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Antioxidant, Antifungal, Water Binding, and Mechanical Properties of Poly(vinyl alcohol) Film Incorporated with Essential Oil as a Potential Wound Dressing Material

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ABSTRACT: In this study, the properties of poly (vinyl alcohol) (PVA) films incorporated with *Zataria multiflora* essential oil (ZMO) as a potential antioxidant/antibacterial material was investigated. PVA films were prepared from PVA solutions (2% w/v) containing different concentrations of ZMO. Water solubility, moisture absorption, water swelling, and water vapor permeability for pure PVA films were 57 ± 1.1, 99 ± 3.2%, 337 ± 8%, and 0.453 ± 0.015 g mm/m^2 h, respectively. Incorporation of ZMO into PVA films caused a significant decrease in water swelling and moisture absorption and increase in solubility and water vapor permeability. Tensile strength, elastic modulus, and elongation at break for pure PVA films were 13.5 ± 0.61 MPa, 15.2 ± 0.8 MPa, and 216 ± 4%, respectively. Incorporation of ZMO into the PVA films caused a significant decrease in tensile strength and elastic modulus and increase in elongation at break of the films. Pure PVA film showed UV-visible light absorbance ranging from 280 to 440 nm with maximum absorbance at 320 nm. Addition of ZMO caused a significant increase in light absorbance and opacity. PVA films exhibited no antioxidant and antifungal activities, whereas PVA/ZMO films exhibited excellent antioxidant and antifungal properties. Although the bioactivity PVA films were improved by the addition of ZMO, however, the mechanical properties and water binding capacity of the films were weaken slightly. Thus, ZMO emulsified in the ethanol not compatible with PVA matrix and more suitable emulsifier was needed in order to obtain strong film with higher mechanical properties. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40937.

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INTRODUCTION

Poly (vinyl alcohol) (PVA) is a water-soluble synthetic polymer of vinyl alcohol with a planar zig-zag structure. It is a versatile polymer with many biomedical applications, and it may be the only synthesized polymer whose backbone is mainly composed of C-C bonds that is biodegradable.[1,2] The excellent chemical resistance and optical and physical properties of PVA resins, have been resulted in its broad biomedical applications.[3–5] PVA also shows great potential for application in the production of biodegradable/biocompatible films, gels, fibers, sponges, beads, and nanoparticles.[6–10] However, the hydrophilicity nature of PVA is its disadvantage when considering the use of PVA film as protective barriers, because they tend to swell or dissolve when contact with the surface with high moisture content. Consequently, the current trends in designing PVA-based biodegradable materials for biomedical applications is focused on the developing films with improved mechanical and water resistance properties via PVA cross-linking with glutaraldehyde and blends production with other polymers.[11–17]

Although PVA films and blends have excellent mechanical and barrier properties, there is no reported antioxidant/antimicrobial activity for these materials. Thus, antimicrobial agents must be employed in the PVA films/blends for inhibit of pathogenic microorganisms in the surface of the materials. There is a growing interest in using plant extracts as natural sources of antioxidant/antimicrobial compounds in the formulation of films.[18] In this context, plant essential oils and their main components are gaining a wide interest in health industry for their potential as antioxidant and antimicrobial agents.[19,20] For example, Jo and others developed a new polymeric film using cinnamon oil and PVA against *Plodia interpunctella*.[21,22] Han and his co-workers developed polypropylene/PVA film incorporated with cinnamon

essential oil with antibacterial activity.[23] Kriegel and his coworkers suggested that electrospun PVA nanofibers could serve as carrier vehicles for micro-emulsions containing solubilized lipophilic functional compounds such as antimicrobials, antioxidants, flavors, and pharmaceuticals.[24] Kanatt and his colleagues suggested that chitosan–PVA film containing mint extract / pomegranate peel extract can be used for development of active film materials with antioxidant and antimicrobial activity.[25]

However, to our knowledge there is no report on the antioxidant and antimicrobial activity of PVA films incorporated with *Zataria multiflora* (ZM) essential oil (ZMO). ZM is a thyme-like plant belonging to the Lamiaceae family that has played an important role in Iranian traditional medicine. It has several traditional uses as an antiseptic, carminative, stimulant, diaphoretic, diuretic, anesthetic, antispasmodic, and analgesic.[26] In the modern pharmacological and clinical investigations, ZM is a valuable medicinal plant that has antimicrobial, antioxidative, anti-inflammatory, spasmolytic, and antinociceptive properties.[27]

The aim of this work was to develop PVA-based films incorporated with different concentrations of ZMO in order to obtain improvements in water solubility, moisture uptake (MU), water swelling, water vapor permeability (WVP), tensile strength, elongation at break, light barrier, antioxidant, and antibacterial properties.

MATERIALS AND METHODS

Materials

PVA with average molecular weight of about 72 kDa and 98% hydrolysis was purchased from Merck chemical (Darmstadt, Germany). All other chemicals including 2, 2′-azino-di (3-ethylbenzthiazoline-6-sulfonate) (ABTS) used in this work were Analytical grade and purchased from Sigma-Aldrich chemical (St. Louis, MO, USA). ZMO was prepared from the air-dried leaves of ZM through hydro-distillation using an all-glass Clevenger-type apparatus (Herbal Exir, Mashhad, Iran). The obtained ZMO was dehydrated over anhydrous sodium sulphate and then was dissolved in two volume of pure ethanol to emulsification.

Preparation of PVA Solutions and Film Casting

To prepare PVA film forming solutions, different concentrations of ZMO (2%, 4%, 6%, 8%, and 10% w/w based on the weight of the PVA powder) were mixed with 2% (w/v) PVA in aqueous solution and stirred for 12 h at 50°C. Glycerol (25% w/w based on the weight of the PVA powder) as plasticizer was added to film forming solutions. The PVA/ZMO solutions were mixed and sonicated using an ultrasound bath (Bandlin, Germany) at 70 W for 30 min at the temperature 50°C. The homogenous mixture was poured onto the Polystyrene Petri dish for film casting. The mixtures were cast onto flat and leveled trays to cast. Once set, the trays were held overnight at 50°C in a vacuum oven (Fan Azma Gostar, Tehran, Iran) for 12 h to yield a uniform thickness in all cases and then cooled to ambient temperature before peeling film off the plate.[11] The films obtained were stored in plastic bags and held in desiccators at 60% relative humidity for further testing. Thicknesses of films were measured with a micrometer (The L.S. Starrett, Great Britain, UK) and the average was taken (in five spots of three films) 85 ± 5 μm.

Scanning Electron Microscopy

The morphology of films was examined through Scanning electron microscopy (SEM). The magnified cross-sectional pictures of the films were taken with a Hitachi 570 SEM (FESEM Hitachi S4160 Japan) in the School of Metallurgy and Materials Engineering University of Tehran, Tehran, Iran. The film samples were fixed on the sample holder and then coated with gold. SEM pictures with 500× magnification were taken with an accelerating voltage of 10 kV.[12]

Water Solubility

The films were cut into 20 mm radius disc and then dried in a vacuum oven (Fan Azma Gostar) at 90°C for 24 h and then weighed to determine the initial dry mass (W_0). Then, the dried films were immersed into 50 mL capped falcon tubes containing 25 mL of distilled water and placed inside the shaker oven (Jal Tajhiz Labtech, Tehran, Iran) for 24 h at 25°C. Thereafter, the solution was filtered thought Whatman filter paper (no. 1) to recover the remaining un-dissolved film. The remaining film pieces were placed in the vacuum oven (Fan Azma Gostar) of 90°C for 24 h and then were weighed to determine the final dry mass of film (W_f). The percentage of weight losing was taken as water solubility (S) and was calculated by the following equation: $S\% = [(W_0 - W_f)/W_0] \times 100$.[28] W_0 was the initial weight of dried film and W_f was the weight of un-dissolved film residue. At least four tests were performed and the average values were reported.

Moisture Absorption

The film samples were cut into 20 mm radius disc and then dried in a vacuum oven (Fan Azma Gostar, Tehran, Iran) at 70°C for 24 h and then weighed to determine the initial dry mass (W_0). Then, the film samples were transferred into desiccators at 100% relative humidity at 37°C for about one week and allowed to absorb moisture until constant weight (equilibrium) was reached. The MU by the film was measured every 8 h up to steady state, by an analytical balance (Acculab, Sartorius group, Germany). Saturation condition (steady state) was checked by observing no changes in successive weight uptake measurements by the film samples. The weight at this stratus recorded as final weight of film (W_f). The percentage of weight gaining was taken as MU and was calculated by using the following equation: $[(W_f - W_0) / W_0] \times 100$. [28] At least four tests were done and the average values were reported.

Swelling Test

The film samples were cut into 20 mm radius disc and then dried in a vacuum oven (Fan Azma Gostar) at 70°C for 24 h and then were weighed to determine the initial dry mass (W_d). The film samples were immersed into a 50 mL falcon tube containing 25 mL of the distilled water. The samples were kept at room temperature for 2 h. Each film samples were taken out of the tubes after 2 h, wiped between filter papers to remove the excess surface water and were weighed to determine final weight of swollen film (W_s). The percentage of weight gaining was taken as water swelling (W_s) percentage and calculated by using

the following equation: SW (%) = $[(W_s - W_d) / W_d] \times 100$.[29] W_s was the weight of the swollen film and W_d was the dry weight of the film. At least four tests were performed and the average values were reported.

Water Vapor Permeability

The film samples were conditioned at 27°C and 60% relative humidity by placing them in desiccators over saturated solution of $Mg(NO_3)_2.6H_2O$ for three days. WVP of the film samples was examined using aluminum cups filled with 20 g silica gel to produce 0% relative humidity under the film. After taking the initial weight of the test cups, they placed in a box over saturated solution of $Mg(NO_3)_2.6H_2O$. The weight gain of the cups was measured at 3 h intervals during one day using electronic balance (Acculab, Sartorius group). A plot of weight gain (g) against time (h) for each cup was prepared. The slope of the linear portion of this plot showed the amount of water vapor diffusion through the film per time unit and expressed as gram unit per time unit (g/h). Water vapor transmission rates (WVTR) of the film was calculated from the slope of the linear portion of this plot per square meter (m^2) and expressed as g/m^2 h. The WVP was calculated by multiplying WVTR by the film thickness (mm) and expressed as g mm/m^2 h.[30] Tests were done in hexaplicate and the average values were provided.

Mechanical Test

Tensile strength, elastic modulus, and elongation at break of the films were measured using an Instron testing machine (Santam, Tehran, Iran). The film samples were cut to 60 mm × 10 mm. However, 20 mm of the films were within the jaws, so the initial length of the film was taken as 40 mm. The initial cross-sectional area of the film samples were 10 mm × 0.085 mm. The thickness of the films was measured at different points with micrometer and the average was taken. The film cuts were conditioned at 27°C and 60% relative humidity by placing them in desiccators over saturated solution of $Mg(NO_3)_2.6H_2O$ for two days.[31] The tensile strength test was then performed by stretch the film samples at speed of 10 mm/min. The nominal stress–strain curves were obtained and tensile strength, elastic modulus and elongation at break values were determined. Tensile strength was calculated by dividing the maximum stress by the initial cross-sectional area and expressed as megapascal (MPa). Elastic modulus was the initial slope of the stress–stain curves at the linear part and expressed as MPa. Elongation at break was calculated by dividing the extension length where the film is torn by the initial length of the film cut and multiplying by 100.[32] All the mechanical tests were carried out at room temperature. A minimum of four tests were performed and the averages were reported.

Light Absorption and Opacity

The film samples were cut to 1 cm × 8 cm and directly placed on two sides of empty spectrophotometer covet. The UV-visible absorption spectrum of the film cuts were measured over a wavelength range from 200 to 700 nm using UV-visible spectrophotometer (Kyoto, Japan). The opacity of the films was calculated by dividing light absorbance value at 320 nm by film thickness (mm).[33,34] A minimum of four tests were performed and the averages were reported.

Antioxidant Release and Antioxidant Activity

The continuous antioxidant release from the films was performed in 12-well plate by monitoring decolorization of ABTS in the presence of the film. The absorbance value of ABTS solution (7 mM ABTS and 2.54 mM potassium persulfate) was adjusted to 0.9 ± 0.1 at 734 nm by diluting the ABTS solution with distilled water. The wells of plate were filled with 2 mL of diluted ABTS solution. The films were cut in to 20 mm disc radius and immediately placed in the wells. The absorbance at 734 nm was recorded up to steady state (absorbance at 734 = 0) was reached for each sample. To determine antioxidant activity the value of light absorbance at 10 min was used. A plot of light absorbance at 734 nm against time (min) for each film was prepared. A standard curve of ascorbic acid ranging from 0.44 to 15.76 mg/mL was prepared. Antioxidant activity was expressed as mg ascorbic acid equivalents (AAE) per gram of films using standard curve.

Antifungal Activity

All microorganisms obtained from the Persian type culture collection (PTCC), Tehran, Iran. The films were individually tested against four fungi; *Paecilomyces variotii*, *Trichoderma harizanum*, *Aspergillus oryzae* and *Aspergillus niger*. The fungi strains suspended in LB media and their densities adjusted to 0.5 McFarland standards at 640 nm (10^8 CFU/ mL) and then diluted to 10^5 CFU/mL with LB. A sample film with 20 mm diameter was placed in a 10 mL liquid culture containing 10 μL microbe cultures. Then, the sample was incubated at 37 °C for 24 h (Shaking Incubator, Jal Tajhiz Labtech). From the incubated samples, a 100 μL solution was taken and diluted with the appropriate dilution factor and the final diluted microbe solution was plated and distributed onto nutrient agar plate (Farazbin Kimia, Tehran, Iran). The plates cultured with the pure PVA film under the same condition were used as control. All plates were incubated at 37°C for 24 h and the numbers of colonies that were formed were counted. The antifungal efficiency of the films was calculated according to the following equation.[32,36] Colony reduction (%) = [(The number of colonies in the presence pure PVA − The number of colonies in the presence of PVA/ZMO)/ Number of colonies in the presence pure PVA] × 100.

Statistical Analysis

All data are representative of at least three independent experiments and expressed as the mean values plus standard deviations. The significant differences between treatments were analyzed by one-way analysis of variance (ANOVA) and Duncan tests at $P < 0.05$ using statistical package for the social sciences (SPSS, Abaus Concepts, Berkeley, CA) software.

RESULTS AND DISCUSSION

Film Morphology

The pure PVA was smooth, transparent and homogeneous surface, because of the excellent film-forming properties of PVA. Addition of ZMO in to the PVA films introduced a solid particle like structure in the film which homogeneously distributed in film structure (Figure 1). The number of the solid particles increased with increase in the essential oil concentration, thus this particles caused by ZMO, which is in accordance with the results of other studies.[25, 37]

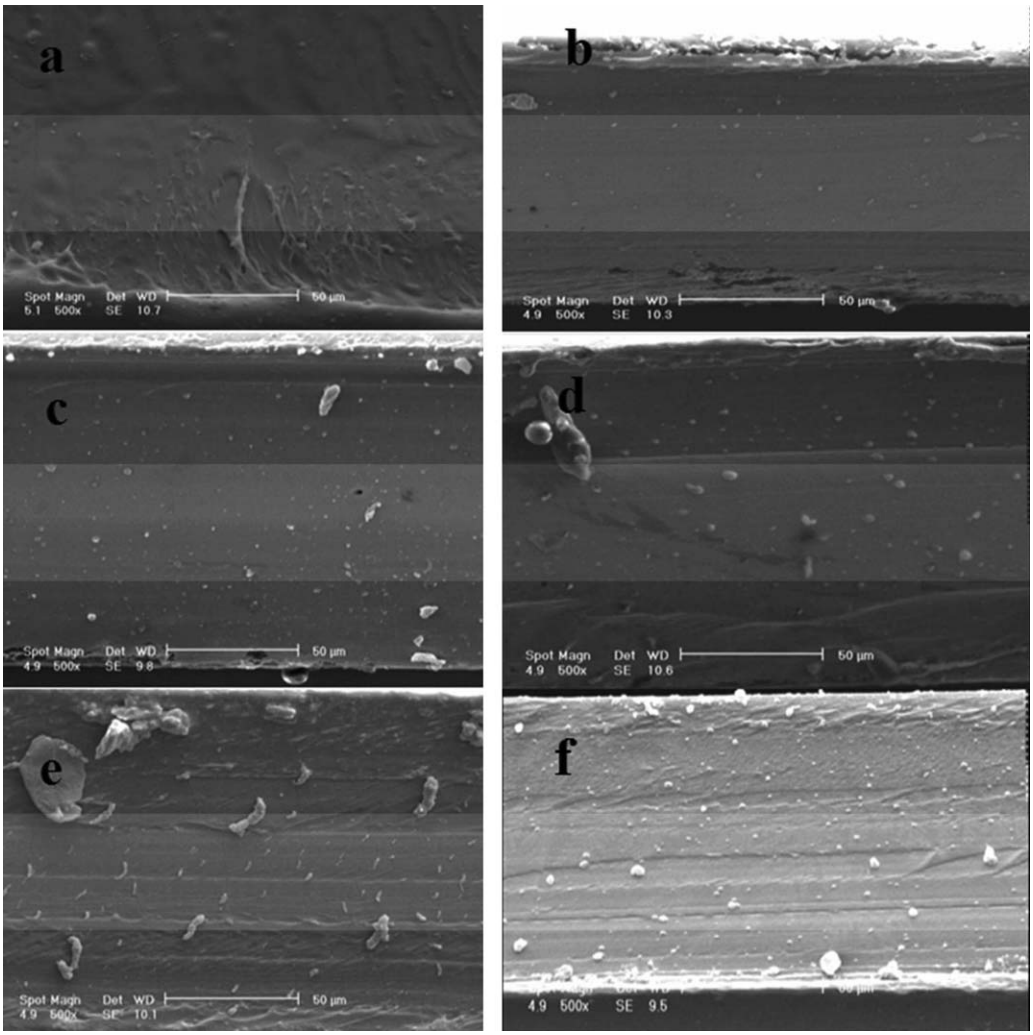

Figure 1. Scanning electron microscopy cross-section pictures of PVA films. (a) Pure PVA film, (b) PVA/ZMO 2% film, (c) PVA/ZMO 4% film, (d) PVA/ZMO 6% film, (e) PVA/ZMO 8% film, and (f) PVA/ZMO 10% film.

Films Solubility

Water resistance (low water solubility) is an important property of biodegradable films for biomedical applications where water activity is high or when film must be contact with water. Pure PVA film showed a solubility value of 57 ± 1.1%, which higher than the values reported by other study.[12] This differential solubility could be related to the absence of cross-linker (glutaraldehyde) in our study. Addition of ZMO (4–10%) to the PVA film caused a significant ($P < 0.05$) increase in the water solubility of the films, dose-dependently (Table I). PVA resins are naturally water soluble, while the solubility of polymeric PVA film reduced to some extent. This water resistance confirmed when film samples maintain their integrity (did not dissolve) in water after 24 h. This indicates that PVA network remained intact in water and only the PVA monomers and other nonbinding material were soluble and the scaffold of PVA network remained

Table I. Water Solubility, Swelling and Water Vapor Permeability of Polyvinyl Alcohol (PVA) Films with *Z. multiflora* Essential Oil (ZMO)

Films	Solubility (%)	Moisture absorption (%)	Swelling (%)	WVP (g mm/m^2 h)
PVA	57 ± 1.1^{e}	99 ± 3.2^{a}	337 ± 8^{a}	0.453 ± 0.015^{f}
PVA + ZMO 2%	59 ± 1.1^{e}	90 ± 1^{b}	304 ± 7^{b}	0.483 ± 0.011^{e}
PVA + ZMO 4%	61 ± 0.7^{d}	86 ± 2^{c}	284 ± 5^{c}	0.517 ± 0.016^{d}
PVA + ZMO 6%	63 ± 1.2^{c}	84 ± 1.5^{cd}	279 ± 6^{cd}	0.543 ± 0.010^{c}
PVA + ZMO 8%	66 ± 1.3^{b}	81 ± 2^{d}	270 ± 5^{d}	0.567 ± 0.014^{b}
PVA + ZMO 10%	69 ± 1.5^{a}	76 ± 2^{e}	251 ± 7^{e}	0.623 ± 0.02^{a}

Mean values with different letters within a column are significantly different as analyzed by Duncan's multiple range tests at ($P < 0.05$).

Table II. Tensile Strength, Elastic Modulus, Elongation at Break and Opacity of Polyvinyl Alcohol (PVA) Films with *Z. multiflora* Essential Oil (ZMO)

Films	Tensile strength (MPa)	Elastic modulus (MPa)	Elongation at break (%)216 ± 4e	Opacity (nm/mm)
PVA	13.5 ± 0.61^{a}	15.2 ± 0.8^{a}	216 ± 4e	12.8 ± 0.8^{e}
PVA + ZMO 2%	12.2 ± 0.36^{b}	11.6 ± 0.7^{bc}	226 ± 3^{d}	13.8 ± 0.7^{de}
PVA + ZMO 4%	11.7 ± 0.26^{b}	11.1 ± 0.8^{cd}	230 ± 4^{cd}	14.1 ± 0.7^{cd}
PVA + ZMO 6%	10.7 ± 0.46^{c}	10.2 ± 0.5^{d}	241 ± 4b	14.9 ± 0.7c
PVA + ZMO 8%	$10.4 \pm .42^{c}$	9.2 ± 0.4^{e}	247 ± 4^{ab}	16.1 ± 0.6^{b}
PVA + ZMO 10%	9.9 ± 0.33^{c}	8.1 ± 0.3^{f}	252 ± 3^{a}	16.8 ± 0.6^{a}

Mean values with different letters within a column are significantly different by Duncan's multiple range tests at ($P < 0.05$).

insoluble.[37] However, incorporation of ZMO into the films caused a significant increase in the solubility. This increase in the water solubility might be attributed to the establishment of PVA–ZMO interactions, which weaken the interactions (mainly hydrogen binding) that stabilize the PVA network.[29] This effect hinders polymer chain-to-chain interactions, reduces the PVA matrix integrity and consequently, causes an increase in the solubility of the films. [7]

Swelling and MU

The swelling capacity and MU of edible films plays an important role in biomedical applications and successful release of antioxidant/antimicrobial and other compounds to the contact surface. Pure PVA film showed a swelling and MU values of 337 ± 8% and 99 ± 3.2%, respectively, which is confirmed by the results of Singh and Pal.[17] Addition of ZMO (2–10%) to the PVA film caused a significant ($P < 0.05$) decrease in the swelling and MU of the films, dose-dependently (Table I). PVA has elevated polarity and can absorb large amount of water molecule probably because of hydroxyl groups. Each monomer has one hydroxyl group that are expected to absorb water molecules.[7,29] In addition, the porous PVA films showed high swelling capacity because the porosity in their network structures that allows more water to enter inside the film.[29] Incorporation of ZMO could reduce swelling capacity of the films which, might be related to hydrophobic nature of ZMO. ZMO can interact with PVA (probably through hydrogen bond) and thereby interfacial interaction between PVA and ZMO was increased. This event saturates PVA network with ZMO thus, water molecules cannot diffuse to PVA network thereby swelling and water uptake are decreased.[15]

Water Vapor Permeability

Pure PVA film showed a low WVP value of 0.453 ± 0.015 g mm/m^2 h, which is confirmed by the results of Alves and his co-workers.[11,12] Addition of ZMO (2–10%) into the PVA film caused a significant ($P < 0.05$) increase in the WVP of the films, dose-dependently (Table I). Generally, incorporation of additive to the films causes a significant change in water vapor transmission through films, whereas the final WVP capacity is related to hydrophobicity/hydrophilicity index of all compounds in the films and porous structure of the film.[17] Pure PVA is a hydrophilic material (because of hydroxyl groups) so strong interaction between these polar groups and water molecules causes a reduction in the water vapor transmission through the film.[15] Our experimental results showed that the WVP of PVA films incorporated with ZMO slightly increased. Components of ZMO can essentially interact with PVA chain and thereby the interfacial interaction between matrix and ZMO was increased. This phenomenon discourages interactions between PVA chains and water molecules in consequence; the water vapor transmission was increased.[14]

Mechanical Properties

The tensile strength is the maximum tensile stress sustained by the films during tension test. The edible film must withstand the normal stress encountered during its application to maintain its integrity. High tensile strength in required, but deformation values must be adjusted according to the intended film applications. Tensile strength, elastic modulus, and elongation at break of the pure PVA film were 13.5 ± 0.61 MPa, 15.2 ± 0.8 MPa and 216 ± 4%, respectively, which are in agreement with previous reports.[14–17] However, incorporation of ZMO (2–10%) caused a significant ($P < 0.05$) decrease in tensile strength and elastic modulus and increase ($P < 0.05$) in elongation at break (Table II). Our results demonstrated the tensile strength of PVA film decreased with the addition of ZMO which is confirmed by previous results on the PVA incorporated with other plant extract.[21,22] PVA films were mainly stabilized by the weak bond including hydrogen bond between hydroxyl groups of monomers. The reduction of the film tensile by addition of ZMO is likely related to the formation of inter molecular interaction (hydrogen bonds) between functional group of PVA and ZMO components. Thus, the original hydrogen bonds between PVA chain that stabilized film matrix could be replaced with new hydrogen bonds between PVA and ZMO, so tensile strength was decreased.[12,32] Elastic modulus is a measure of the stiffness (hardness) of film. A stiff material has high elastic modulus and changes its shape only slightly under elastic loads. A flexible material has low elastic modulus and changes its shape considerably. By the incorporation of ZMO into the films elastic modulus reduced significantly thus the film structure become low stiffer which is in accordance with experimental results of Bonilla et al.[32] Elongation at break is an indication of flexibility and extensibility of film prior to breakage. In the presence of ZMO, the flexibility of the PVA film increased that might be because of the increase in pore sizes and porosity of the films by adding ZMO to the polymer matrix.[28,29] The increasing flexibility of the films by the addition of ZMO could be related to formation of new hydrogen bonds between ZMO and PVA

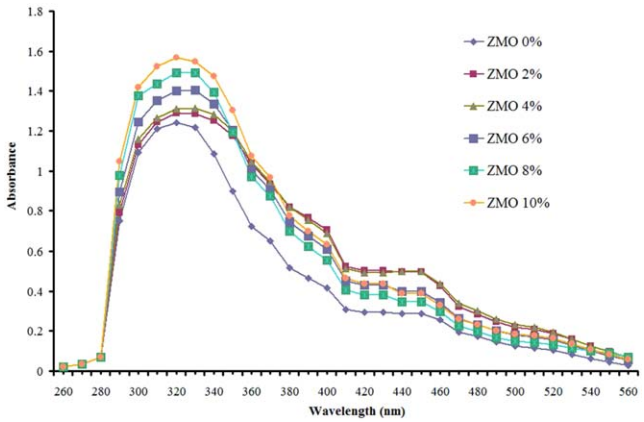

Figure 2. Light absorbance profile of PVA films as a function of *Z. multiflora* essential oil (ZMO) content. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

matrix which caused an increase in the segmental mobility of PVA chain and more sliding effect of PVA chains against each other.[29]

Light Absorption and Opacity

Pure PVA films showed light absorbance in the range between 280 and 440 nm, while maximum absorbance was at 320 nm (Figure 2). The opacity values of PVA films are showed in Table II. Incorporation of ZMO into the PVA films caused a significant increase in the light absorbance and opacity. Thus, the PVA films lost their typical transparent and colorless appearance. However, the resulting PVA/ZMO films gained in light barrier properties, which could be interesting in certain applications for preventing UV-induced lipid peroxidation.[38] The increase in the light absorbance more likely depends on the distribution of ZMO in the PVA matrix as well the interaction between ZMO and PVA. This effect led to differences in film matrix morphology with different light absorbance. The opacity of the PVA film was increased by adding ZMO in the PVA matrix, more likely because of the light scattering effect of ZMO.[13]

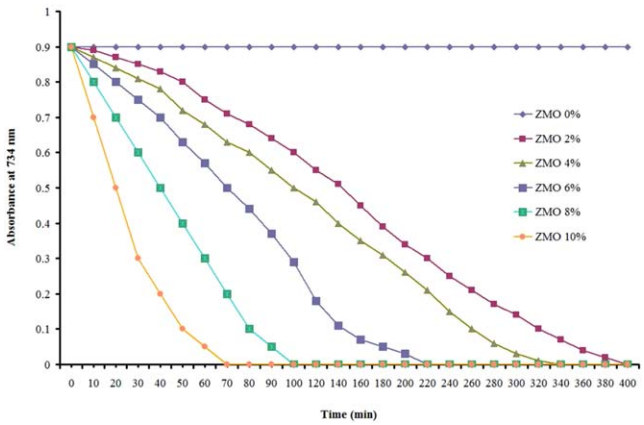

Figure 3. Kinetic release of ZMO from PVA/ZMO films. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Antioxidant Release

The antioxidant release profile from the PVA films was obtained by ABTS decolorization method. The test was based on the radical scavenging of phenolic compound (thymol and carvacrol) of Zataria oil. The antioxidant measurement (reduction of light absorbance at 734 nm) actually reflects the release of antioxidant compound from film into environment. The determination of the absorbance change against time demonstrates the kinetic release of ZMO from film to the surrounding environment. Figure 3 shows the antioxidant releasing behavior of PVA/ZMO. According to the antioxidant release profile, the oil compound released between 70 min (for PVA film containing 10% ZMO) to 400 min (for PVA film containing 2% ZMO). By increase in ZMO in the film the release velocity was increased which is in accordance with the study of Zu et al.[10]

Antioxidant Activity

Antioxidant activity of the PVA films was determined by ABTS decolorization method and expressed as mg ascorbic acid equivalent per gram of the films (Table III). The pure PVA films showed no activity against the ABTS decolorization. The PVA film containing different ZMO concentrations decolorize ABTS, dose-dependently. Addition of ZMO into the PVA films caused a significant increase in the antioxidant activity. Generally, essential oils have been reported as the excellent source of natural antioxidant.[19,20] The total phenolic content and related total antioxidant capacity of some medicinal plant infusions was analyzed, which indicated that there was a significant linear correlation between total phenol content and antioxidant capacity.[39] ZMO is a good source of phenolic monoterpenes (thymol and carvacrol), with a significant antioxidant activity. Thus, antioxidant activity of PVA/ZMO films could be related to its phenolic compound content. The ZMO has been reported possessed antioxidant activity it has also possesses nitric oxide (NO) and malondialdehyde scavenging properties and thus could prevent oxidative/nitrative stress and lipid peroxidation.[40] These results suggested that PVA/ZMO films with excellent antioxidant activity could be promising candidate for safe radical scavenger material.[41]

Antifungal Activity

The antifungal activities of PVA films incorporated with ZMO were examined by viable colony counting assay. The results of colony reduction percentage are summarized in Table IV.

Table III. Antioxidant Activity of Polyvinyl Alcohol (PVA) Films with *Z. multiflora* Essential Oil (ZMO)

Films	Ascorbic acid equivalent (mg/g)
PVA	0 ± 0^{e}
PVA + ZMO 2%	1.32 ± 0.40^{d}
PVA + ZMO 4%	1.72 ± 0.53^{cd}
PVA + ZMO 6%	2.28 ± 0.75^{bc}
PVA + ZMO 8%	4.15 ± 0.72^{ab}
PVA + ZMO 10%	5.55 ± 0.93^{a}

Anti-oxidant activity was expressed as milligram ascorbic acid equivalent (AAE) per gram of the films with ZMO. Mean values with different letters within a column are significantly different as analyzed by Duncan's multiple range tests at ($P < 0.05$).

Table IV. The Antifungal Activities of Polyvinyl Alcohol (PVA) Films with *Z. multiflora* Essential Oil (ZMO)

	Growth inhibition (%)			
Films	*Paecilomyces variotii*	*Trichoderma harizanum*	*Aspergillus oryzae*	*Aspergillus niger*
PVA	0 ± 0^{f}	0 ± 0^{f}	0 ± 0^{f}	0 ± 0^{f}
PVA + ZMO 2%	8 ± 1.6^{e}	6 ± 1.3^{e}	6 ± 1.2^{e}	5 ± 1.5^{e}
PVA + ZMO 4%	32 ± 2.5^{d}	30 ± 2.2^{d}	29 ± 2.6^{d}	22 ± 3^{d}
PVA + ZMO 6%	51 ± 3.2^{c}	48 ± 2.8^{c}	45 ± 3^{c}	40 ± 3.6^{c}
PVA + ZMO 8%	64 ± 4^{b}	61 ± 3.5^{b}	60 ± 4.5^{b}	58 ± 4.2^{b}
PVA + ZMO 10%	82 ± 5^{a}	78 ± 3.6^{a}	75 ± 4.7^{a}	73 ± 5^{a}

Antimicrobial activity was expressed as percentage of growth inhibition in the presence of the films with ZMO. Mean values with different letters within a column are significantly different as analyzed by Duncan's multiple range tests at ($P < 0.05$).

According to the results obtained, pure PVA films showed no antifungal activity against the tested fungi. By addition ZMO into the PVA films antifungal activities positively increased. PVA films incorporated with ZMO are effective against all fungi tested. Previous study suggested that ZMO is a good source of phenolic monoterpenes (thymol and carvacrol), with a significant antimicrobial activity against *Candida*, *Aspergillus*, *Malassezia*, *Fusarium*, and *Saprolegnia* species.[41,42] The antifungal activities of phenolic compounds is related with the attack on the phospholipids present in the cell membranes, which causes increased permeability and leakage of cytoplasm, or in their interaction with enzymes located on the cell wall.[43] The essential oil samples generally displayed potent fungicidal activity and antifungal potencies varied and appeared to be intensified by increasing carvacrol and thymol contents. Carvacrol and thymol could inhibit the growth of fungi through permeabilization and depolarization the cytoplasmic membrane which, ultimately leading to cytoplasmic membrane disruption, induction of dose-dependent Ca^{2+} bursts and inhibition of ergosterol biosynthesis and the disruption of membrane integrity.[44] Furthermore, fungicidal activity of essential oils might be because of the induction of calcium stress and up-regulation of genes involved in metabolic pathways, stress response, autophagy, and drug efflux.[45]

CONCLUSION

PVA/ZMO films were prepared by emulsification of ZMO in PVA solutions. The PVA/ZMO films showed increase in water solubility and WVP and decrease in water swelling and MU as compared to pure PVA films, probably because of hydrophobic nature of ZMO. Incorporation of ZMO caused also a significant decrease in tensile strength and elastic modulus and increase in elongation at break of the films. ZMO particles that were localized in the film matrix increased the opacity of the film, more likely because of the light scattering effect of the essential oil compounds. SEM observations indicate that ZMO were well dispersed in the film matrix and good adhesion between them was obtained which lead to decrease in the tensile strength and increase in water vapor transmission. Pure PVA films exhibited no antioxidant/antifungal activities while, PVA/ZMO films exhibited excellent antioxidant/antifungal properties. Although the bioactivity of PVA films was increased by addition of ZMO, however, the mechanical properties and water-binding capacity of the film decreased, thus ZMO that emulsified in the ethanol not compatible with PVA matrix. Thus, a more favorable emulsifier such as tween 20 or/and other surfactant was needed. Furthermore, in future study we will focus on the applications of such films to *ex vivo* and *in vivo* systems to investigate biocompatibility of the films as a wound dressing.

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REFERENCES

1. Alhosseini, S. N.; Moztarzadeh, F.; Mozafari, M.; Asgari, S.; Dodel, M.; Samadikuchaksaraei, A.; Kargozar, S.; Jalali, N. *Int. J. Nanomed.* **2012**, *7*, 25.
2. Baker, M. I.; Walsh, S. P.; Schwartz, Z.; Boyan, B. D. *J. Biomed. Mater. Res. B* **2012**, *100*, 1451.
3. Deskins, D. L.; Ardestani, S.; Young, P. P. *J. Vis. Exp.* **2012**, *18*, 3885.
4. Lee, C. T.; Kung, P. H.; Lee, Y. D. *Carbohydr. Polym.* **2005**, *61*, 348.
5. Roy, N.; Saha, N.; Kitano, T.; Saha, P. *Carbohydr. Polym.* **2012**, *89*, 346.
6. Russo, M. A.; O'Sullivan, C.; Rounsefell, B.; Halley, P. J.; Truss, R.; Clarke, W. P. *Bioresour. Technol.* **2009**, *100*, 1705.
7. Song, W.; Markel, D. C.; Wang, S.; Shi, T.; Mao, G.; Ren, W. *Nanotechnology* **2012**, *23*, 115101.
8. Tang, X.; Alavi, S. *Carbohydr. Polym.* **2011**, *85*, 7.
9. Tripathi, J.; Keller, J. M.; Das, K.; Tripathi, S.; Fatima, A.; Shripathi, T. *Appl. Surf. Sci.* **2012**, *261*, 481.
10. Zu, Y.; Zhang, Y.; Zhao, X.; Shan, C.; Zu, S.; Wang, K.; Li, Y.; Ge, Y. *Int. J. Biol. Macromol.* **2012**, *50*, 82.
11. Alves, M. H.; Jensen, B. E.; Smith, A. A.; Zelikin, A. N. *Macromol. Biosci.* **2011**, *11*, 1293.
12. Alves, P. M. A.; Carvalho, R. A.; Izabel, C. F.; Moraes, C. G.; Luciano, A. M. Q. B.; Paulo, J. A. S. *Food Hydrocolloids*, **2011**, *25*, 1751.

13. Mansur, H. S.; Sadahira, C. M.; Souza, N. S., Mansur, A. A. P. *Mater. Sci. Eng. C* **2008**, *28*, 539.
14. Pal, K.; Banthia, A. K.; Majumdar, D. K. *AAPS Pharm. Sci. Technol.* **2007**, *8*, 142.
15. Ramaraj, B. *J. Appl. Polym. Sci.* **2007**, *103*, 909.
16. Silva, G. G. D.; Sobral, P. J. A.; Carvalho, R. A.; Bergo, P. V. A.; Mendieta-Taboada; O.; Habitante, A. M. Q. B. *J. Polym. Environ.* **2008**, *16*, 276.
17. Singh, B.; Pal, L. *J. Mech. Behav. Biomed. Mater.* **2012**, *9*, 9.
18. Lucera, A.; Costa, C.; Conte, A.; Del Nobile, M. A. *Front. Microbiol.* **2012**, *3*, 287.
19. Kuorwel, K. K.; Cran, M. J.; Sonneveld, K.; Miltz, J.; Bigger, S. W. *J. Food Sci.* **2011**, *76*, 164.
20. Solorzano-Santos, F.; Miranda-Novales, M. G. *Curr. Opin. Biotechnol.* **2012**, *23*, 136.
21. Jo, H. J.; Park, K. M.; Min, S. C.; Na, J. H.; Park, K. H.; Han, J. *J. Food Sci.* **2013**, *78*, 1713.
22. Kim, I. H.; Han, J.; Na, J. H.; Chang, P. S.; Chung, M. S.; Park, K. H.; Min, S. C. *J. Food Sci.* **2012**, *78*, 229.
23. Han, C.; Wang, J.; Li, Y.; Lu, F.; Cui, Y. *Meat Sci.* **2013**, *96*, 901.
24. Kriegel, C.; Kit, K. M.; McClements, D. J.; Weiss, J. *Langmuir*, **2009**, *25*, 1154.
25. Kanatt, S. R.; Rao, M. S.; Chawla, S. P.; Sharma, A. *Food Hydrocoll.* **2012**, *29*, 290.
26. Iranian Herbal Pharmacopoeia. Ministry of Health and Medical Publications, Tehran. 2002, 51.
27. Sajed, H.; Sahebkar, A.; Iranshahi, M. *J. Ethnopharmacol.* **2013**, *145*, 686.
28. Gonzalez, J.S.; Maiolo, A. S.; Hoppe, C. E.; Alvarez, V. A. *Proc. Mater. Sci.* **2012**, *1*, 483.
29. Vimala, K.; Mohan, Y. M.; Varaprasad, K.; Redd, N. N.; Ravindra, S.; Naidu, N. S.; Raju, K. M. J. *Biomater. Nanobiotechnol.* **2011**, *2*, 55.
30. Kumar, K.; Negi, Y. S.; Bhardwaj, N. K.; Choudhary, V. *Carbohydr. Polym.* **2012**, *88*, 1364.
31. Zhu, H. Y.; Fu, Y. Q.; R. Jiang, R.; Yao, J.; Xiao, L.; Zeng, G. M. *Bioresour. Technol.* **2012**, *105*, 24.
32. Bonilla, I.; Fortunati, E.; Atarés, L.; Chiralt, A.; Kenny, J. M. *Food Hydrocoll.* **2014** *35*, 463.
33. Tripathi, S.; Mehrotra, G. K.; Dutta, P. K. *Int. J. Biol. Macromol.* **2009**, *45*, 372.
34. Tang, X.; Alavi, S. J. *Agricul. Food Chem.* **2012**, *60*, 1954.
35. Kavoosi, G.; Dadfar, S.M.M.; Mohammadi Purfard, A.; Mehrabi, R. *J. Food Saf.* **2013**, *33*, 423.
36. Chen, F.; Shi, Z.; Neoh, K.G.; Kang, E.T. *Biotechnol. Bioeng.* **2009**, *104*, 30.
37. Rescignano, N.; Fortunati, E.; Montesano, S.; Emiliani, C.; Kenny, J. M.; Martino, S.; Armentano, I. *Carbohydr. Polym.* **2014**, *99*, 47.
38. Gomez-Estaca, J.; Lopez-de-Dicastillo, C.; Hernasndez-Munoz, P.; Catala, R.; Gavara, R. *Trend. Food. Sci. Technol.* **2014**, *35*, 42.
39. Miguel, M.G. *Flavour. Fragr. J.* **2010**, *25*, 291.
40. Kavoosi, G.; Teixeira, d.; Saharkhiz, M. J. *J. Pharm. Pharmacol.* **2012**, *64*, 1492.
41. Abdollahi, M.; Hamzehzarghani, H.; Saharkhiz, M. J. *J. Food. Saf.* **2011**, *31*, 424.
42. Khosravi, A. R.; Shokri, H.; Sharifrohani, M.; Mousavi, H. E.; Moosavi, Z. *Foodborne Pathog. Dis.* **2012**, *9*, 674.
43. Vale-Silva, L. A.; Silva, M. J.; Oliveira, D.; Goncalves, M. J.; Cavaleiro, C.; Salgueiro, L. *J. Med. Microbiol.* **2012**, *61*, 252.
44. Ahmad, A.; Khan, A.; Akhtar, F.; Yousuf, S.; Xess, I.; Khan, L. A.; Manzoor, N. *Euro. J. Clin. Microbiol. Infect. Dis.* **2011**, *30*, 41.
45. Rao, A.; Zhang, Y.; Muend, S.; Rao, R. *Antimicrob. Agents Chemother.* **2010**, *54*, 5062.