Synthesis of Biocompatible Polymer Blend for Drug Delivery in Biomedical Applications

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ABSTRACT

Chrysin (CH) and Caffeic acid (CA) loaded polymer films are designed as antibacterial polymer films with the advantages of a sustained release. Polymer blend film was prepared by solution casting method of (polyvinyl alcohol) (PVA) blend with Poly (vinylpyrrolidone) PVP and glycerine (GL) as plasticizer in addition to glutaraldehydeas cross linker. The surface characteristics of film were assessed by scanning electron microscopy .Furthermore, the swelling behavior was followed in DSMO and PBS. PVA/PVP films were loaded with 1, 10 and 50% for either CH or CA. Release kinetics was followed for 24 hr in PBS at 37°C. The cumulative amount released from the polymeric PVA/PVP film loaded with 1, 10 and 50% of CH over the entire period 24 h was found to be 0.087, 0.127 and 0.146 gm, respectively .While the cumulative amount of CA released in same condition was found to be 0.875, 0.766 and 1.13 gm respectively. Antibacterial activity for both CH and CA released from the film was tested against two type of bacterial strains *Staphylococcus aureu*as and *Escherichia coli*.

Keywords : polymer film, Chrysin , Caffeic acid, Polymer blends, Releasing

INTRODUCTION

Polymer blends are widely used in many pharmaceutical and medical applications including drug delivery system, bone repair, enzyme immobilization, wound dressing, they play an important role as a temporary scaffold and temporary barrier [1,2].Poly (vinyl alcohol) PVA and Poly (vinylpyrrolidone) PVP which are currently the objective of considerable attention as biomedical synthetic polymer. Due to both of them are water soluble, biocompatible biodegradable [3, 4] .Moreover, PVA has a remarkable characteristic for film formation with good mechanical properties [5,6]. While the main disadvantage of PVP film is its weak mechanical strength and the material would dissolve in contact with fluids into the human body [7].

Recently, cross linking reaction have been reported as effective routes to bring about the desired modification such as reduce the water solubility and increase or modify the mechanical properties, as well as to obtain useful films for a medium or long term inserts [8]. Both PVA and PVP have been previously used for controlled drug releases as well as many hydrogel applications such as artificial cartilage [9]. The blend has been used in several other applications, such as the delivery of nitric oxide and the replacement of degenerated spinal disks [10]. Film for local short-term release of anti-inflammatory agents post spinal cord Injury was studied [11]. Study by Razzak et al suggested a burn dressing from co-polymeric hydrogels beside on PVA/PVP using x–ray irradiation for cross linking process.[12] Hydrogel as wound dressing was prepared by Park and Chang. In these studies hydrogels were made from a mixture of poly(vinyl alcohol) (PVA)/poly(*N*-vinylpyrrolidone) with aloe vera by freezing and thawing, γ-Ray irradiation, or a two-step process of freezing and thawing and γ-ray irradiation [13].

In recent years, the flavonoid compounds havebeen widely used in biochemical and pharmacological application, As a result of they are extremely safe and associated with low toxicity [14]. Chrysin (5, 7-dihydroxyflavone) a natural flavonoid widely distributed in plants .Has various biological activities, such as antibacterial, anti-inflammation, anticancer, antioxidant and antiallergic activities [15.16]. The other common phenolic compound found in foods of plant is caffeic acid (3,4-Dihydroxycinnamic acid). It has been found in legumes, cereals, fruits, oilseeds, vegetables and herbs [17]. Many studies have been shown to have antioxidant activities and antibacterial activities to *C. perfringens* [18]. Studies by Aziz et al shown inhibited growth of *K. pneumoniae and E. coli at* 300 ppm from caffeic acid .While at 200 ppm form caffeic acid the fungus *Aspergillus* was inhibited [19]. The aim of the present work was to prepare and characterize PVA/PVP blends film cross linking by gluteraldehyde (GA) loaded with caffeic acid and chrysin in order to obtain suitable antibacterial films. Degree of swelling, surface film properties, and releasing study were also investigated.

Material and methods

Materials

Poly (vinylalcohol) (98-99% hydrolyzed) MW~31.000-50.000 (PVA), Glutaraldehyde (25% solution) (GA), Caffeic acid (CA), Glycerol and Chrysin 97% (CH) were provided by Aldrich, Buchs, Switzerland. And Poly (vinyl pyrrolidone) (PVP) MW~ 44.000 was from BDH laboratories, England. Dimethyl sulfoxide (DMSO) was purchased from Fluka A G, Buchs. Phsphote Buffer Saline (PBS) was purchased by Schuchardt, Germany.

Preparation of PVA /PVP film

Aliquot 3 g of PVP was added to completely dissolve of 4.5% (PVA) aqueous solution (w/v) with 1.8 g of glycerol then the solution was mixed using stirrer at 80°C for 1 h. Cross linking reaction was prepared by adding 4 drops of 10% HCl (v/v) and 0.2 ml of 0.0001% GA (v/v) to 20 mL of blend solution, then solution was stirred for 45 min at 60 ºC. Finally, 20 mL from blend solution was poured into petri dish and the film was cast by drying at 45°C for 72 h. The blend film preparation of PVA /PVP was established with same modifications according to studies done by Nho Ch.Y and et al [3].

Preparation of chrysin and Caffeic acid loading film

A piece with approximately (10x10x0.21) mm^3 from blend film was immersed for 24 h in 20 mL of a solution with different concentrations of either CH or CA dissolved in DMSO. The concentrations were 1, 10 and 50 % and prepared at room temperature. The loaded films were then washed in distilled water. Finally, each piece was placed in the hood for 24 h for drying as shown in Figure (1).

Scanning Electron Microscopy Assay

The surface characteristics of PVA/PVP was synthesized before and after loading with 10%of either CH or CA and then evaluated by Scanning Electron Microscope (Stereoscan 360, Cambridge) at 10 kV.

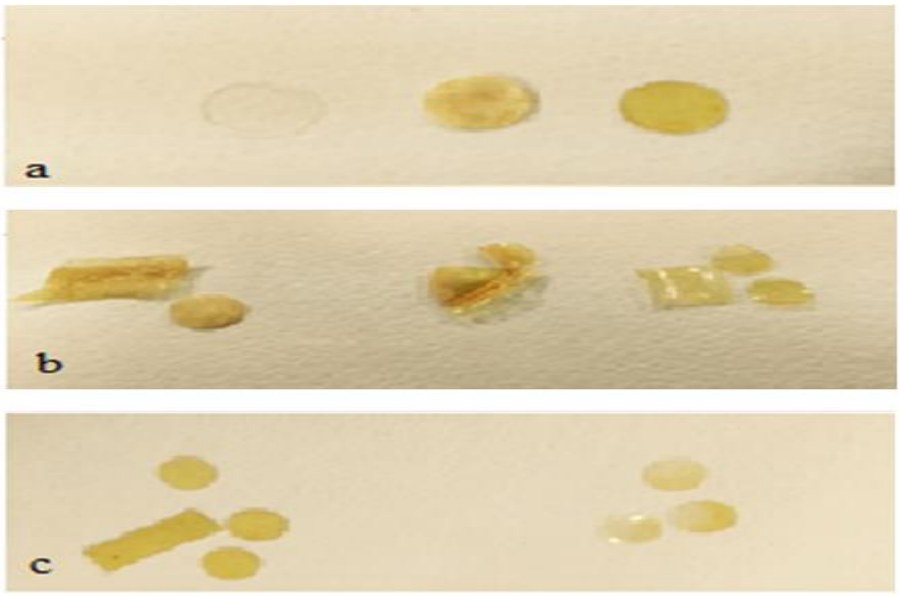
Swelling Assay

For in vitro swelling assay, the films with approximately (10x10x0.21 mm^3) were immersed in DMSO and PBS solution at 37°C. For swelling measurements, the dried films were weighed before being immersed in PBS and in DMSO. After immersion, at different periods of time, the samples were periodically removed from the medium, the excess

swelling medium was gentle remove from the surface of film by using filter paper and the weight of the sample was gravimetrically determined. The degree of swelling could be described as the water absorptivity of the polymer film was calculated according to the following formula.

$$\text{Water Absorptivity}\,(\%) = ((W_S - W_d) / W_d) \times 100$$

Where W_S and W_d are the weight of the blend films in the swollen and dry states, respectively [3].



Figure(1).Cross linked PVA /PVP film in (a) left panel showing only film, then, loading with 50% (w/v) of chrysin and caffeic acid, respectively.(b) left panel showing 50%,10% and 1% (w/v) chrysin loading film. (c) Left panel showing 50% and 10% (w/v) caffeic acid loading film

Characterization Studies

Chrysin and Caffeic Acid Releasing Assay

To detect the maximum absorption, the stock solution of both CH and CA were dissolved in DMSO and their UV–vis scan spectra were determined using spectrophotometer (TG 60UV-Visible spectrophotometer, France) at range from 260 to 800 nm. Furthermore, the loaded samples of either CH or CA were immersed in 20 ml PBS at 37 °C. Different times (5, 10, 20, 30, 60, 120, 180, 240 and 1440 min) sample released into PBS from the polymeric film were scanned by UV–vis spectrophotometer. Amount of released samples were directly predictable from Lambert–Beer curve plotted using several dilute solutions prepared from the stock solutions.

Antibacterial Properties of Releasing Chrysin and Caffeic Acid

The antibacterial properties of CH or CA with were tested against two types of bacterial strains they were *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) by liquid medium method. The bacterial suspension was prepared by adjusting with 0.5McFarland turbidity standard (5×10^7 cell mL^{-1}) tubes. Each bacterial strain was subculture on nutrient broth. To investigate the inhibition rate of the film disks loaded with different concentrations of CH or CA. Then, the broth was inoculated

with 0.2mL of bacterial strains and then with 0.5mL of 1, 10 and 50 % of both compounds were added. The tubes were incubated at 37 °C for 5min, 10 min, 20 min, 30 min, 60 min, 120 min, 180 min and 1440 min. Bacterial growth was measured by optical density at 600nm wavelength. A broth tube containing bacterial strains only, without CH or CA, was used as control. [20]
Furthermore, the effect of prepared blend film disks loaded with the same concentrations used in liquid method was also determined using the disc diffusion method against *E. coli* and *S. aureus*. The bacterial culture was spread on Mueller-Hinton agar plates which were allowed to stand for 5–10 min to allow culture absorption, and then the loaded films of each CH or CA were added. After 48 h incubation at 35 ± 2 ^{o}C, the inhibition zone diameter was measured.

Results and Discussion

Scanning Electron Microscopy

As seen in figure 2 (a,b,c), the surface characteristics of synthesized PVA/PVP film before and after loading with 10% of CH or CA, respectively. The surface of blend film before loading shows homogeneous appearance while the loaded film with CH and CA exhibited rough and uniform surfaces. The rough surface is due to most of CH and CA molecules are distributed over the film.

Swelling studies

Swelling studies are one of great importance for biodegradable materials and for those destined to be used as carriers for bioactive agents, swelling behavior influences releasing and loading [21]. In this study; it was found from Figure (3 A) pure PVA film shows a maximum swelling percentage 85% in PBS after 25 min. Because of dissolving properties of pure film in DMSO swelling behavior was not carried out on pure film. Furthermore, the swelling behavior of the prepared blend film was shown in Figures (3 B).

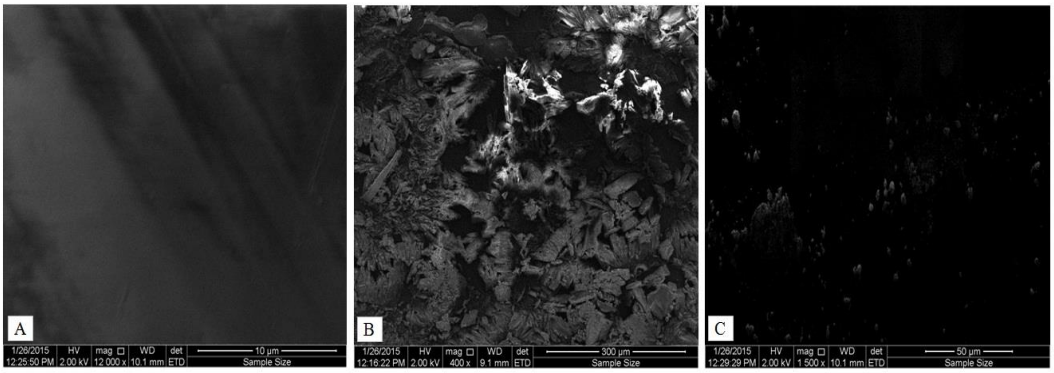


Figure (2) SEM Micrographs of Blend Films as PVA /PVP Only (A), PVA /PVP Loaded with 10% Chrysin (B) and PVA /PVP Loaded with 10% Caffeic acid (C), respectively.

It was observed that film shows 275 % and 200 % as the maximum value of the swelling percentage in PBS and DMSO, respectively after 25 min of treatment. However, this Figure shows that the swelling degree for both PBS and DMSO were increased with time in dependent manner. According to above Figures (3A, 3B) PVA/PVP film shows a good swelling behavior than pure (PVA) film. Thus, must be related to the blending (PVA) with (PVP). In consequence results is in agreement with study done by Bernal and coworkers the chemical structure of (PVP) ring has a proton accepting carbonyl group, whereas (PVA) has hydroxyl functional groups and for that cause, hydrogen bonds have been formed between them. These varieties of interactions have number

effects on the PVA/PVP film properties, as well as the solubility and the mechanical properties [22]. At first the swelling percentage of PVA/PVP film was increased rapidly at ~35 min and then start to slow to reach to the equilibrium swelling degree after ~120 min. The swelling percentage of polymer film one of the most important futures in biomedical applications because of the swelling behavior directly proportional with polymer loading and releasing ability for active material such as drugs [23].

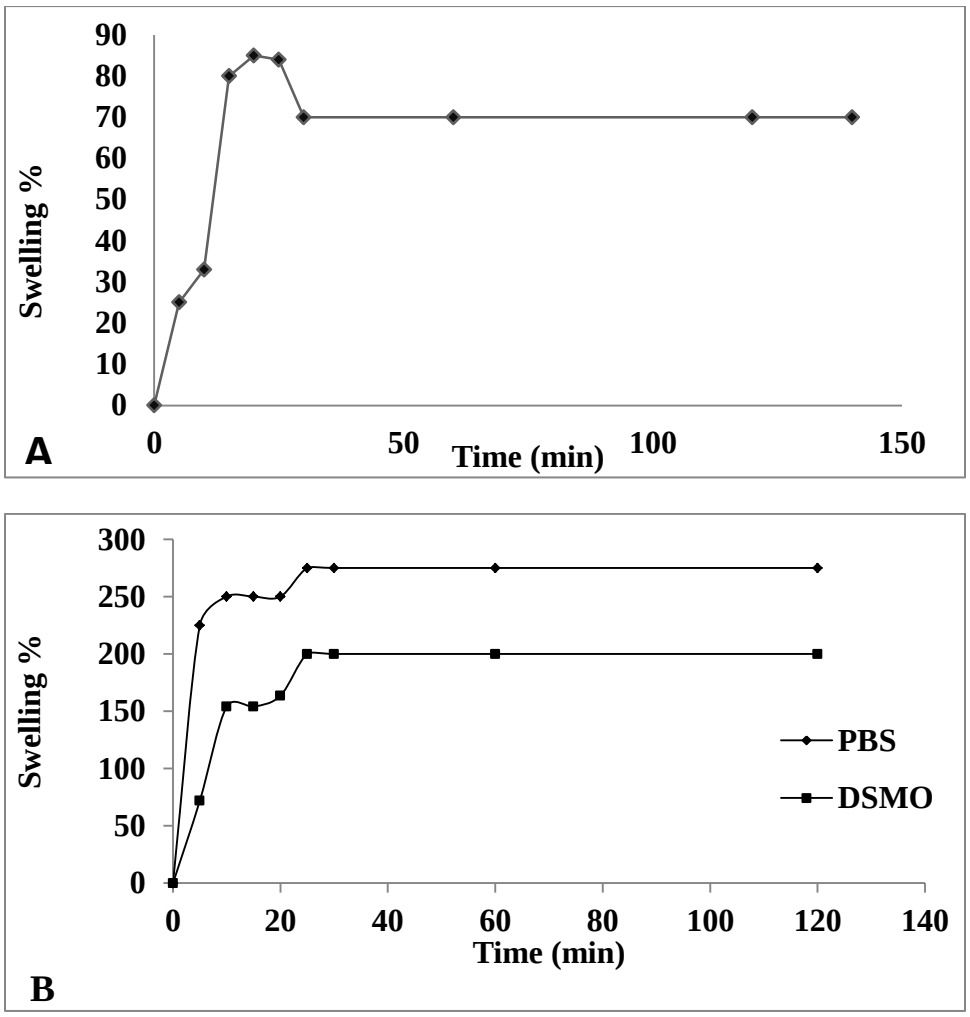


Figure (3).The swelling behaviour of pure PVA film in PBS (A) and PVA /PVP film in PBS and DMSO in (B)

Releasing of Chrysin and Caffeic acid

The UV–vis spectra showed that the λ max of standard stock solution of CH and CA were 348nm and 327 nm, respectively. Amount for both CH and CA was estimated from Lambert–Beer curve that indicated at Figure 5(a, b), respectively. The a cumulative amount that released from the polymeric blend film loaded with 1%, 10% and 50% of CH was obtained at 24 h and were 0.087, 0.127 and 0.146 gm mL^{-1}, respectively (Figure 6). While, in Figure (7) the cumulative amount of CA released at same condition were 0.875, 0.766 and 1.13 gm mL^{-1}, respectively. The differences between

amounts released of CH and CA might relate to the higher solubility of CA that was higher than CH. It was observed the CA solubility was 40 mg mL^{-1} in DMSO while, was 30 mg mL^{-1} for CH. Suggesting, loading amount of CA with polymer film and then released to media was higher than CH [24].

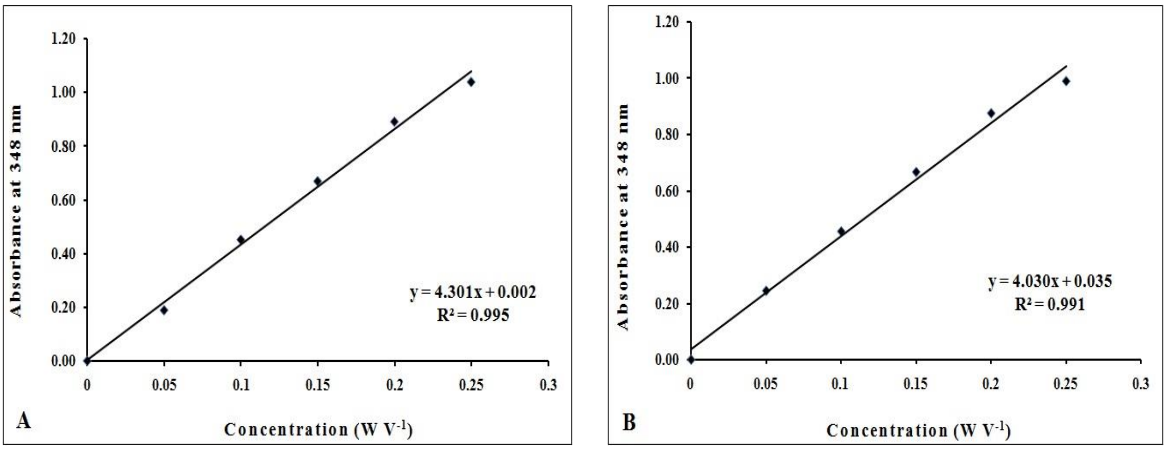


Figure (4).The Beer- Lambert Calibration Curve for Chrysin in (A) and Caffeic acid (B) in DMSO respectively

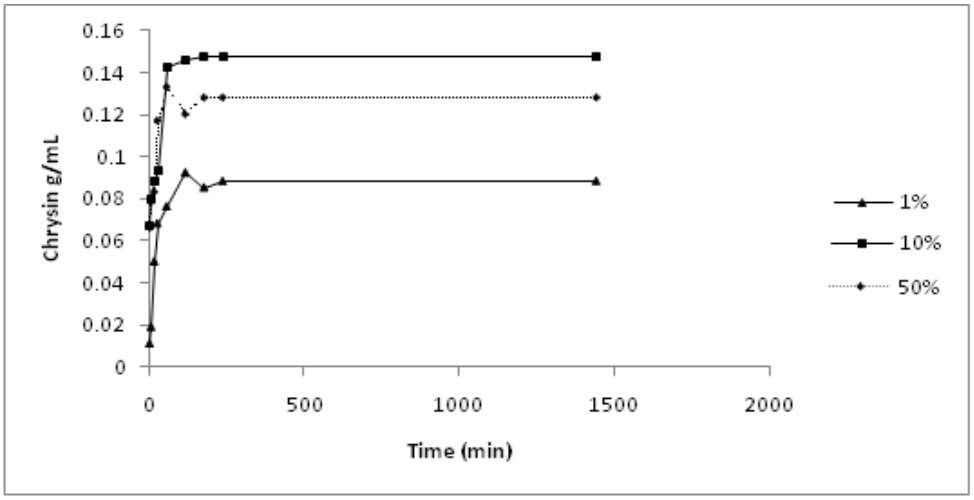


Figure (5).Releasing of Chrysin from Polymer Film

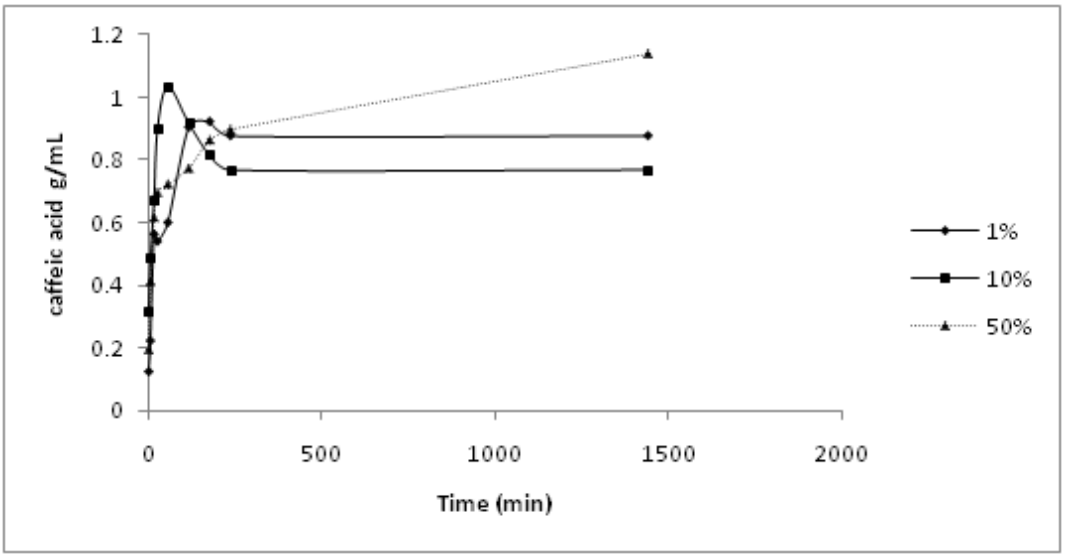


Figure (6).Releasing of Caffeic acid from Polymer Film

Antibacterial activity of released CA and CH

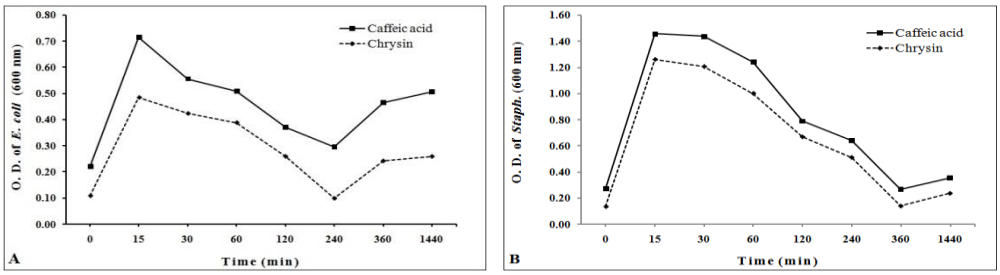
To determine the antibacterial activity of CH or CA, we evaluated its effect on Gram (+) (*S. aureus*) and Gram (-) (*E. coli)* as model bacteria. Various concentrations of the tested material CH or CA loaded with polymer blend film were added to the appropriate bacterial suspension and incubated at 35 ± 2 °C. At various time-points post-treatment the amount of the bacteria was determined by both measuring their optical density 600 nm and the inhibition zone diameter was measured. Disk films loaded with CH and CA showed antibacterial activity against of the gram (-) and (+) bacteria examined, in a concentration dependent manner. The tested material loaded with 50% of CA and CH showed antibacterial activity against both bacterial strain up to the end of the experiment (240 h post-treatment), that indicated at figure 8(a,b), respectively.

However, as determined by measuring the inhibition zone diameter of tested material loaded 50% of CH it shows a slight and weak antibacterial activity against (*S. aureus*) and the zone of inhibition was found 10 mm. While Gram-negative bacteria were not inhibited .As can be seen in the representative results in (Table 1 and Figure 8 (b,c) .Thus, our results is in agreement with those of [16,25], who detected a noticeable action of CH against Gram-positive bacteria and limited activity against Gram-negative ones .The antibacterial activity for the test materials loaded 50% of CA it shows a strong antibacterial activity against both (*S. aureus*) and (*E. coli)* with inhibition zone diameter 15 mm and 10 mm respectively as shown in Table 1 and Figure 8(a,d). Possible explanation in the antibacterial activity difference between CA and CH may reside in the chemical structure for both materials [26].

Table(1) Means of inhibition zones in (mm) of pva/pvp polymer film alone and with 50% of CA and CH against S. aureus and E. coli

Microorganism	**Zone of inhibition (mm)**		
	Control	Caffeic Acid	Chrysin
Staphylococcus aureus	N*	15±1.5	10±1.2
Escherichia coli	N*	10±1.5	N*

* N: no zone of inhibitio



Figure(7) Effect of CA and CH on Gram (+) and Gram (-) bacteria. 50% of were CA and CH added to suspensions of *E. coli* (a) and (b) *S. aureus* and incubated at 37°C .

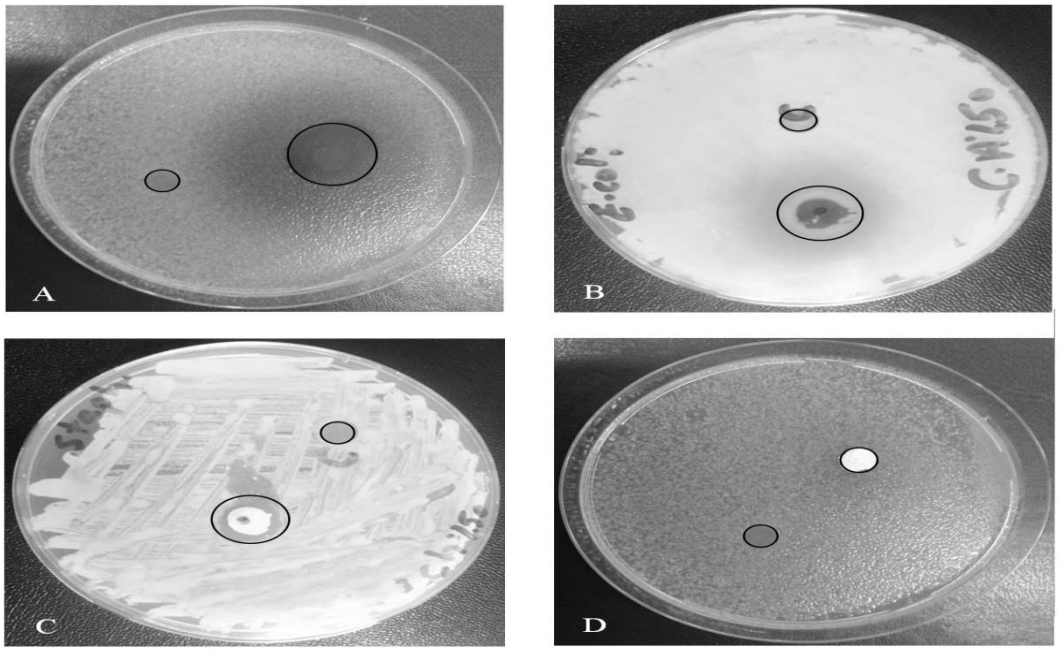


Figure (8) Growth inhibition of *S. aureus* with polymer film loaded with 50% of CA and CH (A,C)), respectively and growth inhibition of *E. coli* with polymer film loaded with 50% of CA and CH (B,D), respectively .

CONCLUSION

In this work, biocompatible PVA and PVP blend film were used with the aim of studying antibacterial delivery systems as an alternative therapy. According to this study using PVA /PVP film in drug delivery system related to swelling and prolongs releasing features. Two kinds of flavonoids (chrysin and caffeic acid) were tested to obtain antibacterial polymer films. In vitro study of test sample loaded with 50% of caffeic acid shows strong antibacterial activity agnist *S. aureus* and *E.coli* than polymer film loaded with 50% chrysin at human body condition. Although the found results in this study delivered a significant role for understanding the in-vitro antibacterial activity of chrysin and caffeic acid, still additional in-vivo study is required for displaying its exact antibacterial activity.

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