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Design and Development of Robust, Daylight-Activated, and Rechargeable Biocidal Polymeric Films as Promising Active Food Packaging Materials

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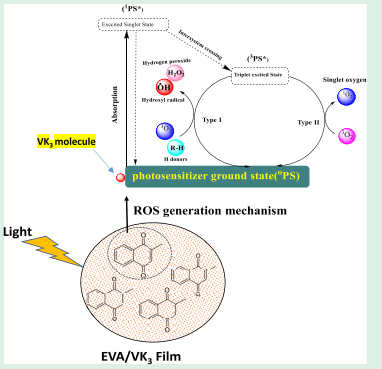
ABSTRACT: The emerging infectious diseases have created one of the major practical needs to develop active packaging materials with durable antibacterial and antiviral properties for the food industry. To meet this demand, the development of new technologies applicable to food contact surfaces is highly desired but challenging. The recent discovery of the photoactive properties of vitamin K (VK) derivatives has raised great expectations as promising candidates in functional film development due to the generation of biocidal reactive oxygen species (ROS) by these compounds. Inspired by the excellent photoactivity of one of the light-stable VK derivatives, menadione (VK_3), under visible daylight irradiation, we demonstrate a protocol for the fabrication of daylight-mediated biocidal packaging materials by incorporating VK_3 into a poly (ethylene-co-vinyl acetate) (EVA) matrix. The VK_3 (i.e., 1−5% w/w) incorporated EVA films successfully demonstrated the production of ROS and antibacterial and antiviral performance against *Escherichia coli*, *Listeria innocua*, and T7 bacteriophage, respectively, under daylight exposure conditions. The results revealed that the addition of a proper percentage of VK_3 significantly enhanced the ROS productivity of the films and created a novel daylight-induced microbial killing performance on the films. The biocidal functions of the films are long-lasting and rechargeable when exposed to light repeatedly, making them a viable contender for replacing currently available conventional packaging films.



KEYWORDS: *photoactivity, packaging films, antibacterial, polymer surfaces, vitamin K derivatives*

1. INTRODUCTION

Increased foodborne microbial illness has raised global concerns because it causes millions of deaths every year and continues to severely jeopardize public health worldwide.[1−3] Consequently, this has motivated massive scientific research and investment in the design and development of novel and versatile antimicrobial materials to overcome microbial invasions and for preventing pathogenic infections.[4−6] Controlling bacterial infections is critical not only for preventing global outbreaks but also for applications ranging from health care to improving daily life quality.[7,8] Over the past few years, there has been a significant increase in consumer interest in high-quality and safe food products.[9,10] Different strategies have been applied to develop new polymeric materials by incorporating antimicrobial agents to minimize the proliferation of microbes observed on various food packages.[11,12] Blending of polymers with conventional antibacterial agents has gained wide attention and has been applied to inhibit microbial growth in food products.[13,14] Data from previous studies clearly show that polymers can be functionalized with N-halamine precursors and related compounds, which could be a useful tool for the decontamination of food pathogens such as Listeria, yeasts, molds, and mesophiles found on solid surfaces.[15−19] However, the leaching of conventional agents and the release of free chlorine from the materials pose a serious threat to human health and the environment.[20,21] Lately, photodynamic inactivation, a relatively novel technology, has emerged as a potential option for preventing microbial inhibition and preserving food quality and shelf-life.[22] Nowadays, photoactive compounds have garnered considerable interest in the development of food packaging films because of their capability to produce oxidative biocide-reactive oxygen species (ROS) in many polymeric materials, durability for repeated uses, and lower-toxicity suitable for food contacts.[23] Under ultraviolet (UVA) exposure, photoactive nanoparticles have shown the ability to produce ROS such as singlet oxygen ($^1O_2$), hydroxyl radical ($^{\cdot}OH$), and hydrogen peroxide (H_2O_2).[24] However, because of

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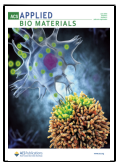

their excitation bandgap energy, these photocatalysts can only be made in the nanoscale range, which is a concern for potential human skin penetration and inhalation.[25] Organic photosensitizers are another family of the compounds that have been found to produce cell-lethal $^1O_2$ when exposed to UVA irradiation; however, due to their short lifetime, $^1O_2$ diffusion to pathogen targets may be limited.[26,27] Many anthraquinone and benzophenone derivatives, despite having the capacity to produce ROS under UVA or daylight, are often associated with undesirable side effects in food packaging.[28,29] However, the structural features of the organic photosensitizers explore the prospects for the discovery of new food-safe compounds with daylight-induced antibacterial and antiviral functions.[30,31] In daily applications of food packaging materials, daylight or UVA irradiation is the most common and safe light source, and the photosensitizers that are able to produce ROS under such exposure conditions are the ones that are mostly desired. Therefore, continuous exploration of safer biocidal agents with less toxicity and novel action-mechanisms is significant for the development of promising packaging substrates.[32]

The main challenge in development of antibacterial food package materials is the identification of novel, naturally occurring food grade compounds as efficient light-activated sensitizers.[33,34] Bio-based agents have gained tremendous applications in the sustainable and biodegradable food packaging industry due to their renewability, availability, biodegradability, and nontoxicity.[32,35] However, the application of natural photosensitizers faces challenges in fulfilling the requirements of food packaging applications due to their low ROS production and chemical instability toward light irradiation.[36] In recent years, investigations into green functional materials have suggested that natural vitamin Ks (VKs) may be capable of generating ROS effectively in various solvent systems under light irradiation.[37] Notably, menadione (VK_3) has demonstrated a robust and long-lasting antibacterial effect in the PBS buffer system and certain polymers when exposed to daylight.[37,38] To explore the potential of utilizing VKs as photoactive agents in developing reusable antiviral personal protective equipment materials, VK-containing photoinduced microbiocidal nanofibrous membranes were fabricated, which exhibited the desired functionality when exposed to both daylight and UVA irradiation.[39] In an effort to apply photodynamic technology to develop practical daylight activated biocidal packaging films for the food sector, we turned our attention to vitamin K_3 as the most promising biocidal photoactive compound among the derivatives. Herein, for the first time, we report design and development of vitamin-based films as promising photoactive food-grade packaging materials. In particular, photoinduced antibacterial and antiviral poly (ethylene-co-vinyl acetate) (EVA) packaging films were prepared by a simple one-step thermal blending extrusion process of the polymer with the nontoxic photoactive VK_3, and the resulting polymer revealed light-activated functions under daylight exposure. EVA is a copolymer of ethylene and vinyl acetate and has been widely used as shrink wrappers, ice bags, and stretch wrap for meat and poultry, as well as other food packaging applications.[40,41] Compared with the native EVA film, VK_3 containing films (EVA/VK_3) could generate sufficient amounts of ROS through both type I and type II mechanisms of photoreactions and demonstrate excellent reusable biocidal functions under normal daylight exposure conditions. More importantly, we observed that only as little as 1% of vitamin K_3 is needed in the polymer blend to achieve ideal reusable daylight-induced biocidal properties for practical use against common foodborne pathogens on surfaces of the films, without obvious negative impact on the optical and mechanical properties of the films.

2. MATERIALS AND METHODS

Menadione (VK_3), ethyl alcohol (EtOH), L-histidine, potassium iodide, sodium hydroxide, ammonium molybdate tetrahydrate, potassium hydrogen phthalate, sodium phosphate dibasic, potassium phosphate monobasic, sodium chloride, potassium chloride, and EVA with 12.8% vinyl acetate were purchased from Sigma-Aldrich (St. Louis, MO, USA). P-Nitroso-*N*,*N*-dimethylaniline (p-NDA) was purchased from TCI Co. Ltd. (Tokyo, Japan). Luria−Bertani (LB) broth, LB agar, tryptic soy broth (TSB), and tryptic soy agar (TSA) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). All chemicals and supplies were used as received, without requiring any further purification.

2.1. Fabrication of EVA/VK_3 Films. The photoactive EVA/VK_3 blends were prepared by using a Brabender Plasti-corder ATR mixer (C W Brabender, USA). EVA polymer beads were mixed with VK_3 in varied amounts (1, 2, 3, 5% w/w). The EVA sample with a melting point of 95 °C and a recommended processing temperature at 160−185 °C was employed in the work. The processing temperature was set up at 160 °C.

The EVA polymer was mixed with VK_3 for 10 min in the mixer to form a homogenous polymer blend. After that, the polymer blend was removed and cut into small pieces for a heat-compression step. The small pieces of the mixtures were sandwiched by two polyester thin films and heat pressed under 160 °C into very thin films in a heat press machine (Model CK220, Amazon). Both pure EVA thin films and EVA/VK_3 films were prepared by the same process.

2.2. Fourier-Transform Infrared Spectroscopy. To collect the Fourier-transform infrared spectroscopy (FT-IR) spectra of the films, a Nicolet 6700 spectrometer (Thermo, USA) was used in the 400−3500 cm^{-1} range. The tensile performance of the films was assessed using an Instron 5565 testing system with a 5 kN load cell, following the ASTM test standard method.

2.3. Measurement of ROS. The ROS production was measured using a Spectrolinker XL-1500 device for daylight irradiation, which was equipped with a D65 standard light source (GE F15T8/D). The sample films were positioned at 16 cm from the lamps. The production of ROS was measured by determining the quantities of $^{\cdot}OH$ and H_2O_2 generated under the daylight irradiation. The yield of $^{\cdot}OH$ was measured through the bleaching of p-NDA. In a typical experiment, a 1.0 × 1.0 cm^2 sample film was submerged in 5.0 mL of p-NDA (50 μM) and exposed to either daylight or darkness for a specified period. The remaining p-NDA in the solution was quantitatively measured by its absorbance at λ_{max} of 440 nm. Likewise, the quantity of H_2O_2 was assessed using an indirect spectrophotometric method. The experiment involved placing a sample film measuring 1.0 × 1.0 cm^2 into 5.0 mL of deionized water and exposing it to either daylight or dark conditions for a specific period. After exposure, 1.0 mL of the sample solution was mixed with 1.0 mL of reagent I, which is composed of an aqueous solution of potassium iodide (66 g L^{-1}), sodium hydroxide (2.0 g L^{-1}), and ammonium molybdate tetrahydrate (0.2 g L^{-1}), as well as 1.0 mL of reagent II, which is composed of an aqueous solution of potassium hydrogen phthalate (20 g L^{-1}). The mixture was stirred for 5 min, and the concentration of H_2O_2 produced in the solution was quantitatively measured by analyzing the absorbance at 351 nm. To test the generation of $^1O_2$ in the films, L-histidine at a concentration of 0.01 M was added to the p-NDA solution. The decrease in p-NDA concentration (ΔCp-NDA_2) was caused by the quenching of p-NDA by hydroxyl radicals and singlet oxygen-oxidized L-histidine. Therefore, the difference between ΔCp-NDA_1 and ΔCp-NDA_2 can be used to evaluate the production of singlet oxygen. The detailed method of singlet oxygen production by the developed films is reported in our previously published literature.[37]

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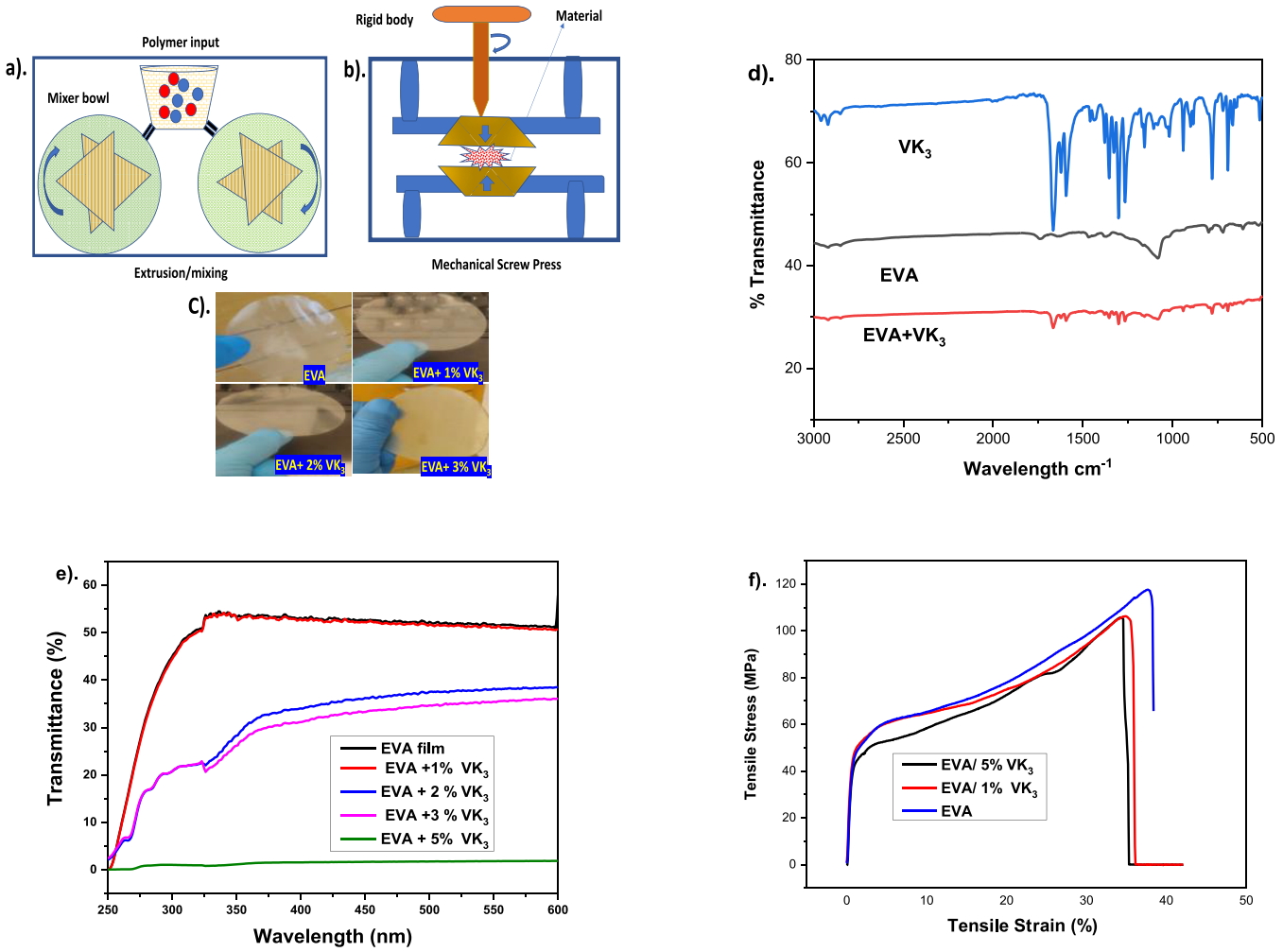

Figure 1. (a) Polymer blending, (b) film formed by heat press, and (c) photos of EVA/VK_3 films; (d) FT-IR spectra of EVA (control) and EVA/VK_3 films; (e) transparency in UV−vis graphs and (f) mechanical properties of the films.

2.4. Antibacterial Test. In this study, two types of bacteria, specifically *Escherichia coli* O157:H7 (ATCC 70072) and *Listeria innocua* (ATCC 33090) were selected to showcase the antibacterial functions of EVA/VK_3 based films under photoinduced conditions. The antibacterial experiments were prepared by culturing an *E. coli* colony in 10 mL of LB broth at 37 °C for 18 h, which resulted in a bacterial suspension containing about 1.0 × 109 CFU mL^{-1}. This suspension was then diluted to around 1.0 × 10^7 CFU mL^{-1} to make a stock bacterial suspension. Similarly, an *L. innocua* colony was grown in 10 mL of TSB broth at 37 °C for at least 24 h, producing a bacterial suspension of approximately 1.0 × 109 CFU mL^{-1}, which was diluted to around 1.0 × 108 CFU mL^{-1} to create a stock bacterial suspension for subsequent photoinduced antibacterial tests.[32] In a typical experiment, a 10 μL bacterial suspension (either *E. coli* or *L. innocua*) was placed onto the surface of control (EVA) or EVA/VK_3 samples that measured 1.0 × 1.0 cm^2. These samples were then subjected to either daylight or darkness for a specified period. At each time interval, the bacterial samples were gathered by combining them with 1.0 mL of deionized water and vortexing. The mixture was then serially diluted (×10^1, ×10^2, ×10^4, and ×10^6) and plated onto LB agar (for *E. coli*) or TSA agar (for *L. innocua*) for bacterial counting. The outcomes were adjusted to a 1.0 × 10^6 CFU initial load and plotted as CFU.[32]

2.5. Antiviral Activity of Developed Films. During the time-dependent daylight-induced antiviral test, a solution of the T7 bacteriophage containing 1 × 107 PFU mL^{-1} was evenly spread onto both control samples and EVA/VK_3 films that were 1 × 1 cm^2 in size, using 10 μL of the solution. These samples were then subjected to different durations of either exposure to daylight or being kept in the dark. At certain time intervals, the T7 phages were collected from the samples by thoroughly mixing them with 3 mL of a maximum recovery diluent. The collected phages were then diluted 100-fold and 100 μL of the diluted phage solution was combined with a suspension of *E. coli* BL21 containing 1 × 109 CFU mL^{-1}. The mixture was incubated at 37 °C for 10 min and then mixed with 3 mL of molten LB agar at 45 °C. The mixture was immediately poured onto a prewarmed LB agar plate, and after the agar solidified, the plates were left to incubate overnight at room temperature. Finally, the phage plaques were counted and adjusted to the initial concentration.[37]

3. RESULTS AND DISCUSSION

3.1. Characterization of EVA-VK_3 Films. Daylight activated food packaging with rechargeable biocidal activity is highly desirable and challenging.[42] In comparison with UVA irradiation, daylight may penetrate in more depth through polymeric films and could induce more photoactive functions on the films. The design and application of daylight-activated molecules can offer comparative advantages and are more realistic in food packaging materials from a practical point of view.[43] For this, the EVA polymer was mixed with VK_3 and then compressed into thin films. VK_3 is thermally stable under 160 °C; however, certain sublimation could occur under higher temperatures and prolonged heating. Figure 1a−c shows the scheme of the preparation process and the appearance of the

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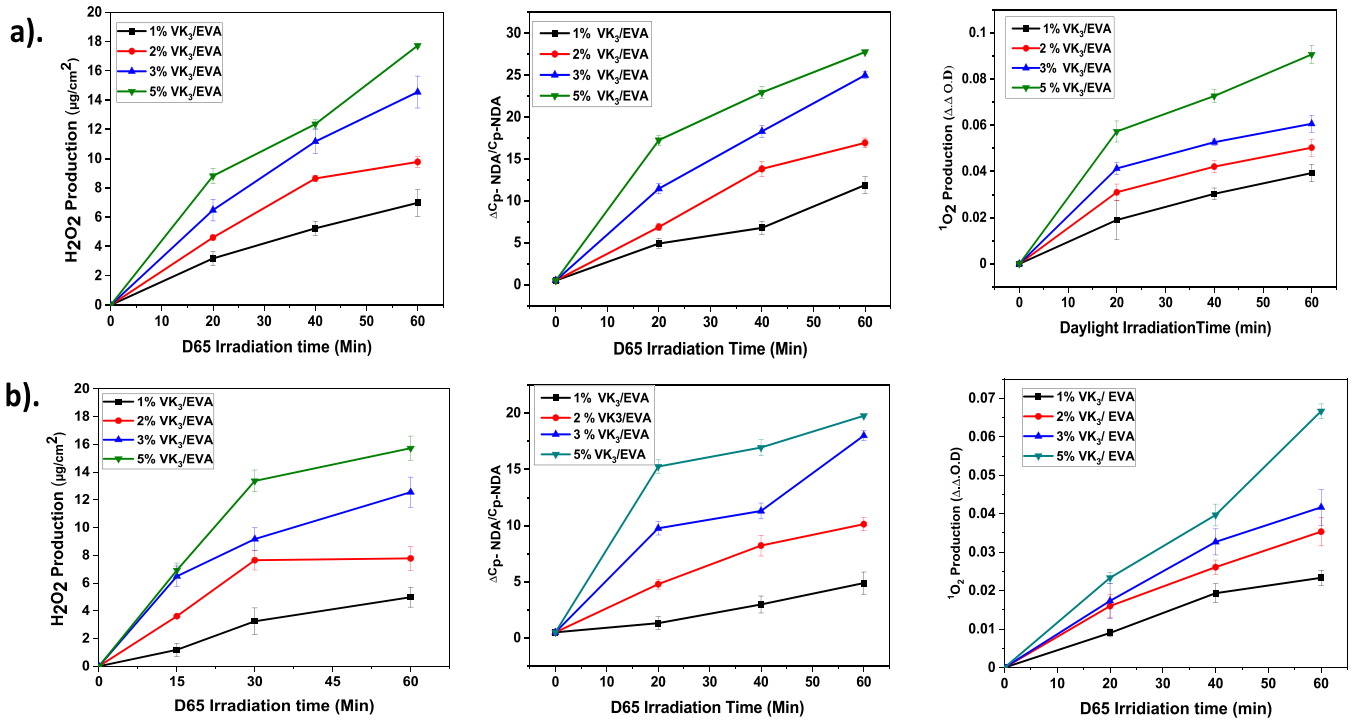

Figure 2. Amounts of H_2O_2, $^{\cdot}OH$, and $^1O_2$ generated by EVA/VK_3 versus time under (a) daylight condition (D65, 25 K lux) and (b) reduced daylight condition (D65, 15 K lux) (VK_3 = 1, 2, 3, 5%).

EVA/VK_3 films with varied amounts of VK_3. The thicknesses of the EVA and VK_3 films were measured to be between 0.20 and 0.24 mm. The optical images of the prepared thin films are shown in Figure 1c. It is worth noting that 1.0% VK_3 could be well blended with EVA, more VK_3 added into the blends leads to formation of films with reduced transparency and increased nonhomogeneous appearance. Some VK_3 molecules could migrate from the blended material to the surfaces, due to the potential sublimation nature of VK_3 under high temperature. The EVA films treated with higher percentages, particularly with 5% VK_3 showed particles on the surfaces of the films and a yellowish color due to the sublimation nature.

Furthermore, to fully characterize the EVA/VK_3 films, FT-IR analyses were performed to examine the chemical structure changes in the EVA after mixing with VK_3. As depicted in Figure 1d, the FT-IR spectrum of pure VK_3 showed a characteristic band at 1720−1715 cm^{-1} (carbonyl group), while peaks at 1550−1140 cm^{-1} correspond to aromatic −C−C− stretches and bands at 3000−3200 cm^{-1} were attributed to −C−H stretches. The major peaks typically observed in the FT-IR spectrum of EVA are also shown in the spectrum. Due to the overall similarity of functional groups, the FT-IR spectra of EVA and EVA/VK_3 showed only slight differences in the absorption intensities and peak positions. As shown in Figure 1d, the characterized IR bands of EVA at around 3000 and 2900 cm^{-1} indicated the presence of ═C−H and −C−H stretching, respectively.[29] The peaks at around 1741 cm^{-1} were attributed to −C═O ester groups of EVA.[44] Bands occurring at 1229, 1013, 2926, 2837, 1450, 1377, and 720 cm^{-1} corresponded to ethylene groups.[31,45] After incorporation of VK_3 into EVA films, the carbonyl band shifted to a lower value due to the presence of conjugated carbonyl C═O groups in the VK_3 structure.[32] The peak intensity of the same band was also enhanced, further confirming the existence of VK_3 molecules in the mixture. New peaks in the region at around 1548−1120 cm^{-1} also appeared, referring to the −C−C stretch in the aromatic ring of the VK_3 molecule in the EVA/VK_3 blends.[33]

The pure EVA film is transparent, and the transparency of the films decreases with the increase of menadione content in the mixtures. Figure 1e displays UV−vis transparency measurements of the resultant films. The control EVA and EVA/VK_3 (1% VK_3) films present very similar transmittances, except a slight reduction in the UVB-UVA (300 and 350 nm) (near visible range), possibly due to the UV absorbance of VK_3, while the transmittance rapidly decreased for films treated with a higher VK_3 content (2−5% VK_3) due to potentially increased crystallization of VK_3 in the films. Both effects contribute to nontransparency of the EVA/VK_3 (5% VK_3) film. In general, the film made of EVA/VK_3 (1% VK_3) meets the requirement of transparent packaging materials.

Figure 1f shows the mechanical performances of the EVA/VK_3 films. In comparison to pure EVA film, the EVA/VK_3 (1 and 5% VK_3) samples showed similar tensile properties, with breaking stress and strain values slightly reduced. The incorporation of 1% VK_3 only induced a little change, indicating that at such a chemical composition the physical properties of EVA are not significantly affected. However, with more VK_3 introduced, the crystallization of VK_3 in the blends could lead to heterogeneous structures in the films and reduced mechanical properties.

3.2. Photoactivity of Developed Films. VK_3 has shown photoactive functions to produce ROS and subsequent antibacterial functions in different solvents and polymer systems.[24−26] The photodynamic antibacterial and antiviral functions on films could lead to applications in packaging materials.[46] The activity of a photosensitizer (PS) in a film is dependent on its potential to produce ROS and photostability under daylight or UVA irradiation, common lighting conditions in stores or residence.[47] The PS will be excited to

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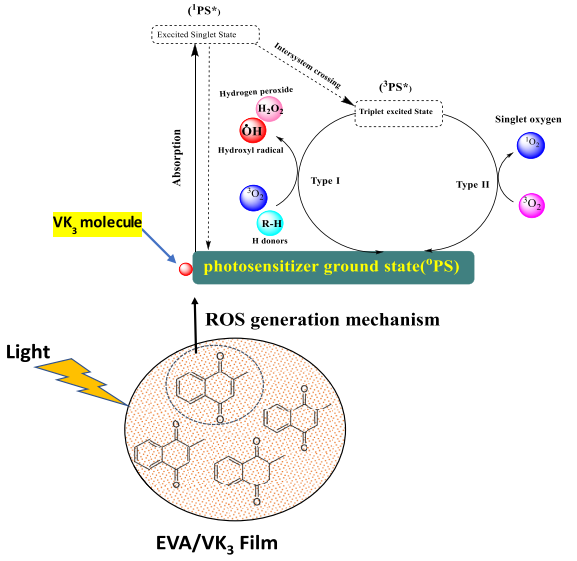
its singlet state (^{1}PS) and then intersystem crossing to its triplet state (^{3}PS), which is the key transient intermediate for the generation of ROS.[23] To demonstrate the ability of generating ROS by EVA/VK_3 films under daylight illumination, a series of measurements of singlet oxygen ($^1O_2$), hydroxyl radical ($^{\cdot}$OH) and hydrogen peroxide (H_2O_2) were conducted, and Figure 2a,b presents the results under two different daylight illumination intensity conditions (lux 25 K and 15 K). Consistent with literature results, VK_3 could generate good amounts of H_2O_2, $^{\cdot}$OH, and singlet oxygen ($^1O_2$) under these two lighting conditions, proving that VK_3 could undergo both path I and path II photoreaction mechanisms, respectively, in the EVA polymer (Scheme 1).[24−26]

Scheme 1. Mechanism of ROS Generation by VK_3 Incorporated EVA Films



The path I photoreaction leads to the formation of H_2O_2 and $^{\cdot}$OH due to a hydrogen abstraction on EVA polymer, while the path II reaction is determined by collision of triplet oxygen molecules with the triplet excited PS. The productions of all three ROS increase with increasing irradiation time and increasing VK_3 content in the films. A higher concentration of VK_3 in EVA resulted in more ROS produced in the films. Under reduced lighting intensity (15 K lux) (Figure 2b), the production yields were not significantly reduced in comparison to the regular one (25 K lux) (Figure 2a). Prolonged light exposure of 60 min did not completely saturate the photoreaction efficiency. The trends of the generations of ROS under the conditions are similar, indicating that both paths I and II are equally effective for VK_3 in EVA films. In another word, EVA is a good hydrogen donor to the path I reaction of VK_3. These results suggest that the EVA/VK_3 films could be potentially effective biocidal functional materials in the food packaging industry. It should be noted that both lighting intensities employed in the experiments are relatively stronger than most regular lighting conditions in superstores and convenient stores, especially areas that are under shaded shelves. However, the results still promising for future development of more practical functional packaging materials.

3.3. Durability and Reusability of Photoactive EVA/VK_3. The photogeneration of ROS by EVA/VK_3 films undergoes two different paths as described in Scheme 1. The path I reaction needs the donation of a hydrogen atom from EVA polymer chains, i.e., a hydrogen abstraction from the polymer, which should be in the vicinal positions of VK_3 molecules. The path II may not have anything to do with the polymer matrix but need effective collision of surface excited VK_3 molecules with oxygen.[48,49] These features may affect the durability and stability of the photoactive functions of EVA/VK_3 films. Figure 3 depicts the photoactivated ROS production of singlet oxygen and $^{\cdot}$OH radicals under D65 for prolonged light exposure of one week. Due to the fact that both $^{\cdot}$OH and H_2O_2 are produced by the same photochemical pathway (Path I), the amounts of $^{\cdot}$OH production by EVA/1% VK_3 films were monitored as a representative of ROS production by the path I reaction. The generation of $^1O_2$ by the polymer is dependent on the reaction path II of VK_3 and oxygen in the environment.[50] After 7 days of exposure to D65 light, the production of $^1O_2$ did not show any substantial change, as shown in Figure 3a. The generation of $^{\cdot}$OH radicals showed a decreasing trend (Figure 3b). Because of the hydrogen abstraction from the polymer, active C−H bonds of vinyl acetate units in EVA should be the dominating groups, playing an important role in the production of $^{\cdot}$OH in the type I photoreaction. Prolonged and repeated light exposures will consume the C−H group, especially in the vicinity of VK3 molecules, leading to certain reductions of generated hydroxyl radicals. However, these results prove the practicability of the

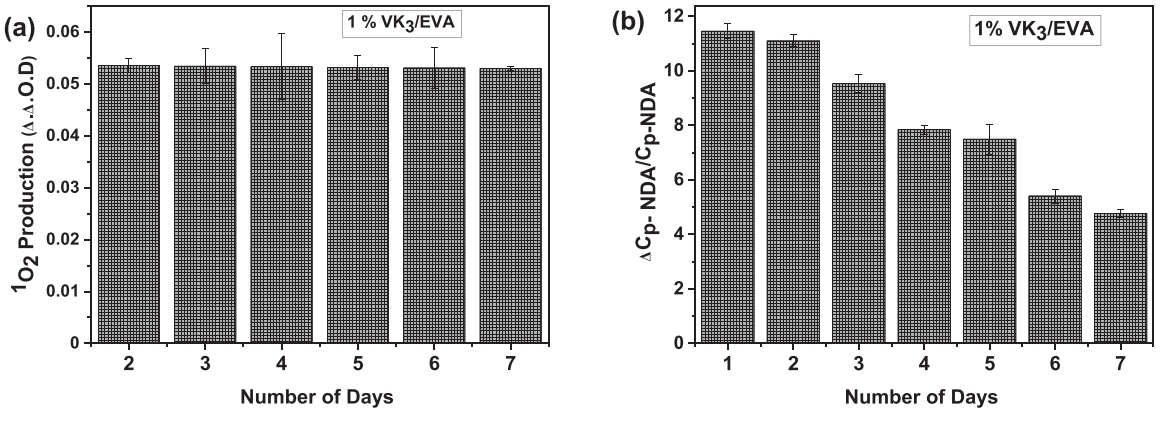


Figure 3. Durability of photoactivity: (a) singlet oxygen and (b) hydroxyl radicals of EVA incorporated with 1% VK_3 up to 1 week.

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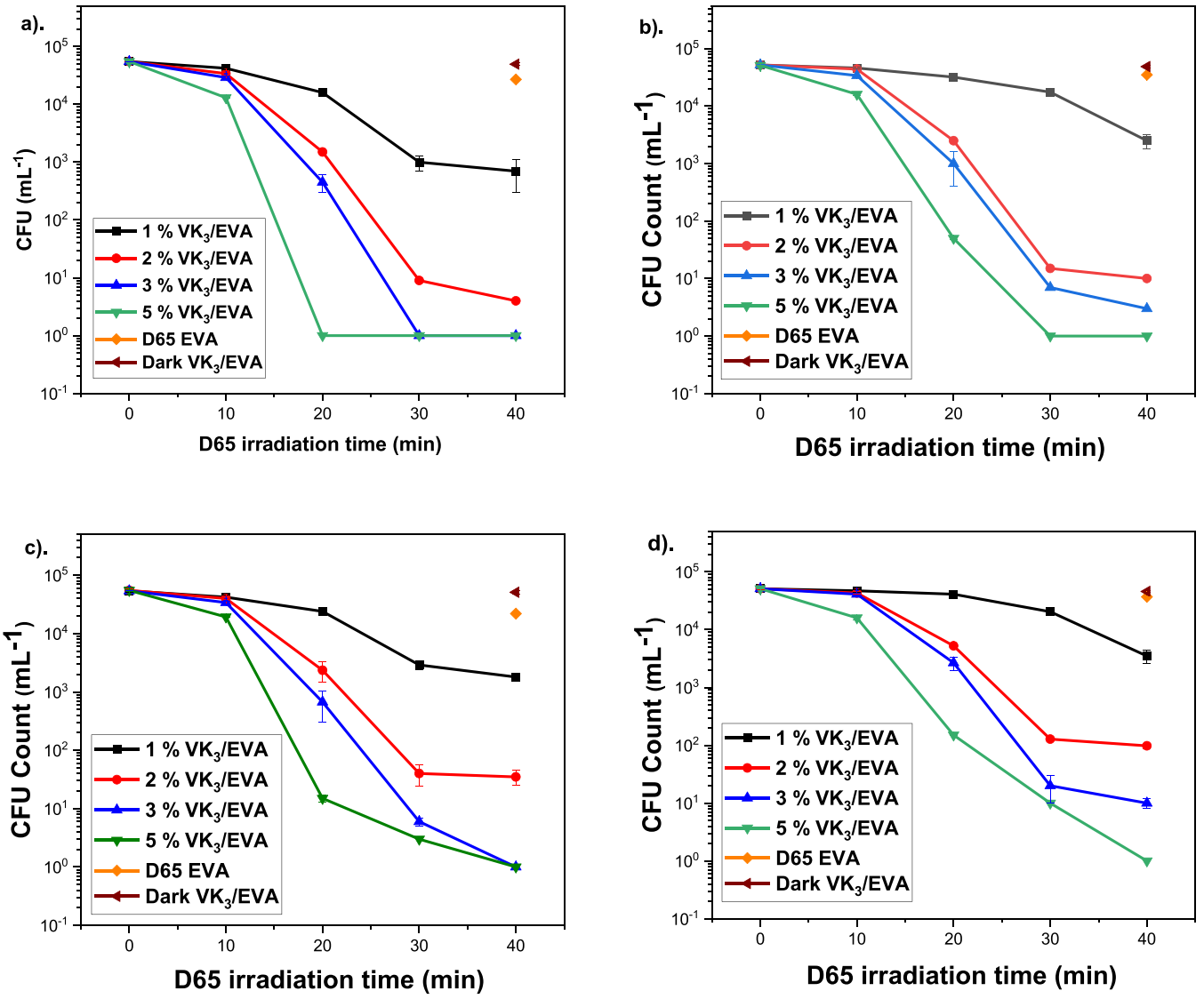

Figure 4. Bactericidal activity *D65 light* against (a). *E. coli* and (b) *L. innocua* under intensity of lux 25 K. Bactericidal activity against (c). *E. coli* and (d) *L. innocua* under intensity of lux 15 K.

prepared films being reused for limited times, maintaining efficient ROS generation capacity.

3.4. Photodynamic Bacterial Inactivation Functions. The photogenerated ROS on surfaces of the EVA/VK_3 films should exhibit antibacterial functions when they are in good contact with microorganisms. The antimicrobial properties of the developed EVA/VK_3 films were evaluated against *E. coli* and *L. innocua*, representatives of Gram-negative and Gram-positive bacteria. The bacteria suspensions were directly placed on the surface of EVA/VK_3 films, which were irradiated under a D65 daylight source for 40 min. An original EVA film without VK_3 under D65 exposure and an EVA/1%VK_3 film under dark for the same duration of 40 min served as controls, both displayed no biocidal activity against *E. coli* and *L. innocua*. However, the VK_3-incorporated films indicated good potency to inactivate both strains. Films containing 2% VK_3 or higher all showed more than 2-log reduction in viability of both bacteria after 30 min of D65 light exposure, corresponding to a killing percentage of >99% (Figure 4a,b). The film containing 1% VK_3 exhibited relatively lower antibacterial functions under 40 min of light exposure, especially against Gram-positive species, consistent with the results observed in the literature.[25] It needs a little longer contact time to demonstrate better performance. These results are exactly same to the measured amounts of ROS generated by the films. Interestingly, the biocidal activity was not decreased significantly under light exposure with reduced intensity (15 K lux) (Figure 4c,d), which is also consistent to the results of the measured ROS under reduced lighting intensity (Figure 2). The antibacterial activity produced by photoactive VK_3 molecules was proven to be more effective against *E. coli* than *L. innocua*. This can be attributed to the thick peptidoglycan layer present in Gram-positive bacteria, which is known to reduce the effectiveness of ROS in killing bacteria. As a result, Gram-positive bacteria have a stronger self-defense mechanism and slower bactericidal activity.[51] According to previous reports published in the literature, ROS molecules attack pathogens by disrupting cellular macromolecules and can exert irreversible changes on their membrane properties and leakage of essential intracellular constituents, which leads to eventual death. To investigate the reusable antibacterial functions of 1% EVA/VK_3 films, a five-

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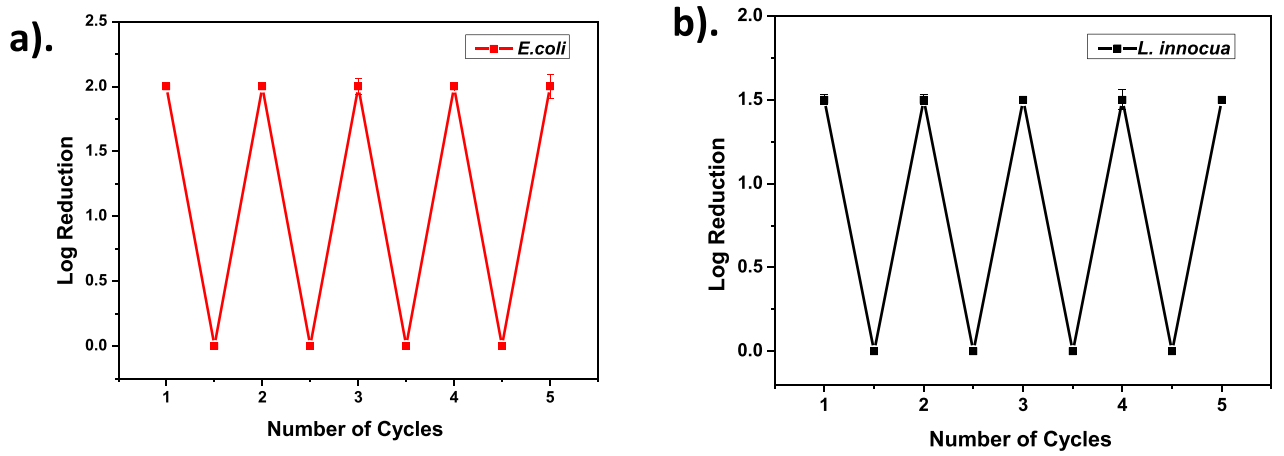

Figure 5. Five cycles of bactericidal activities of EVA/ 1% VK_3 films against (a). *E. coli* and (b) *L. innocua* under D65 exposure at an intensity of 25 K lux. (contact time 40 min).

cycle antibacterial test against both Gram-positive and Gram-negative bacteria was performed on the developed films. As shown in Figure 5a,b, the 1% VK_3 treated films achieved about a 2-log reduction against *E. coli* and *L. inculola*, respectively, even after five test cycles. This confirms that the initial antibacterial activity of the EVA/VK_3 films could easily retain its robust antibacterial functions even after repeated use under daylight conditions. The reusable biocidal functions of the films could be advantageous for food products that require improved protection for longer shelf life. Overall, all VK_3-incorporated films showed highly promising photobactericidal activity, but for real-world applications, 1% VK_3 films might be more preferred for food packaging as the photoactive vitamins cannot leak out of the matrix.

3.5. Antiviral Functions of VK_3 Incorporated EVA Films. The ROS generated on the EVA/VK_3 could endow antiviral functions. As a proof of antiviral performance of the polymer films, a T7 bacteriophage, a nonenveloped double-stranded DNA virus, was selected as a model virus in an established *E. coli*-based stationary phase plating assay. As shown in Figure 6, all EVA/VK_3 films, irrespective of VK_3

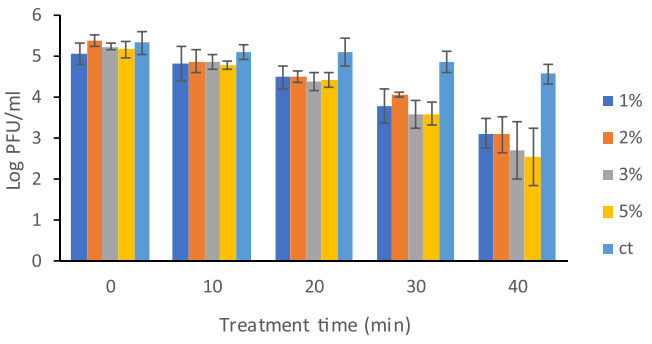

Figure 6. Antiviral activity of the EVA/ VK_3 films.

percentage, showed reductions of the T7 bacteriophage as a function of contact time under daylight exposure. The control film, EVA without any VK_3, showed no significant antiviral activity even after 40 min of contact and light exposure. It should be noted that the EVA/1% VK_3 film demonstrated more than a 2-log reduction under 40 min of daylight exposure. Higher VK_3 content in the films showed faster and more efficient killing activity against T7 bacteriophages. Again, these results are consistent with the results of ROS generation under daylight exposure and antibacterial functions of the films.

4. CONCLUSIONS

In this work, we produced photoactive EVA films by directly mixing with vitamin K_3. The blend films could efficiently and effectively generate ROS such as hydroxyl radicals, hydrogen peroxide, and singlet oxygen following both path I and path II photoreaction mechanisms under daylight illumination. Consequently, the EVA/VK_3 films demonstrated desired daylight-induced antibacterial and antiviral functions suitable for applications as food packaging materials. The efficiency of $^{\cdot}OH$ and H_2O_2 production is higher than that of $^1O_2$ production for the films containing the same amount of VK_3 content, while the production of singlet oxygen is more durable under repeated or prolonged light exposure. The developed EVA/1% VK_3 film showed proper mechanical property, desired transparency, and reasonable antibacterial and antiviral functions under daylight exposure, suitable for practical use. This study proved that such active packaging materials may have great potential for applications in the food packaging industry.

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Notes

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