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Effect of binder on the physical stability and bactericidal property of titanium dioxide (TiO2) nanocoatings on food contact surfaces

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Abstract

TiO_2 is a promising photocatalyst for use in food processing environment as an antimicrobial coating. The purpose of this study was to determine the effect of different binding agents on the physical stability and bactericidal property of TiO_2 nanocoatings created on stainless steel surfaces. A total of six different coating suspensions were prepared by mixing TiO_2 (Aeroxide® P-25) nanoparticles (NPs) with three different types of binders (Shellac (A), polyuretahne (B), and polycrylic (C)) at a 1:4 to 1:16 NP to binder weight ratio. Bactericidal activity of these TiO_2 coatings against *Escherichia coli* O157:H7 (5-strain) was determined at three different UV-A light intensities (0.25, 0.50 and 0.75 mW/cm^2) for 3 h. The type of binder used in the coating had a significant effect on the log reduction of *E.coli* O157:H7. TiO_2 coatings with binder C showed highest reduction (> 4 log CFU/cm^2) followed by TiO_2 coating with binder B and A. Increasing the binder concentration in the formulation from a 1:4 to 1:16 weight ratio decreased the log reduction of *E.coli* O157:H7. Increasing the UV-A light intensity from 0.25 to 0.75 mW/cm^2 increased the log reduction of bacteria for all the TiO_2 coatings. The physical stability of the TiO_2 coatings was determined using ASTM procedures. TiO_2 coatings with binder B showed highest adhesion strength and scratch hardness when compared to coatings with other binders. However, on repeated use experiments (1, 3, 5, and 10 times), TiO_2 coatings with binder C were found to be physically more stable and able to retain their original bactericidal property. The results of this study showed promise in developing durable TiO_2 coatings with strong photocatalytic bactericidal property on food contact surfaces using appropriate binding agents to help ensure safe food processing environment.

Keywords: TiO_2; Antimicrobial coating; Physical stability; Binders; *E. coli* O157:H7.

1. Introduction

Titanium dioxide (TiO_2) is a well-known photocatalyst with excellent antimicrobial properties under UV-A light. It is widely utilized as a self-cleaning and self-sterilizing material for surface coatings in many applications (Fujishima, 2000). TiO_2 is stable, non-toxic, cheap, and capable of repeated use without substantial loss of catalytic ability. TiO_2 photocatalysts have been added to paints, cements, windows, tiles or other building products due to its sterilizing and anti-fouling properties (Lan et al., 2013). Decontamination occurs under ambient conditions utilizing natural oxygen without forming any photo-induced intermediates (Chong et al, 2010). In addition, TiO_2 has been approved by the American Food and Drug Administration (FDA) for use in human food, drugs, cosmetics, and food contact materials (Maneerat & Hayata, 2006).

Since Matsunaga et al. (1985) reported the application of photocatalysis for the destruction of *Lactobacillus acidophilus*, *Saccharomyces cerevisiae*, and *Escherichia coli* using platinum-loaded TiO_2, there has been increased interest in the biological applications of this process. TiO_2 photocatalysts have been studied extensively to inactivate a broad spectrum of microorganisms including viruses, bacteria, fungi, and algae as well as to kill cancer cells (Kim et al., 2003). Foster et al. (2011) presented a more comprehensive review on photocatalytic antimicrobial properties of TiO_2. TiO_2 photocatalysts generate strong oxidizing power when illuminated with UV-A light of wavelength less than 385 nm. The bactericidal properties of TiO_2 are attributed to the high redox potential of the reactive oxygen species (ROS) such as hydroxyl radical ($^{\cdot}OH$), superoxide radical ($O_2^{\cdot-}$), and hydrogen peroxide (H_2O_2) formed by the photo-excitation. TiO_2-mediated photo-oxidation shows promise for the elimination of microorganisms in areas where the use of chemical cleaning agents or biocides is ineffective or is restricted by regulations such as pharmaceutical and food industries (Skorb et al., 2008). In addition, TiO_2

becomes superhydrophilic upon irradiation with UV light and this functionality is reversible and depends on the light exposure (Chen & Mao, 2007). These properties of TiO_2 may help to improve the efficiency of hydrophilic cleaning agents used in the food industry. Thus, TiO_2 photocatalysts offer great potential to develop antimicrobial coatings on food contact and non-food contact surfaces to avoid cross-contamination in the food processing environment.

Studies have reported that immobilized TiO_2 coatings have the ability to disinfect *Listeria monocytogenes* biofilms on stainless steel (Chorianopoulos et al., 2011). Also, TiO_2 coated polypropylene film package can reduce the growth of *E. coli* on cut lettuce (Chawengkijwanich & Hayata, 2008), and *Pencillium expansum* fruit rot on apples and tomatoes (Manreet & Hayata, 2006). However, most of the earlier studies that reported antimicrobial activity of TiO_2 nanocoatings either used complicated approaches for coating or did not fully address the issues of durability of the coatings on usage. In our previous study on nanocoatings, we developed a simple method to create physically stable TiO_2 coatings on stainless steel surfaces using shellac, polyurethane and polycrylic as binding agents (Yemmireddy et al., 2015). For developing antimicrobial TiO_2 nanocoatings on food contact surfaces for the purpose of maintaining a hygienic food processing environment, the binding agents used must be non-toxic. Shellac is an insect-produced natural resin most commonly used in food industry for surface treatment/glazing of confectionary products and citrus fruits to prevent surface damage during handling and storage (Antic et al., 2010). According to FDA, shellac is only approved for indirect food contact use (21 CFR 175.300). However, it is allowed for food contact use due to acceptance petition for GRAS status (Baldwin, 2005). Shellac films show excellent adhesion to a wide variety of surfaces and possess high gloss, hardness and strength. Alternatively, polyurethane has been extensively studied for several industrial applications. Notably, waterborne polyurethanes are

suitable for paints, coatings, and adhesive industries due to their inherent advantages of low volatile compounds, fast drying properties, outstanding flexibility, impact resistance, abrasion resistance, non-flammability, transparency and easy adherence to a variety of substrates (Bhargava et al., 2013). As per FDA (21 CFR 177.1680), polyurethane resins are allowed to use as indirect food additives for use as basic components of single and repeated use food contact surfaces. Similarly, polycrylics are well known for their wide range of applications in several paint formulations. As per our earlier study, TiO_2 coatings created using these binders have shown excellent physical stability. However, the photocatalytic bactericidal property of TiO_2 coatings using these binders is not well understood. Hence, the overall objective of this study was to determine the effect of different binding agents on physical stability and bactericidal property of TiO_2 nanocoatings. Specific objectives include: To determine:

i) The effect of binder on bactericidal property of TiO_2 nanocoatings.

ii) The optimum conditions to create TiO_2 nanocoatings with strong bactericidal property

iii) The durability and bactericidal property of TiO_2 nanocoatings on repeated use.

2. Materials and methods

2.1 Selection of materials

TiO_2 (Aeroxide® P25, Sigma-Aldrich, St. Louis, MO, USA) NPs with an approximate particle size of 21 nm and specific surface area of 50 $m^2 g^{-1}$ as per suppliers specifications were used for developing nanocoatings in this study (Table 1). Three different binders namely, shellac (A), polyurethane (B) and polycrylic (C) were purchased from the local supermarket in Griffin, GA (Table 1). Stainless steel (AISI 304L) coupons having an indentation with 46 x 12.5 x 1.25 mm^3 dimensions and a total surface area of 540 mm^2 were chosen as a model food contact surface for TiO_2 nanocoating. All the coupons were thoroughly cleaned prior to coating first by

washing in acetone followed by ethanol and finally rinsed with deionized water and dried in a hot air oven at 60° C for 30 min.

2.2. Preparation of suspensions for TiO_2 coating

Total six different suspensions of TiO_2 were prepared by mixing TiO_2 NPs with binder A (1:4 or 1:8 weight ratio), binder B (1:8 or 1:16 weight ratio), and binder C (1:8 or 1:16 weight ratio) in a porcelain mortar for about 15 min. The produced viscous suspensions were further treated in an ultrasonic water bath (Model # FS60, Fisher Scientific, Waltham, MA, USA) for about 1 h, in order to avoid formation of TiO_2 aggregates. The resultant viscous paste formulations were used for coating on indented stainless steel coupons.

2.3. Preparation and characterization of TiO_2 nanocoatings

TiO_2 nanocoatings were created on indented stainless steel coupons by following the method described in Yemmireddy et al. (2015). Briefly, a sample of 0.25 ± 0.02 g of coating suspension was weighed into the well of an indented stainless steel (SS) coupon by placing it on a calibrated balance. The deposited coating suspension was evenly spread across the entire area of the indentation by slowly tilting the coupon sideways or if needed using a Crayola paint brush by keeping total amount of deposited coating constant. The coated coupons were air-dried over night at room temperature. The resultant coatings has a thickness of about 50-100 µm when measured using a handheld thickness gauge (Elcometer, Model # 345). The morphology and the microscopic structure of the coating surface was characterized by a variable pressure scanning electron microscope (VPSEM, Zeiss 1450 EP) with accelerating 25 kV. The SEM images were further analyzed using image processing software (Paint. NET) to estimate the area of the coated surface covered by the NPs and the binder.

2.4. Bacterial strains and inoculum preparation

Five strains of *E. coli* O157: H7 isolated from different sources: E009 (beef), EO932 (cattle), O157-1 (beef), O157-4 (human), and O157-5 (human) were used in this study. All bacterial strains were stored at -70 °C in tryptic soy broth (TSB) (Difco, Becton Dickinson, Sparks, MD, USA) containing 20 % glycerol. Prior to the experiment, cultures were activated at least twice by growing them overnight in 10 mL of TSB at 37 °C. Later, each bacterial strain was cultured separately in 10 mL of TSB and kept on a shaking incubator at 230 rpm and 37°C for 16 h. Following the incubation, bacterial cells were harvested by sedimenting at 4000 x g for 12 min and re-suspended in a sterile phosphate-buffered saline (PBS, pH 7.2). An equal volume (2 mL) of each strain suspension was combined to obtain a 10 mL of a five-strain cocktail containing approximately 10^6 CFU/mL. Cell concentration was adjusted by measuring the absorbance of bacterial suspension at 600 nm using a UV/Vis spectrophotometer and confirmed by plating 100 µL portions of the appropriate serial dilution on tryptic soy agar (TSA) (Difco Laboratories) plates incubated at 37 °C for 24 h.

2.5. Photocatalytic disinfection

Prior to photocatalytic disinfection, the TiO_2 coated coupons were pre-sterilized under germicidal UV light (254 nm) in a biosafety hood for about 1 h. The sterilized coupons were placed in 90 mm diameter petri-dishes containing moistened filter paper at the bottom to prevent drying-out of the bacterial culture during the treatment. A 300 µL aliquot of bacterial culture was pipetted into the indented well of the TiO_2 coated coupon and uniformly spread across the entire surface of the TiO_2 coating using a sterile disposable loop. Later, the inoculated samples were illuminated with a UV-A light system fitted with four 40 W lamps (American DJ®, Model # UV Panel HP™, LL-UV P40, Los Angeles, CA , USA) from above. The light intensity reaching on top of the sample was measured using a UV radiometer (UVP®, Upland, CA, USA) with a peak

sensitivity of 365 nm. The light intensity reaching the surface of the sample was adjusted to 0.25, 0.5 or 0.75 mW/cm^2 (±0.05) by changing the distance between the light source and the sample. Plain SS and only binder coated SS coupons under UV-A light were also included as negative and positive controls, respectively. The samples were treated for either 90 or 180 min UV-A light and then immersed in 30 mL of sterile PBS solution containing 0.1% tween 80 and vortexed for 30 s to re-suspend the bacteria. A viability count (log CFU/cm^2) was performed by appropriate dilution and plating on *E.coli* O157:H7 selective Sorbitol-MacConkey agar (SMAC) and incubation at 37 °C for 24 h. All the experiments were conducted in triplicates.

2.6. Measurement of coating physical stability

Hardness of the TiO_2 coatings were assessed with the help of a scratch test, based on ASTM G171-03 method (ASTM, 2009) as described in Yemmireddy et al. (2015) to make a linear scratch of at least 5 mm length with an applied normal force of 2 N at three different locations on each sample. The width of each scratch was measured at three different locations equidistance from each other using a digital microscope pro (20 to 200x magnification, Model # 44308, Celestron LLC,Torrance, CA). Scratch hardness number (HS_p) was calculated as described in the standard and reported in Giga Pascals (GPa). Further, adhesion strength of the coatings was determined with the help of a tape test based on ASTM D3359-02 method-B (ASTM, 2002) as described in Yemmireddy et al. (2015).

2.7. Simulation of repeated use conditions of TiO_2 coatings

In order to determine whether the coatings were able to retain their original bactericidal property and physical stability upon reuse, the coatings were subjected to multiple use conditions. In this procedure, the coatings were subjected to photocatalytic disinfection test conditions as described earlier such as pre-sterilization under germicidal UV light for 1 h

followed by photocatalytic disinfection treatment under UV-A light for 3 h and removal of bacterial cells from the coatings using release buffer for 30 sec were simulated for 1, 3, 5, and 10 times using deionized water in place of actual bacterial culture. After each treatment cycle the coupons were air-dried before proceeding to the next cycle. Finally, the dried coupons after 1, 3, 5, and 10 times simulated use were measured for their bactericidal property and the physical stability as described earlier.

2.8. Statistical analysis

Data were analyzed by the analysis of variance (ANOVA) procedure using Statistical Analysis System (SAS/STAT 9.3, 2011). T-tests were used for pairwise comparisons. Least significant difference of means tests was done for multiple comparisons, and all tests were performed with a level of significance 0.05.

3. Results and discussion

3.1. Effect of type and concentration of binder on the bactericidal activity of TiO_2 nanocoatings

Fig. 1 shows the effect of type of binder on the log reduction of *E.coli* O157:H7 produced by TiO_2 nanocoatings treated for 3 h at 0.5 mW/cm^2 UV-A light intensity. Control samples with plain stainless steel coupons, and only binder A, B, and C coated coupons under UV-A light showed a reduction on *E.coli* O157:H7 population of only 0.17, 0.24, 0.51, and 2.23 log CFU/cm^2, respectively. In addition, when these binder coated coupons were tested in the dark, both binder A and B coatings showed no significant antibacterial activity; while, binder C coating showed a reduction of less than 1 log CFU/cm^2 (data not shown). This shows that under tested conditions, both binder A and B coatings themselves had no significant bactericidal property. However, binder C under the tested UVA intensity showed a significant ($P \leq 0.05$) bacterial reduction. This might be attributed to the possible inherent bactericidal properties of

acrylic paint (i.e. binder C) and its constituents in the presence or absence of UV light. TiO_2 coatings with binders A, B, and C at 1:8 NP to binder weight ratio showed a reduction of 0.96, 3.72, and 3.92 log CFU/cm^2, respectively (Fig 1). Further increasing the concentration of binders A, B, and C in the TiO_2 coating (1:16 NP to binder weight ratio) showed a reduction of only < 0.5 log CFU/cm^2 for coating with binder A (TA16, data not shown), 1.73 log CFU/cm^2 for binder B (TB16) and 3.35 log CFU/cm^2 for binder C (TC16) (Fig. 1). This is almost a 100, 54 and 15 % decrease in the bactericidal efficacy of TiO_2 coatings with binders A, B and C when compared with respective TA8, TB8, and TC8 samples. Alternatively, decreasing the concentration of the binder in the TiO_2 coating to 1:4 NP to binder weight ratio (TA4) resulted in almost 49 % increase in the bactericidal efficacy (0.95 to 1.45 log CFU/cm^2) when compared to TiO_2 coating at 1:8 NP to binder weight ratio (TA8). However, TiO_2 nanocoatings with binders B and C at a 1:4 NP to binder weight ratio is not a feasible formulation for coating by the solution deposition technique used in this study. This indicates that the type of binder used in the TiO_2 coating had significant ($P \leq 0.05$) effect on the photocatalytic bactericidal property. One possible reason for the differences in the antimicrobial activity can be attributed to the differences in the surface characteristics of the individual TiO_2 nanocoatings created with three different binders.

SEM image analysis of the coatings revealed that the number of TiO_2 NPs present on the surface of each coating varied depending on the type of binder used (Table 2). For example, in a given area of nanocoating, the amount of TiO_2 NPs exposed on the surface of coating was only 3, 2, and 3 % for TA8, TB8, and TC8, respectively. While, the corresponding binder coverage was 39, 21, and 39 %, respectively. The remaining percent coverage of the nanocoating can be attributed to the unexposed TiO_2 NPs. The unexposed TiO_2 NPs are believed to be partly

shielded by the binder molecules reducing the ability of UV-A light penetration and bacterial cell contact with the TiO_2 NPs. As per our previous study, the type of binder used in the TiO_2 coating has an effect on the structural properties of the resultant coatings (Yemmireddy et al, 2015). SEM analysis of the coatings revealed that the TiO_2 coating with binder-A was more compact in nature whereas the TiO_2 coatings with binders B and C were porous in nature. The porous structure of the TiO_2 coatings with binders B and C might have helped to carryout efficient oxidation and reduction reactions due to availability of electron donors (H_2O) and the acceptors (O_2) from the immediate environment. This condition helps to generate more ROS for photocatalytic disinfection of bacteria. Thus, the structural characteristics of TiO_2 coating and the number of TiO_2 NPs that are directly in-contact with bacterial cells during photocatalytic disinfection treatment plays an important role in the generation of ROS responsible for the damage of cell walls and eventual cell death. Many studies have reported that close contact between the bacteria and the TiO_2 increases the extent of oxidative damage (Foster et al., 2011). This explains the reason for the high bactericidal activity of TiO_2 coatings with binder B and C when compared to TiO_2 coating with binder A. Based on these results it is clear that increasing the NP concentration in the coatings increased the log reduction of bacteria. However, there exists an optimum level of TiO_2 to binder concentration to exhibit greater bactericidal property depending upon the type of binder used in the coating. TiO_2 coatings with binder C showed the highest bactericidal activity followed by TiO_2 coating with binder B and binder A.

3.2.Effect of light intensity on the bactericidal activity of TiO_2 nanocoatings

The effect of UV-A light intensity on the bactericidal activity of different TiO_2 nanocoatings was shown in Fig 2. When UV-A intensity range from 0.25 to 0.75 mW/cm^2, control samples with plain SS coupon showed a reduction of less than a 1 log CFU/cm^2 after 3 h

treatment. In similar experiments by Chawengkijwanich and Hayata (2008), UV-A light itself showed a 1 log CFU/cm^2 reduction of *E.coli* cells after 3 h treatment at 1 mW/cm^2. Similarly, Kikuchi et al. (1997) reported less than 2 log CFU/cm^2 reduction of *E.coli* cells after 4 h treatment at 1 mW/cm^2. Another study by Krysa et al. (2011), authors reported that increasing UV-A light intensity from 0.2 to 0.6 mW/cm^2, decreased the survival of *E.coli* cells from 77 to 38 % after a 3 h treatment. This can be explained by the fact that UV-A light, with relatively low energy, gradually damages cells through oxidative stress caused by generation of oxygen radicals within the cells (Bock et al, 1998). The oxidative stress caused by UV-A light on bacterial cells might be more pronounced with increasing light intensity and treatment time. This shows that UV-A light itself has minimal bactericidal activity at low intensity levels used in this study.

Increasing the UV-A light intensity from 0.25 to 0.75 mW/cm^2 also increased the bactericidal activity of all TiO_2 coatings (Fig 2). Coating with only binder A has showed a reduction of 0.12, 0.24 and 1.27 log CFU/cm^2 at 0.25, 0.5 and 0.75 mW/cm^2 UVA light intensities, respectively. This indicates that the binder A coating itself has a negligible effect on the reduction of bacteria at lower light intensities of below 0.50 mW/cm^2 and followed the reduction trend of the UV-A control. However, further increasing the light intensity to 0.75 mW/cm^2 increased the bactericidal activity of the binder coating compared to the UVA control. Shellac (i.e. binder A) is a food-grade, insect produced natural resin and widely used as a glazing agent in the food industry. The binder itself is non-toxic and used in several other food applications. Antic et al. (2010) studied the effect of shellac-in-ethanol solutions to reduce the transferability of bacteria from cattle hide to the beef carcass during slaughter operation by immobilizing the bacterial cells on the hide. They reported that shellac itself did not have significant antimicrobial effects while shellac-in-ethanol showed some antibacterial effect.

Similarly, a possible synergistic effect between shellac under UV-A light at 0.75 mW/cm^2 in the current study might have resulted in a slightly increased reduction. Similarly, the binder B (i.e. polyurethane) coating itself under UV-A light had little effect on bactericidal activity (Fig 2). Whereas, binder C (i.e. polycrylic) coating showed significantly ($P \leq 0.05$) higher reduction from 2.5 to 4 log CFU/cm^2 after a 180 min UV-A exposure (Fig 2).

Increasing the intensity of UV light from 0.25 to 0.75 mW/cm^2 for 180 min, increased the bactericidal activity of TiO_2 coating from 0.63 to 1.69 log CFU/cm^2 for binder A (TA8) and from 2.45 to 3.87 log CFU/cm^2 for binder B (TB8). However, no significant ($P > 0.05$) increase in the log reduction was observed for TiO_2 coating using binder C (TC8) (Fig 2). The minimum detection limit for the current test method is 2 log CFU/cm^2. It should be noted that TiO_2 coatings with binder C (TC8) at 0.25 mW/cm^2 already reached the highest possible reductions (4 log CFU/cm^2) for an initial bacterial cell concentration of around 10^6 CFU/cm^2. This is why no additional reduction was achieved for binder C (TC8) at a higher UV intensity. In order to determine UV intensity effect, treatment times for TB8 and TC8 nanocoatings were reduced to 90 min (Fig 2). This treatment step resulted in almost a 51% (for TB8) and 36 % (for TC8) decrease in the bactericidal activity of TiO_2 coatings with binder B and C when compared with treatment for 180 min. This demonstrates that the observed reductions are in-fact due to the pronounced photocatalytic bactericidal effect of TiO_2 coatings. Marolt et al. (2011) reported that the photocatalytic treatment on exposed anatase TiO_2 nanoparticles could result in a reactive species that would destroy the soft organic matter such as binders in the vicinity of NPs, thus exposing even more anatase particles. Increasing the concentration of NPs and UV-A light intensity might have destroyed and removed a certain amount of the superficial binder and of the other degradable paint components from the surface of coating thus increasing the bactericidal

property of TiO_2 nanocoating. Chawengkijwanich and Hayata (2008) reported that increasing UV-A light intensity from 0.05 to 1 mW/cm^2 increased the antimicrobial efficacy of TiO_2 coated polypropylene films from 0.35 to 3 log CFU/cm^2. Similar results were also reported by Krysa et al. (2011) and Dunlop et al. (2010). This indicates that the type of binder, the relative proportion of the NP to the binder, and the intensity of UV light all have a significant effect on the bactericidal property of TiO_2 coatings. However, the photocatalytic activity against pathogens at lower light intensity levels is more relevant to potential real life applications (Foster et al., 2011). Hence extending the photocatalytic bactericidal property of TiO_2 coating towards lower UV-A light intensities or visible light region is more beneficial. Based on the results of the current study, an UV-A light intensity of 0.5 mW/cm^2 was found to be optimum for exhibiting bactericidal property of TiO_2 nanocoatings.

3.3. Bactericidal activity of TiO_2 nanocoatings on repeated use

Fig. 3 shows the bactericidal activity of TiO_2 nanocoatings with binders A, B, and C at 1:8 NP to binder weight ratio after the repeated use experiment. Except for the TiO_2 coatings with binder B and C, there was no significant (P>0.05) loss of photocatalytic bactericidal property of the TiO_2 coatings with binder A was noticed after the multiple use experiment. Originally, TiO_2 coatings with binders A, B, and C (TA8, TB8, and TC8) irradiated for 180 min at 0.5 mW/cm^2 UVA light intensity exhibited a reduction of 0.96, 3.72, and 3.92 log CFU/cm^2, respectively. However, after one time simulated use of coated coupons, no significant difference in the reduction was observed for TiO_2 coating with binder A and the reduction remained around 1 log CFU/cm^2 (TA8-1). Whereas, TiO_2 coatings with binders B (TB8-1) and C (TC8-1) had high initial log reduction but lost almost 73 and 22 % of their original bactericidal property after one time use, respectively. Further, testing the bactericidal property of TiO_2 coatings with binder

C for the 3 (TC8-3), 5 (TC8-5) and 10 (TC8-10) times repeated use experiments did not show significant further reduction in its bactericidal property. Upon repeated use, the change in bactericidal efficacy of TiO_2 nanocoatings can be attributed to the loss of exposed TiO_2 NPs on the surface of coating. This is in part related to the decreased physical stability of the respective coatings when subjected to the repeated use experimental conditions.

3.4. Physical stability of TiO_2 nanocoatings on repeated use

Physical stability results of the TiO_2 coatings with binder A (TA8), B (TB8), and C (TC8) before and after subjecting to the repeated use experiment are shown in Table 3. The thickness of all the TiO_2 coatings decreased after the repeated use experiments. After the one time use experiment, the thickness of coatings TA8, TB8, and TC8 decreased by 31, 29, and 12 %, respectively when compared with the thickness of original coatings. Further subjecting the TiO_2 coating with binder C (TC8) for 3, 5, and 10 times in the repeated use experiment resulted in 38, 48, and 54 % decreases in the thickness of the original coating. Adhesion strength of the TiO_2 coatings was assessed based on ASTM D3359-02 standard method –B. Originally, coatings TA8, TB8, and TC8 showed a mean adhesion rating of 3B, 4B, and 4B, respectively. As per the ASTM standard, adhesion strength is rated from 5B to 0B. Where, 5B means the coatings has superior adhesion with 0% loss of coated area, followed by 4B (<5 %), 3B (5-15%), 2B (15-35%), 1B (35-65%), and 0B (>65%), respectively. It means both the coatings TB8 and TC8 showed good adhesion strength (4B) before subjecting to repeated use. After 1 time repeated use, adhesion strength of TB8 decreased to 3B while no significant change in the adhesion was observed for coatings TA8 and TC8 (Table 3). In addition, TC8 maintained the same original adhesion strength (4B) even after subjecting for 5 times repeated use. However, a decrease in the adhesion (from 4B to 3B) was noticed after the 10 times repeated use experiment for TC8. This

can be attributed to the corresponding decrease in the thickness of the original coating from 97 µm to 45 µm after the 10 times repeated use experiment as described earlier.

Scratch hardness of the TiO_2 coatings with binders A, B, and C before and after the reuse experiment was reported in Table 3. Originally, TiO_2 coating with binder B (TB8) showed the highest scratch resistance (1.08 GPa) followed by TC8 (0.68 GPa) and TA8 (0.14 GPa), respectively. After the one time repeated use experiment, scratch hardness of TB8 and TC8 were reduced to 0.61 GPa and 0.53 GPa, respectively. Whereas, scratch hardness of TA8 increased to 0.42 after one time use. In a similar manner, after the 3, 5, and 10 times repeated use experiments, the scratch hardness of TC8 increased by 32, 32, and 13%, respectively when compared with original coating. These differences in the scratch hardness among different coatings can be partly attributed to the nature of the binders used in the coating. Depending on the nature of binder used in the TiO_2 coating the width of the scratch either increased or decreased after the repeated use experiment. For example, the scratch width of the TiO_2 coating with binder A (TA8) increased from 240 µm to 112 µm after one time repeated use. Since scratch width is inversely proportional to the scratch hardness number (as per the ASTM standard), the scratch hardness of TA8 increased after the one time use experiment. Whereas, the width of the scratch for TB8 (70 to 90 µm) and TC8 (90 to 100 µm) increased after one time use which led to a decreased scratch hardness number. However, as the TiO_2 coating with binder C (TC8) subjected for the 3, 5, and 10 times repeated use experiments, the width of the scratch again decreased to 76, 77, and 82 µm which resulted in an increase in scratch hardness of the coating.

Bhargava et al. (2013) studied the effect of TiO_2 concentration (pigment-to-binder ratio) and dispersing agent on the peel strength of waterborne-polyurethane based coatings on

aluminum substrates. They found that the adhesion strength of the coating decreased with increasing pigment-to-binder ratio. This may explain the reason for the decreased physical stability of TB8 after one time use in the current study. TiO_2 coating with binder B (polyurethane) at a 1:8 NP to binder weight ratio may not be sufficient to impart high physical stability even though it exhibited good bactericidal property originally. Kumar et al. (2012) reported that silicone functionalized TiO_2 based epoxy coatings on carbon steel exhibited higher values of scratch hardness, pull-off adhesion and impact resistance. The synergistic interaction between pigment and polymer matrix through chemical bonding is believed to be the reason for the high mechanical properties of TiO_2 based epoxy coatings. A similar interaction effect might be one possible reason for the increased hardness of TA8 and TC8 even after repeated use. Based on these results, adhesion strength and scratch hardness values of the coating were well correlated with the retention of original bactericidal property of the TiO_2 nanocoatings. Among the tested nanocoatings, TiO_2 coatings with binder C showed high bactericidal property and physical stability after the repeated use experiment. These results indicate that type of binder and the binder-to-nanoparticle concentration used in the coating has a significant effect ($P \leq 0.05$) on the durability and bactericidal property of TiO_2 coatings.

4. Conclusions

As per this study, TiO_2 coatings with polycrylic as binding agent showed the highest bactericidal efficacy followed by TiO_2 coatings with polyurethane, and shellac as binding agents, respectively. Increasing the concentration of binder in the TiO_2 coating decreased the bactericidal efficacy. Increasing the UV-A light intensity from 0.25 to 0.75 mW/cm^2 increased the bactericidal activity of the TiO_2 coatings. However, an intensity of 0.50 mW/cm^2 was found to be optimum to avoid the effect of UV light itself on the bacterial reduction. TiO_2 coating with

polyurethane as binding agent showed the highest adhesion strength and scratch hardness. However, on repeated use experiments, TiO_2 coating with polycrylic was found to be physically more stable and bactericidal when compared with other TiO_2 coatings. The results of this study provide feasibility in development of durable TiO_2 nanocoatings with strong bactericidal properties on food contact surfaces with appropriate binding agents.

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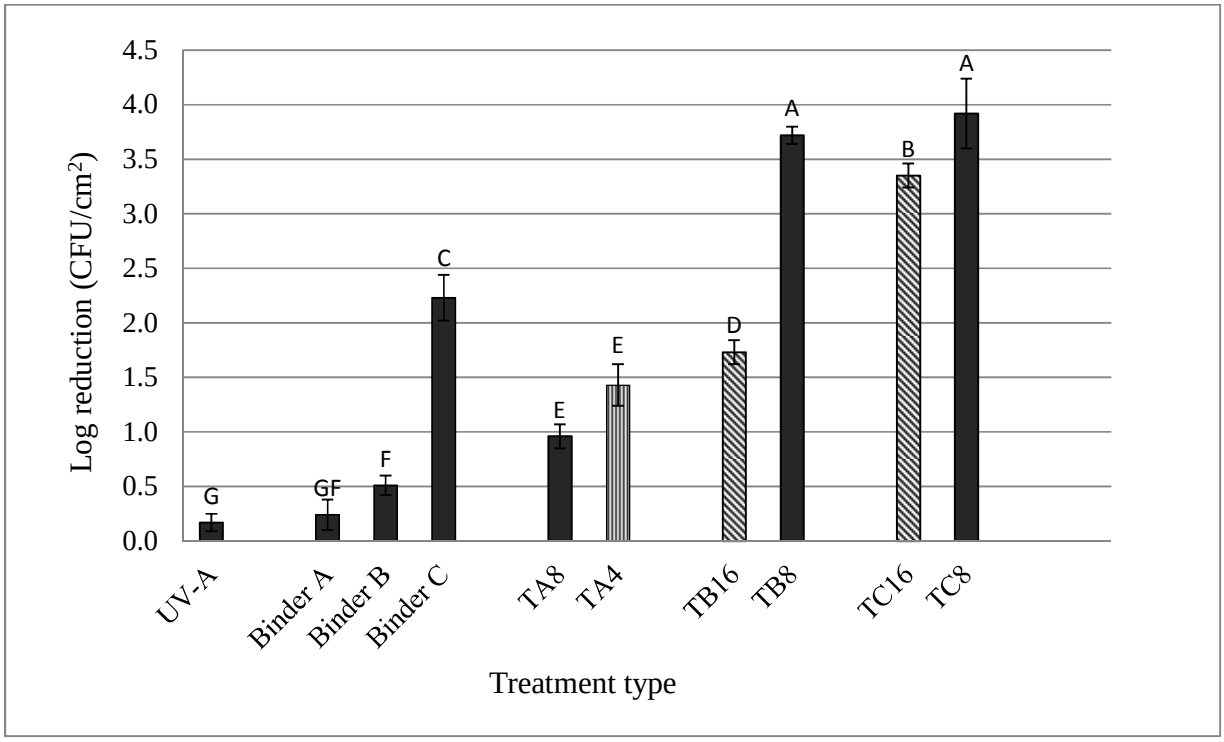
Figures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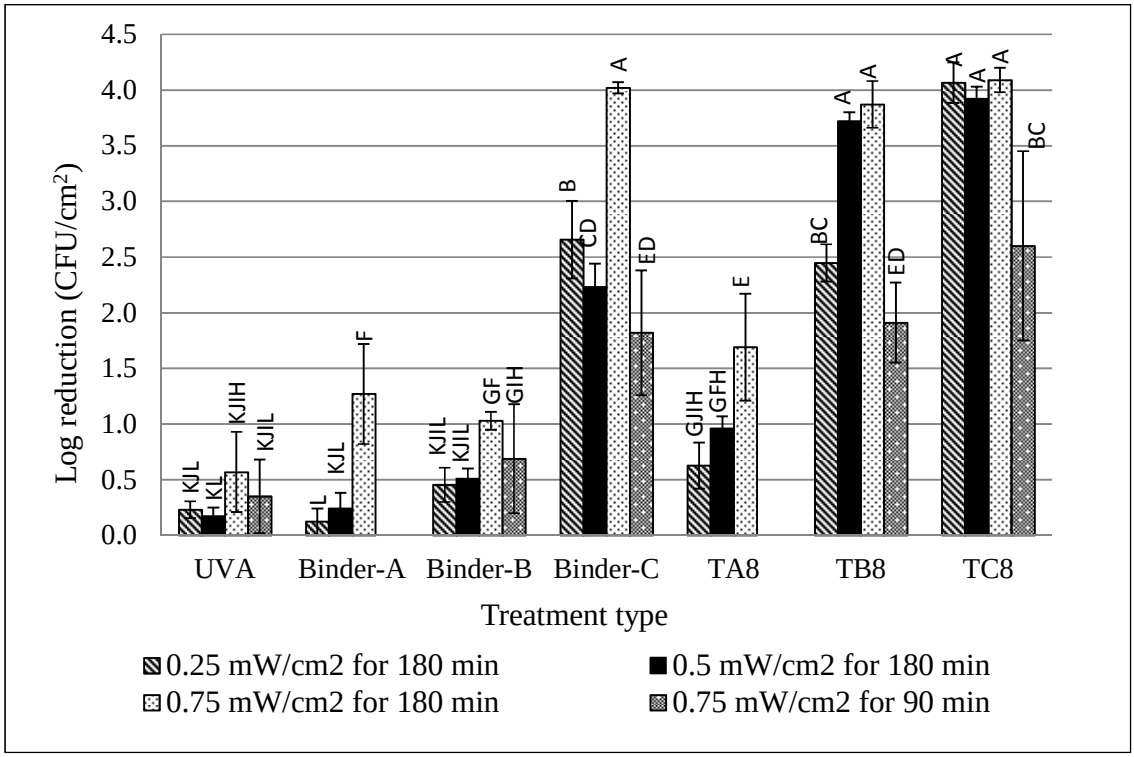
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Log reduction (CFU/cm2)
G
GF
F
C
E
E
D
A
B
A
UV-A
Binder A
Binder B
Binder C
TA8
TA4
TB16
TB8
TC16
TC8
Treatment type


Log reduction (CFU/cm²)
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4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
KJL
KL
KJIH
KJIL
IL
KJL
F
KJIL
KJIL
GF
GIH
B
CD
A
ED
GJIH
GFH
E
BC
A
A
ED
A
A
BC
UVA
Binder-A
Binder-B
Binder-C
TA8
TB8
TC8
Treatment type
0.25 mW/cm2 for 180 min
0.5 mW/cm2 for 180 min
0.75 mW/cm2 for 180 min
0.75 mW/cm2 for 90 min

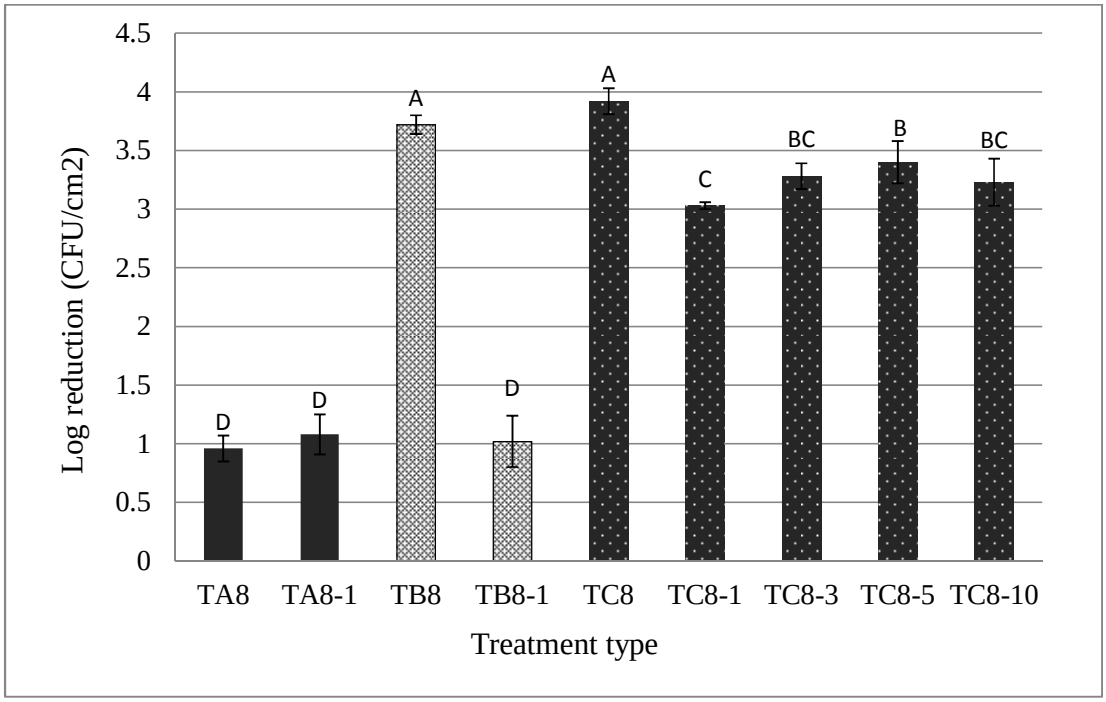
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Log reduction (CFU/cm2)
D
D
A
D
A
C
BC
B
BC
TA8
TA8-1
TB8
TB8-1
TC8
TC8-1
TC8-3
TC8-5
TC8-10
Treatment type

Table 1. Details of the binders and the composition of different TiO_2 nanocoatings

Sample	**Description**
TiO_2	TiO_2 Aeroxide® P25, surface area 50 m^2 g^{-1} and particle size ~21 nm Sigma-Aldrich, St. Louis, MO, USA
Binder A	Shellac a natural resin Zinsser Co., Inc. Somerset, NJ, USA
Binder B	Water based oil modified polyurethane MinWax®, MinWax company, Upper saddle river, NJ, USA
Binder C	Water based polyacrylic MinWax®, MinWax company, Upper saddle river, NJ, USA
TA4	Nanocoating with TiO_2 and binder A at 1:4 weight ratio
TA8	Nanocoating with TiO_2 and binder A at 1:8 weight ratio
TB8	Nanocoating with TiO_2 and binder B at 1:8 weight ratio
TB16	Nanocoating with TiO_2 and binder B at 1:16 weight ratio
TC8	Nanocoating with TiO_2 and binder C at 1:8 weight ratio
TC16	Nanocoating with TiO_2 and binder C at 1:16 weight ratio

Table 2. Estimated surface coverage of the nanocoatings with the binder and the TiO_2 nanoparticles

Sample code[1]	Percent surface coverage based on SEM image analysis (Estimate only)			
	Binder	Exposed TiO_2	Unexposed TiO_2	Total TiO_2
TA4	38	8	54	62
TA8	39	3	58	61
TB8	21	2	77	79
TB16	33	5	62	67
TC8	39	3	58	61
TC16	43	2	55	57

[1] TA4 is the TiO_2 coating with binder A at 1:4 NP to binder weight ratio
TA8, TB8, and TC8 are the TiO_2 coatings with binders A, B, and C at 1:8 NP to binder weight ratio.
TB16, and TC16 are TiO_2 coating with binder B and C at 1:16 NP to binder weight ratio.

Table 3. Physical stability of TiO_2 coatings before and after repeated use experiment

Coating type[1]	**No of times used**	**Thickness (µm)**		**Adhesion rating**		**Hardness (GPa)**	
		Before	**After**	**Before**	**After**	**Before**	**After**
TA8	1	74^{ab}	51^{bcd}	3B	3B	0.14^{f}	0.42^{e}
TB8	1	51^{bcd}	36^{d}	4B	3B	1.08^{a}	0.61^{dce}
TC8	1	97^{a}	85^{a}	4B	4B	0.68^{dc}	0.53^{de}
TC8	3	97^{a}	60^{bc}	4B	4B	0.68^{dc}	0.90^{ba}
TC8	5	97^{a}	50^{cd}	4B	4B	0.68^{dc}	0.90^{ba}
TC8	10	97^{a}	45^{cd}	4B	3B	0.68^{dc}	0.77^{bc}

[1]TA8, TB8, and TC8 are TiO_2 coatings with binders A, B, and C at 1:8 NP to binder weight ratios.
Mean values with same low case superscript within the same variable are not significantly different (P>0.05)