antibacterial materials and diverse, and easy-to-manufacture products are crucial [26]. In this scope, antibacterial hydrogels have high mechanical and biological properties and may be used for various purposes.



Many studies reported that the orange blossom essential oil is responsible for the antibacterial efficiency due to the components contained in the essential oil, such as  $\beta$ -pinene, limonene, linalool, nerylacetate, linalyl acetate, and  $\alpha$ -terpinyl acetate [12]. In this study, pure hydrogel (PSO-0) did not show any antibacterial efficiency against both gram (-) and gram (+) bacteria. PSO-1, PSO-2 and PSO-3 hydrogels have zone inhibition (ZOI) values of 6 mm, 7 mm, and 10 mm against *S. aureus* bacteria (Figure 6). *E. coli* cells, unlike *S. aureus*, have an outer membrane outside the peptidoglycan layer, which may protect them from external antimicrobial compounds to some extent [27]. Therefore, oil-loaded hydrogels indicated less effect on gram (-) bacteria (*E. coli*). While PSO-1 hydrogels don't have any antibacterial efficiency against *E. coli*, PSO-2, and PSO-3 hydrogels have 6 mm, and 8 mm ZOI values, respectively (Table 2). To sum up, it has been confirmed in this study that increasing the concentration of oil increases the antibacterial effect.



**Figure 6.** Antibacterial activity of the hydrogels against (a) *E. coli*, (b) *S. aureus* pathogens

**Table 2.** Antibacterial efficiencies of OBEO-loaded hydrogels

Bacterial strains	Polymer zone diameter (mm)		
	PSO-1	PSO-2	PSO-3
<i>Escherichia coli</i>	0	6	8
<i>Staphylococcus aureus</i>	6	7	10

#### IV. CONCLUSIONS

Orange blossom oil-loaded hydrogels were effectively synthesized via the UV crosslinking method. It has been appointed that the surface morphologies of hydrogels change with the addition of OBEO. Further, some new peaks have formed in the FT-IR spectra. PSO-2 hydrogels ( $\beta$ -CD/OBEO, 1:2) have a maximum compression strength of almost 1 MPa. The antibacterial test results of PSO-3 hydrogels were found to have the highest ZOI value of 10 mm compared to other hydrogels. As a result of the analyses, it was determined that the optimum sample was PSO-2 hydrogels. The findings indicate that the newly synthesized oil-loaded hydrogels will be effective for wound healing in future therapeutic applications.

## ACKNOWLEDGMENT

The author thanks to acknowledge Esra SEVİMLİ and Bursa Technical University Chemistry Department for their kind help in the FT-IR analysis.

## REFERENCES

1. Yu R, Zhang H, Guo B (2022) Conductive biomaterials as bioactive wound dressing for wound healing and skin tissue engineering. *Nanomicro Lett.* 14:1-46. <https://doi.org/10.1007/s40820-021-00751-y>
2. Qu J, Zhao, X, Liang Y, Zhang T, Ma PX, Guo B (2018) Antibacterial adhesive injectable hydrogels with rapid self-healing, extensibility and compressibility as wound dressing for joints skin wound healing. *Biomater* 183:185-199. <https://doi.org/10.1016/j.biomaterials.2018.08.044>
3. Okay O (2020) Self-Healing and Shape-Memory Hydrogels. *Hacettepe Journal of Biology and Chemistry, The 100 Year of Polymers* 48:507-525. <https://doi.org/10.15671/hjbc.797525>
4. Chirani N, Yahia LH, Gritsch L, Motta FL, Chirani S, Farè S (2015) History and applications of hydrogels. *J Biomed Sci* 4:1-23. <https://hdl.handle.net/11311/1170426>
5. Parin FN (2023) Synthesis and Characterisation of PVP-AAm Hydrogels via Hybrid Process: Morphological, Physical, and Antibacterial Activity. *J Adv Res Nat* 9:697-709. <https://doi.org/10.28979/jarnas.1255113>
6. Foudazi R, Zowada R, Manas-Zloczower I, Feke DL (2023) Porous hydrogels: Present challenges and future opportunities. *Langmuir* 39:2092-2111. <https://doi.org/10.1021/acs.langmuir.2c02253>
7. Nascimento LGL, Casanova F, Silva NFN, de Carvalho Teixeira AVN, de Carvalho AF (2020) Casein-based hydrogels: A mini-review. *Food Chem* 314:126063. <https://doi.org/10.1016/j.foodchem.2019.126063>
8. Casanova F, Silva NFN, Gaucheron F, Nogueira MH, Teixeira AV, Perrone IT, Alves MP, Fidelis PC, de Carvalho AF (2017) Stability of casein micelles cross-linked with genipin: A physicochemical study as a function of pH. *Int Dairy J* 68:70-74. <https://doi.org/10.1016/j.idairyj.2016.12.006>
9. Semeniuc CA, Pop CR, Rotar AM (2017) Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. *J Food Drug Anal* 25:403-408. <https://doi.org/10.1016/j.jfda.2016.06.002>
10. Pérez-Recalde M, Arias IER, Hermida ÉB (2018) Could essential oils enhance biopolymers performance for wound healing? A systematic review. *Phytomedicine* 38:57-65. <https://doi.org/10.1016/j.phymed.2017.09.024>
11. Ma J, Lee J, Han SS, Oh KH, Nam KT, & Sun JY (2016) Highly stretchable and notch-insensitive hydrogel based on polyacrylamide and milk protein. *ACS Appl Mater Interfaces* 8:29220-29226. <https://doi.org/10.1021/acsami.6b10912>
12. Ammar AH, Bouajila J, Lebrihi A, Mathieu F, Romdhane M, Zagrouba F (2012) Chemical composition and in vitro antimicrobial and antioxidant activities of Citrus aurantium L. flowers essential oil (Neroli oil). *PJBS* 15:1034-1040. <https://doi.org/10.3923/pjbs.2012.1034.1040>
13. Naik JB, Rajput RL, Narkhede JS, Mujumdar A, Patil PB (2021) Synthesis and evaluation of UV cross-linked Poly (acrylamide) loaded thymol nanogel for antifungal application in oral candidiasis. *J Polym Res* 28:1-13. <https://doi.org/10.1007/s10965-020-02377-x>
14. Moradi M, Barati A, Moradi S, Zarinabadi E. (2024) Synthesis and characterization of starch-based hydrogels containing myrtus oil nanoemulsion for wound dressings. *Polym Bull* 81:3043-3062. <https://doi.org/10.1007/s10965-020-02377-x>
15. Li W, Zhao Y, Sun W, Dong T, Saldaña MD, Sun W (2022) Multi-responsive poly N-isopropylacrylamide/poly N-tert-butylacrylamide nanocomposite hydrogel with the ability to be adsorbed on the chitosan film as an active antibacterial material. *Int J Biol Macromol* 208:1019-1028. <https://doi.org/10.1016/j.ijbiomac.2022.03.198>
16. Zhang K, Feng W, Jin C (2020) Protocol efficiently measuring the swelling rate of hydrogels. *MethodsX* 7:100779. <https://doi.org/10.1016/j.mex.2019.100779>
17. Kundu D, Banerjee T (2020) Development of microcrystalline cellulose based hydrogels for the in vitro delivery of Cephalexin. *Heliyon*, 6:1-10. <https://doi.org/10.1016/j.heliyon.2019.e03027>
18. Popa EG, Rodrigues MT, Coutinho DF et al (2013) Cryopreservation of cell laden natural origin hydrogels for cartilage regeneration strategies. *Soft Matter*, 9:875-885. <https://doi.org/10.1039/C2SM26846A>
19. Magalhães ASG, Almeida Neto MP et al (2012) Application of FTIR in the determination of acrylate content in poly (sodium acrylate-co-acrylamide) superabsorbent hydrogels. *Quím Nova*, 35:1464-1467. <https://doi.org/10.1590/S0100-40422012000700030>

20. Pan K, Zhong Q, Baek SJ (2013) Enhanced dispersibility and bioactivity of curcumin by encapsulation in casein nanocapsules. *J Agric Food Chem* 61:6036-6043. <https://doi.org/10.1021/jf400752a>
21. Abdel-Gaber AM, Hijazi KM, Younes GO, Nsouli B (2017) Comparative study of the inhibitive action between the bitter orange leaf extract and its chemical constituent linalool on the mild steel corrosion in HCl solution. *Quím Nova* 40:395-401. <https://doi.org/10.21577/0100-4042.20170020>
22. Jeannot V, Chahboun J, Russell D, Baret P (2005) Quantification and determination of chemical composition of the essential oil extracted from natural orange blossom water (*Citrus aurantium* L. ssp. *aurantium*). *Int J Aromather* 15:94-97. <https://doi.org/10.1016/j.ijat.2005.03.012>
23. Bhatia S, Al-Harrasi A, Al-Azri MS, Ullah S, Bekhit AEDA, Pratap-Singh A, Chatli MK, Anwer Md K, Aldawsari MF (2022) Preparation and physicochemical characterization of bitter orange oil loaded sodium alginate and casein based edible films. *Polymers* 14:1-15. <https://doi.org/10.3390/polym14183855>
24. Zhang K, Feng W, Jin C (2020) Protocol efficiently measuring the swelling rate of hydrogels. *MethodsX* 7:100779. <https://doi.org/10.1016/j.mex.2019.100779>
25. Kędzierska M, Kudłacik-Kramarczyk S, Jamroży M, Bańkosz M, Walter J, Potemski P, Drabczyk A (2023) Verification of the Influence of the 2-Hydroxy-2-methylpropiohenone (Photoinitiator) Content in Hydrogel Materials on Their Physicochemical Properties and Surface Morphology. *Coatings* 13:1-21 <https://doi.org/10.3390/coatings13010040>
26. Wang X, Song R, Johnson M, He Z, Milne C, Wang X, et al (2021) An injectable chitosan-based self-healable hydrogel system as an antibacterial wound dressing. *Mater* 14:1-11. <https://doi.org/10.3390/ma14205956>
27. Wang J, Li YaoZong L, YongFeng G, ZouFeng, X, MuHua Z, YinYan H et al (2018) Cinnamon oil-loaded composite emulsion hydrogels with antibacterial activity prepared using concentrated emulsion templates. *Ind Crops Prod* 112:281-289. <https://doi.org/10.1016/j.indcrop.2017.12.022>