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SYNTHESIS OF ANTIBACTERIAL PVA-AAM PICKERING EMULSION HYDROGELS (PEHs) FOR MEDICAL APPLICATIONS

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Keywords

PVA/AAm Polymer Blend,
Pickering Emulsion Hydrogels (PEHs),
Medical Applications,
Citronella Essential Oil (CEO),
Antibacterial Efficiency.

Abstract

Citronella essential oil (CEO) has antibacterial characteristics that can be used in medical applications. Polyvinyl alcohol/acrylamide(PVA/AAm) hydrogels including CEO were stabilized utilizing beta-cyclodextrin (β-CD) carbonhydrate-based material. In this context, PVA polymer, and AAm monomer were used as matrix (water phase) whereas CEO and (oil phase) and carbohydrate-based β-cyclodextrin (β-CD) were used in the emulsion system as an active agent and an emulsifier, respectively. The β-CD/CEO inclusion complexes were introduced to PVA/AAm hydrogels in different ratios (1:0.25, 1:0.5, and 1:1% w/v), and to obtain Pickering hydrogels by combining UV and freeze-thawing and anneal-swelling methods, respectively. The influence of different β-CD/CEO ratios on the morphological, physical, hydrophilicity and antibacterial efficiency of PVA/AAm was studied, as well. Although the amount of CEO increased, the hydrophilicity of hydrogels increased, while the swelling properties of hydrogels decreased. Moreover, the hydrogel with the highest amount of CEO showed the highest value of antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus bacteria.* The resulting Pickering hydrogels (PEHs) can be a potential use as tissue expander for many medical field.

TIBBİ UYGULAMALAR İÇİN ANTİBAKTERİYAL PVA/PAAm PICKERING EMÜLSİYON HİDROJELLERİNİN (PEH'LER) SENTEZİ

Anahtar Kelimeler

PVA/AAm Polimer Karışımı,
Pickering Emülsiyon Hidrojeller,
Medikal Uygulamalar,
s-Sitronella Uçucu Yağı (CEO),
Antibakteriyal Etkinlik.

Öz

Sitronella esansiyel yağı (CEO) tıbbi uygulamalarda kullanılabilecek antibakteriyel özelliklere sahiptir. CEO içeren polivinil alkol/akrilamid (PVA/AAm) hidrojeller karbonhidrat bazlı beta-siklodekstrin (β-CD) malzeme ile stabilizeedilmiştir. Bu kapsamda, emülsiyon sisteminde sırasıyla matris (su fazı) olarak PVA ve AAm, CEO etken madde (yağ fazı) ve emülgatör olarak karbonhidrat bazlı β-siklodekstrin (β-CD) kullanılmıştır. β-CD/CEO inklüzyon kompleksleri, PVA/AAm hidrojellerine sırasıyla farklı oranlarda (1: 0.25, 1:0.5 ve 1: 1 w/v) eklenmiştir ve UV ve donma-çözülme ve tavlama-şişme yöntemlerini birleştirerek Pickering hidrojelleri elde edilmiştir. Farklı β-CD/CEO oranlarının PVA/AAm'nin morfolojik, fiziksel, hidrofilikliği ve antibakteriyel etkinliği üzerindeki etkisi de incelenmiştir. CEO miktarı artmasına rağmen hidrojellerin hidrofilikliği artarken hidrojellerin şişme özellikleri azalmıştır. Ayrıca, en yüksek CEO miktarına sahip hidrojel, hem *Escherichia coli* hem de *Staphylococcus aureus* bakterilerine karşı en yüksek antibakteriyel aktivite değerini göstermiştir. Elde edilen Pickering hidrojellerin (PEHs), tıp alanı için doku genişletici olarak potansiyel bir kullanıma olabilmektedir.

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SYNTHESIS OF ANTIBACTERIAL PVA-AAM PICKERING EMULSION HYDROGELS (PEHS) FOR MEDICAL APPLICATIONS

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Highlights

- Citronella essential oil-loaded PVA/PAAm Pickering emulsion hydrogels were synthesis via UV curing, freeze-thawing, and anneal-swelling.
- β-CD is miscible emulsifier to obtain citronella essential oil-loaded PVA/PAAm hydrogels.
- PAC50, PAC100, and PAC200 hydrogels are suitable to use as a wound dressing for both gram (-) *E.coli*, and gram (+) *S.aureus* bacteria.

Purpose and Scope

In this study, it is aimed to synthesize polymer-based hydrogels using hybrid processes.

Design/methodology/approach

In the present study, new antibacterial PVA/PAAm hydrogels were synthesized by UV crosslinking, freeze-thawing and anneal-swelling methods, respectively.

Findings

The β-CD/CO inclusion complexes were introduced to PVA/AAm hydrogels in different ratios (1:0.25, 1:0.5, and 1:1 % w/v), and the amount of CO increased, the hydrophilicity of hydrogels increased, while the swelling properties of hydrogels decreased. Further, the highest amount of CO showed the highest value of antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus bacteria.*

Originality

In the present study, antibacterial PVA/PAAm hydrogels were synthesized by UV crosslinking and freeze-thawing, and anneal-swelling processes. Apart from the previous studies, antibacterial property of the resulting Pickering emulsion hydrogels were achieved by using bio-based citronella natural oil and hydrophilic-based polymer and monomer PVA and AAm, respectively.

1. Introduction

Oil-in-water (O/W) emulsions are commonly utilized to release of hydrophobic substances in a variety of products such as, tissue engineering, health care, food, and medical applications, as well (Lim et al., 2020; Li et al., 2020). Amphiphilic molecules or emulsifiers (e.g., some proteins and surfactants) generallly stabilize the emulsion droplets by reducing the interfacial tension between immiscible phases and increasing steric hindrances or electrostatic repulsion between the dispersed droplets (McClements, 2015). The method called ''Pickering emulsion'', in which stable emulsions are obtained by using solid particles as emulsifiers, has been the focus of researchers (Hu et al., 2018; Souza et al., 2021). Pickering emulsion has higher emulsification stability than conventional emulsions stabilized by emulgators (Li et al., 2020). In this context, Pickering emulsion hydrogels (PEHs) cover the emulsified oil droplets within their three-dimensional network structure, preventing oil droplet flocculation and coalescence. β-yclodextrin (β-CD), a carbohydrate derivative, acts as an excellent Aggregation emulsifier with an internal internal conical structure at room conditions. It may form non-covalent host-guest inclusion complexes with a wide range of hydrophobic substance, such as essential oils, due to hydrophobic interior hole of β-CDs (Li et al., 2022).

PEHs are made up of Pickering emulsions and a hydrogel matrix, which have the properties of both an emulsion and a hydrogel (Zheng et al., 2022; In order to use of hydrogels as biomaterials, it is expected that they will have properties such as biocompatibility, the ability to keep the liquid stably in their structure, non-toxic, and not promote the growth of bacteria and fungi (Wang et al., 2019). Generally, acrylates, acrylamide and derivatives and

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their copolymers are among the monomers that are frequently used in hydrogel synthesis (Begam et al., 2004; He et al., 2016; Bashir et al., 2020; Madduma-Bandarage et al., 2021). In addition to the use of biocompatible monomers for hydrogel synthesis, the use of biomass raw materials to obtain natural building blocks is one of the strategies to reduce our dependence on fossil fuels, reduce greenhouse gas emissions and ultimately reduce our carbon footprint.

Citronella essential oil (CO) has shown antibacterial and antifungal effects in many experimental studies (Lawless et al., 2002; Chaisri et al., 2019; Verma et al., 2020). There are many terpenes in citronella essential oil, such as citronellal, citronellol, geroniol, geraniol acetate (Verma et al., 2009; Verma et al., 2020; Cerceau et al., 2020).
In this study, Pickering emulsion hydrogels were cross-linked (synthesized) with UV and then prepared by freeze-thawing and anneal-swelling. The effects of varying β-CD/citronella complex ratios on the morphological, physical properties, and antibacterial activities in the resulting hydrogel were evaluated.

2. Materials and Method

2.1. Materials

Polyvinyl alcohol (PVA) granules (30.000 M_w, Zag Chemical Company) from Istanbul, Turkey and citronella essential oil (>95% purity) were purchased from Monoville Aromatherapy, Turkey. Acrylamide (AAm) and β-cyclodextrin (β-CD) was kindly granted by Veskim Chemical Company (Turkey) and Wacker Chemical Company, Germany, respectively. 2,2-Dimethoxy-2-phenylacetophenone (99%) and *N,N'*-Methylenebis(acrylamide) (MBAm) (>99.5%) were supplied by Sigma-Aldrich (Germany). In all experiments, distilled water was used.

2.2. Preparation of PVA-based Hydrogels

A homogeneous 10% (w/v) PVA solution was prepared. 4mL of PVA solution was taken into a sealed glass jar and 0.8 g of AAm and 0.08 g of MBAAm (crosslinker) were added into the PVA solution. The polymer solution was mixed homogenously at 25 °C for overnight. A certain amount of β-cyclodextrin (β-CD) was mixed with PVA/AAm solution. Before adding citronella essential oil, photocrosslinker is mixed with polymer solution. Different amounts of citronella were put drop by drop into the polymer solution and thus Pickering emulsions were formed. Pickering emulsions with different citronella ratios were sonicated in ultrasonic homogenizator by applying 60% power for 3 min. Then, these emulsions are UV-cured for 50 min to obtain hydrogel form. Afterwards, the hydrogels with different β-CD/citronella ratios freezed at -20 °C for 18 h, and 6 h of thawing procedure was carried out at 30 °C for two cycles. Therefore, freeze-thawed (FT) hydrogels were prepared. To obtain anneal-swelled hydrogels were produced by drying completely at 80 °C for 2 h and annealing at 120 °C for 1 h. Consequently, these hydrogels were soaked up in distilled water at 25 °C until reached a constant weight.

PVA/AAm hydrogels were synthesized by the same procedure (without the addition of citronella and (β-CD)), as a control sample. PAC0, PAC50, PAC100, and PAC200 were coded to hydrogels containing 0, 50, 100, and 200 µL citronella essential oil, respectively.

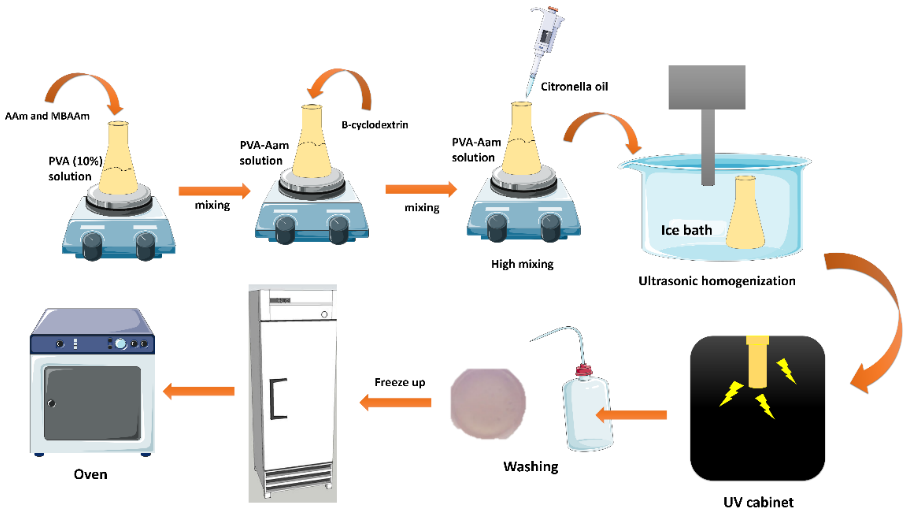

Figure 1. Schematic sketch of Pickering hydrogels.

2.3. Characterization of the Hydrogels

Morphological Analysis of Pickering Emulsions

The morphology of neat polymer (PVA/AAm hydrogel) solution and Pickering emulsions were visualized optical microscope (Leica-M125) with 10X magnification.

Morphological Analysis of Hydrogels

The microstructure of the hydrogel was performed by Scanning Electron Microscope (SEM). SEM micrographs were taken at different magnifications.

Spectroscopic Analysis

FT-IR spectroscopy was used to identify the molecular structure of the hydrogels.

Wettability Test

The wettability of the resulting hydrogels was tested using an optical tensiometer (Biolin Scientific, Gothenburg, Sweden), with 3 µL distilled water. Three replications were carried out for each sample.

Antibacterial Analysis

The standard bacterial strains *Staphylococcus aureus* ATCC® 25923 and *Escherichia coli* ATCC® 25922 were used as positive controls in the study. Bacterial cultures were grown on the solid media. Petri dishes were incubated at 37°C for overnight in an aerobic environment. The density of became stable (Yahya and Abdulsamad, 2020).
The broth suspensions with bacterial colonies were transferred with a 100 l pipette and streaked on the surface of Mueller Hinton Agar at 0.5 McFarland (1108 cfu/mL) turbidity standard.

Hydrogels were placed onto the agar surface. The petri dishes were then incubated for overnight at 37 °C in an aerobic environment. Finally, the diameters of the inhibition zones around any hydrogel disc were measured qualitatively with a calliper (CLSI, 2017).

Swelling Behaviour

Swelling ratios (SR) of all samples were calculated as follows. First, a completely dried hydrogels was immersed in distilled water at room temperature. The hydrogels were wiped out and weighed at periodic intervals. The swelling ratio was calculated using the equation below (Zhang et al., 2020) (Equation 1).

$$SR\ (\%) = \frac{\mathrm{Ws-Wd}}{\mathrm{Wd}} x\ 100 \qquad (1)$$

3. Results and discussions

3.1. Optical Images of Hydrogel Solutions

As presented in Figure 2, citronella droplets with lower than 5 µm were successfully stabilized in a β-CD polymer emulsions. Citronella droplets were reported to be uniformly distributed in PVA/AAm polyblend solutions. The increase in oil concentration increased the distance between the oil droplets (Figure 2D). Especially, oil droplets agglomerated some region in the solution. Neat PVA/AAm polyblend solution showed some bubbles due to the stirring process.

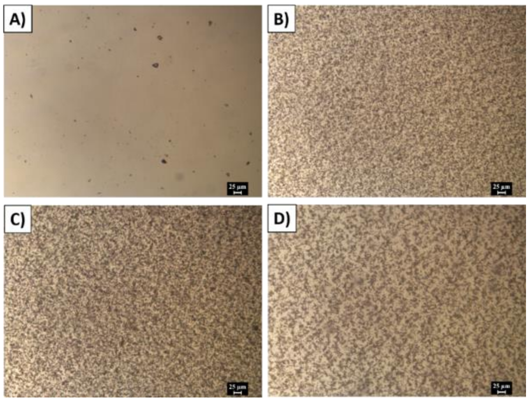

Figure 2. Optical microscobic images of polyblend solutions (A) (PAC0), (B) PAC50, (C) PAC100, (D) PAC200 hydrogels, respectively (Magnification : 5X, scale: 25 µm).

3.2. SEM Analysis of Hydrogels

Figure 3 shows microstructure of neat PVA/AAm hydrogel and Pickering emulsion hydrogels. The difference was noticed in both cross-sectional and surface images of the Pickering hydrogels obtained by introducing oil to the polyblend solutions. The particle size distribution of PAC200 samples was found to be uniform with spherical morphology (Figure 3B). The self-agglomeration of β-cyclodextrin particles at the oil-water interface results during Pickering emulsion formation. The result agreed well with literature (Li et al., 2020).

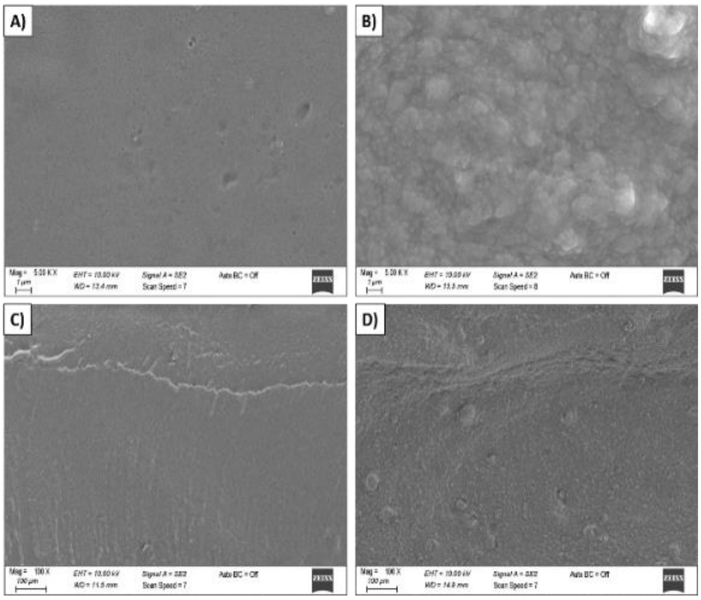

Figure 3. SEM surface micrographs of hydrogels (A) PAC0, (B) PAC200 (Magnification: 5 kX, scale: 1 µm). SEM cross-sectional micrographs of hydrogels (C) PAC0, (D) PAC200 hydrogels, respectively (Magnification: 100 X, scale: 100 µm).

3.3. FT-IR Analysis

FT-IR was used to examine the chemical structure of the neat PVA/AAm hydrogels and Pickering emulsion hydrogels (Figure 4). The strong absorption peaks have been seen at 3180-3335 cm^{-1}, 2852-2935 cm^{-1}, 1598-1650 cm^{-1}, 1416 cm^{-1} and 1095 cm^{-1} which correspond to (-OH) and (-NH) stretching, asymmetric and symmetric (-CH_2) stretching, (-C=O) stretching, C-N stretching) (Blum et al., 2012; Ou et al., 2017; Khoerunnisa et al., 2021). These peaks shows both characteristic peaks of PVA and AAm, individually, and proof of chemical interaction between PVA and AAm.

Citronella essential oil had a vibration peak of 2914 cm^{-1}, indicating C-H bonding (Ramanayaka et al., 2019). The peaks at distinct peaks at 1725 and 1670 cm^{-1}, which might be related to stretching vibrations of aldo, keto, estero, or acido (C = O) groups.

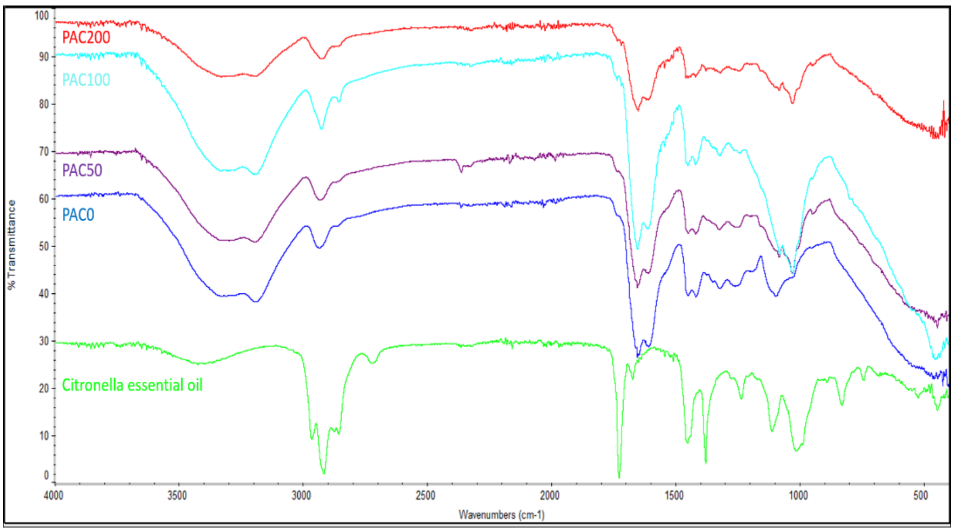

Figure 4. FT-IR analysis of hydrogels and citronella essential oil.

It has been determined that these peaks may be due to the major components of citronella essentail oil (geraniol and citranellal) (Basak et al., 2021). The peaks at 1028 cm^{-1} is related to (-OH bending) owing to the characteristic peak of the citronella. Some changes in the FT-IR spectrum were observed after the addition of β-CD/citronella essential oil inclusion complexes to PVA/AAm solutions.

3.4. Wettability Test

The wettability of a hydrogel surface is affected by its chemical and geometrical structure. Moreover, the curing time can be influence of the contact angle values, as well due to the fact that uncured regions of hydrogels' surface may remain. Figure 5A shows that the contact angle θ of PVA/AAm is around 82.2°. The wettability of colloidal particles is closely associated with the stability of Pickering emulsion stabilized by particles. Therefore, synthesis of the Pickering emulsion hydrogels resulted in a general decrease in contact angle value. This clearly shows that the particles are hydrophilic, with the majority of the particles existing in the aqueous phase to form an oil-in-water emulsion (Li et al., 2020).

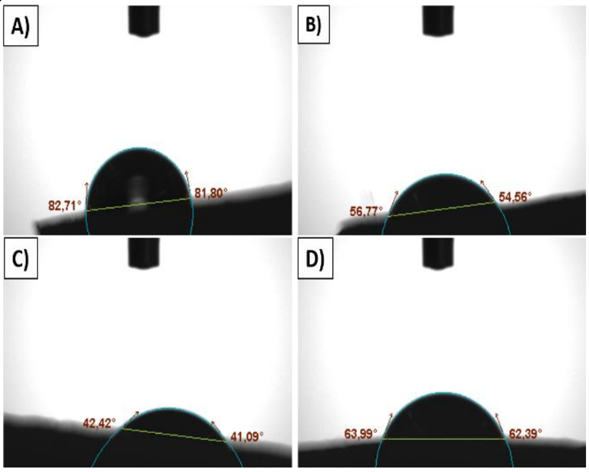

Figure 5. Contact angle images of the (A) PAC0, (B) PAC50, (C) PAC100, and (D) PAC200 hydrogels, respectively.

3.5. Antibacterial Activity

The antibacterial efficiency was determined against gram negative *(E.coli)* and gram positive *(S. aureus)* bacteria using disk diffusion method was applied to all samples. Terpenes show strong biological activities as antibacterial and antiviral agents (Zi et al., 2014) and have important areas of application such as chemicals, flavors, fragrances (Farhat et al. 2019). There are many terpenes in citronella oil, such as citronellal, citronellol, geroniol, geraniol acetate. Several studies have been performed to evaluate the antifungal and antimicrobial properties of citronella essential oil (Wei and Wee, 2013). According to Billerbeck et al. (2001), citronella essential oil of at 400 mg/L dosage could inhhibit 80% of Aspergillus niger growth. Verma et al. (2020) reported inhibition of test bacteria with a minimum inhibitory concentration (MIC) of essential oil in the range of 250-1000 μg /mL. It was also observed that the citronella oil was moderately active against Gram-positive and Gram-negative bacterial strains, and also showed good activity against Candida strains.

In the current study, all Pickering emulsion hydrogels showed antibacterial activity against *E.coli* and *S. aureus* bacteria. Further, PAC200 sample has the highest antibacterial activity (against *E.coli*) with 14 mm zone inhibition (Figure 6). Pure hydrogels did not show any antibacterial activity as expected.

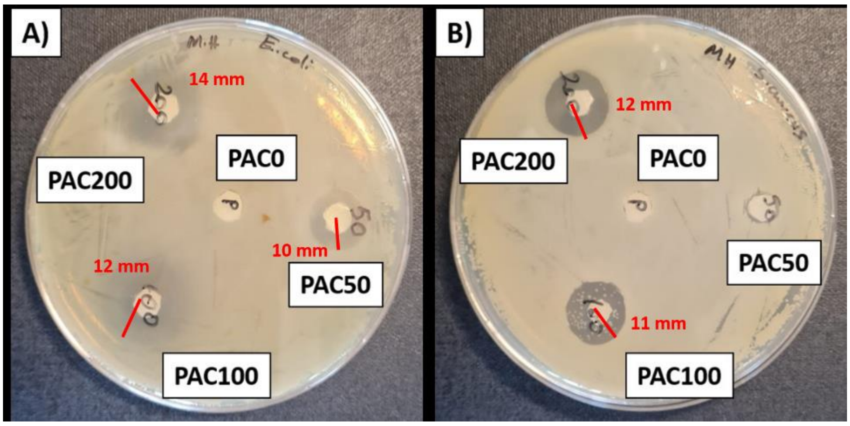

Figure 6. Antibacterial activity of the hydrogels against (A) *E.coli* and B) *S. aureus* bacteria.

3.6. Swelling Behaviour

The swelling ratio findings are shown in Figure 7, and the curves for the hydrogels of pure sample PAC0, and Pickering emulsion hydrogels PAC50, PAC100, PAC200 samples had a similar trend: slight swelling followed by slight shrinking: a dramatic increase in swelling ratios was observed in the first 6 hours. After the 24 h, the swelling ratios of the all hydrogels, PAC0, PAC50, PAC100 and PAC200 samples, approximately 172%, 133%, 135%, and 112%, respectively. Generally, the increase in the amount of essential oil reduced the degree of swelling. The swelling values of PAC50 and PAC100 samples are too close to each other at certain time. This is related to the fact that the increase in the amount of essential oil in the emulsion prevents the water absorbance capacity of the hydrogel.

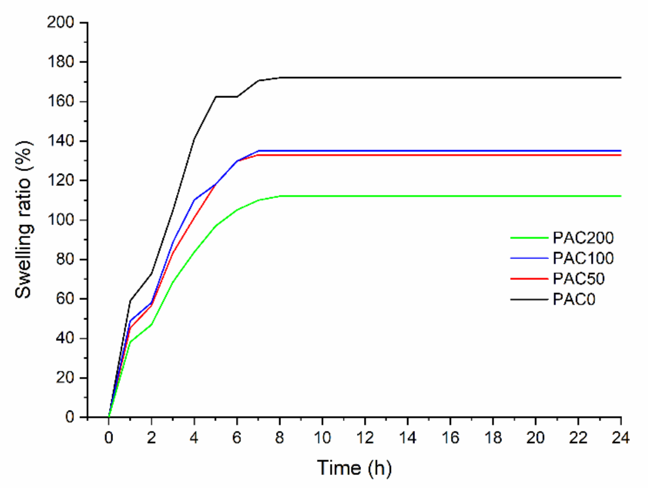

Figure 7. Swelling ratios of all hydrogels in distilled water.

4. Conclusion

In this study, new method was successfully developed to synthesis of emulsion-based hydrogel. In this regard, PVA/AAm hydrogels with citronella essential oil were synthesis via combine 3 system including photopolymerization Pickering emulsions, freeze-thawing and anneal-swelling methods, respectively. The formation of Pickering emulsions was confirmed by optical images. The SEM analysis revealed that adding β-CD/citronella essential oil in polyblend solution, form sphericalstructures on the hydrogel surfaces. Physical interaction between polymer matrices and β-CD/citronella essential oil inclusion complexes was recorded to FT-IR spectrum. Moreover, contact angle decreased as formation of Pickering emulsion hydrogels based on wettability test. On the other hand, in contrast to the surface analysis, swelling ratio values decreased as expected with the formation of emulsion hydrogels (with an increase in oil concentration). Antibacterial efficiency test showed that the obtained Pickering emulsion hydrogels have good antibacterial activity against both Gram (-) and Gram (+) bacteria.

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